BACTERIOLOGICAL REVIEWS, June 1969, p. 264-301 Vol. 33, No. 2
 Printed in U.S.A.

Mechanism of Protein Biosynthesis

PETER LENGYEL AND DIETER SÖLL

Department of Molecular Biophysics, Yale University, New Haven, Connecticut 06520

INTRODUCTION

The amino acid sequence of a particular protein is specified by the sequence of nucleotides in a particular segment of the deoxyribonucleic acid (DNA). The process of protein synthesis consists of two stages. First, the DNA is transcribed into a ribonucleic acid (RNA) intermediate, messenger RNA (mRNA), which has a ribonucleotide sequence complementary to that of the deoxyribonucleotide sequence of one of the strands of the DNA serving as template (transcription) (120). The mRNA becomes attached to cytoplasmic ribonucleoprotein particles (ribosomes) which are the sites of protein synthesis, and there it determines the order of linkage of amino acids into a specific protein (translation) (17, 243, 291). The mRNA is translated in the 5′ to 3′ direction (291). The synthesis of a protein is initiated at the amino-terminal amino acid and proceeds towards the carboxy-terminal amino acid (17, 32*a*, 84*a*, 243, 291). During translation, a group of three adjacent nucleotides in the mRNA (codon) specifies which amino acid is to be linked to the growing peptide chain. It has been established which codons specify each of the 20 amino acids (74). (This is the genetic code). It appears that the sequence of amino acids in a polypeptide chain contains all of the information required for generating the three-dimensional structure of the native protein molecule (chain folding) (13). The topic of this review is restricted to certain aspects of the mechanism of translation, mainly, although not exclusively, as elucidated in microorganisms. The regulation of protein synthesis (99) is not discussed. A collection of significant investigations on mammalian protein synthesis was presented in a volume dedicated to the memory of R. Schweet (14). Similar studies in plant systems have been described recently (5).

Abbreviations

Shorthand writing of oligonucleotides and polynucleotides and abbreviations for nucleotides, amino-acid residues, etc., are as recommended in J. Biol. Chem. **241**:527 (1966).

In addition to those identified in the text, abbreviations are used as follows: RNA capable of accepting, for example, glycine, $tRNA^{Gly}$; aminoacyl-tRNA, AA-tRNA (e.g., Gly-tRNA); mischarged tRNA species, for example, Ala-$tRNA^{Cys}$; different tRNA species capable of accepting the same amino acid, isoaccepting tRNAs; *N*-acetyl AA-tRNA, acAA-tRNA (e.g., acPhe-tRNA); phe-phe-tRNA, diphe-tRNA; phe-phe-phe-tRNA, triphe-tRNA; aminoacyl oligonucleotides derived from AA-tRNA, for example, CpA-Gly or CACCA-acLeu; AA-tRNA synthetase aminoacyladenylate complex, E-AA-AMP; mRNA which is translated into more that one polypeptide, polygenic mRNA; polynucleotides with random sequence (e.g., a polymer containing adenylate, uridylate, and guanylate units), poly (A, U, G). Trinucleotide codons are shown by base initials (e.g., ApUpG, AUG).

Cell-free Protein Synthesizing Systems

In vitro systems have been a major tool for examining the mechanism of protein biosynthesis. They can be prepared (277) by disintegrating cells in aqueous media, removing unbroken cells and cell debris by low-speed centrifugation and small molecules by dialysis. To observe protein synthesis with such extracts, one requires the addition of adenosine triphosphate (ATP), guanosine triphosphate (GTP), an ATP-generating system, proper ions (Mg^{++} and either K^+ or NH_4^+), sulfhydryl compounds (which were found to stabilize the system), and amino acids (some of which are usually radioactively labeled). In such a system, mRNA can be translated into protein. The translation is assayed by following the incorporation of labeled amino acids into protein. The messenger may be present in the extract (endogenous messenger), or it may be added (exogenous messenger). (In the latter case, the endogenous messenger is usually inactivated by incubating the extract before the amino acid incorporation experiment to provide time for the nucleases in the extract to degrade the endogenous mRNA.) The exogenous messenger can be either a natural or a synthetic polyribonucleotide. The use of synthetic polyribonucleotides of known composition or sequence was of utmost significance in deciphering the genetic code (214, 279, 280).

The cell extract can be further fractionated by centrifugation at high speed. The resulting supernatant fraction contains the following compo-

nents of the protein synthesizing machinery: tRNA (about 40 species), AA-tRNA synthetases (at least 20 species), and proteins involved in the elongation and termination of the peptide chain (at least 5 species). The resulting pellet contains: ribosomes (built from at least three kinds of ribosomal RNA and about 50 different kinds of ribosomal protein) and proteins required for peptide chain initiation (at least 3 species). The initiation factors can be removed from the ribosomes by washing with a buffer of high ionic strength. At this time more than 120 different macromolecules are known to be involved in the process of translation.

AA-tRNA SYNTHETASES

In every organism in which this was tested, there exist at least 20 AA-tRNA synthetases which link the correct amino acid presumably to the 3'-hydroxyl (246, 356) of the terminal adenosine of a specific tRNA molecule (*see* section on tRNA). The formation of AA-tRNA catalyzed by the AA-tRNA synthetase is a two-step reaction involving activation and transfer. The product of the activation step is the enzyme-bound amino-pyrophosphate (PP_i) the enzyme catalyzes the breakdown of the complex to ATP and amino acid. The incorporation of $^{32}PP_i$ into ATP is commonly used to estimate the formation of E-AA-AMP (ATP-PP_i exchange). Another assay is the reaction of E-AA-AMP with hydroxylamine to form the hydroxamate of the amino acid and AMP (*see* 307). In the transfer step, the activated amino acid is transferred onto the tRNA (a transacylation) to form AA-tRNA, the production of which can be measured directly.

The specificity of AA-tRNA synthetases, a prerequisite for faithful translation of the genetic message, must be very high (223, 290, 307) and is exercised at the level of amino acid activation as well as in the transfer step. The specificity in the latter step is higher than in the former; i.e., besides activating their cognate amino acids, Ile-tRNA synthetase activates valine and Val-tRNA synthetase activates threonine (21, 29, 157). These "wrong" amino acids are not then transferred to the tRNA, however. Certain amino acid analogues, however, do get charged onto tRNA (*see* 52, 274) and are subsequently incorporated into protein.

Although all of these enzymes perform the same function, there is no selective pressure to ensure that all of these molecules be very similar. Each aminoacyl-tRNA synthetase charges one or very few tRNA species. Consequently, mutations that change the mode of recognition between the enzyme and its substrates might be tolerated by the organism. Thus, the mode of recognition between the particular AA-tRNA synthetases and their substrates need not be the same, and a range of variation in physical and other characteristics of AA-tRNA synthetases does exist.

Since many aspects of AA-tRNA synthetases and amino acid activation have been discussed in

TABLE 1. *Purified AA-tRNA synthetases*

Amino acid	Organism	Mol wt (× 10^3)	($S_{20,w}$)	Reference
Arginine	*Escherichia coli*			253
Arginine	Yeast			233
Glutamic acid	*E. coli*			204
Glutamine	*E. coli*			204
Glycine	*Staphylococcus aureus*			283
Isoleucine	*E. coli*	112	5.9	21
Leucine	Yeast			233
Lysine	*E. coli*	100	5.8	396
Lysine	*E. coli*			362
Methionine	*E. coli*	173	6.5	212
Methionine	*E. coli*	96		47
Phenylalanine	*E. coli*	181	8.6	364
Phenylalanine	Yeast	180	8.2	234
Proline	*E. coli*			247
Serine	Yeast	89	6.7	234
Serine	*E. coli*			174
Threonine	*E. coli*	117		156
Threonine	Rat liver			4
Tyrosine	*E. coli*	95	5.2	53
Tyrosine	*Bacillus subtilis*	88	5.2	53
Valine	*E. coli*		4.3	121
Valine	Yeast	112	5.5	201

more detail previously (290, 307), only a few selected points will be presented here.

Isolation and Properties of AA-tRNA Synthetases

To date, a large number of AA-tRNA synthetases from various sources have been purified (Table 1). Most of the known enzymes have a molecular weight of around 100,000 (*see* Table 1 and reference 269). However, Phe-tRNA synthetases from yeast (234) and *Escherichia coli* (364), as well as Ala-tRNA (269) and Met-tRNA (212) synthetases of *E. coli*, have a molecular weight of about 173,000. The amino acid composition of a number of AA-tRNA synthetases has been determined, but there is much less information about their physical structure. In a few cases, AA-tRNA synthetases have been dissociated by such reagents as urea or guanidinium chloride into subunits of molecular weight about 50,000. It is not known whether the subunits formed are identical or different. For Pro-tRNA synthetase, no enzymatic activity was found in subunits of molecular weight 47,000 (210). The molecular weight of Met-tRNA synthetase purified from *E. coli* K was reported to be 173,000 (212). On incubating this enzyme with a factor isolated from the 100,000-g pellet fraction of the crude extract, it is depolymerized into enzymatically active synthetase molecules of molecular weight 54,000. Treatment of the high-molecular-weight enzyme with dissociating agents (e.g., 8 M urea) results in subunits of molecular weight 43,000 whose enzymatic activity has not yet been determined (65). Met-tRNA synthetase was also isolated (by a method different from the one used for the *E. coli* K enzyme) from another strain of *E. coli* [MRE 244 (47)]. The molecular weight of this enzyme was reported as 96,000.

The kinetic parameters of the AA-tRNA synthetases, in the cases where they have been determined, seem to ve very similar. Measured in the overall reaction (aminoacylation of tRNA) the K_m for the amino acid is between 10^{-6} and 10^{-4} M; for ATP, around 10^{-5} M; and for tRNA, between 10^{-7} and 10^{-6} M. The K_m for ATP and amino acid in the activation step depends upon the nature of the assay and is different for the same AA-tRNA synthetase when measured by ATP-PP_i exchange or by hydroxamate formation (*see* below). The various AA-tRNA synthetases have different optimal requirements for ATP, Mg^{++}, and other ions.

The kinetic methods in current use are still insufficiently precise and too slow to allow detailed unraveling of the exact sequence of reaction steps of amino acid activation (leading to E-AA-AMP formation) and tRNA acylation. In two cases, however, a complex of ATP and the AA-tRNA synthetase has been isolated. This might indicate that ATP is bound to the enzyme before the amino acid (4, 328).

Some lines of evidence suggest that the site for amino acid activation is different from the site for amino acid transfer (64). When *E. coli* Met-tRNA synthetase was treated with parahydroxymercuri benzoate (pHMB) in the presence of methioninyladenylate, a structural analogue of methionyladenylate, the enzyme retained the ability to activate methionine [as tested by ATP-PP_i exchange), but could no longer catalyze the formation of Met-tRNA. The reaction with pHMB did not alter the molecular weight of the enzyme. This suggests that the sites for the two reaction steps are different. "Cold" treatment of *E. coli* Pro-tRNA synthetase was reported to impair the transfer step to a much larger extent than the activation step (300). Whether this is due to dissociation of the enzyme into subunits at low temperatures remains to be determined (210).

Reaction Mechanism

In the activation step the AA-tRNA synthetase catalyzes the formation of the aminoacyladenylate, which remains bound to the enzyme and does not accumulate as a free intermediate. The activation reaction can be followed readily by ATP-PP_i exchange for all synthetases, but the product aminoacyladenylate is not always detected in the alternate assay (hydroxamate formation). This was demonstrated in a comparative study of 14 *E. coli* AA-tRNA synthetases (157). The initial rates of the overall reaction (the formation of AA-tRNA) and of the activation step (the formation of hydroxylamine-sensitive aminoacyladenylate) were measured. For some enzymes, the same rate was observed by each assay, but for others, no hydroxamate formation was detectable. Thr-tRNA synthetase, for instance, forms threonyladenylate (as shown by ATP-PP_i exchange as well as by isolation of the E-Thr-AMP complex), but this does not react with hydroxylamine under standard conditions (156). After the addition of tRNA, threonylhydroxamate is formed by a nonenzymatic reaction of Thr-tRNA with hydroxylamine. It was suggested that the threonyladenylate is buried inside the enzyme molecule, inaccessible to hydroxylamine. Similar lack of hydroxamate formation was shown for Thr-tRNA synthetase of calf liver (219).

Although the formation of E-AA-AMP requires in most cases only the presence of amino acid, ATP, and Mg^{++}, a few cases have been found in which the presence of tRNA is required for amino acid activation. Glu-tRNA synthetase from *E. coli*, pork liver, and yeast (209, 317) catalyzes glutamyladenylate formation only at

very high glutamate concentrations. However, in the presence of tRNA, very low glutamate concentrations are sufficient for synthesis. The $tRNA^{Gln}$ is strictly required under any conditions for the formation of glutaminyladenylate by its cognate enzyme in the same three organisms. The same is true for *E. coli* Arg-tRNA synthetase (248) and rat liver Gly-tRNA synthetase (105). In the latter case, however, the activation was measured only by hydroxamate formation, and thus the conclusion may be invalidated by the limitations of the hydroxamate method discussed above. It was reported that tRNA activates arginyl-adenylate formation catalyzed by Arg-tRNA synthetase (252). It is possible that the apparent need for tRNA in amino acid activation in the case of certain synthetases, but not in that of others, reflects that the order of binding of the substrates (ATP, amino acid, tRNA) to the synthetases may not be the same for all enzymes.

The E-AA-AMP complex has been isolated by gel filtration for a variety of enzymes (4, 6, 33, 136, 200, 289, 397). The isolated complex can be used in the elucidation of the reaction mechanism of the transfer step. Differences between enzymes are apparent in the requirement for inclusion of divalent metal ions in the reaction mixture in which the transfer of the amino acid from the complex onto the tRNA is tested. The transfer proceeds without metal ions in the case of *E. coli* Ile-tRNA (289) and Lys-tRNA (397) synthetase as well as yeast Val-tRNA (200) synthetase. The transfer requires Mg^{++} ions in the case of Thr-tRNA synthetase from rat liver (4) and from *E. coli* (156), Ile-tRNA synthetase from *Bacillus stearothermophilus* (136), and Ser-tRNA synthetase from yeast (33). Some other divalent metal ions may substitute for Mg^{++} (156).

Complexes Between AA-tRNA Synthetases and tRNA

Stable complexes between tRNA and its cognate AA-tRNA synthetase have been obtained with yeast Val-tRNA synthetase (199, 200) and Leu-tRNA synthetase (341), as well as with Ile- and Tyr-tRNA synthetase from *E. coli* (418) and Arg-, Gly-, and Val-tRNA synthetase from *B. stearothermophilus* (294). The tRNA—AA-tRNA synthetase complexes, isolated by gel filtration on Sephadex G-100, by sucrose density gradient centrifugation, and by electrophoresis, have been shown to be enzymatically active. If the stable complex is isolated on a membrane filter (Millipore Corp., Bedford, Mass.), however, the enzyme is completely inactivated (418). The complex formation is usually specific for the tRNA and its cognate AA-tRNA synthetase. Lack of specificity has been observed (199), however, at low salt concentrations. Complex formation between synthetases and tRNA from heterologous systems also occurs (294).

Whether the acceptor end of the tRNA molecule is involved in this complex formation remains controversial (200, 293, 294). In some cases, periodate-oxidized tRNA will not bind to or inhibit AA-tRNA synthetase, whereas the opposite is true in other cases. These findings suggest that the process of recognition between tRNA and its cognate AA-tRNA synthetase may vary from case to case.

Genetics of Synthetases

Mutants for a number of AA-tRNA synthetases have been isolated from or found in *E. coli*, *Salmonella typhimurium*, and yeast (*see* Table 2). The genetic loci of the *E. coli* AA-tRNA synthetases (as far as they have been determined) are scattered all over the chromosome. The temperature-sensitive synthetase mutants display little activity in in vitro tests even at low temperature, and some possess a decreased affinity for amino acid, ATP, or both. In some cases, they can be protected against heat denaturation by the presence of amino acid, ATP, or Mg^{++}. Most of the present data on *E. coli* are consistent with the existence of only one AA-tRNA synthetase for each amino acid. It remains to be seen whether reports of several AA-tRNA synthetases for the same amino acid in *E. coli* (66, 420) could possibly be attributed to aggregates or to tRNA—AA-tRNA synthetase complexes (341). In higher organisms, the occurrence of multiple synthetases (22, 164, 393) is partly accounted for by the recent discovery (24, 48) of different AA-tRNA synthetases (specific for the same amino acid) in the mitochondria and the cytoplasm of the same cell. It has been shown that a mitochondrial Leu-tRNA synthetase is specified by a nuclear gene (137).

TRANSFER RNA

Sequence of tRNA

Since the elucidation of the first tRNA sequence (160), great progress in tRNA fractionation techniques (126, 127) and sequencing methods (334) stimulated the pace of tRNA sequence analysis. At present, the primary sequences of 17 tRNA species from bakers' (90, 160, 231, 273, 312) and brewers' (423) yeast, *E. coli* K-12 (76, 89, 129) and B (M. Uziel, *unpublished data*; U. L. RajBhandary, *unpublished data*), *Torulopsis utilis* (379, 380), rat liver (360), and wheat germ (90) have been established. The chain length of the

TABLE 2. *AA-tRNA synthetase mutants*

Amino acid	Organism	Genetic location	Reference
Alanine	*Escherichia coli*	Between *purC* and *thy*	416
Arginine	*E. coli*	between *aroD* and *his*	158
Glycine	*E. coli*	Near *xyl*	35
Histidine	*E. coli*		268
Histidine	*Salmonella*	Near *strB*	326
Isoleucine	*E. coli*		374
Isoleucine	Yeast		148
Phenylalanine	*E. coli*	At min 33[a]	92, 104, 173
Serine	*E. coli*	Between min 17 and 19[a]	159
Tryptophan	*E. coli*	Between *str* and *malA*	88
Valine	*E. coli*	At min 87[a]	92, 173, 415

[a] On the Taylor map (384).

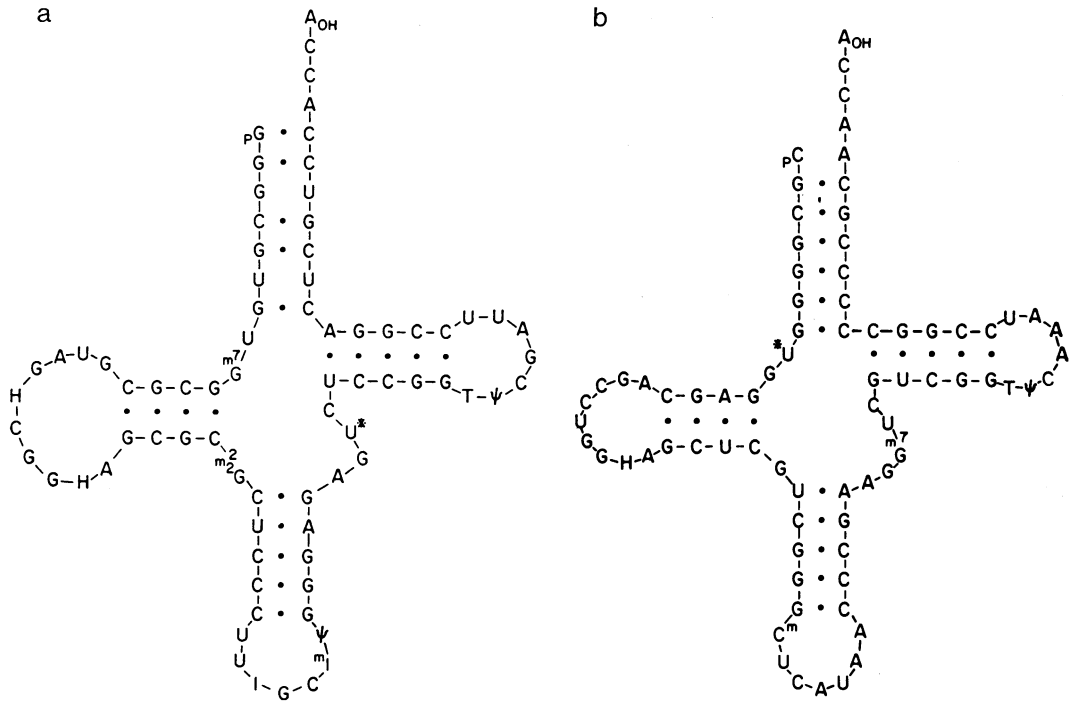

FIG. 1. *(a) Nucleotide sequence of a yeast tRNA^{Ala} (84). (b) Nucleotide sequence of an E. coli tRNA^{fMet} (94). Both sequences are shown in a cloverleaf-type arrangement. Minor nucleosides: G^{m7},N^{7}-methylguanosine; H, dihydrouridine; G$^{m_2^2}$, N^2-dimethyl-guanosine; ^mI,methylinosine; ψ, pseudouridine; U* (Fig. 1a), a mixture of U and H; U* (Fig. 1b), nature of base still in doubt; T, ribothymidine; C^m,2'O-methylcytidine.*

known tRNA molecules varies between 75 and 85 nucleotides, and their content of minor bases (143) between 10 and 20%. The primary sequences of all these tRNAs can be arranged (satisfying base-pairing requirements) in a cloverleaf model (230; Fig. 1). The additional bases of the larger tRNA are taken up in the minor loop. With the exception of the anticodon and the CCA-acceptor end, no structural features can yet be assigned to the other functions of the tRNA molecule. However, tRNA^{fMet} exhibits unusual base pairing near the 5' and 3' end of the molecule. In all other tRNAs of known sequence (e.g., in tRNA^{Ala} from yeast, whose base sequence is shown in Fig. 1a), base pairing starts at base number 5, measured from the amino acid acceptor end, whereas in tRNA^{fMet} (Fig. 1b) base pairing starts at base number 6 (89). Many attempts to determine the secondary (85, 275) and tertiary (202) structure of tRNA have been

made (114), so far providing no really convincing evidence for any model. Recent progress in the crystallization of tRNA from *E. coli* and yeast (69, 78, 113, 146, 180) has encouraged the hope that the tertiary structure of tRNA will be determined by X-ray crystallography. Crystallization of the complex of tRNA and its cognate AA-tRNA synthetase may prove even more illuminating.

Genetics of tRNA

So far tRNA has been accessible for genetic studies only in very rare cases. Nonsense mutations of the amber and ochre type are known to cause premature peptide chain termination (117), and their suppression in *E. coli* has been shown to be due in some cases to a suppressor tRNA (59, 97, 129, 130, 352, 411). The anticodons of the tyrosine suppressor tRNA and of the corresponding $tRNA^{tyr}$ species from a strain not carrying the suppressor mutation differ in a single nucleotide (129). This provides evidence for the assumption that the suppressor mutation occurred in the structural gene for the tRNA. The genetic loci for the suppressor tRNA are distributed all over the chromosome. Work on the regulation of histidine biosynthesis in *S. typhimurium* has revealed a class of mutants believed to be mutants in $tRNA^{his}$ (347).

Recognition of tRNA by the AA-tRNA Synthetase

The question of the specific recognition site on the tRNA for its cognate AA-tRNA synthetase is the oldest and still foremost open question in tRNA research. Whereas some earlier experiments were taken to support the view that the anticodon is a specific recognition site for the AA-tRNA synthetase (254), there is mounting evidence that this may not be so, at least not for all tRNA. The *E. coli* tyrosine amber suppressor tRNA, which differs by a single base in the anticodon from its "parental" $tRNA^{tyr}$ (129), is active in in vivo and in vitro suppression (119). This shows that the tRNA with a changed anticodon is still recognized by the AA-tRNA synthetase. Chemical modification of pure tRNA species with known sequences also supports this conclusion. *Torulopsis* $tRNA^{tyr}$ (150), cyanoethylated only in the pseudouridine residue of the anticodon, could be charged by purified Tyr-tRNA synthetase to the same extent as unmodified tRNA, albeit at a much slower rate. Similar results were obtained in experiments with yeast $tRNA^{ala}$ (419). Treatment of a $tRNA^{gly}$ with nitrous acid resulted in a change in its coding specificity (60). The tRNA (modified presumably in the anticodon) could be charged by the Gly-tRNA synthetase, although at a slower rate (61). Modification of a base next to the anticodon [i.e., the isopentenyladenosine (ipA) residue in $tRNA^{ser}$ (106) and $tRNA^{tyr}$ (119)], or the elimination of a base (386), do not impair the ability of the tRNA to be charged. The tRNA partially or fully lost their ability, however, to participate in protein synthesis, probably because of a different conformation of the anticodon loop. Experiments with isoaccepting *E. coli* $tRNA^{ser}$ species also support the view that the anticodon need not be the specific recognition site for the AA-tRNA synthetase (372). Two $tRNA^{Ser}$ species specified by entirely unrelated codons (UCA and AGU) could be charged to the same extent and at the same rate by a purified *E. coli* Ser-tRNA synthetase preparation. The two tRNA species competed for the Ser-tRNA synthetase, indicating that they were recognized by the same enzyme. In a different kind of experiment, pure yeast $tRNA^{Val}$ was split by T_1-ribonuclease next to I in the anticodon sequence. The separated half-molecules did not possess acceptor activity. The mixture of both halves, however, accepted almost the theoretical amount of valine despite the break in the polynucleotide chain (25). This shows that the anticodon does not need to be intact for recognition by the AA-tRNA synthetase.

According to a recent hypothesis (345) based on studies of ultraviolet-inactivation of $tRNA^{Ala}$, the three base pairs closest to the acceptor end of a tRNA molecule represent the specific recognition site for the AA-tRNA synthetase. Two sets of experiments, however, cast some doubt on the general validity of this hypothesis. Rat liver $tRNA^{Ser}$ can be charged by yeast Ser-tRNA synthetase as well as by the homologous enzyme, although the nucleotide sequences in the presumed recognition site differ in two out of three base pairs in rat liver and yeast $tRNA^{Ser}$ (360). Likewise, yeast $tRNA^{fMet}$ with a sequence different from the corresponding *E. coli* species can be charged by the *E. coli* Met-tRNA synthetase (313). However, in both cases, tRNA was charged by heterologous enzymes and, therefore, these results may not serve as a firm basis for invalidating the above hypothesis. The large degree of homology in the structures of the various tRNA makes it difficult to recognize a specific contiguous nucleotide sequence in an identical position in all tRNA as recognition site for the AA-tRNA synthetase. The process may involve the recognition of features of tertiary structure, as has been suggested (77).

Minor and Redundant tRNA Species

E. coli tRNA can be separated into a large number of distinct fractions by chromatographic

techniques (264, 408). Artifacts of preparation and chromatography resulting in tRNA aggregates (222, 354, 422) and in inactive (118, 217) and partially modified tRNA species may account for some of the fractions. However, there is cause to believe that many fractions are distinct minor tRNA species. In a few cases, these have been shown to be redundant tRNA (multiple isoaccepting tRNA specific for the same codon) (353, 354). The existence of redundant tRNA may explain certain mechanisms of nonsense or missense suppression (129, 139). At least two species of *E. coli* $tRNA^{Tyr}$ recognize the same codons. A mutation in one of the $tRNA^{Tyr}$ genes resulting in a change in the anticodon of a $tRNA^{Tyr}$ has been shown to be the origin of the tyrosine suppressor tRNA (129). This tRNA no longer recognizes the normal tyrosine codons, but reads an amber triplet as though it were a tyrosine codon. The remaining $tRNA^{Tyr}$ species (not affected by this mutation) are sufficient for supporting normal protein synthesis. Observations on the number of different tRNA species are valid only in haploid organisms (having only one chromosome per cell), since mutations in one of duplicate tRNA genes could lead to redundant tRNA.

The composition of the tRNA population may vary in cells from different stages of growth (187; J. Bartz and D. Söll, *unpublished data*) and in cells grown in different conditions (86, 198). This poses the fundamental questions of how tRNA synthesis is regulated and why an organism maintains the production of minor redundant tRNA. It is possible that the latter species are used for yet unknown functions of tRNA in regulating and maintaining cell growth.

Virus Infection and tRNA

The changes in tRNA after infection of *E. coli* B with T2 or T4 phage have been studied extensively (370). Alterations in tRNA depend on protein synthesis; they are not observed if chloramphenicol is added prior to phage infection. The changes after infection are displayed in the same fashion whether the tRNA is charged with AA-tRNA synthetases derived from infected or uninfected cells, and they can be divided into two classes: inactivation of host-specific tRNA and appearance of phage-coded tRNA.

Inactivation of host tRNA. A change in the Leu-$tRNA^{leu}$ pattern on Methylated Albumin-kieselguhr (MAK) chromatography has been shown after T2 infection (172, 370). More recent studies involving fractionation of Leu-$tRNA^{leu}$ by reverse phase chromatography provide evidence for the selective inactivation of the major $tRNA^{Leu}$ species (399, 400) and may support the notion (400) that this is due to the action of a phage-induced nuclease specific for this species. Concomitant with the inactivation of the major $tRNA^{Leu}$ is the appearance of new, possibly phage-coded, $tRNA^{Leu}$ species, as revealed by reverse phase chromatography (170, 172). Binding studies with fractions of Leu-$tRNA^{Leu}$ from infected and uninfected cells were not completely successful in elucidating the coding response of the various $tRNA^{Leu}$ species (171).

Phage-coded tRNA. The studies of cysteine-dependent thiolation of tRNA (163) provided another tool for testing tRNA after phage infection. In uninfected and phage-infected cells grown on ^{35}S-sodium sulfate, all sulfur-containing RNA proved to be 4*S* RNA, the elution profiles of which on MAK columns were different for tRNA from infected and uninfected cells (163). The material of the major new peak from infected cells does not hybridize with *E. coli* DNA, but it hybridizes with T4 DNA; T4 mRNA and *E. coli* tRNA do not compete in this reaction (407). Using the elegant technique of hybridizing charged tRNA at low temperature, it was shown that at least one species of $tRNA^{Leu}$ and of $tRNA^{Pro}$ was coded by T2 phage (407).

Only sulfur-containing tRNA species can be detected by the above method. The failure to observe gross tRNA changes in cells infected with T-odd, MS2, or ϕX 174 phages, therefore, does not exclude the possibility that tRNA species are coded by these phages.

Herpes simplex virus was shown to specify at least one species of $tRNA^{Arg}$ (367, 368). The multiplication of the oncogenic BS1 virus was reported to be associated with the appearance of virus-specific tRNA (391).

Cytokinins and tRNA

The cytokinins are substances which promote cell division, growth, and organ formation in plants. Their activity is usually measured by the increase caused in the cell mass of tobacco stem (151). Of all the cellular components, tRNA is uniquely associated with cytokinin activity. Such activity was found in the tRNA of bacteria, plants, and mammals.

Since the discovery of ipA, a very potent cytokinin, as a constituent of yeast tRNA (31, 145), many examples of cytokinin-active nucleotides in crude and purified tRNA preparations have been reported (119, 144, 147, 231, 324, 348, 360, 379, 423). For instance, the derivative of ipA, N^6-(*cis*-4-hydroxy-3-methyl-but-2-enyl) adenosine, occurs in the tRNA of plant tissues (144) and its 2-meth-

ylthio-derivative has been found in *E. coli* tRNA (51). Fractionation of crude *E. coli* tRNA revealed cytokinin activity in $tRNA^{Try}$, $tRNA^{Tyr}$, $tRNA^{phe}$, $tRNA^{Ser}$, and $tRNA^{Leu}$ (16). It is interesting to note that the cytokinin-active *E. coli* tRNA species all recognize codons beginning with U. In the cytokinin-active tRNA of known sequence, ipA or its methylthio derivative is located adjacent to the anticodon (119, 231, 360, 379, 423; M. Uziel, *unpublished data*, and U. L. RajBhandary, *unpublished data*). The participation of the ipA residue in maintaining the fidelity of translation and the tertiary structure of the tRNA is evident in the following experiments. Treatment of $tRNA^{Ser}$ with iodine leads to a tRNA with a modified ipA residue (106). The modified tRNA showed decreased binding to ribosomes in the presence of the appropriate mRNA, but retained the ability to be charged by its cognate AA-tRNA synthetase. *E. coli* suppressor $tRNA^{tyr}$ was obtained in several forms which differ in the base adjacent to the 3′ nucleotide of the anticodon. The tRNA containing A or ipA in this position were much less active in in vitro suppression than was the tRNA with 2-methylthio-ipA (119). Whether these effects on coding properties play a role in the mechanism of action of the cytokinins remains to be determined.

Treatment of tRNA with $KMnO_4$ converts ipA residues to A residues; tRNA treated with $KMnO_4$ was added to rat liver or yeast extract supplemented with labeled mevalonate or isopentenylpyrophosphate. It was established that in the extract isopentenyl side chains became linked to A residues of the treated tRNA. This linkage is catalyzed by an enzyme which is specific for certain A residues in selected tRNA molecules (107, 185).

Cell Wall Synthesis and tRNA

A novel role for tRNA has recently been discovered: it participates in the synthesis of bacterial cell walls and aminoacylphosphatidylglycerols, compounds found in certain bacteria. In in vitro experiments with four different bacterial strains, a strict requirement has been shown for tRNA in the biosynthesis of the pentapeptide bridges linking the peptidoglycan strands of bacterial cell walls (49, 242, 308, 322, 323). Gly-tRNA in *Staphylococcus aureus* (49, 242), Thr-tRNA in *Micrococcus roseus* (323), Ser- and Gly-tRNA in *S. epidermidis* (308), and Ala-tRNA in *Arthrobacter crystallopoietes* (322) transfer their respective amino acids into the bridge. This reaction is catalyzed by a particulate enzyme fraction. The process is specific for the amino acid. Heterologous tRNA can replace the homologous tRNA in certain cases (242, 308, 322, 323). The substrate specificity of the enzyme for the tRNA was demonstrated in the following experiment: Ala-$tRNA^{Cys}$ [prepared by desulfurization with Raney nickel from Cys-$tRNA^{Cys}$ (67)] did not substitute for Ala-$tRNA^{Ala}$ in the bridge peptide formation as catalyzed by the enzyme fraction from *A. crystallopoietes* (322). The role of tRNA was further clarified by fractionation of crude tRNA. Gly-tRNA, Ser-tRNA, and Thr-tRNA obtained from *S. aureus*, *S. epidermidis*, and *M. roseus*, respectively, were fractionated into a number of isoaccepting tRNA species with different coding properties. All these tRNA species participated in in vitro peptide bridge formation. Some of them, however, did not bind to ribosomes in the presence of the corresponding trinucleotide codons and did not support in vitro protein synthesis directed by synthetic and natural messengers. Thus, it seems unlikely that the anticodon is involved in the tRNA specificity.

Studies with a purified Gly-tRNA synthetase from *S. aureus* have indicated that the same enzyme charges tRNA for protein synthesis and for peptidoglycan synthesis (283). The mechanism of tRNA participation in the peptide bridge formation is not clear. The process is distinctly different from protein biosynthesis since it proceeds apparently without ribosomes and in the presence of antibiotics like puromycin or chloramphenicol, which are known to block protein synthesis. The synthesis of lysylphosphatidylglycerol in *S. aureus* requires Lys-tRNA, and also proceeds with heterologous tRNA (215). The high specificity of the aminoacylphosphatidylglycerol synthetases for tRNA and also for the amino acid has been shown in experiments with a series of chemically or enzymatically produced "mischarged" tRNA species (134, 276) which all possessed acceptor activity after modification. Thus, in the synthesis of alanylphosphatidylglycerol in extracts of *Clostridium welchii*, *N*-acetyl-Ala-$tRNA^{Ala}$, lactyl-$tRNA^{Ala}$, Ala-$tRNA^{Cys}$, Cys-$tRNA^{Cys}$, and Phe-$tRNA^{Ala}$ could not substitute for Ala-$tRNA^{Ala}$ (134).

Differentiated Cells and tRNA

Transfer RNA has been found in mitochondria isolated from yeast (412), *Neurospora* (23), *Tetrahymena* (373), and rat liver (48, 216). Differences have been shown in chromatographic properties between isoaccepting tRNA of mitochondrial and cytoplasmic origin from the same organism. It remains to be seen whether mitochondrial tRNA is transcribed from nuclear or from mitochondrial DNA (373).

Differences have also been found between the

chromatographic patterns of the tRNA extracted from various differentiated cells (208, 413), malignant cells (382), and cells involved in specific antibody response (414).

Other tRNA Reactions

Several reactions are known which involve modification of the aminoacyl residue of AA-tRNA. One is the formylation of Met-tRNA^{fMet} to yield fMet-tRNA^{fMet} (237). Another reaction is the conversion of glutamic acid attached to tRNA to glutamine in *B. megatherium* (410). A still uncharacterized enzyme catalyzes the formation of Glu-tRNA^{Gln}, which is converted subsequently to Gln-tRNA^{Gln} by a transamidase in the presence of an amide donor. The significance of this reaction is not known. The cyclization of the glutamine residue of *E. coli* Gln-tRNA^{Gln} to pyrrolidone carboxylate-tRNA^{Gln} by an enzyme from papaya latex has been reported recently (30). The enzyme displays no specificity for tRNA since it also cyclizes N-terminal Gln-residues of peptides.

RIBOSOMES

Ribosomes are large ribonucleoprotein particles on which the actual process of translation takes place. There are two functionally different subunits of *E. coli* ribosomes, the 30*S* and the 50*S* subunits. The 30*S* subunit consists of about 20 different proteins and one species of RNA (16*S*), whereas the 50*S* subunit contains about 30 different proteins (G. R. Craven and C. Kurland, *unpublished data*) and two species of RNA (5*S* and 23*S*). Heavy subunits of mammalian ribosomes have been reported to contain an additional RNA species (110, 304). The most dramatic progress in this field during the past 2 years has been the physical and chemical characterization of the components of *E. coli* ribosomes and the in vitro reconstitution of active ribosomes from separated nucleic acid and protein components. This discussion is restricted to the above subjects. Other aspects of the formation and structure of bacterial ribosomes have been summarized recently (298). The biosynthesis of ribosomes in animal cells has been discussed in a recent review (82).

Ribosomal Proteins of the 30S Subunit

Twenty-one protein components have been separated from the *E. coli* 30*S* subunits by column chromatography and by polyacrylamide gel electrophoresis (79, 109, 261, 389). Nineteen of these are different proteins since each of them has a unique amino acid composition and fingerprint pattern of its tryptic peptides. The molecular weights of the different ribosomal proteins vary from 5,000 to 63,000, although the exact molecular weight of each protein and the stoichiometry of these species in a 30*S* subunit is not yet agreed upon. The question of molecular weight is very important; if the molecular weights and stoichiometry reported by one group of investigators were correct, then the sum of the weights of individual proteins would be larger than the total amount of protein in a 30*S* subunit. The implication from these results would then be that there is more than one kind of ribosome (79).

The acrylamide gel electrophoretic patterns of the proteins from 30*S* subunits of *E. coli* strain K-12 and strain B differ in the position of a single band (205). The two proteins corresponding to these characteristic bands have similar molecular weights of about 20,000, but the proteins differ in at least 10 amino acid residues (32).

Studies comparing the ribosomal proteins of procaryotic and eucaryotic organisms are under way (68, 169, 363).

Ribosomal RNA

5S RNA. 5*S* RNA can be prepared by phenol extraction of whole cells (327) or from isolated ribosomes (122). With the exception of tRNA, it is the smallest RNA, having a chain length of 120 nucleotides and lacking minor bases. The nucleotide sequence of 5*S* RNA from *E. coli* (46) and from a human tumor line [KB (111)] has been established, and it is interesting to note that a part of the nucleotide sequence is duplicated in each of the two 5*S* RNA species. The sedimentation coefficient of 5*S* RNA varies from 4.4 to 4.7, depending upon salt and Mg^{++} concentration (36, 122). Several conformations have been suggested for this polynucleotide (18, 36, 46, 55). No function is known for the 5*S* RNA, although there is a recent report (181) according to which its presence stimulates the phage f2 RNA-directed protein synthesis in vitro.

16S and 23S RNA. Studies on the terminal sequences of 16*S* and 23*S* ribosomal RNA have been performed on RNA from a variety of organisms (230). A heterogeneity in the 3′ terminal nucleotide of the 23*S* ribosomal RNA has been reported. Ribosomal RNA contain small amounts of methylated bases, and the sequences around these minor bases have been examined (108). The methylation occurs only at a small number of sites. The sequences in the neighborhood of methylated nucleotides occur twice in each 23*S* RNA molecule.

Reconstitution of Active Ribosomes from RNA and Protein

In solutions of high ionic strength, 30*S* and 50*S* ribosomal subunits dissociate into smaller ribonucleoprotein particles (core particles) and proteins. By combining these components in properly selected conditions, ribosomes active in in vitro protein synthesis were reconstituted several years ago (287, 359). Recently, 23*S* core particles from the 30*S* subunit have been dissociated into free 16*S* RNA and proteins. The separated RNA and core proteins were mixed at 37C, cooled to 4C, and the additional protein fractions from the 30*S* subunit were added (390). The reconstituted particles had the same sedimentation coefficient as native 30*S* subunits, and when combined with 50*S* subunits were fully active in in vitro protein synthesis directed by poly U or phage f2 RNA. No difference in the number or in the nature of the protein components was found between reconstituted and native 30*S* subunits. Proteins from 50*S* subunits could not substitute for those from 30*S* subunits in the reconstitution of functionally active 30*S* subunits. In similar reconstitution experiments with the 30*S* subunit proteins from *E. coli*, the intact 16*S* *E. coli* RNA could not be replaced by degraded *E. coli* ribosomal RNA, 16*S* yeast ribosomal RNA, or 18*S* rat liver ribosomal RNA. However, 16*S* RNA from either *Azotobacter vinelandii* or *B. stearothermophilus* did substitute for *E. coli* 16*S* RNA (288).

The availability of techniques for separating all the ribosomal proteins has opened the way for elucidating the function of the individual proteins. In reconstitution experiments with isolated proteins and RNA from streptomycin-resistant, streptomycin-dependent, and streptomycin-sensitive 30*S* subunits, a particular protein (of molecular weight 14,000) was shown to be the target of streptomycin action; 30*S* subunits were reconstituted from the 16*S* RNA and a mixture of proteins lacking the streptomycin target protein. To the incomplete particles obtained, streptomycin target protein was added, which was obtained from either streptomycin-resistant (299), streptomycin-sensitive (299), or streptomycin-dependent (C. Kurland, *unpublished data*) ribosomes. The behavior of the reconstituted 30*S* subunit toward streptomycin was determined by the origin of the streptomycin target protein used in this experiment.

PEPTIDE CHAIN INITIATION

Much of our knowledge about initiation has come from studies on *E. coli* and a few other microorganisms. The components known to be involved in this process include an initiator tRNA (fMet-tRNA$_F$) responding to initiator codons, several initiation factors, ribosomal subunits, and GTP.

About 40% of the *E. coli* proteins have methionine as the N-terminal amino acids, although methionine constitutes only 2.5% of the total amino acid in proteins. Methionine, alanine, serine, and threonine together account for about 95% of the N-terminal amino acids in these proteins (398). Over 60% of the Met residues attached to *E. coli* tRNA have their α-amino group formylated. No other *N*-formylated AA-tRNA has been detected. The formylation of the α-amino group of methionine occurs after the amino acid has been esterified by tRNA; *N*-formylmethionine itself does not become linked to tRNA (239). The formyl donor is N^{10}-formyltetrahydrofolate (2, 84, 237). The formylating enzyme, Met-tRNA transformylase, has been purified from *E. coli* (84).

There are at least two classes of methionine-accepting tRNA species in *E. coli*; tRNA$_F$ and tRNA$_M$. Met-tRNA$_F$ can be formylated enzymatically, whereas Met-tRNA$_M$ cannot (70, 71). The tRNA$_F$ exhibits unusual base pairing near the 3′ and 5′ ends (89). This uniquely distinguishes it from all other tRNA of known sequence (cf. Sequence of tRNA).

Initiator of Peptide Chains: fMet-tRNA

The RNA of bacteriophage f2 and R17 can serve as mRNA and, as such, direct the synthesis of at least three virus-specific proteins in the cell-free amino acid-incorporating system from *E. coli* (59, 272). Each of these proteins formed in vitro have *N*-formylmethionine as the N-terminal amino acid (2, 221, 394, 402). This indicates that fMet-tRNA can serve as a peptide chain initiator for each of the proteins programmed by a polygenic mRNA.

The dependence of in vitro protein synthesis directed by f2 RNA on fMet-tRNA was demonstrated in the following way. To deplete *E. coli* of fMet-tRNA and the formyl donors (i.e., formyltetrahydrofolate) the synthesis of their precursor (tetrahydrofolate) was blocked by Trimethoprim, an inhibitor of dihydrofolate reductase (50). In the cell-free extract of Trimethoprim-treated *E. coli* cells, amino acid incorporation directed by f2 RNA strictly depends upon either added fMet-tRNA or formyltetrahydrofolate [which makes possible the formation of fMet-tRNA in the extract (96)]. This dependence is found only at low Mg^{++} concentration (4 to 8 mM). At high Mg^{++} concentration, the incorporation is only partially or not at all dependent on fMet-tRNA (96, 188).

The following result indicates that fMet-tRNA is the major, if not the only, peptide chain initiator in *E. coli*. In a cell-free extract of *E. coli* in which protein synthesis was directed by endogenous mRNA, one fMet residue was incorporated for approximately 150 amino acids, and formylmethionine was the only formyl-amino acid (56).

Fate of the Formyl and the fMet Residues

As stated earlier, the majority of *E. coli* proteins have methionine, alanine, serine, or threonine as the N-terminal amino acid. On the other hand, most, if not all, of the *E. coli* proteins are initiated by *N*-formylmethionine. This suggests that *E. coli* (and presumably other bacteria) have an enzyme or enzymes which remove the formyl and in certain cases also the Met residue from the N-terminal end of the peptide chain. Extracts from certain microorganisms [*E. coli*, *B. stearothermophilus*, and *B. subtilis* (1, 115, 378)] were found to contain enzymes cleaving fMet-peptides into formate and Met-peptides. A protein fraction from *E. coli* which contained such a peptide deformylase was found to liberate formate from proteins synthesized in vitro. The rate of hydrolysis of fMet-peptides by this protein fraction was greater than that of other formyl-peptides, acetyl-Met-alanine or *N*-formylmethionine. Such characteristics are those expected from an enzyme whose physiological function is to remove formyl residues from nascent protein (1). The fMet-phe-tRNA is a substrate for the deformylase, whereas fMet-tRNA is not. This suggests that the deformylation may occur soon after the incorporation of the fMet residue into a fMet-AA-tRNA intermediate (220). The peptide deformylase of *E. coli* is very labile in vitro and is inhibited strongly by compounds with sulphydryl groups. The proteins synthesized in vitro retain their formyl residue presumably because the deformylase does not survive the procedures used in preparing and testing the cell-free extract (1).

Since the peptide deformylase removes only formyl residues, it is expected that other enzymes are responsible for removing the Met residues. Extracts from *E. coli* and *B. subtilis* contain an aminopeptidase capable of removing a Met residue from Met-puromycin (but not from fMet-puromycin), and from the N-terminal hexapeptide of f2 coat protein (but only after the formyl residue has been cleaved off by treatment with the deformylase). These observations indicate that if a fMet residue is removed from a nascent protein, this takes place in two steps: first, the formyl residue is cleaved off, and subsequently, the Met residue (378).

E. coli extracts can also cleave *N*-formylmethionine (409). This cleavage is believed to be due to acetylornithine deacetylase action. In the extract of an *E. coli* mutant lacking acetylornithine deacetylase, the rate of hydrolysis of *N*-formylmethionine was only 1% of that observed in the extract of wild-type *E. coli* (115).

The amino acid adjacent to fMet is not unique in the proteins isolated from an *E. coli* extract in which protein synthesis was directed by endogenous mRNA. Alanine, serine, and, to a smaller extent, other amino acids were found in the position adjacent to the N-terminal residue of the peptide chain (56).

It remains to be seen if the amino acid adjacent to the fMet residue determines whether the Met residue is removed by the aminopeptidase or if other factors (e.g., the secondary structure of the protein) are decisive.

Coding Specificity and Functions of $tRNA_F$ and $tRNA_M$

One of the methods used in deciphering the code was based on the fact that particular ribotrinucleotides or ribopolynucleotides promote the binding of different AA-tRNA to ribosomes. This binding can be conveniently tested since free AA-tRNAs are not held on nitrocellulose membrane filters, whereas those whose binding to ribosomes is promoted by the proper codon are retained [the so called "binding assay" (278)]. The binding assay and the promotion of amino acid incorporation by mRNA of defined sequence were the techniques used in studies with cell-free systems on the coding specificities and functions of the two methionine-accepting tRNA species. It was concluded that, whereas fMet-tRNA serves as a source of N-terminal Met residues, Met-$tRNA_M$ provides Met residues for internal and C-terminal positions of the polypeptide chains (71, 124, 331). The codons specifying $tRNA_F$ are AUG and GUG; the codon specifying $tRNA_M$ is AUG (71, 124, 331, 371).

Phasing Activity of Initiator Codons

If an AUG codon is at or near the 5′ end of a polynucleotide, it decreases the binding (of AA-tRNA to ribosomes) by the codons partially overlapping with the AUG sequence and increases the binding by the codons adjacent to the 3′ side of AUG. For example, $AUG(U)_{15}$ stimulates the binding of Met- and Phe-tRNA, but not of Val-tRNA. (UUU codes for phenylalanine, GUU for valine.) In contrast, $AUGG(U)_{16}$ stimulates the binding of Met- and Val-tRNA, whereas the binding of Phe-tRNA is reduced to a low level. AUG thus seems to set the phase of reading. This phasing activity is maximal at low Mg^{++} concentration. At higher Mg^{++} concentration, this effect of the AUG

codon diminishes, and the selection of the reading frame is random (371, 385).

The in vitro translation of synthetic messengers not having an AUG codon at or near the 5′ end apparently starts mostly at the second triplet (351). Messengers with AUG at the 5′ end, however, are translated from the 5′ end (361). Since neither AUG nor GUG is part of the sequence of the first 10 nucleotides in the RNA of an RNA bacteriophage (83), it may be presumed that a signal (perhaps AUG or GUG) is present in the phage RNA for initiating the translation at a proper site in the correct reading frame. In polygenic mRNA, such signals must occur at several sites.

Translation of Polygenic mRNA

The structural genes for the 10 enzymes of histidine biosynthesis, constituting the histidine operon, are adjacent to each other on the *Salmonella typhimurium* chromosome. The operon is transcribed into a single polygenic mRNA. The synthesis of all 10 enzymes can be repressed by histidine. When histidine-requiring mutants are grown on limiting amounts of histidine, the level of each enzyme increases about 25-fold. The rate of synthesis of the individual histidine biosynthetic enzymes was followed in cells which were first grown in a medium with excess histidine and then transferred into a medium in which histidine was limiting (derepression). Two modes of derepression were observed. About 20 min elapsed between the increase in the rate of synthesis of the first and the last enzyme when the level of the formyltetrahydrofolate pool (available for formylation of Met-$tRNA_F$) was low. In these conditions, the order of the increase in the rate of synthesis of the individual enzymes was the same as the order of the corresponding genes on the chromosome [sequential translation (27, 28)]. Sequential translation of enzymes of the lactose (10, 77, 211), galactose (249), and tryptophan (165, 263) operons was also reported (99). This mode of gene expression presumably reflects translation starting from a single initiation site at the 5′ end of the RNA. This may be a consequence of (i) the sequential transcription of the polygenic mRNA starting at the 5′ end, (ii) the unavailability of internal initiation sites for ribosome attachment due, for example, to secondary structure of the mRNA, or (iii) the lower efficiency of the internal initiation sites than that of the one near the 5′ end; furthermore (iv), it might be that not each peptide chain initiation site is a ribosome attachment site as well, and that signals for ribosome attachment onto mRNA may include longer nucleotide sequences than do the signals for peptide chain initiation.

Remarkably, the increase in the rate of synthesis of all enzymes of histidine biosynthesis apparently occurred simultaneously under conditions in which the level of the formyltetrahydrofolate pool in the cells was high (27, 28). It has been shown that the histidine operon is transcribed into a single mRNA in conditions of either sequential or simultaneous translation (392). This observation, and the fact that it was the size of the formyltetrahydrofolate pool (presumably regulating the amount of fMet-tRNA) which determined whether the mode of translation was sequential or simultaneous, suggests that in this case the cause of the difference between the two modes of translation involves primarily the translation process itself and might, for example, be the consequence of having more efficient initiation sites at the 5′ end of the mRNA than in internal positions. Simultaneous initiation of the translation of two different polypeptides was also observed upon adding f2 RNA to the in vitro *E. coli* system (221). The simultaneous mode of translation seems to require that ribosomes do attach simultaneously to several sites of the polygenic mRNA. Consequently, a free 5′ end of the mRNA may not be a prerequisite for this attachment. In support of this conclusion, it has been shown that a circular messenger (i.e., one with no ends) promotes the binding of fMet-tRNA and amino acid incorporation into polypeptides (39). In the experiment proving this point, use was made of the curious fact that in the presence of certain antibiotics single-stranded DNA can be translated in vitro without prior transcription into RNA (244); the molecule used as messenger was actually the circular DNA of the bacteriophage fd.

Initiation Factors

The high nuclease activity in extracts from *E. coli* causes fast degradation of added synthetic mRNA. A system with somewhat lower nuclease activity can be prepared by washing the ribosomal pellet (resulting from the centrifugation of the cell extract at high speed) with ammonium chloride (0.5 to 2 M), purifying the washed ribosomes by chromatography on diethylaminoethyl cellulose, and mixing the purified ribosomes with the high-speed supernatant fraction (361). Such a system incorporates amino acids in response to synthetic messengers with or without initiator codons, at high Mg^{++} concentration; it is not active, however, at low Mg^{++} concentrations with any kind of messenger. Adding the ammonium chloride solution in which the ribosomal pellet was washed makes the system responsive at low Mg^{++} concentration to certain natural

mRNA or synthetic mRNA with initiator codons. Fractionation of the ribosomal washing solution led to the discovery of several complementary factors (presumably of protein nature) which are required at low Mg^{++} concentration for the translation of mRNA with initiator codons. These factors are designated initiation factors (361; *see* also 37, 94, 319). Their function in translation will be discussed in subsequent sections.

Exchange of Ribosomal Subunits During Protein Synthesis

The 30*S* and 50*S* ribosomal subunits (separated from each other) have a unique role in initiation. These subunits are metabolically stable and remain intact in growing *E. coli* during several generations. The ribosomes undergo exchange of their 30*S* and 50*S* subunits. This exchange has been demonstrated by centrifugal analysis of the distribution of isotopic labels among ribosomes and their subunits after the transfer of a growing culture from a medium containing heavy isotopes to one containing light isotopes (167). Rapid and extensive subunit exchange also takes place during protein synthesis in a cell-free system. The exchange is dependent upon protein synthesis and is blocked by inhibitors of protein synthesis. The kinetics of the exchange suggest that ribosomes exchange their subunits after each passage over a polygenic mRNA or even over a single gene of the mRNA (166).

As an explanation for the exchange, it was presumed that ribosomes dissociate into subunits after finishing the synthesis of a protein molecule and are reformed by coupling of subunits when initiating the synthesis of a new protein molecule (166; *see* also 235). In line with this hypothesis (235) free 30*S* and 50*S* subunits have been found in lysates of *E. coli* cells along with 70*S* ribosomes and polyribosomes [i.e., structures in which several ribosomes are attached to and are translating a single mRNA molecule (108a, 186, 235, 309, 336*a*, 337)].

Role of 30S Subunits in Initiation

An early intermediate in initiation is apparently a complex including the 30*S* subunit, mRNA, and fMet-$tRNA_F$. Some of the experimental results and considerations on which this conclusion is based are the following. Protein synthesis in vitro at low Mg^{++} concentration (4 to 9 mM) seems to require proper chain initiation, i.e., fMet-$tRNA_F$, initiation factors, and mRNA with properly located initiator codons. (In the absence of any one of these prerequisites, e.g., in the case of polyphenylalanine synthesis as promoted by poly U, a higher Mg^{++} concentration is mandatory.)

At low Mg^{++} concentration in the presence of initiation factors, f2 RNA or poly (A, U, G_t) promote the binding of fMet-$tRNA_F$ to 30*S* subunits. In the same conditions, the binding of other AA-tRNAs (e.g., Val-tRNA) to 30*S* subunits is not stimulated by poly (A, U, G), although this messenger is rich in valine codons. [To obtain binding of Val-tRNA, 50*S* subunits also have to be added (285; *see* also 95, 155).] The complex containing a 30*S* subunit and fMet-$tRNA_F$ is a compulsory intermediate for initiation, as shown by the following observations. "Heavy" ribosomes (70*S*) were prepared from *E. coli* (grown in a D_2O- and ^{15}N-containing medium) by extracting the cells in conditions in which a large part of the ribosomes sediment as 70*S* components. These 70*S* "heavy" ribosomes were included in a reaction mixture containing, among other components, "light" 50*S* ribosomal subunits, poly (A, U, G), fMet-$tRNA_F$, Val-tRNA, and initiation factors. After analyzing the reaction mixture after incubation by density gradient centrifugation, it was established that the majority of the fMet-$tRNA_F$ was attached to hybrid 70*S* ribosomes, whereas most of the Val-tRNA was bound to "heavy" 70*S* ribosomes. These results indicate that 70*S* ribosomes must dissociate into their subunits preceding fMet-$tRNA_F$ binding, although not preceding Val-tRNA binding (141).

Process of Initiation

Steps in initiation (Fig. 2). As indicated in the previous section, a complex including fMet-$tRNA_F$, mRNA, and a 30*S* subunit [(285); complex I] is apparently one of the early intermediates in peptide chain initiation. The formation of complex I requires GTP and initiation factors (9, 11, 95, 141, 153, 155, 207, 227, 265, 285, 331). Subsequently, a 50*S* subunit seems to attach to complex I (complex II). The fMet-$tRNA_F$ in complex II is thought to be bound at the hypothetical site A of the ribosome (123, 189, 190, 265, 286, 336). In the next step, fMet-$tRNA_F$ is believed to be translocated to site P, a second hypothetical site on the ribosome (complex III). There are indications that GTP may be cleaved into guanosine diphosphate (GDP) and orthophosphate (P_i) in the course of the transformation of complex II to III (187). The formation of complex III is the last step in chain initiation.

Characteristics of initiation complexes. The fMet-$tRNA_F$ and mRNA bound to ribosomes (e.g., in complexes I, II, and III) may be distinguished from the free species by filtration through nitrocellulose filters; the bound species are retained on the filters, whereas the free species

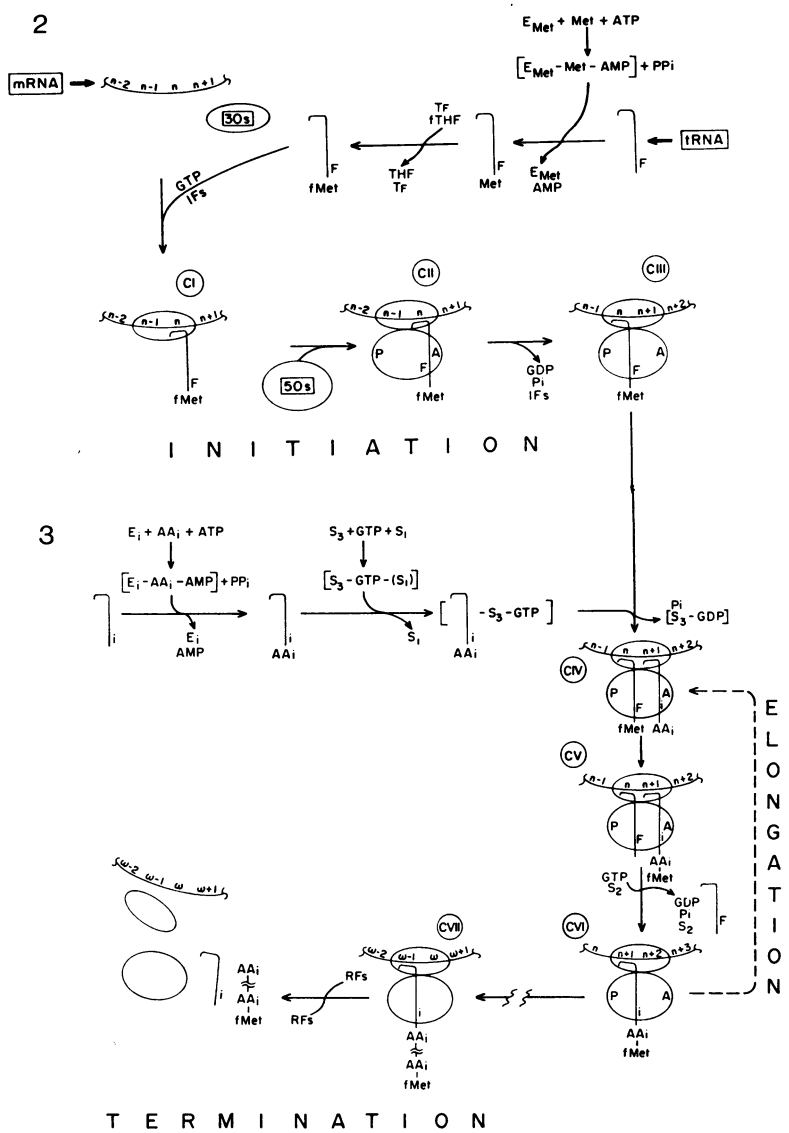

FIG. 2. *Schematic outline of steps in peptide chain initiation. (The steps are described in the text.) Symbols: n − 2, n − 1, n, n + 1, n + 2, a series of adjacent codons in the mRNA segment shown; n, initiator codon; "bottomless bracket," tRNA^{fMet}; T_F, N^{10}-formyltetrahydrofolate-Met-tRNA$_F$-transformylase; fTHF, N^{10}-formyltetrahydrofolate; THF, tetrahydrofolate; IF$_s$, initiation factors; E$_{Met}$, Met-tRNA synthetase. The oval shapes represent ribosomal subunits. A and P indicated in the 50S ribosomal subunit are hypothetical tRNA binding sites. CI (circled), etc., indicate the hypothetical intermediates described in the text.*

FIG. 3. *Schematic outline of steps in peptide chain elongation and termination. (The steps are described in the text.) Symbols: S_1, S_2, S_3, elongation factors; "bottomless bracket," tRNA accepting AA$_i$; ω − 2, ω − 1, ω, ω + 1, a series of adjacent codons in the mRNA segment shown; ω, a terminator codon; RF$_s$, release factors. In C VII (circled), a polypeptidyl residue is attached to the tRNA. For other abbreviations, see the legend to Fig. 2.*

are not (278). Free fMet-tRNA$_F$, free mRNA, and complex I can also be separated from each other and from complexes II and III by centrifugation through a sucrose gradient.

The filtration assay is much faster than the one involving ultracentrifugation. This may account for the fact that a loose complex which is displayed as a complex in filtration may dissociate

at least partially into its components in the course of ultracentrifugation (190). Using these techniques, it was established that the coupling of 30*S* and 50*S* subunits to form 70*S* ribosomes (i.e., complexes II or III) strictly depends at 5 mM Mg^{++} on mRNA (actually, f2 RNA was used), fMet-$tRNA_F$ initiation factors, and GTP (190).

5'-Guanylyl - methylene - diphosphonate (GMPPCP), an analogue of GTP, can substitute for the latter in the formation of complex I (11, 265) and in the subsequent attachment of the 50*S* subunit to this complex (189, 190). GMPPCP has a methylene bridge between the β and γ phosphorus atoms and, thus, cannot undergo enzymatic cleavage into GDP and P_i (152). The fact that it can substitute for GTP indicates that GTP cleavage need not occur before or during the initial attachment of a 50*S* subunit to complex I. The complex formed in the presence of GMPPCP and the complex formed in the presence of GTP behave similarly in the filtration test. However, the complex formed in the presence of GMPPCP largely dissociates in the course of centrifugation through a sucrose gradient (190) and does not react with puromycin (292), whereas the complex formed in the presence of GTP largely persists in the sucrose gradient test (190) and does react with puromycin, giving rise to fMet-puromycin (42, 206, 292, 331).

Puromycin and the tRNA binding sites of the ribosome. Puromycin is an inhibitor of protein synthesis which may be considered an analogue of the terminal aminoacyladenosine portion of AA-tRNA (417). Puromycin can react with peptidyl-tRNA (125) in the presence of ribosomes (262), giving rise to peptidyl-puromycin and free tRNA (258). This reaction may serve as a basis for defining different tRNA binding sites on the ribosome (388).

Polyphe-tRNA, whose synthesis is directed by poly U in the in vitro system, remains bound to the ribosomes (125). Ribosomes with bound peptidyl-tRNA can exist in two states. (i) In one state, they can react with puromycin (forming peptidyl-puromycin) without further additions. It is customary to define that such ribosomes have the peptidyl-tRNA attached to the peptidyl donor tRNA binding site (P site). (ii) Ribosomes with bound peptidyl-tRNA in the other state require addition of GTP and of the high-speed supernatant fraction for reacting with puromycin. Such ribosomes are said to have the peptidyl-tRNA bound to the AA-tRNA receptor site (A site). The supernatant fraction and GTP are required for catalyzing the translocation of the peptidyl-tRNA from site A to site P (258). In the course of this step, GTP is apparently cleaved into GDP and P_i (*see* Translocation).

Site of binding of fMet-$tRNA_F$ to Ribosomes. FMet-$tRNA_F$ in the initiation complex formed in the presence of GMPPCP (as well as mRNA and initiation factors) does not react with puromycin (292). This seems to suggest that the initiator tRNA is not bound to site P of the ribosome in these conditions. Furthermore, tetracycline, an antibiotic inhibiting the binding of AA-tRNA to the A site (133), was found to block the binding of fMet-$tRNA_F$ to ribosomes in the presence of GTP, mRNA, and initiation factors (336). This suggests that fMet-tRNA is bound, at least initially, to site A.

FMet-$tRNA_F$ in the initiation complex formed in the presence of GTP (as well as mRNA and initiation factors) does react with puromycin (42, 206, 292, 331). This reveals that in these conditions fMet-$tRNA_F$ is bound to the P site [(388), complex III].

The above results, and the fact that the guanosine triphosphatase activity of an initiation factor (F2) is stimulated by fMet-$tRNA_F$, 30*S* and 50*S* subunits, mRNA, and other initiation factors, are consistent with, although they do not necessarily prove, the following sequence of events. FMet-$tRNA_F$ is attached to the A site first (complex II) and is subsequently translocated to the P site (complex III). GTP is cleaved before or during this translocation (187).

Role of initiation factors. Several complementary initiation factors have been isolated from crude preparations (45, 187, 232, 291, 301, 320). The relation of all the factors described by the various investigators remains to be established. The highest number of complementary factors reported from a single laboratory is four; some of these appear to consist of several nonidentical subunits (291).

At least some of the initiation factors can be found attached to native 30*S* subunits (95, 301). The same factors do not occur on either 70*S* ribosomes or free 50*S* subunits (301). This suggests that the factors are released from the 30*S* subunits sometime after the 50*S* subunit becomes attached to the 30*S* subunit in the course of initiation. Since initiation factors were not found in the high-speed supernatant fraction of the cell extract, it may be assumed that they are present in short supply, and as soon as they are released from 70*S* ribosomes they become bound to 30*S* subunits.

The order in which the components of complex I are associated is not known. The data available are in line with, but do not prove, the view that the binding of a 30*S* subunit to mRNA occurs first and is followed by the attachment of fMet-$tRNA_F$ (12, 45). Apparently, one or two factors (in addition to GTP) are involved in the attach-

ment of mRNA to the 30S subunit (45, 187, 291, 320).

It remains to be seen whether the positioning of a ribosome on an initiation sequence of the mRNA is assisted by fMet-tRNA$_F$ or by initiation factors. It was reported that some of the initiation factors are needed for the translation of certain natural messengers, but not for that of synthetic messengers with AUG or GUG at or near the 5′ end (291, 320). This raises the interesting possibility that the factors may recognize an initiation sequence longer than AUG or GUG. If such longer initiation sequences exist, they may serve as a basis for explaining the postulated occurrence of initiation sequences with different efficiencies (241).

There is no agreement between reports concerning, for example, the following problems (291, 320). Is the binding of mRNA and of fMet-tRNA$_F$ to the 30S subunit mediated by the same initiation factor or by different ones? Is the attachment of the 50S subunit to complex I promoted by a factor also needed in forming complex I? It may be expected that with the availability of pure factors, these and other apparent discrepancies will be resolved.

Role of the formyl residue and of tRNA$_F$ in initiation. The following observations indicate that unformylated Met-tRNA$_F$ cannot initiate protein synthesis in *E. coli*. Met-tRNA$_F$ does not substitute for fMet-tRNA$_F$ in allowing translation of f2 RNA in vitro at low Mg^{++} concentration (96, 188). Protein synthesis in vivo stops if the formylation of Met-tRNA$_F$ is inhibited (343). These results may be the immediate consequence of the fact that the initiation factors which promote the binding of fMet-tRNA$_F$ to ribosomes in the presence of GTP and initiator triplets do not promote the binding of unformylated Met-tRNA$_F$ (332). The ultimate basis for blocking the α-amino group of the initiator tRNA is, however, presumably the substrate specificity of the enzyme catalyzing peptide bond formation (257).

AA-tRNA with a blocked α-amino group (other than fMet-tRNA$_F$) can substitute for fMet-tRNA$_F$ in making amino acid incorporation at low Mg^{++} concentration possible in vitro. In such conditions, amino acid incorporation directed, for example, by poly (A, U) depends strictly on added peptidyl-tRNA. It should be noted that the α-amino group of the C-terminal AA-residue is blocked in peptidyl-tRNA (267; *see* also 284). Poly U-directed polyphenylalanine formation at 4 mM Mg^{++} concentration was found to take place only if *N*-acetyl-Phe-tRNA, as well as GTP and initiation factors, were present in the reaction mixture (227). This result and the fact that initiation factors do not recognize unformylated Met-tRNA$_F$ as their substrate indicate that the factors or the components with which they interact recognize whether the α-amino group is blocked or not.

Transfer RNA$_F$ and its derivatives are distinguished from other tRNA and their derivatives by the following enzymes: (i) the transformylase which formylates Met-tRNA$_F$, but not Met-tRNA$_M$ (84, 237); (ii) the initiation factors and the 30S ribosomal subunit which appear to form a more stable complex with fMet-tRNA$_F$ than with acetyl-Phe-tRNA (138); (iii) factors involved in peptide chain elongation which do not recognize fMet-tRNA$_F$ or Met-tRNA$_F$ as their substrate (295); (iv) an enzyme hydrolyzing *N*-acyl-AA-tRNAs (81), which hydrolyzes fMet-tRNA$_M$ (to *N*-formylmethionine and tRNA$_M$) but does not attack fMet-tRNA$_F$ or acetyl-Met-tRNA$_F$ (192, 395). This enzyme also hydrolyzes peptidyl-tRNA [at least one with a blocked α-amino residue (192)]. A possible function of this hydrolase is to destroy analogues of fMet-tRNA$_F$ and to thus avoid false chain initiation.

Possible Involvement of fMet-tRNA$_F$ in the Regulation of RNA Synthesis

In *E. coli*, the rate of net RNA synthesis (e.g., as measured by the incorporation of uracil from the medium into RNA) decreases to less than 10% of its normal value after the removal from the medium of an amino acid which cannot be synthesized by the cell. It is said, therefore, that net RNA synthesis is under stringent control of amino acids (RC^{str}). *E. coli* mutants exist in which RNA synthesis continues at about the normal rate after removal of a required amino acid. In mutants of this type, the control of RNA synthesis is apparently relaxed (RC^{rel}). The inhibition of the charging of an amino acid onto tRNA affects RNA synthesis just like the removal of a required amino acid (91).

Either of two compounds which inhibit peptide chain initiation by preventing the formylation of Met-tRNA$_F$ were also found to inhibit net RNA synthesis in RC^{str} *E. coli*, but not in RC^{rel} *E. coli*. The two compounds are Trimethoprim and hydroxylamine, and each of these blocks fMet-tRNA$_F$ synthesis by depleting the formyltetrahydrofolate pool. These results may indicate that net RNA synthesis in RC^{str} *E. coli* depends on the availability of all AA-tRNAs as well as fMet-tRNA$_F$ (182, 343). Since the only process which is known to require all the above compounds is protein synthesis, it may be that net RNA synthesis is regulated by protein synthesis or by a step or intermediate in this process (293; *see* also 262*a*, 324*a*).

Peptide Chain Initiation in Various Organisms

Procaryotic cells. At least some of the proteins specified by bacteriophage T4-infected *E. coli* are initiated with *N*-formylmethionine (183, 184, 251).

Some of the procaryotic cells, other than *E. coli*, in which the occurrence of fMet-tRNA is established are the following: *B. subtilis* (20, 162, 378), *B. stearothermophilus* (1, 295), *Micrococcus lysodeicticus*, *Pseudomonas aeruginosa*, *Anacystis nidulans* (a blue-green alga) (20), *Mycoplasma laidlawii* B, *M. gallisepticum* A 5969, and *Mycoplasma* species (caprine strain; 149). The dependence of the derepression pattern on the formylating capacity in *Salmonella typhimurium* (27) and the fact that Met-tRNA from *E. coli* B can be formylated by extracts of *Lactobacillus leichmanii*, *Pseudomonas* species, *Streptomyces antibioticus*, and *Clostridium tetanomorphum* (84) suggest, but do not prove, the involvement of fMet-tRNA in protein synthesis in these organisms.

Eucaryotic cells. *N*-formylmethionine was reported as the N-terminal residue of the viral coat protein synthesized in a system containing *Euglena gracilis* chloroplast ribosomes, *E. gracilis* high-speed supernatant fraction, and f2 bacteriophage RNA (338). FMet-tRNA has also been found in mitochondria from yeast and rat liver (350). Protein synthesis in chloroplasts and mitochondria resembles that in bacteria in several ways. Thus, chloramphenicol and some other antibiotics inhibit chloroplast (93, 240, 303) and mitochondrial protein synthesis in vivo and in vitro (73, 193, 318), but do not seem to affect the cytoplasmic system from yeast and rat liver. Similarity among ribosomes from chloroplasts, mitochondria, and bacteria is suggested by the finding that all these particles have a sedimentation coefficient of about 70*S*, whereas that of the ribosomes from the cytoplasm is about 80*S* (34, 72, 194, 228, 365, 383). The existence of fMet-tRNA in mitochondrial, chloroplast, and bacterial systems may indicate that the same initiator is characteristic of 70*S* ribosomes in general.

FMet-tRNA has not been detected in the cytoplasmic system, and the initiator serving 80*S* ribosomes is not known.

Pyrrolidone-2-carboxylic acid has been shown to be an N-terminal residue in several proteins (311). It is an intriguing possibility that pyrrolidone-2-carboxylic acid may arise by the cyclization of a specific Gln-tRNA, which in turn serves as an initiator (30, 255). In view of the easy cyclization of glutamine to pyrrolidone-2-carboxylic acid, careful studies will be needed to examine this possibility. A number of proteins have *N*-acetylated amino acids as the N-terminal residue. An enzyme has been isolated from chicken reticulocytes catalyzing the transfer of the acetyl residue from acetyl-coenzyme A to certain proteins. This finding indicates that masking of the α-amino group of the N-terminal amino acid may occur after protein synthesis (236).

PEPTIDE CHAIN ELONGATION

Studies on peptide chain elongation in microbial extracts were greatly facilitated by the fact that at relatively high (presumably unphysiological) Mg^{++} concentration incorporation of aminoacyl residues from AA-tRNA into polypeptidyl-tRNA can be directed by natural or synthetic messengers (even if the latter lack initiator codons), and does not require either fMet-$tRNA_F$ or initiation factors (96, 213, 218). The need for "proper" chain initiation is obviated at high Mg^{++} concentration, probably because in such conditions AA-tRNAs may attach to both the P and A sites of ribosomes bound to mRNA. If two AA-tRNA are attached to the same ribosome, dipeptidyl-tRNA may be formed since the catalyst for peptide bond formation is part of the ribosome (*see* Peptide Bond Formation). Dipeptidyl-tRNA, in turn, is an analogue of fMet-$tRNA_F$ and may serve as a chain initiator (218, 267; *see* Role of the formyl residue and of $tRNA_F$ in initiation).

A simple system convenient for experiments on elongation is one in which the synthesis of a homopolypeptidyl-tRNA (e.g., polyphe-tRNA) is directed by a homopolyribonucleotide [e.g., poly U (279)]. In addition to the mRNA, AA-tRNA, proper ions, and thiols, such systems need to include ribosomes, GTP (176), and components of the high-speed supernatant fraction of the cell extract (271). To observe a strict dependence of amino acid incorporation on the high-speed supernatant fraction, the ribosomes have to be thoroughly washed. This treatment apparently removes from the ribosomes (among other substances) the factors required in elongation (271).

It should be noted that the large majority of elongation factor activity is in the high-speed supernatant fraction of microbial extracts (271). This is in marked contrast with the initiation factor activity which, as stated earlier, is exclusively located in the pellet obtained by centrifuging the extract at high speed.

Elongation Factors

Elongation factors were purified from the high-speed supernatant fraction of the extract of a number of bacteria and from yeast. Initially, two factors were found to be required for polyphe-tRNA synthesis in *E. coli* extract, factor T and

factor G (7). These two factors were also obtained in crystalline form (175, 302). More recently, the T factors from *E. coli* and from *P. fluorescens* have been divided into two active components, both of which are needed (together with factor G) in polyphe-tRNA synthesis; one component is unstable (T_u), the other is stable (T_s ; 226). Separated T_u and T_s form a complex with each other. In the presence of GTP, this complex apparently dissociates into a T_u-GTP complex and T_s (103, 250).

Three elongation factors (S_1, S_2, and S_3) were purified from the extract of a thermophilic organism, *B. stearothermophilus*. S_1 apparently corresponds to T_s, S_2 to G, and S_3 to T_u. All three partially purified *B. stearothermophilus* elongation factors are stable (349).

Elongation factors were prepared from *E. coli* extract also in the presence of phenylmethylsulfonylfluoride, an inhibitor of proteolysis. In these conditions, two elongation factors were isolated, both of which are required in polyphe-tRNA synthesis: factor E and a heat-stable protein. The exact relationship of these two factors to factors T and G is not known. It seems, however, that factor E contains some components of both factors T and G (161). Two elongation factors were obtained from the high-speed supernatant fraction of an *E. coli* extract which was treated with pHMB, a compound known to react with SH groups. One of the two factors is a protein which retains its activity even after boiling in acid. The factor is apparently not identical with either T_u, T_s, or G (421).

The variety of complementary elongation factors obtained from *E. coli* extracts treated in different ways may indicate that these factors are in a complex unit in vivo which can be artificially divided into sets of components in several ways. This possibility and the fact that a thorough and lengthy washing of the ribosomes is required in order to remove from them all of the elongation factor activity should admonish us to caution in interpreting the results obtained in the in vitro system. It may well be that the artificially disrupted system in which elongation is studied in vitro does not reflect faithfully the functioning of the elongation machinery in vivo. Complementary elongation factors were also isolated from yeast extract (19, 321).

Process of Elongation

Outline of the steps in elongation. A hypothetical scheme of steps in chain elongation is shown in Fig. 3. This scheme is based mainly on experiments with the T_u, T_s, and G factors from *E. coli* and the S_1, S_2, and S_3 factors from *B. stearothermophilus*. (Subsequently, whenever possible, the S_1, S_2, S_3 nomenclature will be used.)

The last step in peptide chain initiation is apparently the translocation of fMet-$tRNA_F$ from site A to site P on the 70*S* ribosome-mRNA complex (cf. Steps in initiation). The first composite step in the next phase (i.e., peptide chain elongation) is presumably the attachment (to the vacant A site in complex III) of AA-tRNA specified by the codon adjacent to the 3′ side of the initiator codon [AA-tRNA binding (168, 266, 278)]. The product of this step will be designated as complex IV. The attachment of AA-tRNA to complex III (which leads to the formation of complex IV) requires GTP and involves the factors S_3 and S_1 (102, 224, 314, 349). Results of in vitro experiments seem to indicate that GTP is cleaved to GDP and P_i in this step (296, 344).

Subsequently, the carboxyl group of the formylmethionyl residue of the fMet-$tRNA_F$ is released from its linkage to $tRNA_F$ and is linked in a peptide bond with the α-amino group of the AA-tRNA (peptide bond formation). In the product of this step (complex V), the fMet-AA-tRNA is located at site A (100, 101, 142). Peptidyl transferase, the enzyme catalyzing this reaction, apparently is part of the 50*S* subunit (256).

In the course of the next composite step (translocation), the following events are presumed to occur: (i) the discharged tRNA (in site P) is released from the ribosome, (ii) the fMet-AA-tRNA is shifted from site A to site P, and (iii) the ribosome moves the length of one codon along the mRNA in the 5′-to-3′ direction (401). Translocation is catalyzed by an elongation factor (S_2). When studied in vitro the step also requires GTP which is cleaved to GDP and P_i (44, 100, 101, 142, 306, 381).

Thus, both AA-tRNA binding and translocation require the cleavage of GTP when studied in vitro. It remains to be seen, however, whether in vivo the cleavage of two GTP molecules is needed for the two steps or if cleavage of one GTP suffices.

After translocation, the stage is set for the addition of another amino-acyl residue to the fMet-AA-tRNA. Each addition requires the following cycle of events: AA-tRNA binding, peptide bond formation, and translocation. These events will be discussed in more detail in subsequent sections.

In the second cycle and in all the subsequent ones, peptidyl-tRNAs are taking the place of fMet-tRNA. The cycles are repeated and the peptide chain is growing, presumably until a terminator codon in mRNA is reached by the A site of the ribosome.

AA-tRNA Binding

The need for GTP and a factor (from the high-speed supernatant fraction of the cell extract) in attaching AA-tRNA to the mRNA-ribosome complex was first established in a cell-free system from reticulocytes (15). The requirement for GTP and a factor in catalyzing the same step in microbial systems was not revealed for quite a while. This delay was a consequence of the following facts. Chain elongation was studied mainly with poly U, which promotes polyphe-tRNA synthesis only at high Mg^{++} concentration. At high Mg^{++} concentration, in turn, there is a strong AA-tRNA binding which is independent of factors and GTP, and which may obscure the factor- and GTP-dependent binding (197, 314).

Recent binding experiments in the *E. coli* system (314) and the yeast system (19, 321) were performed, however, at lower Mg^{++} concentrations; under such conditions there is a need for GTP and supernatant factors in binding phe-tRNA to the ribosome-poly U complex (314). Apparently, two factors (S_1 and S_3) are involved in this step (102, 142, 349).

The following events are presumed to precede the binding of AA-tRNA to the ribosome. First, S_1 and S_3 form a complex with GTP (complex A). Complex A can be assayed conveniently since it is retained on nitrocellulose filters (8, 132, 315). The complex can be separated from free GTP by gel filtration (8, 349). Both S_1 and S_3 are required in complex A formation (102, 349); apparently the amount of S_3 determines the quantity of GTP bound, whereas the amount of S_1 determines the rate of complex formation (103). S_3 is present in complex A, but it has not been definitely established whether S_1 is also part of the complex. ATP is not bound by S_1 and S_3, whereas GDP is.

In a subsequent step, complex A apparently binds AA-tRNA and is transformed into a GTP–AA-tRNA–S_3 complex (complex B) (132, 349). The formation of complex B from its components requires S_1 (349). There are indications that S_1 may not be part of complex B (316). This complex, in contrast to complex A, is not retained on nitrocellulose filters (132, 315). It can be separated from unbound GTP and AA-tRNA by gel filtration (349). The ratio of GTP and AA-tRNA in complex B is approximately 1:1 (131, 295). Binding of AA-tRNA in the complex depends strictly on GTP. GTP cleavage was not observed in the course of the formation of complexes A and B (131, 349). Most, and probably all, AA-tRNA species do form the complex (295). (For an important exception, *see* Problems Arising from the Dual Specificities of the AUG and GUG Codons.) The existence of competition between different AA-tRNA in binding indicates that the same S_3 factor can bind different AA-tRNA species (295). Uncharged tRNAs is not bound in the complex and an excess of uncharged tRNA does not compete with AA-tRNA for binding (131, 295, 349). GDP and ATP do not substitute for GTP in making the binding of AA-tRNA possible (349).

There are indications that the GTP molecule in complex B is cleaved to GDP and P_i after the binding of phe-tRNA to the poly U-ribosome complex, but before peptide bond formation (296, 344). According to one report (296), the same partially purified S_1 and S_3 fractions from *B. stearothermophilus* which promote the binding of AA-tRNA to ribosomes also catalyze the cleavage of GTP to GDP and P_i in the presence of ribosomes. This cleavage is promoted by the simultaneous presence of poly U and phe-tRNA. The ratio of GTP cleaved to phe-tRNA bound is approximately 1. Since it is known that in the presence of ribosomes the factor S_2 also catalyzes the cleavage of GTP, it was ascertained that the GTP cleavage promoted by partially purified S_1 and S_3 is not due to S_2 contaminating these factors; it was shown that fusidic acid, an antibiotic inhibiting the guanosine triphosphatase activity of S_2, has no effect on the GTP cleavage catalyzed by S_1 and S_3 (296).

In another study (344), partially purified S_1 and S_3 factors from *E. coli* were used to form complex B with GTP and phe-tRNA. This complex was added to a reaction mixture containing poly U and ribosomes. Upon incubating this mixture, phe-tRNA became bound to ribosomes and about an equimolar amount of GTP was cleaved to GDP and P_i. The resulting GDP was found in a complex with S_3.

The following facts are consistent with the hypothesis that GTP cleavage occurs after the binding of AA-tRNA to the mRNA-ribosome complex but that it precedes the next step of chain elongation, i.e., peptide bond formation. (i) If GMPPCP (the analogue of GTP which cannot be cleaved enzymatically to GDP and P_i) is substituted for GTP, phe-tRNA is bound to the ribosome-poly U complex in the presence of S_1 and S_3 but peptide bond formation does not take place, whereas in the presence of GTP it does (142, 296; *see* also 347). (ii) Sparsomycin, an antibiotic which blocks peptide bond formation (128, 260), does not inhibit either AA-tRNA binding (224, 296) or GTP cleavage catalyzed by the factors S_1 and S_3 (296).

It might have been expected that a compound such as tetracycline, which inhibits the binding of AA-tRNA to the mRNA-ribosome complex (133,

154, 336, 366), would also inhibit the promotion of the guanosine triphosphatase activity (of S_1 and S_3 in the presence of ribosomes) by poly U and phe-tRNA. Somewhat unexpectedly, it was found that tetracycline had no effect on the GTP cleavage (296). It remains to be seen whether this lack of inhibition is indicative of the existence of a loose binding of AA-tRNA to the ribosome which may occur in the presence of tetracycline and which is sufficient to promote the guanosine triphosphatase activity of S_1 and S_3.

S_3 may be recovered bound to the ribosomes if incubated in a reaction mixture including poly U, phe-tRNA, S_1, and GMPPCP. If, however, GTP is substituted for GMPPCP, S_3 cannot be found attached to ribosomes. These observations are consistent with the possibility that S_3 may become attached to the mRNA-ribosome complex together with GTP and AA-tRNA and is released from the complex after the GTP has been cleaved but prior to peptide bond formation. Since GMPPCP is not cleaved, S_3 remains attached (A. Skoultchi, Y. Ono, and P. Lengyel, *in preparation*).

GTP cleavage in the course of AA-tRNA binding to the mRNA-ribosome complex was first reported long ago by Schweet and his collaborators in experiments on the reticulocyte system (15; *see* also 245).

Peptide Bond Formation

After the attachment of AA-tRNA to the fMet-$tRNA_F$-ribosome-mRNA complex in the first cycle of chain elongation (or to a peptidyl-tRNA-ribosome-mRNA complex in all subsequent cycles), the stage is set for peptide bond formation (Fig. 3). The peptide bond is formed between the carboxyl group of the fMet-$tRNA_F$ (or of the carboxy terminal aminoacyl-residue of the peptidyl-tRNA) and the α-amino group of the AA-tRNA.

Some of the characteristics of the peptide bond-forming reaction are the following. Supernatant proteins and GTP are probably not directly involved (229, 256, 305, 388). Peptidyl transferase, the hypothetical enzyme thought to catalyze it, is apparently an integral part of the 50*S* subunit (229, 256, 258, 388). The presence of di- and monovalent cations (Mg^{++} and K^+ or NH_4^+) is required for the reaction (259, 388). CpCpA, the 3′ terminal nucleotide sequence common to all species of tRNA, seems to be involved in the interaction with peptidyl transferase of both the peptidyl-tRNA (in site P; 257) and the AA-tRNA (in site A; 330).

Most of these conclusions were derived from studies with substrate analogues which lack certain functional parts of the tRNA molecules but retain others. Puromycin, the most widely used of these, is an analogue of the terminal aminoacyl-adenosine moiety of the AA-tRNA. It contains that part of the tRNA which participates in peptidyl transfer, but lacks those parts which are involved in the codon specific binding to mRNA and binding to ribosomes. The peptidyl moiety of peptidyl-tRNA can be linked in a peptide bond to puromycin instead of to AA-tRNA. The resulting peptidyl-puromycin is released from the ribosomes (3, 125, 258, 262, 270, 417). Polyphe-tRNA, the synthesis of which in vitro is directed by poly U, remains attached to ribosomes (125). Ribosomes, with attached polyphe-tRNA, retain the ability to react with puromycin after having been washed with salt solutions under conditions similar to those which make ribosomes dependent upon the elongation factors and GTP for polypeptide synthesis (229). This suggests that the elongation factors and GTP are not directly involved in peptide bond formation. This conclusion is reinforced in studies with ribosomes isolated free of mRNA and peptidyl-tRNA. Such ribosomes, even after thorough washing with salt solution, are still able to catalyze the reaction of puromycin with polylysyl-tRNA (in the presence of poly A; 329) or with fMet-tRNA (in the presence of, for example, the AUG triplet; 42, 424).

Lowering the Mg^{++} concentration of a solution containing ribosomes with poly U and polyphe-tRNA attached causes dissociation of the complex into 30*S* subunits and 50*S* subunits with bound polyphe-tRNA (125). The fact that such charged 50*S* subunits can react with puromycin indicates that peptidyl-transferase, the enzyme catalyzing the reaction, is located in the 50*S* subunit (388). This conclusion is supported by experiments in which isolated, washed 50*S* subunits were found to catalyze the reaction of puromycin with CAACCA-fMet, a 3′ terminal fragment of fMet-$tRNA_F$ (256). The reaction of puromycin with this fragment evidently takes place by the same mechanism as peptide bond formation in protein synthesis, since both reactions require the same mono- and divalent cations and the fragment reaction is inhibited by certain antibiotics [e.g., chloramphenicol, gougerotin, and sparsomycin (406)] known to inhibit in vivo and in vitro protein biosynthesis (128, 256).

The fragment reaction requires the presence of alcohol (e.g., ethyl alcohol) for reasons not known at the present time (259).

The substrate specificity at the P site was studied by comparing the activity of various analogues of peptidyl-tRNA in the puromycin reaction (257). Various AA-oligonucleotides and acyl-AA-oligonucleotides were isolated from par-

tial nuclease digests of AA-tRNA or acyl-AA-tRNA. It was observed that the donor must have a CCA nucleotide sequence for the puromycin reaction to take place: e.g., CCA-fMet and CCA-acLeu are active as peptidyl donors, whereas CA-fMet and A-fMet are inactive. The nucleotides in the next three positions seem to affect the reaction only slightly, since the activity of CCA-fMet is about the same as that of CAACCA-fMet. Acyl-AA-tRNAs (e.g., acLeu-tRNA) are more active than the corresponding fragments (e.g., CCA-acLeu), suggesting that other moieties of the tRNA molecule besides the terminal CCA interact with the 50*S* subunit (257).

The substrate specificity at the A site was examined by comparing the activity of various AA-tRNA analogues as peptidyl acceptors. The role of the 3′ terminal nucleotide grouping in peptidyl acceptor substrates is indicated by the following results. Polylys-tRNA is released from ribosomes and the bond between the polylysyl residue and tRNA is cleaved by CpA-Gly (forming, presumably, CpA-Gly-$(Lys)_n$), whereas UpA-Gly has little releasing activity and UpU-Gly and pA-Gly have none (330).

In the peptidyl-tRNA bound at the P site in chain elongation, the α-amino group of the carboxy terminal aminoacyl residue is blocked (by the carboxyl group of the adjacent AA-residue of the peptide chain). Consequently, it is not surprising that AA-tRNA or AA-oligonucleotides with a blocked α-amino group (e.g., acPhe-tRNA or CACCA-acLeu) are much more reactive with puromycin than the corresponding AA-tRNAs or AA-oligonucleotides with a free α-amino group (42, 207, 257). The requirement (or at least preference) of the peptidyl transferase for a blocked α-amino group in the aminoacyl residue attached to the donor tRNA may be the ultimate cause for having the α-amino group in the chain initiator ($fMet\text{-}tRNA_F$) blocked.

Translocation

After peptide bond formation, the newly formed peptidyl-tRNA (fMet-AA-tRNA in the first cycle of chain elongation) is located in site A (100, 101, 142; *see* also 218, 388, 401), and the discharged tRNA, which remains bound to the ribosome (225), probably in site P (complex V in Fig. 3). Translocation (represented schematically by the conversion of complex V to complex VI in Fig. 3) is a composite step catalyzed by the S_2 factor. Translocation requires GTP, which is apparently cleaved to GDP and P_i (44, 75, 100, 101, 142, 281, 282, 306, 388). Complex VI is analogous to complex III: the P site is occupied in both by peptidyl-tRNA (actually by fMet-tRNA in complex III) and the A site is free.

The results and considerations on which these conclusions are based include the following. The acPhe-tRNA was bound to a ribosome-poly U complex in the absence of initiation and elongation factors [at relatively high Mg^{++} concentration (142)]. The acPhe-tRNA ribosome-poly U complex was incubated with phe-tRNA, GTP, S_1, and S_3, and subsequently the ribosomes with the attached components were isolated from the reaction mixture by centrifugation. It was established (i) that the reisolated ribosomes have acdiPhe-tRNA attached, but no (or little) actriPhe-tRNA. (ActriPhe-tRNA was not found in the supernatant fraction of the incubation mixture, either.); (ii) The large majority of the acdiPhe-tRNA did not react with added puromycin, indicating that it was bound at the A site. (iii) Discharged tRNA was sticking to the ribosomes (225), probably at the P site. These characteristics suggest that the above complex resembled complex V.

Upon incubating the isolated complex with GTP and S_2, the discharged tRNA was released from the ribosomes (225; *see* also 195, 196, 340, 375) and most of the acdiPhe-tRNA became reactive with added puromycin, indicating that it was now located at the P site (142).

A portion of the complex was reisolated after the incubation with GTP and S_2. After incubating this reisolated complex with S_1, S_3, Phe-tRNA, and GTP, the formation of actriPhe-tRNA was observed. Thus, the incubation with S_2 and GTP made the A site available for binding Phe-tRNA, which in turn made tripeptidyl-tRNA synthesis possible (142).

In another study, AUGUUU and AUGUUU-UUU were used as mRNA in the *E. coli* system. The fMet-Phe-tRNA was synthesized in the presence of GTP, initiation factors, S_1, and S_3 (the last two not resolved from each other) in the presence of either mRNA; the formation of fMet-Phe-Phe-tRNA, however, also required S_2 factor and occurred only in the presence of the tricodon mRNA (AUGUUUUUU). The fact that the synthesis of fMet-Phe-Phe-tRNA required a tricodon as the messenger is consistent with the view that translocation involves a movement of the ribosome along the mRNA (100, 101).

These findings indicate that S_2 is not needed for dipeptidyl-tRNA formation but that it is required for tripeptidyl-tRNA formation. The data are in accord with the view that the action of S_2 results in (i) release of the discharged tRNA, (ii) translocation of the peptidyl-tRNA from the A site to the P site, and (iii) movement of the ribosome along the mRNA the length of one codon (218, 401). There is no cause to assume that translation

of codons beyond the third would require additional factors.

The studies cited so far in this section provided some insight into the order of events in tripeptide synthesis and the role of the S_2 factor in this process. For assaying only S_2 activity, however, less-involved procedures are available. Each of these is based on one of the following observations. (i) As stated earlier, S_2 factor and GTP increase the fraction of ribosome-bound polyphe-tRNA which reacts with puromycin (388). (ii) At relatively high Mg^{++} concentration, phe-tRNA may be attached to ribosome-poly U complexes in the absence of elongation factors. The fraction of the bound phe-tRNA, which is reactive with added puromycin, is increased several fold by incubating the complex with S_2 and GTP (44). (iii) At relatively high Mg^{++} concentration in a system including ribosomes, poly U, and phe-tRNA, diphe-tRNA is synthesized in the absence of elongation factors, whereas the extensive synthesis of oligophe-tRNA (with more than two phe residues per molecule) depends on S_2 factor and GTP (306). (iv) S_2 factor, in the presence of ribosomes, cleaves GTP into GDP and P_i; this activity is stimulated by poly U and tRNA (75, 281, 282).

The following are some of the results and considerations which support the view that GTP cleavage by S_2 and ribosomes is connected to protein synthesis. (i) Fusidic acid, a steroidal antibiotic, inhibits both ribosome-dependent guanosine triphosphatase activity of S_2 factor and translocation (306, 381). Fusidic acid-resistant *E. coli* mutants were obtained, and it was established that fusidic acid sensitivity or resistance is localized in the S_2 factor (179). (ii) GMPPCP (the GTP analogue with a methylene bridge between the β and γ phosphorus atoms) does not substitute for GTP in translocation; actually, in the presence of GTP, it acts as an inhibitor (44, 101, 142, 152, 306).

The incubation of ribosomes at elevated temperatures prior to using them in the guanosine triphosphatase assay in the presence of the S_2 factor results in the uncoupling of GTP cleavage from peptide synthesis; the rate of GTP cleavage increases slightly, whereas the rate of protein synthesis decreases sharply (75, 282).

Results were presented in an earlier section to indicate that GTP is cleaved in the course of peptide chain elongation after the attachment of AA-tRNA to the ribosomes but before peptide bond formation (296, 344). This guanosine triphosphatase activity of the S_1 and S_3 factors was first examined in a system free of S_2 activity (296). In more recent experiments, S_2 was also included in the reaction mixtures. It was established that the GTP added to the system in the form of the S_3-GTP-AA-tRNA complex does not make translocation possible (297). Thus, at least in the in vitro system with purified separated elongation factors, not less than two molecules of GTP are cleaved for the addition of each aminoacyl residue to the peptidyl-tRNA chain.

Efforts to establish the existence of a high-energy intermediate, whose formation is catalyzed by GTP and S_2 factor, have so far not been successful (281).

The availability of fusidic acid-resistant *E. coli* mutants (179) and other mutants with a thermosensitive S_2 factor (387) will soon lead to the mapping of the chromosomal site of the gene specifying S_2.

Problems Arising from the Dual Specificities of the AUG and GUG Codons

As initiation codons, AUG and GUG specify fMet-tRNA$_F$ in the *E. coli* system. As codons for internal aminoacyl residues of the peptide chain, however, AUG stands for Met-tRNA$_M$ and GUG for Val-tRNA (357). The dual specificities of AUG and GUG raise the question of how mixups of Val-tRNA, fMet-tRNA$_F$, and Met-tRNA$_F$ are avoided in protein synthesis. The outline of the answer is apparently the following. Val-tRNA and Met-tRNA$_F$ do not serve as initiators since they are not recognized by initiation factors. Furthermore, fMet-tRNA$_F$ does not participate in elongation; having its α-amino group blocked, it cannot serve as a peptidyl residue acceptor. A more difficult problem arises with the methionyl residues (in Met-tRNA$_F$) which have a free α-amino group and, in principle, could thus be mistakenly incorporated in the place of valine in response to GUG codons in chain elongation. Studies with in vitro systems [in which random poly(U, G) or poly(U, A, G) were used as mRNA] indicate, however, that Met-tRNA$_F$ (unformylated) does not serve as a source of methionyl residues for internal positions of the peptide chain (238).

The avoidance of this mistake apparently may be accounted for by the substrate specificity of the chain elongation factors S_3 and S_1; most and possibly all AA-tRNAs, including Met-tRNA$_M$, do form complexes with the factors S_3 and S_1 in the presence of GTP, whereas fMet-tRNA$_F$ and Met-tRNA$_F$ do not (295). The AA-tRNA–GTP–S_3 complexes are intermediates in AA-tRNA binding to the ribosomes. Thus, the lack of complex formation with Met-tRNA$_F$ may explain why Met-residues (from Met-tRNA$_F$) are not inserted into internal positions of the peptide chain in response to GUG codons.

The fact that Met-tRNA$_M$ does form a com-

plex with the elongation factors, whereas Met-$tRNA_F$ does not, indicates that the factors discriminate against $tRNA_F$.

PEPTIDE CHAIN TERMINATION

During chain elongation, the growing polypeptide chain remains linked to tRNA and bound to the mRNA-ribosome complex. After completion, the polypeptide is released from both of these bonds in the course of a composite process called peptide chain termination (116, 191, 203, 376, 425). Termination is apparently triggered when in the course of the movement of the ribosome along the mRNA a chain termination signal is reached at the A site of the 30S subunit (Fig. 3). It is believed that the codons UAA, UAG, and UGA (which are designated as nonsense codons) may serve as chain termination signals (38, 333, 404, 405). Two release factors (R_1 and R_2) were found to be involved in this process. R_1 is specific for termination coded by UAA or UAG, and R_2 for that coded by UAA or UGA (58, 63, 339). After release of the polypeptidyl-tRNA, the mRNA-ribosome complex falls apart, giving rise to subunits (337, 403). There are some indications, although no conclusive evidence, that ribosomes may actually be released from the complex as free 70S ribosomes (2*a*, 186, 228*a*, 325) which are subsequently dissociated into subunits, probably by the action of a dissociation factor (369). Some of the results and considerations on which the above scheme is based are presented in the subsequent sections.

Termination Signals

The codons UAA, UAG, and UGA do not seem to specify any amino acid in *E. coli* strains [unless the strains carry certain suppressor mutations (117)]. These codons do not promote the binding of any AA-tRNA to ribosomes (43, 355), and a series of adjacent UAG or UGA codons in a polynucleotide; e.g., poly (U-A-G) or poly (U-G-A) does not direct in vitro amino acid incorporation (178). [Actually, these two polynucleotides do direct the formation of two homopeptides each, but these homopeptides are specified by the other codons in the RNA, (i.e., AGU, GUA, GAU, and AUG).] When occurring in mRNA, the nonsense codons specify termination; e.g., the synthetic oligonucleotide AUG-UUUUAA directs the formation of fMet-phenylalanine [not linked to tRNA (203)]. RNA from mutants of the bacteriophages f2 or R17 with a UAG codon within the gene specifying the coat protein promotes the synthesis in vitro of a coat protein fragment (402), whereas RNA from the corresponding wild-type phages directs the formation of the complete coat protein in vitro just as it does in vivo (272). The approaches used in identifying the nonsense codons and in deciphering their nucleotide sequences were reviewed recently (117).

Release Factors and the Mechanism of Termination

Most of the information about the mechanism of chain termination was obtained in two assay systems. In one of these, RNA from a mutant R17 or f2 phage with a UAG nonsense codon early in the coat protein gene was used as the messenger. In vitro, this messenger directs the synthesis of the free (not tRNA-linked) amino terminal hexapeptide of the coat protein (40, 58, 425). A cell-free system was prepared including only those AA-tRNA species which are needed for forming the hexapeptide. In this system, RNA from wild-type phage directs the formation of hexapeptidyl-tRNA, whereas RNA from the nonsense mutant promotes free hexapeptide synthesis (40, 58). Thus, if a codon is untranslatable in consequence of the lack of a required AA-tRNA, this leads to the stoppage of peptide chain propagation but not to chain termination. The fact that, in the presence of the mRNA from a nonsense mutant, chain termination occurs even in the absence of all tRNAs except the six species added suggests that if RNA is involved in chain termination, it is not contained in the tRNA fraction (40, 58, 112, 352).

A substrate for studying the mechanism of termination was prepared in the following way (58). The formation of a hexapeptidyl-tRNA as specified by the phage mutant RNA was blocked at the pentapeptidyl-tRNA stage by omitting from the in vitro system the amino acid coded by codon six. The ribosome-mRNA-pentapeptidyl-tRNA complex was then separated from the supernatant fraction by centrifugation and the AA-tRNA needed to complete the hexapeptidyl-tRNA was added in the presence of GTP. The last amino acid became then added to the coat protein fragment. The resulting hexapeptidyl-tRNA remained attached to the mRNA-ribosome complex. This product made possible the study of the unique requirements of the release step. It was found that the release of free hexapeptide from this complex depends on a component from the supernatant fraction. This component was designated release factor (R factor) (58).

Recently, another convenient termination assay was developed. AUG, fMet-tRNA, and ribosomes were incubated to form an AUG-fMet-tRNA-ribosome complex. The release of formylmethionine from this complex is promoted in the presence of a crude R-factor preparation by each

of the three nonsense codons added in the form of triplets (63). By using this test, the R-factor preparation was separated into two components; R_1 catalyzes chain termination in response to UAA or UAG, and R_2 in response to UAA or UGA (339). The release of formylmethionine from fMet-tRNA by the R factors depends strictly on ribosomes.

The codon recognition pattern of R_1 resembles that found with some species of tRNA. The molecule interacting with A at the 3′ terminal nucleotide position of the mRNA codon also interacts with G in this position. However, the codon recognition by R_2 has a pattern not found with any tRNA species so far. The R factors are nondialyzable and are inactivated on incubation with trypsin and *N*-ethylmaleimide, but not by ribonuclease. Thus, R_1 and R_2 behave like proteins with free sulfhydryl groups (339; *see also* 400).

The fact that the formylmethionine release from the ribosome in response to terminator codons is inhibited by tetracycline may suggest that these codons exert their terminating activity at the same ribosomal site of the 30*S* subunit where, in the course of elongation, AA-tRNA is attached. The lack of effect of fusidic acid on the release indicates that translocation, as catalyzed by the S_2 factor, is not involved in the process (339).

Fate of the mRNA-Ribosome Complex After Chain Termination

The fate of the mRNA-ribosome complex after chain termination was examined in vitro (403). The messenger in the system was ^{32}P-labeled RNA from either wild-type f2 phage or from a mutant f2 phage carrying a UAG nonsense codon in a known position of the coat protein-specifying gene. The technique used makes possible the monitoring of the translation of the mRNA by a single ribosome. First, asparaginase is added to the system to destroy asparagine. Subsequent addition of ^{32}P-labeled phage RNA results in the formation of a stable phage RNA-ribosome-peptidyl-tRNA complex (with the ribosome stuck on the mRNA at a coding site for an asparagine residue on the coat protein gene). Further binding of ^{32}P-RNA to ribosomes is prevented by adding either an excess of unlabeled phage RNA or aurin-tricarboxylic acid. [The latter compound blocks attachment of ribosomes to mRNA and, consequently, initiation of new peptide chains, but it does not affect peptide chain elongation (135).] Subsequently, asparagine is added and, thus, synchronous translation of the coat protein gene ensues. The fate of the ^{32}P-f2-RNA-ribosome complexes can be monitored by determining the amount of labeled RNA sedimenting as a complex with ribosomes (at the 80*S* region) and the amount sedimenting as free ^{32}P-RNA or ^{32}P-RNA attached to the 30*S* subunit (30*S* region and lighter). By using this technique, it was established that phage RNA and the polypeptide being synthesized rapidly disappear from the complex with the ribosome after translation of nonsense codons in the coat protein gene.

These observations seem to indicate that termination results in dissociation of the mRNA from the ribosome or, at least, dissociation of the 50*S* subunit, leaving behind an mRNA-30*S* subunit complex.

The release of the f2 RNA from the complex is rapid, but only partial (about 20%), after termination at the end of the coat protein gene (in experiments in which the reattachment of ^{32}P-RNA to ribosomes is blocked by added excess unlabeled f2 RNA). It is possible that this incomplete release reflects the translation of a subsequent gene on the mRNA by the same ribosome (or at least the same 30*S* subunit) which just completed the translation of the previous gene. Incomplete release occurs, perhaps, if the termination signal is near to an initiator codon (403). (For a discussion of related subjects, see references 57, 98, 225, 335, and 426.)

The hypothetical schemes concerning the fate of ribosomes in the chain termination complex (complex VII in Fig. 3) after the release of the completed polypeptide can be divided arbitrarily into two classes: (i) ribosomes released as free subunits and (ii) ribosomes released as 70*S* particles which subsequently dissociate into subunits (2*a*, 186, 337). If, among the products of chain termination, free 70*S* ribosomes (i.e., free of mRNA, peptidyl-tRNA, and fMet-tRNA) could be demonstrated, this would rule out the first class and be consistent with the second. 70*S* particles (in addition to subunits and polyribosomes) have been detected in cell lysates (186, 228*a*, 309, 310, 325, 337). However, although a 70*S* particle (at least in principle) may be a free ribosome, it may also be a monosome (i.e., a complex of mRNA with a single ribosome with attached peptidyl-tRNA) or an initiation complex (a complex of mRNA with a single ribosome with attached fMet-tRNA).

According to some reports, 70*S* particles accumulate in cells that are incubated without a carbon source or treated with actinomycin (186). The latter treatment is believed to deplete the polysomes by blocking RNA synthesis. The fact that such 70*S* particles dissociate fully into subunits at an Mg^{++} concentration (1 mM) at which monosomes dissociate only partially was taken as a support for the view that the 70*S* par-

ticles are free ribosomes (325). [Puromycin-treated 70S monosomes also dissociated at 1 mM Mg^{++} (325), and such ribosomes may carry attached discharged tRNA (225)]. Adding a protein fraction obtained from a 1 M NH_4Cl solution used to wash native 30S subunits causes rapid dissociation of the presumed free ribosomes (369). The active component of the protein fraction was designated as a dissociation factor and appears to act on the ribosomes in a stoichiometric rather than in a catalytic manner. The dissociation factor resembles initiation factors at least in one aspect: it is present on native 30S subunits but not on 70S ribosomes (369). On the basis of these results, it was postulated that after chain termination ribosomes are released as free 70S particles (2a, 369) which are dissociated to subunits after reacting with a dissociation factor which combines with the 30S subunit. Apparently, the dissociation factor is released from the 30S subunit some time after the latter combines with a 50S subunit to form a 70S initiation complex (369).

According to other reports (309, 310), larger amounts of 70S particles can be detected in a lysate if it was prepared in the presence of K^+ or NH_4^+ ions. However, such particles, or at least the majority of them, are artifacts; i.e. they are formed after cell lysis, probably by association of 30S and 50S subunits to form initiation complexes. Few or no 70S particles are detectable in lysates in which Na^+ or Li^+ are the monovalent cations, although preexisting 70S particles do not seem to dissociate in these conditions. Although these results seem to be in line with the first scheme, they cannot rule out the transient existence of free 70S ribosomes as intermediates in chain termination.

Further studies on the fate of the termination complex, which are underway in several laboratories, will help to resolve these apparent discrepancies.

PROBLEMS AND CONCLUSIONS

The following are a few of the outstanding problems in protein synthesis.

AA-tRNA synthetases. The principal aim is to understand the molecular basis of the high specificity of these enzymes for their cognate amino acid and tRNA. X-ray diffraction analysis of the enzymes of tRNAs, and, hopefully, of AA-tRNA synthetase of tRNA complexes may contribute to the solution of this problem. The detailed kinetics of the sequence of steps leading to the amino-acylation of tRNA remains to be determined. It is noteworthy that, although the reactions catalyzed by all the AA-tRNA synthetases are analogous, the various enzymes in this group differ in many properties (e.g., molecular weight, effect of SH-reagents, etc.).

Transfer RNA. Although the nucleotide sequences of more than a dozen tRNA species have been determined, the anticodon and the CCA terminus are still the only regions of the molecule whose functions are established. The sites on the tRNA involved in recognition by the AA-tRNA synthetase, by the various tRNA binding sites of the ribosome, and by initiation and elongation factors all remain to be identified. In addition to X-ray diffraction studies, experiments with chemically or genetically altered tRNA molecules may facilitate the identification.

Much remains to be learned about the biosynthesis of tRNA. The chemical synthesis of tRNA-specifying genes (62, 342) may provide the basis for extensive studies on the transcription of these genes and on the modification of the products of the transcription (presumably the tRNA precursors) by the various modifying enzymes.

Further studies are needed on the role of tRNA in the regulation of protein synthesis, on the function of virus-specified tRNAs in the metabolism of virus-infected cells, and on the possible role of tRNA in differentiation and hormone action. The involvement of tRNA in bacterial cell wall synthesis proves that the function of this class of macromolecules is not restricted to protein synthesis.

Ribosomes. The chemical characterization of ribosomal proteins, together with the reconstitution of the 30S subunit, may provide the basis for the elucidation of the specific roles of the particular ribosomal proteins in ribosome function. It is probable that a number of these proteins are serving as binding sites for AA-tRNA and peptidyl-tRNA and for initiation, elongation, and release factors. It is likely that eventually the techniques of electron microscopy and X-ray diffraction will become applicable to ribosome structure analysis.

Peptide chain initiation. Sequencing of natural mRNAs and of the RNAs of single-stranded RNA bacteriophages may be required for establishing the nucleotide sequence of ribosome attachment sites, peptide chain initiation and termination signals, and intercistronic regions. Instead of determining the complete nucleotide sequence of these RNAs, it may suffice to sequence those regions to which the ribosomes attach when initiating translation at various initiation sites of polygenic mRNAs. Presumably, attached ribosomes may protect the attachment sites on the mRNA against digestion by nucleases (377), and this may make the selective sequencing of such sites possible. Which component of the

protein-synthesizing machinery (ribosomal protein, initiation factors, fMet-tRNA, etc.) recognizes the initiation signals remains to be established.

The availability of pure initiation factors from *E. coli* should assist in determining the intermediate steps in initation.

The process of initiation, the nature of the initiator tRNA, and the initiation signals in the cytoplasm of eukaryotic cells are not known at this time.

Peptide chain elongation. The requirement for GTP and the cleavage of GTP to GDP and P_i in the course of the addition of each aminoacyl residue to the growing peptide chain has been known for some time. Energetically, there seems to be no need for the free energy of GTP for peptide bond formation since the ΔF of the peptidyl donor-peptidyl-tRNA (estimated to be -7 kcal/mole) is ample for the formation of the peptide bond [ΔF, -3 kcal/mole (218)]. Indeed, GTP is apparently not needed for the actual formation of the peptide bond in vitro.

The step in elongation that was first shown to require GTP is translocation (15, 75, 142, 218, 281). There is cause to believe that the GTP molecule needed for this step is cleaved prior to, or concomitantly with, the events in translocation. Recent data indicate that GTP is also required for AA-tRNA binding to the ribosome-mRNA complex and that cleavage of this GTP molecule may be a prerequisite for peptide bond formation (296, 344). Results of in vitro experiments suggest that the GTP cleaved in the course of AA-tRNA binding cannot serve in the translocation; i.e., at least two GTP molecules may be cleaved in the in vitro system for each aminoacyl residue incorporated into the growing polypeptide (297).

It may be too early to conclude that two molecules of GTP are cleaved for each peptide bond formed in vivo, too. One of the causes warranting this caution is that in vitro conditions may create artifacts: The guanosine triphosphatase activity of S_2 (the translocase) as studied in vitro is apparently uncoupled from peptide bond formation. [More precisely, the S_2 factor catalyzes the cleavage of GTP just in the presence of ribosomes) (75)]. It may well be that in vivo this is not the case.

It is an intriguing possibility, however, that translocation is a two-step process and that the cleavage of the first GTP may be needed to drive its first step. In the first step of such a hypothetical translocation model (after AA-tRNA binding and concomitantly with peptide bond formation), the 30*S* subunit, schematically speaking, moves along the mRNA the length of one codon, leaving the 50*S* subunit behind. (In the resulting intermediate, the discharged tRNA may be bound to the P site of the 50*S* subunit and the peptidyl-tRNA to the P site of the 30*S* subunit and to the A site of the 50*S* subunit.) In the second step (in which S_2 factor is involved and a second molecule of GTP is cleaved) the discharged tRNA is released, the 50*S* subunit is realigned with the 30*S* subunit, and the peptidyl-tRNA is shifted (from the A site to the P site of the 50*S* subunit). A two-step translocation model involving an intermediate similar to the one in this outline was proposed recently (41).

It remains to be established whether the GTP molecule(s) cleaved in the course of peptide chain elongation donate P_i or GDP to form a high-energy intermediate with a component of the translation machinery or whether the GTP(s) acts as an allosteric effector. It should be noted that the free energy of GTP could, in principle, drive ribosome movement even in an allosteric fashion (i.e., with no high energy intermediate of the $X \sim P$ or $GDP \sim X$ type involved). If binding of GTP would cause a conformational change of a protein and the cleavage of GTP would reverse the change, then a ratchet-type unidirectional movement could result from the repeated binding of GTP and its removal by cleavage to GDP and P_i.

Whatever the case, the mechanism of the movement of the ribosome relative to the mRNA and the role of GTP in this process are perhaps the most exciting unsolved problems in translation. A hypothetical model of ribosome function was proposed some time ago (358).

Peptide chain termination. Both genetic and biochemical studies indicate that each of the UAA, UAG, and UGA codons can serve as a signal in mRNA for chain termination. It is believed, however, that the normal and frequently used termination codon in vivo is UAA, and that UAG and UGA are rarely used, if at all. This assumption is based on the following facts and considerations. By genetic manipulation (more specifically, by introduction of nonsense suppressors) *E. coli* strains can be constructed in which UAG or UGA is frequently read as a chain elongation codon specifying an amino acid, instead of serving as a termination signal. Nevertheless, such strains grow normally. It is argued that if UAG or UGA would be principal chain termination signals, the growth of such strains would be adversely affected.

On the other hand, no strains have been found in which UAA would be translated with a high probability as an amino acid. Thus, it is believed that such strains may not be viable and, consequently, that UAA may be indispensable as a chain terminator codon (117).

The fact that two release factors are maintained in the cell, one of which is specific for UAG (in addition to UAA) and the other for UGA (in addition to UAA), may suggest that UAG and UGA are used as normal termination signals, even if rarely (339).

Sequencing of natural mRNAs will be needed to establish definitely the nature of the termination signals.

It also remains to be seen whether the release factors do recognize the termination signals directly, or whether other molecules, which in turn interact with the appropriate species of release factors (339), are involved in this process.

In conclusion, it should be noted that the time is ripe for the application of the powerful methods of genetic analysis to the protein-synthesizing machinery. The first steps in this direction have been taken (129, 140, 179, 274, 337, 387). The selection of specific mutants, a prerequisite for such genetic studies, shall be greatly facilitated by the use of antibiotics [known to affect particular components of the machinery as selective agents (406)].

The scope of this review has been restricted to microbial protein synthesis. It is obvious that the knowledge obtained by examining this pathway in microorganisms should accelerate the progress of similar investigations in higher organisms. The latter may contribute ultimately to the understanding of such diverse phenomena as antibody synthesis, hormone action, and differentiation.

ACKNOWLEDGMENTS

We thank J. R. Knowles, Y. Ono, A. Skoultchi, and J. Waterson for reading parts of the manuscript, and Nancy Benedict and Dorothy La Marche for patiently and repeatedly typing it.

These investigations were supported by Public Health Service grants GM 13707 and GM 15401 from the National Institute of General Medical Sciences and by grant GB 7269 from the National Science Foundation.

LITERATURE CITED

1. Adams, J. M. 1968. On the release of the formyl group from nascent protein. J. Mol. Biol. 33:571–589.
2. Adams, J. M., and M. R. Capecchi. 1966. *N*-formylmethionyl-sRNA as the initiator of protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 55:147–155.
2a. Algranati, I. D., N. S. Gonzalez, and E. G. Bade. 1969. Physiological role of 70*S* ribosomes in bacteria. Proc. Nat. Acad. Sci. U.S.A. 62:574–580.
3. Allen, D. E., and P. C. Zamecnik. 1962. The effect of puromycin on rabbit reticulocyte ribosomes. Biochim. Biophys. Acta 55:865–874.
4. Allende, C. C., J. E. Allende, M. Gatica, J. Celis, G. Mora, and M. Matamala. 1966. The aminoacyl ribonucleic acid synthetases. I. Properties of the threonyladenylate-enzyme complex. J. Biol. Chem. 241:2245–2251.
5. Allende, J. E. Protein biosynthesis in plant systems. *In* P. N. Campbell, and J. R. Sargent (ed.), Techniques of protein synthesis, vol. 2. Academic Press Inc., New York, *in preparation*.
6. Allende, J. E., C. C. Allende, M. Gatica, and M. Matamala. 1964. Isolation of threonyl-adenylate-enzyme complex. Biochem. Biophys. Res. Commun. 16:342–346.
7. Allende, J. E., R. Monro, and F. Lipmann. 1964. Resolution of the *E. coli* amino-acyl sRNA transfer factor into two complementary fractions. Proc. Nat. Acad. Sci. U.S.A. 51:1211–1216.
8. Allende, J. E., N. W. Seeds, T. W. Conway, and H. Weissbach. 1967. Guanosine triphosphate interaction with an amino acid polymerization factor from *E. coli*. Proc. Nat. Acad. Sci. U.S.A. 58:1566–1573.
9. Allende, J. E., and H. Weissbach. 1967. GTP interaction with a protein synthesis initiation factor preparation from *Escherichia coli*. Biochem. Biophys. Res. Commun. 28: 82–88.
10. Alpers, D. H., and G. M. Tomkins. 1965. The order of induction and deinduction of the enzymes of the lactose operon in *E. coli*. Proc. Nat. Acad. Sci. U.S.A. 53: 797–803.
11. Anderson, J. S., M. S. Bretscher, B. F. C. Clark, and K. A. Marcker. 1967. A GTP requirement for binding initiator tRNA to ribosomes. Nature 215:490–492.
12. Anderson, J., J. E. Dahlberg, M. S. Bretscher, M. Revel, and B. F. C. Clark. 1967. GTP-stimulated binding of initiator-tRNA to ribosomes directed by f2 bacteriophage RNA. Nature 216:1072–1076.
13. Anfinsen, C. B. 1967. The formation of the tertiary structure of proteins, p. 95–116. *In* Harvey lectures. Ser. 61. Academic Press Inc., New York.
14. Archives of Biochemistry and Biophysics. 1968. Vol. 125(2) Issue dedicated to the memory of Richard S. Schweet.
15. Arlinghaus, R., J. Schaeffer, and R. Schweet. 1964. Mechanism of peptide bond formation in polypeptide synthesis. Proc. Nat. Acad. Sci. U.S.A. 51:1291–1299.
16. Armstrong, D., J. Burrows, F. Skoog, K. L. Roy, and D. Söll. 1969. Cytokinins: distribution in *Escherichia coli* tRNA species. Proc. Nat. Acad. Sci. U.S.A., *in press*.
17. Attardi, G. 1967. The mechanism of protein synthesis. Ann. Rev. Microbiol. 21:383–416.
18. Aubert, M., J. F. Scott, M. Reynier, and R. Monier. 1968. Rearrangement of the conformation of *Escherichia coli* 5*S* RNA. Proc. Nat. Acad. Sci. U.S.A. 61:292–299.
19. Ayuso, M. S., and C. F. Heredia. 1968. Guanosine triphosphate dependent enzymatic binding of aminoacyl transfer ribonucleic acid to yeast ribosomes. Eur. J. Biochem. 7: 111–118.
20. Bachmayer, H., and G. Kreil. 1968. The formation of *N*-formyl-methionyl-puromycin by intact cells of four different bacteria and blue-green alga. Biochim. Biophys. Acta 169:95–102.
21. Baldwin, A. N., and P. Berg. 1966. Purification and properties of isoleucyl ribonucleic acid synthetase from *Escherichia coli*. J. Biol. Chem. 241:831–838.
22. Barnett, W. E. 1965. Interspecies aminoacyl-sRNA formation: fractionation of *Neurospora* enzymes involved in anomalous aminoacylation. Proc. Nat. Acad. Sci. U.S.A. 53:1462–1467.
23. Barnett, W. E., and D. H. Brown. 1967. Mitochondrial transfer ribonucleic acids. Proc. Nat. Acad. Sci. U.S.A. 57:452–458.
24. Barnett, W. E., D. H. Brown, and J. L. Epler. 1967. Mitochondrial-specific aminoacyl-RNA synthetases. Proc. Nat. Acad. Sci. U.S.A. 57:1775–1781.
25. Bayev, A. A., I. Fodor, A. D. Mirsabekov, V. D. Axelrod, and L. Ya. Kasarinova. 1967. Functional studies of fragments of yeast valine tRNA molecules. Mol. Biol. (Russia) 1:859–871.
26. Bayev, A. A., T. V. Venkstern, A. D. Mirzabekov, A. I. Krutilina, L. Li, and V. D. Axelrod. 1967. The primary structure of valine tRNA of bakers' yeast. Mol. Biol. (Russia) 1:754–766.
27. Berberich, M. A., J. S. Kovach, and R. F. Goldberger. 1967.

Chain initiation in a polycistronic message: sequential versus simultaneous derepression of the enzymes for histidine biosynthesis in *Salmonella typhimurium*. Proc. Nat. Acad. Sci. U.S.A. 57:1857–1864.

28. Berberich, M. A., P. Venetianer, and R. F. Goldberger. 1966. Alternative modes of derepression of the histidine operon observed in *Salmonella typhimurium*. J. Biol. Chem. 241: 4426–4433.
29. Bergmann, F. A., P. Berg, and M. Dieckmann. 1961. The enzymic synthesis of amino-acyl derivatives of ribonucleic acid. II. The preparation of leucyl-, valyl- isoleucyl- and methionyl ribonucleic acid synthetases from *Escherichia coli*. J. Biol. Chem. 236:1735–1740.
30. Bernfield, M. R., and L. Nestor. 1968. The enzymatic conversion of glutaminyl-tRNA to pyrrolidone carboxylate-tRNA. Biochem. Biophys. Res. Commun. 33:843–849.
31. Biemann, K., S. Tsunakawa, J. Feldmann, D. Dütting, and H. G. Zachau. 1966. Struktur eines ungewöhnlichen Nucleosids aus serin-spezifischer Transfer Ribonucleinsäure. Angew Chem. Int. Ed. Engl. 78:600–601.
32. Birge, E. A., G. R. Craven, S. J. S. Hardy, C. G. Kurland, and P. Voynow. 1969. The K locus is the structural determinant of a 30*S* ribosomal protein. J. Biol. Chem., *in press*.

32a. Bishop, J., J. Leahy, and R. Schweet. 1960. Formation of the peptide chain of hemoglobin. Proc. Nat. Acad. Sci. U.S.A. 46:1030–1038.

33. Bluestein, H. G., C. C. Allende, J. E. Allende, and G. Cantoni. 1968. Seryl transfer ribonucleic acid synthetase from bakers' yeast. III. The seryl-adenylate-enzyme complex and its interaction with transfer ribonucleic acids. J. Biol. Chem. 243:4693–4699.
34. Boardman, N. K., R. I. B. Francki, and S. A. Wildman. 1966. Protein synthesis by cell-free extracts of tobacco leaves. III. Comparison of the physical properties and protein synthesizing activities of 70*S* chloroplast and 80*S* cytoplasmic ribosomes. J. Mol. Biol. 17:470–489.
35. Böck, A., and F. C. Neidhardt. 1966. Location of the structrual gene for glycyl ribonucleic acid synthetase by means of a strain of *Escherichia coli* possessing an unusual enzyme. Z. Vererbungsl. 98:187–192.
36. Boedtker, H., and D. G. Kelling. 1967. The ordered structure of 5*S* RNA. Biochem. Biophys. Res. Commun. 29:758–766.
37. Brawerman, G., and J. Eisenstadt. 1966. A factor from *Escherichia coli* concerned with the stimulation of cell-free polypeptide synthesis by exogenous ribonucleic acid. II. Characteristics of the reaction promoted by the stimulation factor. Biochemistry 5:2784–2789.
38. Brenner, S., A. O. W. Stretton, and S. Kaplan. 1965. Genetic code: the "nonsense" triplets for chain termination and their suppression. Nature 206:994–998.
39. Bretscher, M. S. 1968. Direct translation of a circular messenger DNA. Nature 220:1088–1091.
40. Bretscher, M. S. 1968. Polypeptide chain termination: an active process. J. Mol. Biol. 34:131–136.
41. Bretscher, M. S. 1968. Translocation in protein synthesis: a hybrid structure model. Nature 218:675–677.
42. Bretscher, M. S., and K. A. Marcker. 1966. Polypeptidyl-sRibonucleic acid and aminoacyl-sRibonucleic acid binding sites on ribosomes. Nature 211:380–384.
43. Brimacombe, R., J. Trupin, M. Nirenberg, P. Leder, M. Bernfield, and T. Jaouni. 1965. RNA codewords and protein synthesis. VIII. Nucleotide sequences of synonym codons for arginine, valine, cysteine and alanine. Proc. Nat. Acad. Sci. U.S.A. 54:954–960.
44. Brot, N., R. Ertel, and H. Weissbach. 1968. Effect of a soluble transfer factor on the reaction of aminoacyl-tRNA with puromycin. Biochem. Biophys. Res. Commun. 31:563–570.
45. Brown, J. C., and P. Doty. 1968. Protein factor requirement for binding of messenger RNA to ribosomes. Biochem. Biophys. Res. Commun. 30:284–291.
46. Brownlee, G. G., F. Sanger, and B. G. Barrell. 1967. Nucleotide sequence of 5*S*-ribosomal RNA from *Escherichia coli*. Nature 215:735–736.
47. Bruton, C. J., and B. S. Hartley. 1968. Sub-unit structure and specificity of methionyl-transfer-ribonucleic-acid synthetase from *Escherichia coli*. Biochem. J. 108:281–288.
48. Buck, C. A., and M. M. K. Nass. 1968. Differences between mitochondrial and cytoplasmic transfer RNA and aminoacyl transfer RNA synthetases from rat liver. Proc. Nat. Acad. Sci. U.S.A. 60:1045–1052.
49. Bumsted, R. M., J. L. Dahl, D. Söll, and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. X. Further study of the glycyl transfer ribonucleic acids active in peptidoglycan synthesis in *Staphylococcus aureus*. J. Biol. Chem. 243:779–782.
50. Burchall, J. J., and G. H. Hitchings. 1965. Inhibitor binding analysis of dihydrofolate reductases from various species. Mol. Pharmacol. 1:126–136.
51. Burrows, W. J., D. J. Armstrong, F. Skoog, S. M. Hecht, J. T. A. Boyle, N. J. Leonard, and J. Occolowitz. 1968. Cytokinins from soluble RNA of *Escherichia coli*: 6-(3-methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine. Science 161:691–693.
52. Calendar, R., and P. Berg. 1966. The catalytic properties of tyrosyl ribonucleic acid synthetases from *Escherichia coli* and *Bacillus subtilis*. Biochemistry 5:1690–1695.
53. Calendar, R., and P. Berg. 1966. Purification and physical characterization of tyrosyl ribonculeic acid synthetases from *Escherichia coli* and *Bacillus subtilis*. Biochemistry 5:1681–1690.
54. Cameron, H. J., and G. R. Julian. 1968. The effect of chloramphenicol on the polysome formation of starved stringent *Escherichia coli*. Biochim. Biophys. Acta 169:373–380.
55. Cantor, C. R. 1967. Possible conformations of 5*S* ribosomal RNA. Nature 216:513–514.
56. Capecchi, M. R. 1966. Initiation of *E. coli* proteins. Proc. Nat. Acad. Sci. U.S.A. 55:1517–1524.
57. Capecchi, M. R. 1967. Polarity *in vitro*. J. Mol. Biol. 30: 213–217.
58. Capecchi, M. R. 1967. Polypeptide chain termination *in vitro*: isolation of a release factor. Proc. Nat. Acad. Sci. U.S.A. 58:1144–1151.
59. Capecchi, M. R., and G. N. Gussin. 1965. Suppression in vitro: identification of a serine-sRNA as a "nonsense" suppressor. Science 149:417–422.
60. Carbon, J., and J. B. Curry. 1968. A change in the specificity of transfer RNA after partial deamination with nitrous acid. Proc. Nat. Acad. Sci. U.S.A. 59:467–474.
61. Carbon, J., and J. B. Curry. 1968. Genetically and chemically derived missense suppressor transfer RNA with altered enzymic aminoacylation rates. J. Mol. Biol. 38:201–216.
62. Caruthers, M. H., H. Büchi, A. Kumar, E. Ohtsuka, J. H. van de Sande, and H. Weber. 1969. Progress in the total synthesis of the gene for yeast alanine transfer RNA. I. Chemical synthesis of deoxypolynucleotide segments. Fed. Proc. 28:532.
63. Caskey, C. T., R. Tompkins, E. Scolnick, T. Caryk, and M. Nirenberg. 1968. Sequential translation of trinucleotide codons for the initiation and termination of protein synthesis. Science 162:135–138.
64. Cassio, D. 1968. Etude de la methionyl tRNA synthetase de *Escherichia coli*. 2. Inactivation selective et reversible de la capacite de charger le tRNA. Eur. J. Biochem. 4:222–224.
65. Cassio, D., and J. P. Waller. 1968. Etude de la methionyl-tRNA synthetase d'*E. coli*. Dissociation en sous-unites actives par action d'un facteur extrinsique. Eur. J. Biochem. 5:33–41.
66. Cerhova, M., and I. Rychlik. 1967. Isolation of two methi-

onyl-tRNA synthetases from *Escherichia coli*. Collection Czech. Chem. Commun. 32:3808–3811.
67. Chapeville, F., F. Lipmann, G. von Ehrenstein, B. Weisblum, W. J. Ray, and S. Benzer. 1962. On the role of soluble ribonucleic acid in coding for amino acids. Proc. Nat. Acad. Sci. U.S.A. 48:1086–1092.
68. Chersi, A., M. Dzionara, D. Donner, and H. G. Wittmann. 1968. Ribosomal proteins. IV. Isolation, amino acid compositions, peptide maps and molecular weights of yeast ribosomal proteins. Mol. Gen. Genet. 101:82–88.
69. Clark, B. F. C., B. P. Doctor, K. C. Holmes, A. Klug, K. A. Marcker, S. J. Morris, and H. Paradies. 1968. Crystallization of transfer RNA. Nature 219:1222–1224.
70. Clark, B. F. C., and K. A. Marcker. 1965. Coding response of *N*-formyl-methionyl-sRNA to UUG. Nature 207: 1038–1039.
71. Clark, B. F. C., and K. A. Marcker. 1966. The role of *N*-formyl-methionyl-sRNA in protein biosynthesis. J. Mol. Biol. 17:394–406.
72. Clark, M. F., R. E. F. Matthews, and R. K. Ralph. 1964. Ribosomes and polyribosomes in *Brassica pekinensis*. Biochim. Biophys. Acta 91:289–304.
73. Clark-Walker, G. D., and A. W. Linnane. 1966. *In vivo* differentiation of yeast cytoplasmic and mitochondrial protein synthesis with antibiotics. Biochem. Biophys. Res. Commun. 25:8–13.
74. Cold Spring Harbor Symposia on Quantitative Biology. 1966. The genetic code, vol. 31.
75. Conway, T. W., and F. Lipmann. 1964. Characterization of a ribosome-linked guanosine triphosphatase in *Escherichia coli* extracts. Proc. Nat. Acad. Sci. U.S.A. 52:1462–1469.
76. Cory, S., K. A. Marcker, S. K. Dube, and B. F. C. Clark. 1968. Primary structure of a methionine transfer RNA from *Escherichia coli*. Nature 220:1039–1040.
77. Cramer, F., H. Doepner, F. v. d. Haar, E. Schlimme, and H. Seidel. 1968. On the conformation of transfer RNA. Proc. Nat. Acad. Sci. U.S.A. 61:1384–1391.
78. Cramer, F., F. v. d. Haar, W. Saenger, and E. Schlimme. 1968. Einkristalle von phenylalaninspezifischer Transfer-Ribonucleinsäure. Angew. Chem. Int. Ed. Engl. 80: 969–970.
79. Craven, G. R., P. Voynow, S. J. S. Hardy, and C. G. Kurland. 1969. The ribosomal proteins of *Escherichia coli* II. Chemical and physical characterization of the 30*S* ribosomal proteins. J. Biol. Chem., *in press*.
80. Cundliffe, E. 1967. Antibiotics and polyribosomes. Chlortetracycline and polyribosomes of *Bacillus megatherium*. Mol. Pharmacol. 3:401–411.
81. Cuzin, F. N., N. Kretchmer, R. E. Greenberg, R. Hurwitz, and F. Chapeville. 1967. Enzymatic hydrolysis of *N*-substituted aminoacyl-tRNA. Proc. Nat. Acad. Sci. U.S.A. 58:2079–2086.
82. Darnell J. E., Jr., 1968. Ribonucleic acids from animal cells. Bacteriol. Rev. 32:262–290.
83. DeWachter, R., and W. Fiers. 1969. Sequences at the 5'-terminus of bacteriophage Qβ RNA. Nature 221:233–235.
84. Dickerman, H. W., E. Steers, Jr., B. G. Redfield, and H. Weissbach. 1967. Methionyl soluble ribonucleic acid transformylase. I. Purification and partial characterization. J. Biol. Chem. 242:1522–1525.
84*a*. Dintzis, H. M. 1961. Assembly of the peptide chains of hemoglobin. Proc. Nat. Acad. Sci. U.S.A. 47:247–261.
85. Doepner, H., H. Seidel, and F. Cramer. 1966. Untersuchungen zur Sekundärstruktur Löslicher Ribonucleinsäuren. Angew. Chem. 78:601.
86. Doi, R. H., I. Kaneko, and B. Goehler. 1966. Regulation of a serine transfer RNA of *Bacillus subtilis* under two growth conditions. Proc. Nat. Acad. Sci. U.S.A. 56:1548–1551.
87. Doi, R. H., I. Kaneko, and R. T. Igarashi. 1968. Pattern of valine transfer ribonucleic acid of *Bacillus subtilis* under different growth conditions. J. Biol. Chem. 243:945–951.
88. Doolittle, W. F., and C. Yanofsky. 1968. Mutants of *Escherichia coli* with an altered tryptophanyl-transfer ribonucleic acid synthetase. J. Bacteriol. 95:1283–1294.
89. Dube, S. K., K. A. Marcker, B. F. C. Clark, and S. Cory. 1968. Nucleotide sequence of *N*-formyl-methionyl-transfer RNA. Nature 218:232–233.
90. Dudock, B. S., G. Katz, E. K. Taylor, and R. W. Holley. 1969. Primary structure of wheat germ phenylalanine transfer RNA. Proc. Nat. Acad. Sci. U.S.A. 62:941–945.
91. Edlin, G., and P. Broda. 1968. Physiology and genetics of the "ribonucleic acid control" locus in *Escherichia coli*. Bacteriol. Rev. 32:206–226.
92. Eidlic, L., and F. C. Neidhardt. 1965. Protein and nucleic acid synthesis in two mutants of *Escherichia coli* with temperature-sensitive aminoacyl ribonucleic acid synthetases. J. Bacteriol. 89:706–711.
93. Eisenstadt, J. M., and G. Brawerman. 1964. The protein-synthesizing systems from the cytoplasm and the chloroplasts of *Euglena gracilis*. J. Mol. Biol. 10:392–402.
94. Eisenstadt, J. M., and G. Brawerman. 1966. A factor from *Escherichia coli* concerned with the stimulation of cell-free polypeptide synthesis by exogenous ribonucleic acid. I. Evidence for the occurrence of a stimulation factor. Biochemistry 5:2777–2783.
95. Eisenstadt, J. M., and G. Brawerman. 1967. The role of the native subribosomal particles of *Escherichia coli* in polypeptide chain initiation. Proc. Nat. Acad. Sci. U.S.A. 58:1560–1565.
96. Eisenstadt, J., and P. Lengyel. 1966. Formylmethionyl-tRNA dependent amino acid incorporation in extracts of trimethoprim-treated *Escherichia coli*. Science 154:524–527.
97. Engelhardt, D. L., R. E. Webster, R. C. Wilhelm, and N. D. Zinder. 1965. *In vitro* studies on the mechanism of suppression of a nonsense mutation. Proc. Nat. Acad. Sci. U.S.A. 54:1791–1797.
98. Engelhardt, D. L., R. D. Webster, and N. D. Zinder. 1967. Amber mutants and polarity *in vitro*. J. Mol. Biol. 27: 45–58.
99. Epstein, W., and J. R. Beckwith. 1968. Regulation of gene expression. Ann. Rev. Biochem. 37:411–436.
100. Erbe, R. W., and P. Leder. 1968. Initiation and protein synthesis: translation of di- and tri-codon messengers. Biochem. Biophys. Res. Commun. 31:789–803.
101. Erbe, R. H., M. Nau, and P. Leder. 1969. Translation and translocation of defined RNA messengers. J. Mol. Biol. 39:441–460.
102. Ertel, R., N. Brot, B. Redfield, J. E. Allende, and H. Weissbach. 1968. Binding of guanosine 5'-triphosphate by soluble factors required for polypeptide synthesis. Proc. Nat. Acad. Sci. U.S.A. 59:861–868.
103. Ertel, R., B. Redfield, N. Brot, and H. Weissbach. 1968. Role of GTP in protein synthesis: interaction of GTP with soluble transfer factors from *E. coli*. Arch. Biochem. Biophys. 128:331–338.
104. Fangman, W. L., and F. C. Neidhardt. 1964. Demonstration of an altered aminoacyl ribonucleic acid synthetase in a mutant of *E. coli*. J. Biol. Chem. 239:1839–1843.
105. Favorova, O. O., T. N. Spasokukotskaya, and L. L. Kisselev. 1968. Two forms of glycyl-RNA-synthetase activities in rat liver supernatant fraction. Mol. Biol. 2: 69–78.
106. Fittler, F., and R. H. Hall. 1966. Selective modification of yeast seryl-tRNA and its effect on the acceptance and binding functions. Biochem. Biophys. Res. Commun. 25:441–446.
107. Fittler, F., L. Kline, and R. H. Hall. 1968. N^6-(Δ^2-isopentenyl) adenosine: biosynthesis in vitro by an enzyme extract from yeast and rat liver. Biochem. Biophys. Res. Commun. 31:571–576.
108. Fellner, P., and F. Sanger. 1968. Sequence analysis of specific

areas of the 16S and 23S ribosomal RNAs. Nature 219: 236–238.
108a. Flessel, C. P., P. Ralph, and A. Rich. 1967. Polyribosomes of growing bacteria. Science 158:658–660.
109. Fogel, S., and P. S. Sypherd. 1968. Chemical basis for heterogeneity of ribosomal proteins. Proc. Nat. Acad. Sci. U.S.A. 59:1329–1336.
110. Forget, B. G., and S. Weissman. 1967. Low molecular weight RNA components from KB cells. Nature 213:878–882.
111. Forget, B. G., and S. M. Weissman. 1967. Nucleotide sequence of KB cell 5S RNA. Science 158:1695–1699.
112. Fox, J. L., and M. C. Ganoza. 1968. Chain termination *in vitro*: studies on the specificity of amber and ochre triplets. Biochem. Biophys. Res. Commun. 32:1064–1070.
113. Fresco, J. R., R. D. Blake, and R. Langridge. 1968. Crystallization of transfer ribonucleic acids from unfractionated mixtures. Nature 220:1285–1287.
114. Fresco, J. R., and T. Lindahl. 1969. Transfer RNA. Ann. Rev. Biochem. 38:, *in press*.
115. Fry, K. T., and M. R. Lamborg. 1968. Aminohydrolase activity of *Escherichia coli* extracts with formylated amino acids and dipeptides as substrates. J. Mol. Biol. 28:423–433.
116. Ganoza, M. C. 1966. Polypeptide chain termination in cell-free extracts of *E. coli*. Cold Spring Harbor Symp. Quant. Biol. 36:273–278.
117. Garen, A. 1968. Sense and nonsense in the genetic code. Science 160:149–159.
118. Gartland, W. J., and N. Sueoka. 1966. Two interconvertible forms of tryptophanyl sRNA in *E. coli*. Proc. Nat. Acad. Sci. U.S.A. 55:948–956.
119. Gefter, M. L., and R. L. Russell. 1969. Role of modifications in tyrosine transfer RNA: A modified base affecting ribosome binding. J. Mol. Biol. 39:145–157.
120. Geiduschek, P., and R. Haselkorn. 1969. Messenger RNA. Ann. Rev. Biochem., *in press*.
121. George, H., and A. Meister. 1967. Purification and properties of L-valyl-sRNA synthetase from *Escherichia coli*. Biochim. Biophys. Acta 132:165–174.
122. Geroch, M. E., E. G. Richards, and G. A. Davies. 1968. 5S RNA. I. Preparation and characterization of highly purified 5S RNA from *Escherichia coli*. Eur. J. Biochem. 6:325–330.
123. Ghosh, H. P., and H. G. Khorana. 1967. Studies on polynucleotides. LXXXIV. On the role of ribosomal subunits in protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 58: 2455–2461.
124. Ghosh, H. P., D. Söll, and H. G. Khorana. 1967. Studies on polynucleotides. LXVII. Initiation of protein synthesis *in vitro* as studied by using ribopolynucleotides with repeating nucleotide sequences as messengers. J. Mol. Biol. 25:275–298.
125. Gilbert, W. 1963. Polypeptide synthesis in *Escherichia coli*. II. The polypeptide chain and sRNA. J. Mol. Biol. 6: 389–403.
126. Gillam, I., D. Blew, R. C. Warrington, M. von Tigerstrom, and G. M. Tener. 1968. General procedure for the isolation of specific transfer ribonucleic acids. Biochemistry 7:3459–3468.
127. Gillam, I., S. Millward, D. Blew, M. von Tigerstrom, E. Wimmer, and G. M. Tener. 1967. Separation of soluble ribonucleic acids on benzoylated diethylaminoethylcellulose. Biochemistry 6:3043–3056.
128. Goldberg, I. H., and K. Mitsugi. 1967. Sparsomycin inhibition of polypeptide synthesis promoted by synthetic and natural polynucleotides. Biochemistry 6:372–383.
129. Goodman, H. M., J. Abelson, A. Landy, S. Brenner, and J. D. Smith. 1968. Amber suppression: a nucleotide change in the anticodon of a tyrosine transfer RNA. Nature 217: 1019–1024.
130. Gopinathan, K. P., and A. Garen. 1969. A leucyl-transfer RNA specified by the amber suppressor gene Su6. J. Mol. Biol., *submitted for publication*.
131. Gordon, J. 1967. Interaction of guanosine 5'-triphosphate with a supernatant fraction from *E. coli* and aminoacyl-sRNA. Proc. Nat. Acad. Sci. U.S.A. 58:1574–1578.
132. Gordon, J. 1968. A stepwise reaction yielding a complex between a supernatant fraction from *E. coli*, guanosine-5'-triphosphate and aminoacyl-sRNA. Proc. Nat. Acad. Sci. U.S.A. 59:179–183.
133. Gottesman, M. 1967. Reaction of ribosome-bound peptidyl transfer ribonucleic acid with aminoacyl transfer ribonucleic acid or puromycin. J. Biol. Chem. 242:5564–5571.
134. Gould, R. M., M. P. Thornton, V. Liepkalns, and W. J. Lennarz. 1968. Participation of aminoacyl transfer ribonucleic acid in aminoacyl phosphatidylglycerol synthesis. II. Specificity of alanyl phosphatidylglycerol synthetase. J. Biol. Chem. 243:3096–3104.
135. Grollman, A. P., and M. L. Stewart. 1968. Inhibition of the attachment of messenger ribonucleic acid to ribosomes. Proc. Nat. Acad. Sci. U.S.A. 61:719–725.
136. Grosjean, H., J. Charlier, and J. Vanhumbeeck. 1968. Magnesium requirement in the transacylation reaction catalysed by the isoleucyl-RNA synthetase of *Bacillus stearothermophilus*. Biochem. Biophys. Res. Commun. 32:935–939.
137. Gross, S. R., M. T. McCoy, and E. B. Gilmore. 1968. Evidence for the involvement of a nuclear gene in the production of the mitochonrial leucyl-tRNA synthetase of *Neurospora*. Proc. Nat. Acad. Sci. U.S.A. 61:253–260.
138. Grunberg-Manago, M., B. F. C. Clark, M. Revel, P. S. Rudland, and J. Dondon. 1969. Stability of different ribosomal complexes with initiator transfer RNA and synthetic messenger RNA. J. Mol. Biol. 40:33–44.
139. Gupta, N. K., and H. G. Khorana. 1966. Missense suppression of the tryptophan synthetase A-protein mutant A78. Proc. Nat. Acad. Sci. U.S.A. 56:772–779.
140. Guthrie, C., H. Nashimoto, and M. Nomura. 1969. Structure and function of *E. coli* ribosomes VIII. Cold-sensitive mutants defective in ribosome assembly. Proc. Nat. Acad. Sci. U.S.A., *in press*
141. Guthrie, C., and M. Nomura. 1968. Initiation of protein synthesis: a critical test of the 30S subunit model. Nature 219:232–235.
142. Haenni, A. L., and J. Lucas-Lenard. 1968. Stepwise synthesis of a tri-peptide. Proc. Nat. Acad. Sci. U.S.A. 61:1363–1369.
143. Hall, R. H. 1969. The modified nucleosides in nucleic acids. Columbia University Press, New York.
144. Hall, R. H., L. Csonka, H. David, and B. McLannan. 1967. Cytokinins in the soluble RNA of plant tissues. Science 156:69–71.
145. Hall, R. H., M. J. Robins, L. Stasiuk, and R. Thedford. 1966. Isolation of N^6-(γ, γ-dimethylallyl) adenosine from soluble ribonucleic acid. J. Amer. Chem. Soc. 88:2614–2615.
146. Hampel, A., M. Labanauskas, P. G. Connors, L. Kirkegard, U. L. RajBhandary, P. B. Sigler, and R. M. Bock. 1968. Single crystals of transfer RNA from formylmethionine and phenylalanine transfer RNAs. Science 162:1384–1387.
147. Harada, F., H. J. Gross, F. Kimura, S. H. Chang, S. Nishimura, and U. L. RajBhandary. 1968. 2-Methylthio-N^6-(Δ^2-isopentenyl) adenosine: A component of *E. coli* tyrosine transfer RNA. Biochem. Biophys. Res. Commun. 33:299–306.
148. Hartwell, L. H., and C. S. McLaughlin. 1968. Mutants of yeast with temperature-sensitive isoleucyl-tRNA synthetases. Proc. Nat. Acad. Sci. U.S.A. 59:422–428.
149. Hayashi, H., H. Fisher, and D. Söll. 1969. tRNA from Mycoplasma. Biochemistry, *in press*.
150. Hayashi, H., and K. Miura. Chemical modification of tyrosine tRNA from *Torulopsis utilis*. J. Mol. Biol., *in preparation*.
151. Helgeson, J. P. 1968. The cytokinins. Science 161:974–981.

152. Hershey, J. W. B., and R. E. Monro. 1966 A competitive inhibitor of the GTP reaction in protein synthesis. J. Mol. Biol. 18:68–76.
153. Hershey, J. W. B., and R. E. Thach. 1967. Role of guanosine 5′-triphosphate in the initiation of peptide synthesis. I. Synthesis of formylmethionyl-puromycin. Proc. Nat. Acad. Sci. U.S.A. 57:759–766.
154. Hierowsky, M. 1965. Inhibition of protein synthesis by chlorotetracycline in the *E. coli in vitro* system. Proc. Nat. Acad. Sci. U.S.A. 53:594–599.
155. Hille, M. B., M. J. Miller, K. Iwasaki, and A. J. Wahba. 1967. Translation of the genetic message. VI. The role of ribosomal subunits in binding of formylmethionyl-tRNA and its reaction with puromycin. Proc. Nat. Acad. Sci. U.S.A. 58:1652–1654.
156. Hirsh, D. I. 1968. A study of the threonyl adenylate complex with threonyl transfer ribonucleic acid synthetase and its reaction with hydroxylamine. J. Biol. Chem. 243:5731–5738.
157. Hirsh, D. I., and F. Lipmann. 1968. The divergence in reactivity of aminoacyl transfer ribonucleic acid synthesis of *Escherichia coli* with hydroxylamine. J. Biol. Chem. 243: 5724–5730.
158. Hirshfield, I. N., R. DeDeken, P. C. Horn, D. A. Hopwood, and W. K. Maas. 1968. Studies on the mechanism of repression of arginine biosynthesis in *Escherichia coli*. III. Repression of enzymes of arginine biosynthesis in arginyl-tRNA synthetase mutants. J. Mol. Biol. 35:83–93.
159. Hoffman, E., R. C. Wilhelm, W. Konigsberg, and J. R. Katze. 1969. A structural gene for seryl-tRNA synthetase in *Escherichia coli. In preparation.*
160. Holley, R. W., J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, and A. Zamir. 1965. Structure of a ribonucleic acid. Science 147:1462–1465.
161. Hollis, V. W., Jr., and A. V. Furano. 1968. The fractionation of transfer factors in the presence of a proteolytic inhibitor. J. Biol. Chem. 243:4926–4930.
162. Horikoshi, K., and R. H. Doi. 1967. Synthesis of *N*-formylmethionyl-sRNA by *Bacillus subtilis* extracts. Arch. Biochem. Biophys. 122:685–693.
163. Hsu, W. T., J. W. Foft, and S. B. Weiss. 1967. Effect of bacteriophage infection on the sulfur-labeling of sRNA. Proc. Nat. Acad. Sci. U.S.A. 58:2028–2035.
164. Imamoto, F., T. Yamane, and N. Sueoka. 1965. Existence of two phenylalanyl-sRNA synthetases in *Neurospora* crassa. Proc. Nat. Acad. Sci. U.S.A. 53:1456–1462.
165. Ito, J., and F. Imamoto. 1968. Sequential derepression and repression of the tryptophan operon in *E. coli*. Nature 220:441–444.
166. Kaempfer, R. 1968. Ribosomal subunit exchange during protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 61:106–113.
167. Kaempfer, R. O. R., M. Meselson, and H. J. Raskas. 1968. Cyclic dissociation into stable subunits and re-formation ribosomes during bacterial growth. J. Mol. Biol. 31:277–289.
168. Kaji, A., and H. Kaji. 1963. Specific interaction of soluble RNA with polyribonucleic acid induced polysomes. Biochem. Biophys. Res. Commun. 13:186–192.
169. Kaltschmidt, E., M. Dzionara, D. Donner, and H. G. Wittmann. 1967. Ribosomal proteins. I. Isolation, amino acid composition, molecular weights and peptide mapping of proteins from *E. coli* ribosomes. Mol. Gen. Genet. 100: 364–373.
170. Kan, J., T. Kano-Sueoka, and N. Sueoka. 1968. Characterization of leucine transfer ribonucleic acid in *Escherichia coli* following infection with bacteriphage T2. J. Biol. Chem. 243:5584–5590.
171. Kano-Sueoka, T., M. Nirenberg, and N. Sueoka. 1968. Effect of bacteriophage infection upon the specificity of leucine transfer RNA for RNA codewords. J. Mol. Biol. 35:1–12.
172. Kano-Sueoka, T., and N. Sueoka. 1966. Modification of leucyl-sRNA after bacteriophage infection. J. Mol. Biol. 20:183–209.
173. Kaplan, S., and D. Anderson. 1968. Selection of temperature-sensitive activating enzyme mutants in *Escherichia coli*. J. Bacteriol. 95:991–997.
174. Katze, J. R., and W. Konigsberg. Purification and properties of seryl tRNA synthetase from *Escherichia coli. In preparation.*
175. Kaziro, Y., and N. Inoue. 1968. Crystalline G factor from *Escherichia coli*. J. Biochem. (Tokyo) 64:423–425.
176. Keller, E. B., and P. C. Zamecnik. 1956. The effect of guanosine diphosphate and triphosphate on the incorporation of labeled amino acids into proteins. J. Biol. Chem. 221: 45–59.
177. Kepes, A. 1967. Sequential transcription and translation in the lactose operon of *Escherichia coli*. Biochim. Biophys. Acta 138:107–123.
178. Khorana, H. G. 1968. Polynucleotide synthesis and the genetic code, p. 79–105. Harvey lectures. Ser. 62. Academic Press Inc., New York.
179. Kinoshita, K., G. Kawano, and N. Tanaka. 1968. Association of fusidic acid sensitivity with G factor in a protein-synthesizing system. Biochem. Biophys. Res. Commun. 33:769–773.
180. Kim, S. H., and A. Rich. 1968. Single crystals of transfer RNA: an X-ray diffraction study. Science 162:1381–1384.
181. Kirtikar, M. W., and A. Kaji. 1968. Stimulation of phage ribonucleic acid-dependent incorporation of amino acids by 5*S* ribonucleic acid. J. Biol. Chem. 243:5345–5353.
182. Klein, A., A. Eisenstadt, J. Eisenstadt, and P. Lengyel. 1969. Inhibition of peptide chain initiation in *Escherichia coli* by hydroxylamine and effects on RNA synthesis. *In preparation.*
183. Klein, A., C. Mathews, J. Eisenstadt, and P. Lengyel. 1967. On the requirement for formyl residues in the synthesis of bacteriophage T_4 proteins. Biochim. Biophys. Acta 149: 622–624.
184. Klein, A., C. Mathews, J. Eisenstadt, and P. Lengyel. 1968. Further studies on the requirement for formyl residues in the synthesis of bacteriophage T_4 proteins. Biochim. Biophys. Acta 169:543–545.
185. Kline, L. K., F. Fittler, and R. H. Hall. 1969. Biosynthesis of N^{6}-(Δ^{2} isopentenyl) adenosine in tRNA: Purification and characteristics of an enzyme that catalyzes attachment of the Δ^{2}-isopententyl group to tRNA. Biochemistry, *in press.*
186. Kohler, R. E., E. Z. Ron, and B. D. Davis. 1968. Significance of the free 70*S* ribosomes in *E. coli* extracts. J. Mol. Biol. 36:71–82.
187. Kolakofsky, D., K. F. Dewey, J. W. B. Hershey, and R. E. Thach. 1968. Guanosine 5′-triphosphatase activity of initiation factor f2. Proc. Nat. Acad. Sci. U.S.A. 61: 1066–1070.
188. Kolakofsky, D., and T. Nakamoto. 1966. The initiation of viral protein synthesis in *E. coli* extracts. Proc. Nat. Acad. Sci. U.S.A. 56:1786–1793.
189. Kolakofsky, D., T. Ohta, and R. E. Thach. 1968. Junction of the 50*S* ribosomal subunit with the 30*S* initiation complex. Nature 220:244–247.
190. Kondo, M., G. Eggertsson, J. Eisenstadt, and P. Lengyel. 1968. Ribosome formation from subunits: dependence on formylmethionyl-tRNA in extracts from *E. coli*. Nature 220:368–370.
191. Kössel, H. 1968. Studies on polynucleotides. LXXXIII. Synthesis *in vitro* of the tripeptide valyl-seryl-lysine by poly (G-U-A-A). Biochim. Biophys. Acta 157:91–96.
192. Kössel, H., and U. RajBhandary. 1968. Studies on polynucleotides. LXXXVI. Enzymic hydrolysis of *N*-acylaminoacyl-transfer RNA. J. Mol. Biol. 35:539–560.

193. Kroon, A. M. 1965. Protein synthesis in mitochondria. III. On the effects of inhibitors on the incorporation of amino acids into protein by intact mitochondria and digitonin fractions. Biochim. Biophys. Acta 108:275–284.
194. Küntzel, H., and H. Noll. 1967. Mitochondrial and cytoplasmic polysomes from *Neurospora crassa*. Nature 215: 1340–1345.
195. Kuriki, Y., H. Ishitsuka, and A. Kaji. 1969. Factor and GTP dependent release of tRNA from 70*S* ribosomes. Fed. Proc. 28:598.
196. Kuriki, Y., and A. Kaji. 1968. Factor and guanosine 5′-triphosphate-dependent release of deacylated transfer RNA from 70*S* ribosomes. Proc. Nat. Acad. Sci. U.S.A. 61:1399–1405.
197. Kurland, C. G. 1966. The requirements for specific sRNA binding by ribosomes. J. Mol. Biol. 18:90–108.
198. Kwan, C., D. Apirion, and D. Schlessinger. 1968. Anaerobiosis induced changes in an isoleucyl transfer ribonucleic acid and the 50*S* ribosomes of *Escherichia coli*. Biochemistry 7:427–433.
199. Lagerkvist, U., and L. Rymo. 1969. Structure and function of transfer ribonucleic acid. III. Some properties of a complex between valyl ribonucleic acid synthetase and transfer ribonucleic acid specific for valine. J. Biol. Chem., 244:2476–2483.
200. Lagerkvist, U., L. Rymo, and J. Waldenström. 1966. Structure and function of transfer ribonucleic acid. II. Enzyme-substrate complexes with valyl ribonucleic acid synthetase from yeast. J. Biol. Chem. 241:5391–5400.
201. Lagerkvist, U., and J. Waldenström. 1967. Purification and some properties of valyl ribonucleic acid synthetase from yeast. J. Biol. Chem. 242:3021–3025.
202. Lake, J. A., and W. W. Beeman. 1968. On the conformation of yeast transfer RNA. J. Mol. Biol. 31:115–125.
203. Last, J. A., W. M. Stanley, M. S. Salas, M. B. Hille, A. J. Wahba, and S. Ochoa. 1967. Translation of the genetic message. IV. UAA as chain terminator codon. Proc. Nat. Acad. Sci. U.S.A. 57:1062–1067.
204. Lazzarini, R. A., and A. H. Mehler. 1966. Glutamyl- and Glutaminyl-sRNA synthetases from *Escherichia coli*, p. 409. *In* G. Cantoni, and D. R. Davis (ed.), Procedures in nucleic acid research. Harper & Row, New York.
205. Leboy, P. S., E. C. Cox, and J. G. Flasks. 1964. The chromosomal site specifying a ribosomal protein in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 52:1367–1374.
206. Leder, P., and H. Bursztyn. 1966. Initiation of protein synthesis. II. A convenient assay for the ribosome-dependent synthesis of *N*-formyl-C^{14}-methionylpuromycin. Biochem. Biophys. Res. Commun. 25:233–238.
207. Leder, P., and M. M. Nau. 1967. Initiation of protein synthesis. III. Factor GTP-codon-dependent binding of F-met-tRNA to ribosomes. Proc. Nat. Acad. Sci. U.S.A. 58: 774–781.
208. Lee, J. C., and V. M. Ingram. 1967. Erythrocyte transfer RNA: change during chick development. Science 158: 1330–1332.
209. Lee, L. W., J. M. Ravel, and W. Shive. 1967. A general involvement of acceptor ribonucleic acid in the inital activation step of glutamic acid and glutamine. Arch. Biochem. Biophys. 121:614–618.
210. Lee, M., and K. H. Muench. 1969. Prolyl transfer ribonucleic acid synthetase of *Escherichia coli*. J. Biol. Chem. 244:223–230.
211. Leive, L., and V. J. Kollin. 1967. Synthesis, utilization and degradation of lactose operon mRNA in *Escherichia coli*. J. Mol. Biol. 24:247–259.
212. Lemoine, F., J. P. Waller, and R. van Rapenbusch. 1968. Studies on methionyl transfer RNA synthetase. 1. Purification and some properties of methionyl transfer RNA synthetase from *Escherichia coli* K-12. Eur. J. Biochem. 4:213–221.
213. Lengyel. P. 1967. On peptide chain initiation, p. 193–212. *In* J. H. Taylor (ed.), Molecular genetics. Part II. Academic Press Inc., New York.
214. Lengyel, P., J. F. Speyer, and S. Ochoa. 1961. Synthetic polynucleotides and the amino acid code. Proc. Nat. Acad. Sci. U.S.A. 47:1936–1942.
215. Lennarz, W. J., J. A. Nesbitt, III, and J. Reiss. 1966. The participation of sRNA in the enzymatic synthesis of *O*-L-lysyl phosphatidylglycerol in *Staphylococcus aureus*. Proc. Nat. Acad. Sci. U.S.A. 55:934–941.
216. Lietman, P. S. 1968. Enzymatic acylation of phenylalanyl transfer ribonucleic acids from mitochondria and cytosol of rat liver. J. Biol. Chem. 243:2837–2839.
217. Lindahl, T., A. Adams, and J. R. Fresco. 1966. Renaturation of transfer ribonucleic acids through site binding of magesium. Proc. Nat. Acad. Sci. U.S.A. 55:941–948.
218. Lipmann, F. 1967. Peptide bond formation in protein biosynthesis in regulation of nucleic acid and protein biosynthesis, p. 177. V. V. Koningsberger and L. Bosch. (ed.), Biochimica et Biophysica Acta Library, vol. 10. Elsevier Publishing Co., New York.
219. Lipmann, F., W. C. Hülsmann, G. Hartmann, H. G. Boman, and G. Acs. 1959. Amino acid activation and protein synthesis. J. Cell. Comp. Physiol. 54(Suppl. 1):75–89.
220. Livingston, D. M., and P. Leder. 1969. Deformylation and protein biosynthesis. Biochemistry 8:435–443.
221. Lodish, H. 1968. Bacteriophage f2 RNA: control of translation and gene order. Nature 200:345–350.
222. Loehr, J. S., and S. B. Keller. 1968. Dimers of alanine transfer RNA with acceptor activity. Proc. Nat. Acad. Sci. U.S.A. 61:1115–1122.
223. Loftfield, R. B., and E. A. Eigner. 1966. The specificity of enzymic reactions. Aminoacyl-soluble RNA ligases. Biochim. Biophys. Acta 130:426–448.
224. Lucas-Lenard, J., and A. Haenni. 1968. Requirement of guanosine 5′-triphosphate for ribosomal binding of aminoacyl-sRNA. Proc. Nat. Acad. Sci. U.S.A. 59:554–560.
225. Lucas-Lenard, J., and A. Haenni. 1969. Release of tRNA during peptide chain elongation. Proc. Nat. Acad. Sci. U.S.A., *in press*.
226. Lucas-Lenard, J., and F. Lipmann. 1966. Separation of three microbial amino acid polymerization factors. Proc. Nat. Acad. Sci. U.S.A. 55:1562–1566.
227. Lucas-Lenard, J., and F. Lipmann. 1967. Initiation of polyphenylalanine synthesis by *N*-acetylphenylalanyl-sRNA. Proc. Nat. Acad. Sci. U.S.A. 57:1050–1057.
228. Lyttleton, J. W. 1962. Isolation of ribosomes from spinach chloroplasts. Exp. Cell Res. 26:312–317.
228*a*. MacDonald, R. E., and D. P. Yeater. 1968. Extraction of polyribosomes and ribosomal subunits from exponentially growing *Echerichia coli*. Bacteriol. Proc. p. 113.
229. Maden, B. E. H., R. R. Traut, and R. E. Monro. 1968. Ribosome-catalysed peptidyl-transfer: the polyphenylalanine system. J. Mol. Biol. 35:333–345.
230. Madison, J. T. 1968. Primary structure of RNA. Ann. Rev. Biochem. 37:131–148.
231. Madison, J. T., G. A. Everett, and H. Kung. 1966. Nucleotide sequence of a yeast tyrosine transfer RNA. Science 153: 531–534.
232. Maitra, U., and J. Dubnoff. 1968. Protein factors involved in the *in vitro* transcription and translation of mRNA. Fed. Proc. 27:398.
233. Makman, M. H. 1966. Seryl, phenylalanyl, arginyl, and leucyl sRNA synthetases from bakers' yeast, p. 424. *In* G. Cantoni, and D. R. Davis (ed.), Procedures in nucleic acid research. Harper & Row, New York.
234. Makman, M. H., and G. L. Cantoni. 1965. Isolation of seryl and phenylalanyl ribonucleic acid synthetases from bakers' yeast. Biochemistry 4:1434–1442.
235. Mangiarotti, G., and D. Schlessinger. 1966. Polyribosome metabolism in *Escherichia coli*. Extraction of polyribosomes and ribosomal subunits from fragile, growing *Escherichia coli*. J. Mol. Biol. 20:123–143.

236. Marchis-Mouren, G., and F. Lipmann. 1965. On the mechanism of acetylation of fetal and chicken hemoglobins. Proc. Nat. Acad. Sci. U.S.A. 53:1147–1154.
237. Marcker, K. 1965. The formation of *N*-formyl-methionyl-sRNA. J. Mol. Biol. 14:63–70.
238. Marcker, K. A., B. F. C. Clark, and J. S. Anderson. 1966. *N*-formyl-methionyl-sRNA and its relation to protein biosynthesis. Cold Spring Harbor Symp. Quant. Biol. 31:279.
239. Marcker, K., and F. Sanger. 1964. *N*-formyl-methionyl-s-RNA. J. Mol. Biol. 8:835–840.
240. Margulies, M. 1964. Effect of chloramphenicol on light-dependent synthesis of proteins and enzymes of leaves and chloroplasts of Phaseolus vulgaris. Plant Physiol. 39:579–585.
241. Martin, R. G., H. J. Whitfield, Jr., D. B. Berkowitz, and M. J. Voll. 1966. A molecular model of the phenomenon of polarity. Cold Spring Harbor Symp. Quant. Biol. 31:215–220.
242. Matsuhashi, M., C. P. Dietrich, and J. L. Strominger. 1967. Biosynthesis of the peptidoglycan of bacterial cell walls. III. The role of soluble ribonucleic acid and of lipid intermediates in glycine incorporated in *Staphylococcus aureus*. J. Biol. Chem. 242:3191–3206.
243. Matthaei, H., G. Sander, D. Swan, T. Kreuzer, H. Caffier, and A. Parmeggiani. 1968. Reaktionsschritte der Polypeptidsynthese and Ribosomen: Mechanismen der Proteinsynthese. Naturwissenschaften 55:281–294.
244. McCarthy, B. J., and J. J. Holland. 1965. Denatured DNA as a direct template for *in vitro* protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 54:880–886.
245. McKeehan, W., P. Sepulveda, S. Liu, and B. Hardesty. 1969. Two distinct transfer enzymes from rabbit reticulocytes with ribosome dependent guanosine triphosphate phosphohydrolase activity. Biochem. Biophys. Res. Commun. 34:668–672.
246. McLaughlin, C. S., and V. M. Ingram. 1964. Aminoacyl position in aminoacyl-sRNA. Science. 145:942–943.
247. Mehler, A. H., and C. Jesensky. 1966. Prolyl sRNA synthetase of *Escherichia coli*, p. 420–433. *In* G. Cantoni, and D. R. Davis (ed.), Procedures in nucleic acid research. Harper & Row, New York.
248. Mehler, A. H., and S. K. Mitra. 1967. The activation of arginyl transfer ribonucleic acid synthetase by transfer ribonucleic acid. J. Biol. Chem. 242:5495–5499.
249. Michaelis, G., and P. Starlinger. 1967. Sequential appearance of the galactose enzymes in *E. coli*. Mol. Gen. Genet. 100: 210–215.
250. Miller, D. L., and H. Weissbach. 1969. An interaction between the transfer factors required for protein synthesis. *Submitted for publication.*
251. Miovic, M., and L. I. Pizer. 1968. Protein initiation following virulent bacteriophage infection. Proc. Nat. Acad. Sci. U.S.A. 61:192–199.
252. Mitra, S. K., and A. H. Mehler. 1966. The role of transfer ribonucleic acid in the pyrophosphate exchange reaction of arginine-transfer ribonucleic acid synthetase. J. Biol. Chem. 241:5161–5162.
253. Mitra, S. K., and A. H. Mehler. 1967. The arginyl transfer ribonucleic acid synthetase of *Escherichia coli*. J. Biol. Chem. 242:5490–5494.
254. Miura, K. 1967. Specificity in the structure of transfer RNA, p. 39–82. *In* J. N. Davidson, and W. E. Cohn. (ed.), Progress in nucleic acid research. Vol. 6. Academic Press Inc., New York.
255. Moav, B., and T. N. Harris. 1967. Pyrrolid-2-one-5 carboxylic acid involvement in the biosynthesis of rabbit immunoglobulin. Biochem. Biophys. Res. Commun. 29: 773–776.
256. Monro, R. E. 1967. Catalysis of peptide bond formation by 50*S* ribosomal subunits from *Escherichia coli*. J. Mol. Biol. 26:147–151.
257. Monro, R. E., J. Cerna, and K. A. Marcker. 1968. Ribosome-catalyzed peptidyl transfer-: substrate specificity at the P-site. Proc. Nat. Acad. Sci. U.S.A. 61:1042–1049.
258. Monro, R. E., B. E. H. Maden, and R. R. Traut. 1967. The mechanism of peptide bond formation in protein biosynthesis, p. 179–202. *In* D. Shugar (ed.), Genetic elements: properties and function. Academic Press Inc., New York.
259. Monro, R. E., and K. A. Marcker. 1967. Ribosome-catalysed reaction of puromycin with a formylmethionine-containing oligonucleotide. J. Mol. Biol. 25:347–350.
260. Monro, R. E., and D. Vazquez. 1967. Ribosome-catalyzed peptidyl-transfer: effect of some inhibitors of protein synthesis. J. Mol. Biol. 28:161–165.
261. Moore, P. B., R. R. Traut, H. Noller, P. Pearson, and H. Delius. 1968. Ribosomal proteins of *Escherichia coli*. II. Proteins from the 30*S* subunit. J. Mol. Biol. 31:441–461.
262. Morris, A., and R. Scheet. 1961. Release of soluble protein from reticulocyte ribosomes. Biochim. Biophys. Acta 47: 415–416.
262*a*. Morris, D. W., and J. A. DeMoss. 1966. Polysome transitions and the regulation of ribonucleic acid synthesis in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 56:262–268.
263. Morse, D. E., R. F. Baker, and C. Yanofsky. 1968. Translation of the tryptophan messenger RNA of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 60:1428–1435.
264. Muench, K. H., and P. A. Safille. 1968. Transfer ribonucleic acids in *Escherichia coli*. Multiplicity and variation. Biochemistry 7:2799–2808.
265. Mukundan, M. A., J. W. B. Hershey, K. E. Dewey, and R. E. Thach. 1968. Binding of formylmethionyl-tRNA to 30*S* ribosomal subunits. Nature 217:1013–1016.
266. Nakamoto, T., T. W. Conway, J. E. Allende, G. J. Spyrides, and F. Lipmann. 1963. Formation of peptide bonds. I. Peptide bond formation from aminoacyl-sRNA. Cold Spring Harbor Symp. Quant. Biol. 28:227.
267. Nakamoto, T., and D. Kolakofsky. 1966. A possible mechanism for initiation of protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 55:606–613.
268. Nass, G. 1967. Regulation of histidine biosynthetic enzymes in a mutant of *Escherichia coli* with an altered histidyl-tRNA synthetase. Mol. Gen. Genet. 100:216–224.
269. Nass, G., and G. Stöffler. 1967. Molecular weight distribution of the aminoacyl-tRNA-synthetases of *Escherichia coli* by gel filtration. Mol. Gen. Genet. 100:378–382.
270. Nathans, D. 1964. Inhibition of protein synthesis by puromycin. Fed. Proc. 23:984–989.
271. Nathans, D., and F. Lipmann. 1961. Amino acid transfer from aminoacyl-ribonucleic acids to protein on ribosomes of *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 47:497–504.
272. Nathans, D., G. Notani, J. H. Schwartz, and N. D. Zinder. 1962. Biosynthesis of the coat protein of coliphage f2 by *E. coli* extracts. Proc. Nat. Acad. Sci. U.S.A. 48:1424–1431.
273. Neelon, F. A., M. Molinaro, H. Ishikura, L. B. Sheiner, and G. L. Cantoni. Oligonucleotides produced by digestion of bakers' yeast serine transfer ribonucleic acid with specific nucleases. J. Biol. Chem. 242:4515–4522.
274. Neidhardt, F. C. 1966. Roles of amino acid activating enzymes in cellular physiology. Bacteriol. Rev. 30:701–719.
275. Nelson, J. A., C. C. Ristow, and R. W. Holley. 1967. Studies on the secondary structure of yeast alanine tRNA: reaction with *N*-bromosuccinimide and with nitrous acid. Biochim. Biophys. Acta 149:590–593.
276. Nesbitt, J. A., III, and W. J. Lennarz. 1968. Participation of aminoacyl transfer ribonucleic acid in aminoacyl phosphatidylglycerol synthesis. I. Specificity of lysyl phosphatidylglycerol synthetase. J. Biol. Chem. 243:3088–3095.
277. Nirenberg, M. W. 1964. Cell-free protein synthesis directed by messenger RNA, p. 17–23. *In* S. P. Colowick and N. O. Kaplan, (ed.), Methods in enzymology. Vol. 6. Academic Press Inc., New York.
278. Nirenberg, M., and P. Leder. 1964. RNA codewords and protein synthesis. Science 145:1399–1407.

279. Nirenberg, M. W., and H. Matthaei. 1961. The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polynucleotides. Proc. Nat. Acad. Sci. U.S.A. 47:1588–1602.
280. Nishimura, S., D. S. Jones, E. Ohtsuka, H. Hayatsu, T. M. Jacob, and H. G. Khorana. Studies on polynucleotides. XLVII. The *in vitro* synthesis of homopeptides as directed by a ribopolynucleotide containing a repeating trinucleotide sequence. New codon sequences for lysine, glutamic acid and arginine. J. Mol. Biol. 13:283–301.
281. Nishizuka, Y., and F. Lipmann. 1966. The interrelationship between guanosine triphosphate and amino acid polymerization. Arch. Biochem. Biophys. 116:344–351.
282. Nishizuka, Y., and F. Lipmann. 1966. Comparison of guanosine triphosphate split and polypeptide synthesis with a purified *E. coli* system. Proc. Nat. Acad. Sci. U.S.A. 55: 212–219.
283. Niyomporn, B., J. L. Dahl, and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. IX. Purification and properties of glycyl transfer ribonucleic acid synthetase from *Staphylococcus aureus*. J. Biol. Chem. 243:773–778.
284. Noll, H. 1966. Chain initiation and control of protein synthesis. Science 151:1241–1245.
285. Nomura, M., and C. V. Lowry. 1967. Phage f2 RNA-directed binding of formylmethionyl-tRNA to ribosomes and the role of 30S ribosomal subunits in initiation of protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 58:946–953.
286. Nomura, M., C. V. Lowry, and C. Guthrie. 1967. The initiation of protein synthesis: Joining of the 50S ribosomal subunit to the initiation complex. Proc. Nat. Acad. Sci. U.S.A. 58:1487–1493.
287. Nomura, M., and P. Traub. 1967. Structure and function of ribosomes and subribosomal particles, p. 459–476. *In* H. J. Vogel, J. O. Lampen, and V. Bryson (ed.), Organizational biosynthesis. Academic Press Inc., New York.
288. Nomura, M., P. Traub, and H. Bechmann. 1968. Hybrid 30S ribosomal particles reconstituted from components of different bacterial origins. Nature 219:793–799.
289. Norris, A. T., and P. Berg. 1964. Mechanism of aminoacyl adenylate complexes of isoleucyl RNA synthetase. Proc. Nat. Acad. Sci. U.S.A. 52:330–337.
290. Novelli, G. D. 1967. Amino acid activation for protein synthesis. Ann. Rev. Biochem. 36:449–484.
291. Ochoa, S. 1968. Translation of the genetic message. Naturwissenschaften 55:505–514.
292. Ohta, T., S. Sarkar, and R. E. Thach. 1967. The role of guanosine 5′-triphosphate in the initiation of peptide synthesis. III. Binding of formylmethionyl-tRNA to ribosomes. Proc. Nat. Acad. Sci. U.S.A. 58:1638–1644.
293. Ohta, T., I. Shimada, and K. Imahori. 1967. Conformational change of tyrosyl-RNA synthetase induced by its specific transfer RNA. J. Mol. Biol. 26:519–524.
294. Okamoto, T., and Y. Kawade. 1967. Electrophoretic separation of complexes of aminoacyl-tRNA synthetase and transfer RNA. Biochim. Biophys. Acta 145:613–620.
295. Ono, Y., A. Skoultchi, A. Klein, and P. Lengyel. 1968. Peptide chain elongation: discrimination against the initiator transfer RNA by microbial amino-acid polymerization factors. Nature 220:1304–1307.
296. Ono, Y., A. Skoultchi, J. Waterson, and P. Lengyel. 1969. Peptide chain elongation: GTP cleavage catalyzed by factors binding aminoacyl-transfer RNA to the ribosome. Nature, 222:645–648.
297. Ono, Y., A. Skoultchi, J. Waterson, and P. Lengyel. 1969. Peptide chain elongation. IV. On the stoichiometry of aminoacyl-tRNA binding and GTP cleavage and on translocation. *In preparation.*
298. Osawa, S. 1968. Ribosome formation and structure. Ann. Rev. Biochem. 37:109–130.
299. Ozaki, M., S. Mizushima, and M. Nomura. 1969. Identification and functional characterization of the protein controlled by the streptomycin-resistant locus in *Escherichia coli*. J. Mol. Biol., *in press.*
300. Papas, T. S., and A. H. Mehler. 1968. Modification of the transfer function of proline transfer ribonucleic acid synthetase by temperature. J. Biol. Chem. 243:3767–3769.
301. Parenti-Rosina, R., A. Eisenstadt, and J. M. Eisenstadt. 1969. Isolation of protein initiation factors from 30S ribosomal subunits. Nature 221:363–365.
302. Parmeggiani, A. 1968. Crystalline transfer factors from *Escherichia coli*. Biochem. Biophys. Res. Commun. 30:613–619.
303. Parthier, B. 1965. Chloramphenicol-wirkung auf eine durch Licht stimulierte Proteinsynthese in Blättern und in isolierten Chloroplasten. Z. Naturforsch. 20b:1191–1197.
304. Pene, J. J., E. Knight, and J. E. Darnell. 1968. Characterization of a new low molecular weight RNA in HeLa cell ribosomes. J. Mol. Biol. 33:609–623.
305. Pestka, S. 1968. Studies on the formation of transfer ribonucleic acid-ribosome complexes. J. Biol. Chem. 243: 2810–2820.
306. Pestka, S. 1968. Studies on the formation of transfer ribonucleic acid-ribosome complexes. V. On the function of a soluble transfer factor in protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 61:726–733.
307. Peterson, P. J. 1967. Amino acid selection in protein biosynthesis. Biol. Rev. 42:552–613.
308. Petit, J. F., J. L. Strominger, and D. Söll. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. VII. Incorporation of serine and glycine into interpeptide bridges in *Staphylococcus epidermidis*. J. Biol. Chem. 243:757–767.
309. Phillips, L. A., B. Hotham-Iglewski, and R. M. Franklin. 1969. Polyribosomes of *Escherichia coli*. I. Effects of monovalent cations on the distribution of polysomes, ribosomes and ribosomal subunits. J. Mol. Biol. 40:279–288.
310. Phillips, L. A., B. Hotham-Iglewski, and R. M. Franklin. 1969. Polyribosomes of *Escherichia coli*. II. Experiments to determine the *in vivo* distribution of polysomes, ribosomes and ribosomal subunits. *Submitted for publication.*
311. Press, E. M., P. J. Piggot, and R. R. Porter. 1966. The N- and C-terminal amino acid sequences of the heavy chain from a pathological human immunoglobulin IgG. Biochem. J. 99:356–366.
312. RajBhandary, U. L., S. H. Chang, A. Stuart, R. D. Faulkner, R. M. Hoskinson, and H. G. Khorana. 1967. Studies on polynucleotides. LXVIII. The primary structure of yeast phenylalanine transfer RNA. Proc. Nat. Acad. Sci. U.S.A. 57: 751–758.
313. RajBhandary, U. L., and H. P. Ghosh. 1969. Studies on polynucleotides. XCI. Yeast methionine transfer ribonucleic acid: purification, properties, and terminal nucleotide sequences. J. Biol. Chem. 244:1104–1113.
314. Ravel, J. M. 1967. Demonstration of a guanosine triphosphate-dependent enzymatic binding of aminoacyl-ribonucleic acid to *Escherichia coli* ribosomes. Proc. Nat. Acad. Sci. U.S.A. 57:1811–1816.
315. Ravel, J. M., R. L. Shorey, and W. Shive. 1967. Evidence for a guanine nucleotide-aminoacyl-RNA complex as an intermediate in the enzymatic transfer of aminoacyl-RNA to ribosomes. Biochem. Biophys. Res. Commun. 29:68–73.
316. Ravel, J. M., R. L. Shorey, and W. Shive. 1968. The composition of the active intermediate in the transfer of aminoacyl-RNA to ribosomes. Biochem. Biophys. Res. Commun. 32:9–14.
317. Ravel, J. M., S. F. Wang, C. Heinemeyer, and W. Shive. 1965. Glutamyl and glutaminyl ribonucleic acid synthetases of *E. coli* W. Separation, properties and stimulation of adenosine triphosphate-pyrophosphate exchange by acceptor ribonucleic acid. J. Biol. Chem. 240:432–438.
318. Rendi, R. 1959. The effect of chloramphenicol on the incorporation of labeled amino acids into proteins by isolated subcellular fractions from rat liver. Expt Cell Res. 18:187–189.
319. Revel, M., and F. Gros. 1966. A factor from *E. coli* required

for the translation of natural messenger RNA. Biochem. Biophys. Res. Commun. 25:124–132.

320. Revel, M., J. C. Lelong, G. Brawerman, and F. Gros. 1968. Function of three protein factors and ribosomal subunits in the initiation of protein synthesis in *E. coli*. Nature 219: 1016–1021.
321. Richter, D., and F. Klink. 1968. Amino acyl-tRNA-dependent interaction of a transfer enzyme from yeast with a complex formed by two other transfer factors with guanosine triphosphate. Fed. Eur. Biochem. Soc. Letters 2:49–52.
322. Roberts, W. S. L., J. F. Petit, and J. L. Strominger. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. VIII. Specificity in the utilization of L-alanyl transfer ribonucleic acid for interpeptide bridge synthesis in *Arthrobacter crystallopoietes*. J. Biol. Chem. 243:768–772.
323. Roberts, W. S. L., J. L. Strominger, and D. Söll. 1968. Biosynthesis of the peptidoglycan of bacterial cell walls. VI. Incorporation of L-threonine into interpeptide bridges in *Micrococcus roseus*. J. Biol. Chem. 243:749–756.
324. Robins, M. H., R. H. Hall, and R. Thedford. 1967. N^6-(Δ^2-isopentenyl) adenosine. A component of the transfer ribonucleic acid of yeast and of mammalian tissue. Methods of isolation and characterization. Biochemistry 6:1837–1848.
324*a*. Ron, E. Z., R. E. Kohler, and B. D. Davis. 1966. Increased stability of polysomes in an *Escherichia coli* mutant with relaxed control of RNA synthesis. Proc. Nat. Acad. Sci. U.S.A. 56:471–475.
325. Ron, E. Z., R. E. Kohler, and B. D. Davis. 1968. Magnesium dependence of free and polysomal ribosomes from *E. coli*. J. Mol. Biol. 36:83–89.
326. Roth, J. R., and B. N. Ames. 1966. Histidine regulatory mutants in *Salmonella typhimurium*. II. Histidine regulatory mutants having altered histidyl-tRNA synthetase. J. Mol. Biol. 22:325–334.
327. Rosset, R., and R. Monier. 1963. A propos de la presence d'acide ribonucleique de faible poids moleculaires dans les ribosomes d'*Escherichia coli*. Biochim. Biophys. Acta 68:653–656.
328. Rouget, P., and F. Chapeville. 1968. Reaction sequence of leucine activation catalyzed by leucyl-RNA synthetase. 2. Formation of complexes between the enzyme and substrates. Eur. J. Biochem. 4:310–314.
329. Rychlik, I. 1966. Release of lysine peptides by puromycin from polylysyl-transfer ribonucleic acid in the presence of ribosomes. Biochim. Biophys. Acta 114:425–427.
330. Rychlik, I., S. Chladek, and J. Zemlicka. 1963. Release of peptide chains from the polylysyl-tRNA-ribosome complex by cytidylyl-(3′ -5′) -2′ (3′-)-*O*-glycyladenosine. Biochim. Biophys. Acta 138:640–642.
331. Salas, M., M. B. Hille, J. A. Last, A. J. Wahba, and S. Ochoa. 1967. Translation of the genetic message. II. Effect of initiation factors on the binding of formyl methionyl tRNA to ribosomes. Proc. Nat. Acad. Sci. U.S.A. 57:387–394.
332. Salas, M., M. J. Miller, A. J. Wahba, and S. Ochoa. 1967. Translation of the genetic message. V. Effect of Mg^{++} and formylation of methionine in protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 57:1865–1869.
333. Sambrook, J. F., D. P. Fan, and S. Brenner. 1967. A strong suppressor specific for UGA. Nature 214:452–453.
334. Sanger, F., G. G. Brownlee, and B. G. Barrell. 1965. A two-dimensional fractionation procedure for radioactive nucleotides. J. Mol. Biol. 13:373–398.
335. Sarabhai, A., and S. Brenner. 1967. A mutant which reinitiates the polypeptide chain after chain termination. J. Mol. Biol. 27:145–162.
336. Sarkar, S., and R. E. Thach. 1968. Inhibition of formylmethionyl-transfer RNA binding to ribosomes by tetracycline. Proc. Nat. Acad. Sci. U.S.A. 60:1479–1486.
336*a*. Schaechter, M., E. P. Previc, and M. E. Gillespie. 1965. Messenger RNA and polyribosomes in *Bacillus megaterium*. J. Mol. Biol. 12:119–129.
337. Schlessinger, D., and D. Apirion. 1969. *Escherichia coli* ribosomes: recent developments. Ann. Rev. Microbiol., *in press*.
338. Schwartz, J. H., R. Meyer, J. Eisenstadt, and G. Brawerman. 1967. Involvement of *N*-formylmethionine in initiation of protein synthesis in cell-free extracts of *Euglena gracilis*. J. Mol. Biol. 25:571–574.
339. Scolnick, E., R. Tompkins, T. Caskey, and M. Nirenberg. 1968. Release factors differing in specificity for terminator codons. Proc. Nat. Acad. Sci. U.S.A. 61:768–774.
340. Seeds, N. W., and T. W. Conway. 1966. Reversal by GTP of soluble RNA inhibition of polyphenylalanine synthesis. Biochem. Biophys. Res. Commun. 23:111–116.
341. Seifert, W., G. Nass, and W. Zillig. 1968. Electrophoretic separation of tRNA-bound leucyl-tRNA synthetase from *Escherichia coli* extracts. J. Mol. Biol. 33:507–511.
342. Sgaramella, V., N. Gupta, H. G. Khorana. 1969. Progress in the total synthesis of the gene for yeast alanine transfer RNA. II. Enzymatic joining of chemically synthesized segments. Fed. Proc. 28:532.
343. Shih, A., J. Eisenstadt, and P. Lengyel. 1966. On the relation between ribonucleic acid synthesis and peptide chain initiation in *E. coli*. Proc. Nat. Acad. Sci. U.S.A. 56:1599–1605.
344. Shorey, R. L., J. M. Ravel, C. W. Garner, and W. Shive. Properties of the active intermediate in the enzymatic transfer of Aminoacyl ribonucleic acid to ribosomes. *Submitted for publication*.
345. Shulman, L. H., and R. W. Chambers. 1968. Transfer RNA. II. A structural basis for alanine acceptor activity. Proc. Nat. Acad. Sci. U.S.A. 61:308–315.
346. Silbert, D. F., G. R. Fink, and B. N. Ames. 1966. Histidine regulatory mutants in *Salmonella typhimurium*. III. Class of regulatory mutants deficient in tRNA for histidine. J. Mol. Biol. 22:335–347.
347. Skogerson, L., and K. Moldave. 1968. Evidence for aminoacyl-tRNA binding, peptide bond synthesis, and translocase activities in the aminoacyl transfer reaction. Arch. Biochem. Biophys. 125:497–505.
348. Skoog, F., D. Armstrong, J. D. Cherayil, A. E. Hampel, and R. M. Bock. 1966. Cytokinin activity: localization in transfer RNA preparations. Science 154:1354–1356.
349. Skoultchi, A., Y. Ono, H. M. Moon, and P. Lengyel. 1968. On three complementary amino acid polymerization factors from *Bacillus stearothermophilus*: separation of a complex containing two of the factors, guanosine 5′-triphosphate and aminoacyl-transfer RNA. Proc. Nat. Acad. Sci. U.S.A. 60:675–682.
350. Smith, A. E., and K. A. Marcker. 1968. *N* formylmethionyl transfer RNA in mitochondria from yeast and rat liver. J. Mol. Biol. 38:241–243.
351. Smith, M. A., M. Salas, W. M. Stanley, Jr., A. J. Wahba, and S. Ochoa. 1966. Direction of reading of the genetic message. II. Proc. Nat. Acad. Sci. U.S.A. 55:141–147.
352. Söll, D. 1968. Studies on polynucleotides. LXXXV. Partial purification of an amber suppressor tRNA and studies on *in vitro* suppression. J. Mol. Biol. 34:175–187.
353. Söll, D., J. D. Cherayil, and R. M. Bock. 1967. Studies on polynucleotides. LXXV. Specificity of tRNA for codon recognition as studied by the ribosomal binding technique. J. Mol. Biol. 29:97–112.
354. Söll, D., D. S. Jones, E. Ohtsuka, R. D. Faulkner, R. Lohrmann, H. Hayatsu, H. G. Khorana, J. D. Cherayil, A. Hampel, and R. M. Bock. 1966. Specificity of sRNA for recognition of codons as studied by the ribosomal binding technique. J. Mol. Biol. 19:556–573.
355. Söll, D., E. Ohtsuka, D. S. Jones, R. Lohrmann, H. Hayatsu, S. Nishimura, and H. G. Khorana. 1965. Studies on polynucleotides. XLIX. Stimulation of the binding of aminoacyl-sRNA to ribosomes by ribo-trinucleotides and a survey of codon assignments for twenty amino acids. Proc. Nat. Acad. Sci. U.S.A. 54:1378–1385.
356. Sonnenbichler, J., H. Feldmann, and H. G. Zachau. 1963. Identifizierung der Aminoacyl-sRNA als 3′-Ester des terminalen Adenosins. Z. Physiol. Chem. 334:283–286.

357. Speyer, J. F. 1967. The genetic code, p. 137–191. *In* J. H. Taylor (ed.), Molecular genetics. Part II. Academic Press Inc., New York.
358. Spirin, A. S. 1968. How does the ribosome work? A hypothesis based on the two subunit construction of the ribosome, p. 115–127. *In* Currents in modern biology, vol. 2. North-Holland Publishing Co., Amsterdam.
359. Staehelin, T., H. Raskas, and M. Meselson. 1967. Taking the ribosome apart and putting it back together again, p. 443–457. *In* H. J. Vogel, J. O. Lampen, and V. Bryson. (ed.), Organizational biosynthesis. Academic Press Inc., New York.
360. Staehelin, M., H. Rogg, B. C. Baguley, T. Ginsberg, and W. Wehrli. 1968. Structure of a mammalian serine tRNA. Nature 219:1363–1365.
361. Stanley, W. M., Jr., M. Salas, A. J. Wahba, and S. Ochoa. 1966. Translation of the genetic message: factors involved in the initiation of protein synthesis. Proc. Nat. Acad. Sci. U.S.A. 56:290–295.
362. Stern, R., and A. H. Mehler. 1965. Lysyl-sRNA synthetase from *Escherichia coli*. Biochem. Z. 342:400–409.
363. Stöffler, G. V. Rudloff, and H. G. Wittmann. 1968. Ribosomal proteins. III. Preparative separation of *Escherichia coli* and yeast ribosomal proteins by means of gel filtration. Mol. Gen. Genet. 101:70–81.
364. Stulberg, M. P. 1967. The isolation and properties of phenylalanyl ribonucleic acid synthetase from *Escherichia coli* B. J. Biol. Chem. 242:1060–1064.
365. Stutz, E., and H. Noll. 1967. Characterization of cytoplasmic and chloroplast polysomes in plants: evidence for three classes of ribosomal RNA in nature. Proc. Nat. Acad. Sci. U.S.A. 57:774–781.
366. Suarez, G., and D. Nathans. 1965. Inhibition of aminoacyl-sRNA binding to ribosomes by tetracycline. Biochem. Biophys. Res. Commun. 18:743–750.
367. Subak-Sharpe, H., and J. Hay. 1965. An animal virus with DNA of high guanine + cytosine content which codes for sRNA. J. Mol. Biol. 12:924–928.
368. Subak-Sharpe, H., W. M. Shepherd, and J. Hay. 1966. Studies on sRNA coded by herpes virus. Cold Spring Harbor Symp. Quant. Biol. 31:583–594.
369. Subramanian, A. R., E. Z. Ron, and B. D. Davis. 1968. A factor required for ribosome dissociation in *Escherichia coli*. Proc. Nat. Acad. Sci. U.S.A. 61:761–767.
370. Sueoka, N., and T. Kano-Sueoka. 1964. A specific modification of leucyl-sRNA of *Escherichia coli* after phage T2 infection. Proc. Nat. Acad. Sci. U.S.A. 52:1535–1540.
371. Sundararajan, T., and R. Thach. 1966. Role of the formylmethionine codon AUG in phasing translation of synthetic messenger RNA. J. Mol. Biol. 19:74–90.
372. Sundharadas, G., J. R. Katze, D. Söll, W. Konigsberg, and P. Lengyel. 1968. On the recognition of serine transfer RNA specific for unrelated codons by the same seryltransfer RNA synthetase. Proc. Nat. Acad. Sci. U.S.A. 61:693–700.
373. Suyama, Y. 1967. The origins of mitochondrial ribonucleic acids in *Tetrahymena pyriformis*. Biochemistry 6:2829–2839.
374. Szentirmai, A., M. Szentirmai, and H. E. Umbarger. 1968. Isoleucine and valine metabolism of *Escherichia coli*. XV. Biochemical properties of mutants resistant to thiaisoleucine. J. Bacteriol. 95:1672–1679.
375. Takanami, M. 1962. Transfer of amino acids from soluble ribonucleic acid to ribosome. III. Further studies on the interaction between ribosome and soluble ribonucleic acid. Biochim. Biophys. Acta 61:432–444.
376. Takanami, M., and Y. Yan. 1965. The release of polypeptide chains from ribosomes in cell-free amino acid incorporating systems by specific combinations of bases in synthetic polyribonucleotides. Proc. Nat. Acad. Sci. U.S.A. 54: 1450–1458.
377. Takanami, M., Y. Yan, and T. H. Jukes. 1965. Studies on the site of ribosomal binding of f2 bacteriophage RNA. J. Mol. Biol. 12:761–773.
378. Takeda, M., and R. E. Webster. 1968. Protein chain initiation and deformylation in *B. subtilis* homogenates. Proc. Nat. Acad. Sci. U.S.A. 60:1487–1494.
379. Takemura, S., M. Miyazaki, S. Hashimoto, and M. Murakami. 1969. The primary structure of iso-leucine and tyrosine-tRNA from *T. utilis*. J. Biochem. (Tokyo), *in press*.
380. Takemura, S., T. Mizutani, and M. Miyazaki. 1968. The primary structure of valine i Transfer ribonucleic acid from *Torulopsis utilis*. J. Biochem. (Tokyo) 63:277–278.
381. Tanaka, N., T. Kinoshita, and H. Masukawa. 1968. Mechanism of protein synthesis inhibition by fusidic acid and related antibiotics. Biochem. Biophys. Res. Commun. 30: 278–283.
382. Taylor, M. W., G. A. Granger, C. A. Buck, and J. J. Holland. 1967. Similarities and differences among specific tRNA in mammalian tissues. Proc. Nat. Acad. Sci. U.S.A. 57:1712–1719.
383. Taylor, M. M., and R. Storck. 1964. Uniqueness of bacterial ribosomes. Proc. Nat. Acad. Sci. U.S.A. 52:958–965.
384. Taylor, A. L., and C. D. Trotter. 1967. Revised linkage map of *Escherichia coli*. Bacteriol. Rev. 31:332–353.
385. Thach, R. E., and K. F. Dewey, J. C. Brown, and P. Doty. 1966. Formylmethionine codon AUG as an initiator of polypeptide synthesis. Science 153:416–418.
386. Thiebe, R., and H. G. Zachau. 1968. A specific modification next to the anticodon of phenylalanine transfer ribonucleic acid. Eur. J. Biochem. 5:546–555.
387. Tocchini-Valentini, G. P., and E. Mattoccia. 1968. A mutant of *E. coli* with an altered supernatant factor. Proc. Nat. Acad. Sci. U.S.A. 61:146–151.
388. Traut, R. R., and R. E. Monro. 1964. The puromycin reaction and its relation to protein synthesis. J. Mol. Biol. 10: 63–72.
389. Traut, R. R., P. B. Moore, H. Delius, H. Noller, and A. Tissieres. 1967. Ribosomal proteins of *Escherichia coli*. I. Demonstration of different primary structures. Proc. Nat. Acad. Sci. U.S.A. 57:1294–1301.
390. Traub, P., and M. Nomura. 1968. Structure and function of *E. coli* ribosomes. V. Reconstitution of functionally active 30*S* ribosomal particles from RNA and proteins. Proc. Nat. Acad. Sci. U.S.A. 59:777–784.
391. Travnicek, M. 1968. RNA with amino acid-acceptor activity isolated from an oncogenic virus. Biochim. Biophys. Acta 166:757–759.
392. Venetianer, P., M. A. Berberich, and R. F. Goldberger. 1968. Studies on the size of the messenger RNA transcribed from the histidine operon during simultaneous and sequential derepression. Biochim. Biophys. Acta 166:124–133.
393. Vescia, A. 1967. Separation of two leucyl-ribonucleic acid synthetases from rat liver. Biochem. Biophys. Res. Commun. 29:496–500.
394. Vinuela, E., M. Salas, and S. Ochoa. 1967. Translation of the genetic message. III. Formylmethionine as initiator of proteins programmed by polycistronic messenger RNA. Proc. Nat. Acad. Sci. U.S.A. 57:729–734.
395. Vogel, Z., A. Zamir, and D. Elson. 1968. On the specificity and stability of an enzyme that hydrolyzes N-substituted aminoacyl-transfer RNA. Proc. Nat. Acad. Sci. U.S.A. 61:701–707.
396. Waldenström, J. 1968. Purification and some properties of lysyl ribonucleic acid synthetase from *Escherichia coli*. Eur. J. Biochem. 3:483–487.
397. Waldenström, J. 1968. Some properties of lysyl ribonucleic acid synthetase from *E. coli*. Eur. J. Biochem. 5:239–245.
398. Waller, J. 1963. The NH_2-terminal residues of the proteins from cell-free extracts of *E. coli*. J. Mol. Biol. 7:483–496.
399. Waters, L. C., and G. D. Novelli. 1967. A new change in leucine transfer RNA observed in *Escherichia coli* infected with bacteriophage T2. Proc. Nat. Acad. Sci. U.S.A. 57: 979–985.
400. Waters, L. C., and G. Novelli. 1968. The early change in *E.*

coli leucine tRNA after infection with bacteriophage T2. Biochem. Biophys. Res. Commun. 32:971–976.

401. Watson, J. D. 1964. The synthesis of proteins upon ribosomes. Bull. Soc. Chim. Biol. 46:1399–1425.
402. Webster, R. E., D. L. Engelhardt, and N. D. Zinder. 1966. *In vitro* protein synthesis: chain initiation. Proc. Nat. Acad. Sci. U.S.A. 55:155–161.
403. Webster, R. E., and N. D. Zinder. 1969. The fate of the message-ribosome complex upon translation of termination signals. J. Mol. Biol., *in press*.
404. Weigert, M. G., and A. Garen. 1965. Base composition of nonsense codons in *E. coli*. Nature 206:992–994.
405. Weigert, M. G., E. Lanka, and A. Garen. 1967. Base composition of nonsense codons in *Escherichia coli*. II. The N2 codon UAA. J. Mol. Biol. 23:391–400.
406. Weisblum, B., and J. Davies. 1968. Antibiotic inhibitors of the bacterial ribosome. Bacteriol. Rev. 32:493–528.
407. Weiss, S. B., W. T. Hsu, J. W. Foft, and N. H. Scherberg. 1968. Transfer RNA coded by the T_4 bacteriophage genome. Proc. Nat. Acad. Sci. U.S.A. 61:114–121.
408. Weiss, J. F., and A. D. Kelmers. 1967. A new chromatographic system for increased resolution of transfer ribonucleic acids. Biochemistry 6:2507–2513.
409. Weissbach, H., and B. Redfield. 1967. Deformylation of *N*-formylmethionine by *Escherichia coli* extracts. Biochem. Biophys. Res. Commun. 27:7–11.
410. Wilcox, M., and M. W. Nirenberg. 1968. Transfer RNA as a cofactor coupling amino acid synthesis with that of protein. Proc. Nat. Acad. Sci. U.S.A. 61:229–236.
411. Wilhelm, R. C. 1966. Discussion. Cold Spring Harbor Symp. Quant. Biol. 31:496.
412. Wintersberger, E. 1964. Proteinsynthese in isolierten Hefe-Mitochondrien. Biochem. Z. 341:409–419.
413. Yang, S. S., and D. G. Comb. 1968. Distribution of multiple forms of lysyl transfer RNA during early embryogenesis of sea urchin, *Lytechinus variegatus*. J. Mol. Biol. 31:139–142.
414. Yang, W., and G. D. Novelli. 1968. Isoaccepting tRNA in mouse plasma cell tumors that synthesize different myeloma protein. Biochem. Biophys. Res. Commun. 31:534–539.
415. Yaniv, M., F. Jacob, and F. Gros. 1965. Mutations thermosensibles des systemes activant la valine chez *E. coli*. Bull. Soc. Chim. Biol. 47:1609–1626.
416. Yaniv, M., and F. Gros. 1966. Studies on temperature conditional mutants of *Escherichia coli* with a modified aminoacyl RNA synthetase, p. 157–178. *In* D. Shugar (ed.), Genetic elements. Academic Press Inc., New York.
417. Yarmolinski, M., and G. L. de la Haba. 1959. Inhibition by puromycin of amino acid incorporation into protein. Proc. Nat. Acad. Sci. U.S.A. 45:1721–1729.
418. Yarus, M., and P. Berg. 1967. Recognition of tRNA by aminoacyl tRNA synthetases. J. Mol. Biol. 28:479–490.
419. Yoshida, M., T. Furuichi, Y. Kaziro, and T. Ukita. 1968. The modification of nucleosides and nucleotides. IX. Inactivation of coding response of yeast tRNA containing inosine residue by cyanoethylation. Biochim. Biophys. Acta 166:636–645.
420. Yu, C. T., and H. P. Rappaport. 1966. Multiple fractions of leucyl-transfer ribonucleic acid synthetase activity from *E. coli*. Biochim. Biophys. Acta 123:134–141.
421. Zabos, P., F. Hatfaludi, and R. Machovich. 1967. A heat-resistant factor from *E. coli* involved in the "transfer reaction" of protein synthesis. Acta Biochim. Biophys. Acad. Sci. Hung. 2:263–278.
422. Zachau, H. G. 1968. Serine specific transfer ribonucleic acids. 16. Aggregation of serine-specific transfer ribonucleic acids. Eur. J. Biochem. 5:559–566.
423. Zachau, H. G., D. Dütting, and H. Feldman. 1966. Nucleotidsequenzen zweier serinspezifischer transfer-ribonucleinsäuren. Angew. Chem. 78:392–393.
424. Zamir, A., Leder, P., and D. Elson. 1966. A ribosome-catalyzed reaction between *N*-formylmethionyl-tRNA and puromycin. Proc. Nat. Acad. Sci. U.S.A. 56:1794–1801.
425. Zinder, N. D., D. L. Engelhardt, and R. E. Webster. 1966. Punctuation in the genetic code. Cold Spring Harbor Symp. Quant. Biol. 31:251–256.
426. Zipser, D. 1969. Polar mutations and operon function. Nature 221:21–25.