Polypeptide Chain Elongation in Protein Biosynthesis

A protein grows by single unit addition on the ribosome-reactor with messenger RNA as conveyor belt.

Fritz Lipmann

The three-dimensional structure that a protein assumes after it leaves the synthetic assembly line results from a spontaneous folding caused by interaction between amino acid sequences. What is produced as an essentially one-dimensional string of peptidically linked amino acids is, in this manner, converted into a precisely designed, catalytically active enzyme protein. The one-dimensional polypeptide is formed by an accurate translation of a base triplet sequence in the messenger ribonucleic acid (mRNA) into amino acid sequence of the peptide (*1*, *2*).

Proteins are built by the condensation of a variable number of the 20 basal amino acids into a polypeptide chain. The 20 amino acids are derivatives of the smallest of the group, glycine.

$$\overset{\alpha}{C}H_2NH_2\ COOH$$

One of the two hydrogens on the α-carbon of glycine is replaced by a variety of substituents which make the derivatives asymmetrical, normally of the L-configuration. The amino acids are linked by peptidic bonds between the carboxyl group of one and the α-amino group of the next. A backbone structure is thereby created which, if stretched into a ribbon (Fig. 1), would carry the substituents sideways on each α-carbon. The substituents are listed and sorted in Fig. 2.

The three-dimensional characteristics of a protein are dominantly determined by forces between the side chains. The polypeptide backbone loops and folds due to the attraction and repulsion of these annexes between each other and the aqueous medium. When reconstructed from x-ray diagrams, the polypeptide chain looks curiously contorted (*3*). The amino acids from which proteins are made have remained unchanged in kind and number for probably billions of years of evolution (*4*), from bacteria to man. The magic 20, in specifically folded structures of properly sequenced polypeptide chains, yield the essential parts of the molecular technology of all the living organisms.

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Amino Acid Activation and a Survey of Polymerization

To learn about the mechanism of a polymerization, it is essential to use preactivated units in the joining reaction. Therefore, in our studies, amino-acyl-transfer ribonucleic acids (tRNA's) were used as amino acid precursors. Amino acids are activated by amino acid-specific enzymes. In the initial reaction, aminoacyl adenylate is formed by displacement of the terminal pyrophosphoryl in adenosine triphosphate (ATP). Then the amino acid is shifted on the same enzyme to the RNA. Hence, the aminoacyl-tRNA synthetase fulfills a double function; it activates the amino acid for peptide bonding by esterifying it to the 3′-terminal of its tRNA (Fig. 3) which, midway in its chain of 70-odd nucleotides, contains an anticodon triplet specific for the amino acid it carries (*2*). The anticodon base triplet aims to hydrogen bond with a codon triplet in mRNA, thereby placing the amino acids in proper sequence on the growing polypeptide chain. By joining the amino acids to their specific tRNA's, the aminoacyl-tRNA synthetases function as translators of amino acid specificity into base-pairing specificity (*5*). The reactor on which mRNA, aminoacyl-tRNA, and complementary reagents are convened is the ribosome. Bacterial ribosomes have a molecular weight of approximately 2×10^6, and sediment at 70*S*. They are composed of two subunits which sediment at 30*S* and 50*S*. The progress of polymerization on the mRNA-charged ribosome is surveyed in Fig. 4 (*6*).

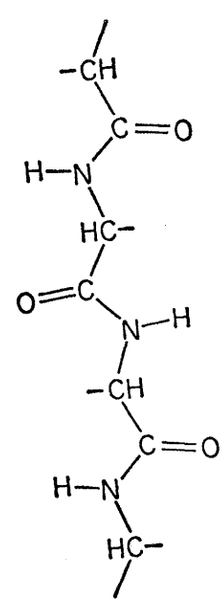


Fig. 1. Polypeptide backbone stretched to one-dimensional ribbon. From the carbon neighboring the CO in the peptide link the side chains listed in Fig. 2 emerge, as indicated by the open dashes.

Chain Elongation in Escherichia coli Homogenates

Both the start and the finish of a normal protein are complex phases involving a variety of specific mechanisms. Through elimination of these complications by the use of phenylalanine polymerization on polyuridylic acid, we focused on polypeptide chain elongation and isolated from the supernatant fraction two proteins, T and G, the bacterial elongation complements. Fraction G is a single protein, whereas T is composed of two subfractions, T_u and T_s (*7*). In the following paragraphs we will concern ourselves primarily with the elongation functions of these proteins and their relation to guanosine triphosphate (GTP) (*8*). The use of

GTP-derived energy in protein synthesis has moved more and more into the center of the problem and will have to be dealt with extensively.

The discovery by Nirenberg and Matthaei (*9*) that polyuridylic acid can act as a template for synthesis of polyphenylalanine simplified the study of protein synthesis; it made it possible to use a synthetic polymer to specify the synthesis of a uniform polypeptide. As we now know, the optimum conditions worked out for this system inadvertently singled out chain elongation. Fortunately, the essential components of the elongation reaction are the same for synthesis of polyphenylalanine and of complex proteins. These components are ribosomes; several proteins isolated from particle-free supernatant fractions; the ions Mg^{++}, K^{+}, or NH_4^{+}; GTP; and a sulfhydryl source. At a Mg^{++} concentration of about $10^{-2}M$, no special chain initiator is needed because phenylalanyl-tRNA (Phe-tRNA) can serve as a starter of new chains (*10, 11*). The final phase, termination, was also obviated, because elongation proceeds to near exhaustion of the added Phe-tRNA without release of peptide chains from tRNA (*12*).

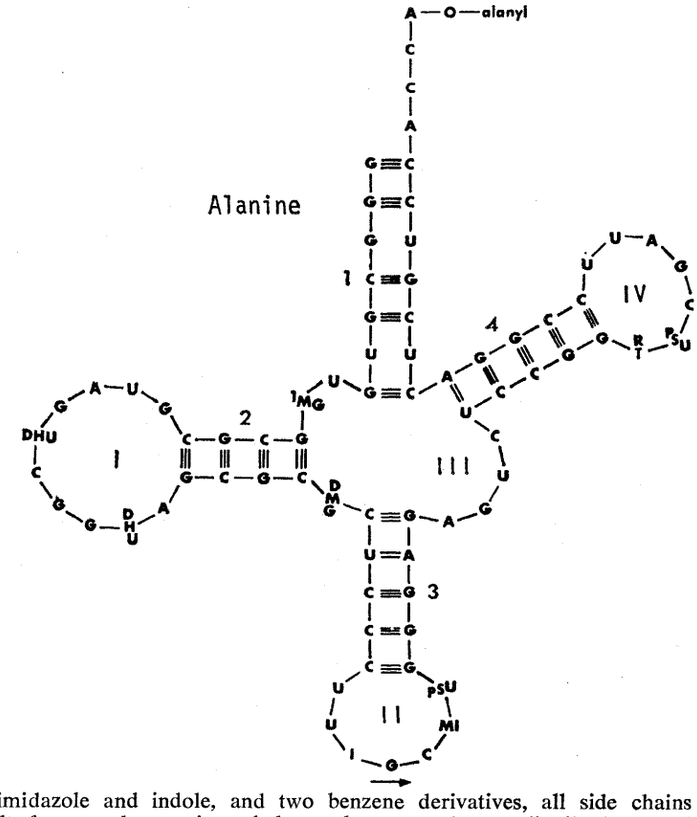
Table 1. The bacterial complements for initiation, elongation, and termination of polypeptide chains. For references, see text.

Factor	Origin	Function
		Initiation
F_3 (B)	Ribosome wash	Binding of mRNA to ribosomes (30*S*)
F_1–F_2 (A-C) and GTP	Ribosome wash	Binding to ribosome of *N*-blocked aminoacyl-tRNA
		Elongation cycles
T_u } T T_s }	Supernatant fraction	Binding of GTP and aminoacyl-tRNA; transfer to ribosomes
Peptidyl transferase	50*S* ribosomal protein	Peptidyl transfer to aminoacyl-tRNA
G and GTP	Supernatant fraction	Translocation of peptidyl-tRNA; release of P_i from GTP
		Termination
R_1–R_2	Supernatant fraction	Release of finished polypeptide from tRNA due to codons UAA, UAG, or UGA

Coordination of the complementary factors with the three phases, initiation, elongation, and termination, is illustrated in Table 1 as a brief summary of the overall process of making a protein. To start the synthesis, mRNA is bound to a 30*S* ribosomal subunit (*13*), and the initiator formylmethionyl-tRNA (fMet-tRNA) connects to its codon AUG (*2*), and concomitantly the 50*S* subunit joins the 30*S*. A special set of complements, the initiation factors, function in this phase. They were isolated from ribosomes by extraction with $1M$ NH_4Cl (*14*), although they are not ribosome constituents in the proper sense. The fractions F_1, F_2, and F_3 of Iwasaki *et al.* (*15*) are considered analogous to

No.	Amino acid	Side chain
1	Glycine	$-H$
2	Alanine	$-CH_3$
3	Valine	$-CH(CH_3)_2$
4	Isoleucine	$-CH(CH_3)-CH_2-CH_3$
5	Leucine	$-CH_2-CH(CH_3)_2$
6	Proline	$-CH_2-CH_2-CH_2-$
7	Phenylalanine	$-CH_2-C_6H_5$
8	Serine	$-CH_2OH$
9	Threonine	$-CH(OH)-CH_3$
10	Tyrosine	$-CH_2-C_6H_4OH$
11	Cysteine	$-CH_2SH$
12	Methionine	$-(CH_2)_2-S-CH_3$
13	Lysine	$-(CH_2)_4-NH_2$
14	Arginine	$-(CH_2)_3-NH-C(=NH)-NH_2$
15	Histidine	$-CH_2-C_3H_3N_2$
16	Tryptophan	$-CH_2-C_8H_6N$
17	Aspartic acid	$-CH_2-COOH$
18	Asparagine	$-CH_2-CONH_2$
19	Glutamic acid	$-(CH_2)_2-COOH$
20	Glutamine	$-(CH_2)_2-CONH_2$

Fig. 2 (left). The side chain characteristics have been subdivided into five groups. The first and largest group presents the hydrophobic contingent. The hydrophobicity of the side chains should not be compared to that of the free amino acid. Blocking of the amino and carboxyl groups in the peptide chain must increase the hydrophobicity considerably; it also modifies the characteristics of functional groups in the side chains, taking as example the hydroxyl group of serine in many hydrolases. The four other groups carry functional annexes, many of which are hydrophilic. They are marked by heavy print and are self-explanatory. Except for the two heterocyclic bases, imidazole and indole, and two benzene derivatives, all side chains are aliphatic. The functional versatility of proteins must result from a harmonious balance between charge distribution, and hydrophobic and hydrophilic dominance. Proteins with enzymatic function seem to assume a grossly globular structure with hydrophilic sites oriented outward and hydrophobic sites looping inward, including strategically placed functional side chains for catalytic action. Fig. 3 (right). Alanyl-tRNA from yeast. Cloverleaf structure: on top, the *O*-terminal adenosine is ester-linked to the carboxyl group of alanine. The bottom loop contains the anticodon · I-G-C · for alanine (*2*).



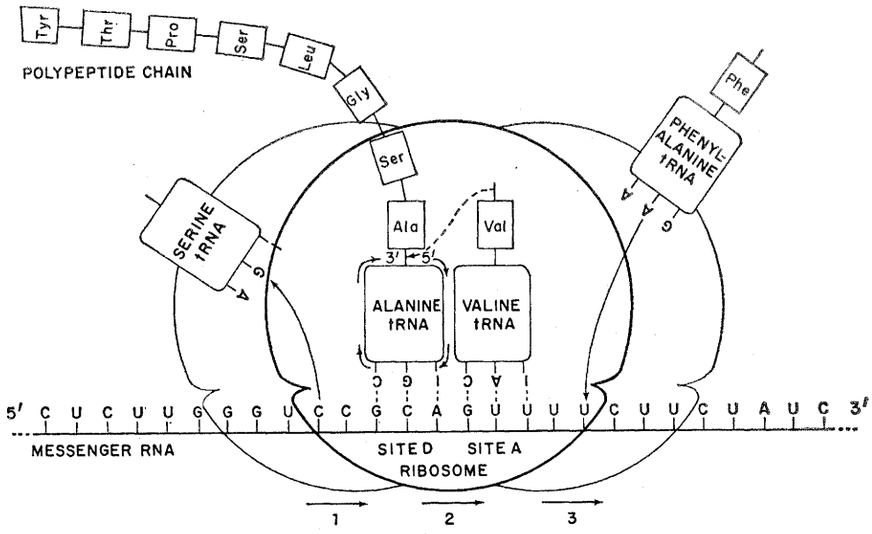


Fig. 4. This survey of sequential addition of amino acids is adapted from Crick (*6*). The mRNA is gliding along on the 30*S* part; the peptidyl and aminoacyl terminals of the two interacting tRNA's are bound to the 50*S* unit, and through anticodon-codon interaction, are hydrogen-bonded to mRNA on the 30*S*. On the left side of the figure, after peptidyl transfer, the tRNA, freed of its charged serine, is shown leaving. In the middle, transpeptidation to the newly adding valyl-tRNA is shown taking place and, on the right, elongation continues with phenylalanyl-tRNA aiming to connect its anticodon with the succeeding codon triplet on mRNA. The 5′ → 3′ arrows around the alanine tRNA emphasize the antiparallel nature of the binding between anticodon on tRNA and codon on mRNA. *Site D*, donor site; *site A*, acceptor site.

fractions A, C, and B of Revel *et al.* (*16, 17*). The F_3 fraction functions in the binding of mRNA when f2 phage RNA is tested (*15*), but seems not to be needed for polyuridylic acid binding. The fractions F_1 and F_2 and GTP promote the binding of the bacterial initiator fMet-tRNA (*18*) and other *N*-acylated tRNA's (*11*). Separately, fraction F_1 is inactive but stimulates F_2, which generally shows activity alone. It appears that during initiation of a full synthetic cycle, the 70*S* ribosomal unit forms by the association of free 30*S* and 50*S* subunits (*13*), and after termination, separates into subunits (*19*). The initiation phase is followed by elongation, which is the central topic of this article.

Elongation stops when one of the termination triplets UAA, UAG, or UGA appears on mRNA next to a peptidyl-tRNA (*2*). The finished product is released, that is, hydrolyzed, from the tRNA in a manner not yet understood. That termination is connected with factors in the supernatant was indicated by early experiments of Ganoza (*20*), when she was working with the synthetic polymer of uridylic and adenylic acids which contained a high proportion of the UAA triplet. Working with a prematurely terminating mutant of an *E. coli* phage of the f2 type (*21*), Capecchi (*22*) separated a release factor R from the elongation factors. Recently, Nirenberg and his collaborators (*23*) found a release of fMet when fMet-tRNA was linked to AUG-charged ribosomes in the presence of termination triplets and supernatant factor R (*22*). They separated R into subfractions R_1 and R_2 which are specific for UAA and UAG, and UAA and UGA, respectively. Recent experiments by Ganoza (*24*) seem to indicate that there is an additional complement necessary for termination and that it is tightly bound to ribosomes.

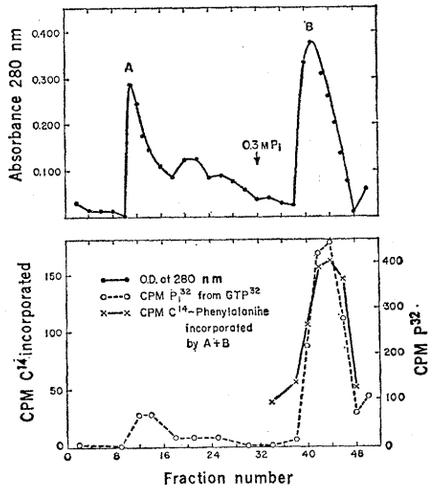


Fig. 5. Overlap of ribosome-linked guanosine triphosphatase and polymerization activity of fraction B (*64*). The DEAE-Sephadex column was developed and the incorporation assay was performed in the presence of factor A. Guanosine triphosphatase activity was measured under similar assay conditions except that polyuridylic acid, phenylalanyl-tRNA, and factor A were omitted. The tracer was GTP (γ^{32}P) and production of inorganic phosphate was used as an index of enzyme activity; the values plotted have been corrected for a ribosome blank of 110 counts per minute (*CPM*).

Separation of the Bacterial Elongation Factors

From the supernatant of *E. coli*, Nathans and Lipmann (*25*) first isolated by DEAE-cellulose chromatography a single peak containing all high molecular complements for polymerization. One of the more interesting results at this stage was that, working with endogenous mRNA, the peak fraction promoted incorporation of a variety of amino acids attached to the tRNA fraction of *E. coli*. This indicated that the process was not amino acid-specific at the stage of condensation from aminoacyl-tRNA's into polypeptide chains.

Indications had appeared that the DEAE-cellulose peak could be further subdivided (*26*), and Allende *et al.* (*27*), now using the polyuridylic acid-directed polyphenylalanine synthesis, succeeded in separating two complementary fractions with the use of DEAE-Sephadex, rather than DEAE-cellulose, and a stepwise elution with 0.2 and 0.3*M* phosphate buffer (Fig. 5). A stable fraction (A) eluted at the lower concentration, and an unstable fraction (B) at the higher concentration. The B fraction coincided with a guanosine triphosphatase. The method of separation was improved by Nishizuka and Lipmann (*28*), again using DEAE-Sephadex but with a KCl gradient instead of stepwise separation (Fig. 6). Three peaks appeared; the earliest contained a factor similar to A, now called factor T, the second a mixture of both, and the third a factor similar to B, now called factor G. The

distinct middle peak indicates a complex between the two that deserves more attention (*29*). The last fraction, factor G, complemented factor T for polymerization and coincided with a ribosome-linked guanosine triphosphatase (*26, 30, 31*) (Fig. 7). This factor is rather stable, in contrast to the instability of the guanosine triphosphatase-linked B fraction (*27*). On the other hand, fraction T, which eluted early with KCl, is very unstable but otherwise parallels stable A (*27*). The solution to the puzzle of the switch of instability from late to early eluate led to a subdivision of factor T (Fig. 8) into unstable T_u and stable T_s (*7*). A comparison of the KCl gradient procedure (*28*) with the stepwise elution procedure (*30*) showed that only factor T_s eluted at low phosphate concentration, yielding the stable A fraction, but that at higher phosphate concentration, factor G eluted together with the T_u factor yielding an unstable B fraction.

The Nishizuka procedure (*28*) has recently been shown to yield nearly pure factor G. With a slight modification, Kaziro and Inoue (*32*) were able to isolate a homogeneous fraction from which the G factor was obtained in crystalline form. The G factor as well as the factor T complex had been crystallized earlier by Parmeggiani (*33*) (Fig. 9). Parmeggiani has now obtained two types of T factor crystals, plaques and needles; the latter probably contain factors T_u and T_s in a different proportion (*33, 34*).

The G factor complemented ribosomes for polymerization as well as for a specific split of GTP to GDP and inorganic phosphate (*30*). This seemed to indicate a functional connection of G factor with energy delivery from the terminal high-energy phosphate bond of GTP. The energy was thought at first to boost the carboxyl activation of amino acids. However, the energy required for linking a peptide bond appears to be well covered by the energy-rich ester link between the amino acid or peptidylcarboxyl group and the 3′-terminal adenosine in tRNA (*35, 36*). It therefore seemed likely that GTP-derived energy was applied elsewhere.

The Elongation Cycle

Every amino acid addition proceeds through a reaction cycle that may be divided into four phases (Fig. 10) (*35*).

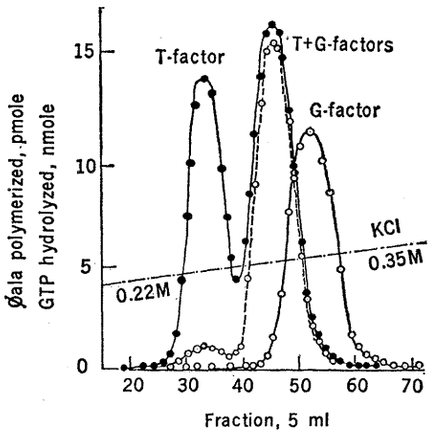


Fig. 6. Separation of factors T and G by DEAE-Sephadex column chromatography. A portion of each fraction was assayed for activity of polyphenylalanine synthesis and guanosine triphosphatase, under standard conditions. ●—●, Phenylalanine polymerizing activity assayed in the presence of added factor G; ○---○, phenylalanine polymerizing activity in the absence of factor G; ○—○, guanosine triphosphatase activity. For further details, see (*28*).

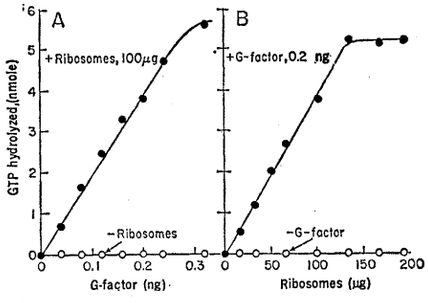


Fig. 7. The G factor assayed by ribosome-linked guanosine triphosphatase. (A) Assay of factor G with excess ribosomes; (B) effect of ribosomes with excess factor G (*35*).

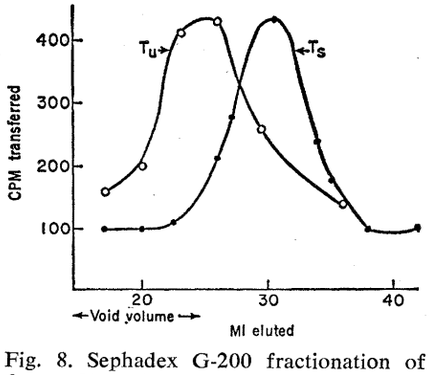


Fig. 8. Sephadex G-200 fractionation of factors T_u and T_s. ●—●, Factor T_s was measured with sucrose-washed ribosomes (0.2 mg per 0.25 ml of reaction mixture). ○—○, Factor T_u activity was determined in the presence of 20 µg of factor G and 40 µg of factor T_s. For further details, see (*6*).

(i) In the starting phase a peptidyl-tRNA occupies the donor (peptidyl) site on the ribosome, and the acceptor (aminoacyl) site is open. (ii) The codon-anticodon, which is occupied by peptidyl-tRNA, appears to signal the appropriate aminoacyl-tRNA to bind to the open codon next to it. This binding is catalyzed by factor T + GTP, and initiates peptidyl transfer which is catalyzed by a ribosomal transferase (*37*). (iii) After peptidyl transfer, the tRNA, now free of the attached peptidyl, remains on the donor site and the newly elongated peptidyl-tRNA on the acceptor site, both ready to be translocated. (iv) In the last phase, the complex between peptidyl-tRNA and mRNA has moved from the acceptor to the donor site and, at the same time, the free tRNA has been displaced from the donor site. We have argued (*35, 36*) that, for this translocation, GTP-derived energy is transmitted through factor G and is used to carry the complex one triplet forward [compare (*i*) and (*iv*) in Fig. 10]. There is a certain similarity in such a movement to the ATP-linked stepwise interaction between actin and myosin in muscle contraction, as discussed by Huxley (*38*); this similarity suggested to us (*35*) that the ribosome-linked split of GTP that is catalyzed by factor G might function in translocation.

Peptidyl Transferase

This enzyme has often been assumed to be supplied by the supernatant (*39*). However, for bacterial systems, Monro *et al.* (*40*) have shown that peptidyl transferase, which catalyzes peptide synthesis, is one of the constituent proteins of the 50*S* part of the ribosome. They tested for the transfer of fMet from fMet-tRNA to the amino group of the chain-terminating antibiotic, puromycin, an analog of the aminoacyl-adenosine terminal of tRNA. This model reaction proved very useful in the study of transpeptidation and the observations demonstrate that peptidyl transfer is intrinsically independent of supernatant factors or GTP.

Peptidyl transferase apparently is built into the 50*S* ribosome subunit to which the two reacting tRNA's link specifically; the peptidyl (or fMet) tRNA links to the donor site, and the aminoacyl-tRNA to the acceptor site (Fig. 10). Therefore, in order to transact peptidyl transfer, the enzyme should be

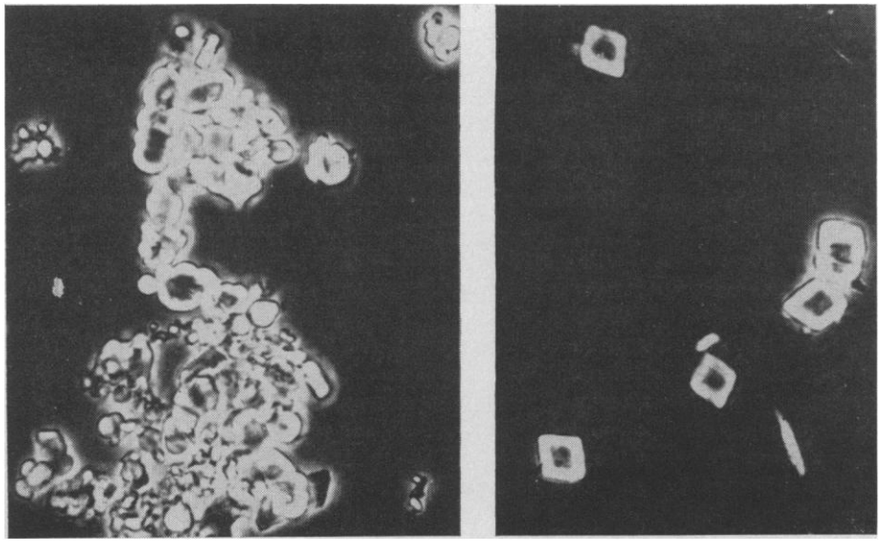


Fig. 9. Crystals of factors G (left) and T (right) prepared by Parmeggiani (*33*).

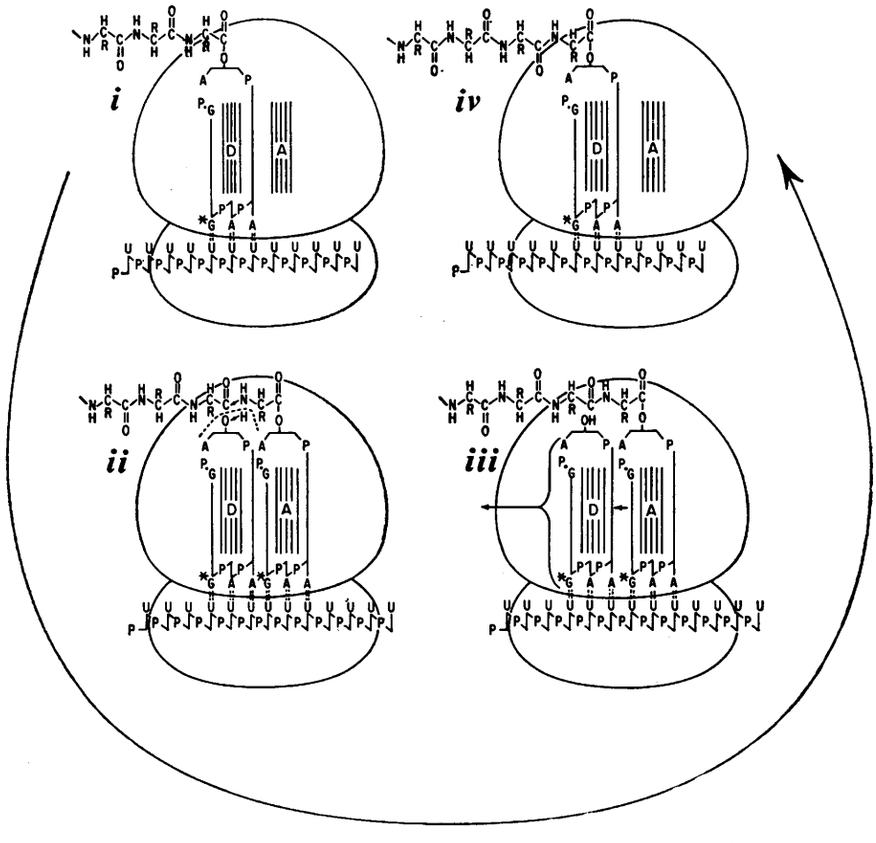


Fig. 10. Peptide condensation cycle. (*i*) In the starting phase (upper left) a peptidyl-tRNA has just been transferred to the donor site (*D*) on the ribosomes; (*ii*) (lower left), the acceptor site (*A*) becomes occupied by an aminoacyl-tRNA, whose anticodon matches mRNA's codon triplet next to the one occupied by the peptidyl-tRNA. The binding is a function of factor T (T_u and T_s) + GTP; it initiates transpeptidation from peptidyl-tRNA to the free amino group of aminoacyl-tRNA and frees the tRNA formerly linked to peptidyl-tRNA. To open the donor site for translocation of the newly extended peptidyl-tRNA, now situated on the acceptor site, factor G + GTP simultaneously promote in (*iii*) a displacement of free tRNA from, and transfer of extended peptidyl-tRNA to, the donor site. With translocation completed in (*iv*), the peptidyl-tRNA returns to the starting position of (*i*) after it has been extended by one amino acid; it carries mRNA to the left by the length of one codon, and exposes under the donor site a new codon for attachment of the next aminoacyl-tRNA.

centered between the donor and acceptor sites, and polarized in such a manner as to transfer the peptidylcarboxyl group from donor to the amino acceptor on the acceptor site. Similarly, Skogerson and Moldave (*41*) have recently proposed that, in mammalian systems, peptidyl transfer is a ribosomal function.

In the overall complex of ribosomal amino acid addition, the peptidyl-tRNA and aminoacyl-tRNA are connected to mRNA on the 30*S* which imposes further limits on the geometry of the positioning of the two tRNA's. This has to be kept in mind as we discuss the mechanism of binding of each new aminoacyl-tRNA as it coordinates with peptide synthesis and is followed by translocation. The scheme for elongation in Fig. 10 is only a coarse projection of binding and translocation onto the ribosome image. More detailed, interesting, but largely theoretical propositions for translocation have been made by Bretscher (*42*) and by Spirin (*43*). A detailed molecular model for codon-anticodon interaction of the two neighboring tRNA's with mRNA yielding two short double-helical stretches was proposed by Fuller and Hodgson (*44*). These propositions should not be looked upon as definite but rather as thought-provoking, welcome starters toward the resolution of a complex geometrical and kinetic problem.

Complexing of T Factor with GTP and Aminoacyl-tRNA

Schweet and his colleagues (*39*) characterized in the reticulocyte system two supernatant factors, TF-1 and TF-2. The TF-1 factor was shown to be connected with a GTP-dependent binding of aminoacyl-tRNA to ribosomes. In *E. coli*, however, such binding seemed to be independent of GTP or supernatant. Thus the experiments for mapping the code by binding aminoacyl-tRNA's to ribosomes charged with base triplets (*45, 46*) or polymers (*9, 47*) were all done without supernatant factors or GTP. However, it was realized only recently that, in the microbial system, a requirement of GTP in various stages had been obscured by the use of high Mg^{++} concentrations. For example, all the binding experiments for mapping the code had been done at 20 m*M* Mg^{++} or more. The need for GTP appeared only when

Table 2. Effect of factors T_s and T_u, and GTP on ^{3}H-Phe-tRNA binding to ribosomes. The ribosomes were charged with *N*-acetyl-Phe-tRNA; the Millipore technique was used (*45*) to measure the amount of Phe-tRNA bound.

Additions	Phe-tRNA (pmole)
None	2.8
T_s + GTP	2.8
T_u + GTP	5.2
T_u + T_s + GTP	11.6

a low Mg++ concentration (4 to 5 m*M*) was used, and, most clearly, in binding assays for initiating *N*-blocked aminoacyl-tRNA (*10, 11*). As an example, Fig. 11 shows binding of the artificial initiator *N*-acetylphenylalanyl-tRNA (*N*-acetyl-Phe-tRNA) (*11*) at low and high Mg++ concentrations. Both GTP and the special initiation factors F_1 and F_2 (*14, 16*) are needed at 4 m*M* Mg++ but not at 10 m*M*.

In following up the need for GTP in the binding of fMet-tRNA to ribosomes with factors F_1 and F_2 (*48, 49*), Allende and Weissbach (*50*) found that the crude ribosomal wash used for the isolation of factors F_1 and F_2 (*14*) contained a protein that caused a retention of GTP on the Millipore filter. They thought at first that this was caused by a complex between factor F_2 and GTP. With gel filtration through Sephadex G-50 as the assay, however, Gordon (*51*) observed that the GTP was bound to factor T (Fig. 12)—a contaminant in the Allende and Weissbach test (*50*)—and that the binding was strongly stimulated by aminoacyl-tRNA [Fig. 1 in (*51*)]. At the same time, Allende *et al.* (*52*) confirmed that factor T rather than factor F_2 was the GTP-binding protein; the lack of effect of aminoacyl-tRNA on GTP binding to factor T by the Millipore technique was explained by Gordon (*53*), who found that, when the three constituents were added together to the Millipore, most of the complex slipped through the filter and appeared in the filtrate. This led to the formulation of a two-step reaction in the formation of the ternary complex.

$$T + GTP \rightleftharpoons T\text{-}GTP$$

$$T\text{-}GTP + \text{aminoacyl-tRNA} \rightleftharpoons GTP\text{-}T\text{-}\text{aminoacyl-tRNA}$$

The ternary complex contained GTP and aminoacyl-tRNA in stoichiometric amounts (*53, 54*). Significantly, neither *N*-acetyl-Phe-tRNA (*53, 54*) nor fMet-tRNA (*55*) reacted with factor T and GTP. This Phe-tRNA-T-GTP complex was shown (Fig. 13) to react very rapidly with ribosomes (upper curve), contrasting with a much slower binding if the components were supplied separately (lower curve). Therefore, one may consider that factor T carries the aminoacyl-tRNA to the ribosome.

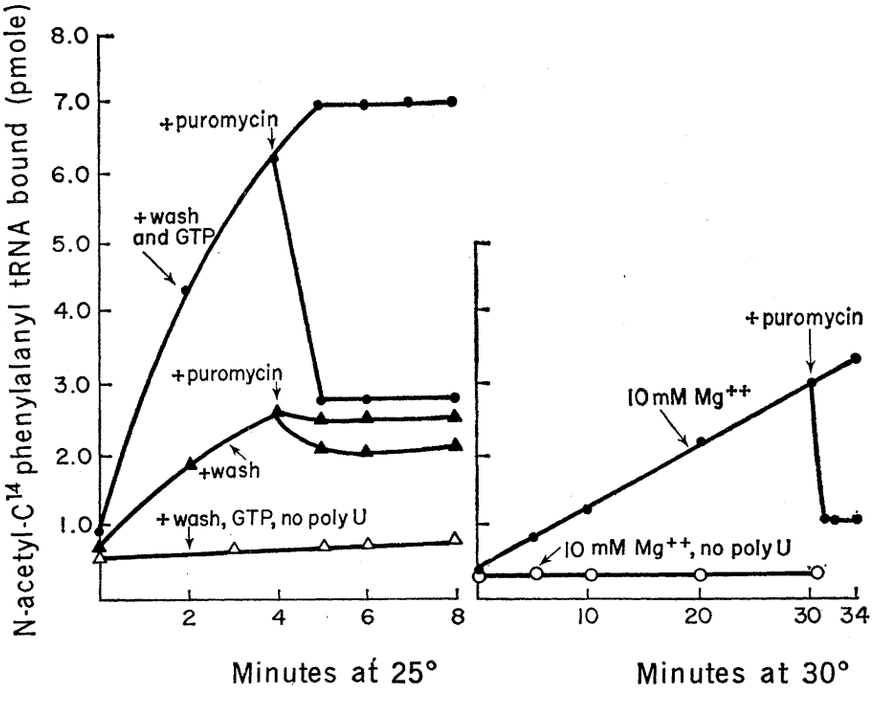


Fig. 11. Binding of *N*-acetyl-Phe-tRNA to ribosomes washed in 1*M* NH_4Cl at low and high Mg^{++} concentrations; (left) 4 m*M* Mg^{++}; (right) 10 m*M* Mg^{++}. The wash added at left contains the initiation factors F_1 and F_2 of Stanley *et al.* (*14*). For further details, see (*11*).

Table 3. Effect of GTP and GMP-PCP on factor T-promoted binding of ^{3}H-Phe-tRNA to ribosomes and on *N*-acetyl-Phe-Phe-tRNA formation. Ribosomes carrying prebound *N*-acetyl-^{14}C-Phe-tRNA were incubated (with factor T, either GTP or GMP-PCP, and ^{3}H-Phe-tRNA) and were centrifuged. The products bound to the ribosomal pellets through tRNA were freed by alkaline hydrolysis and analyzed by paper electrophoresis (*56*).

Additions	Picomoles bound		
	N-Acetyl-Phe-tRNA (^{14}C)	*N*-Acetyl-Phe-Phe-tRNA (^{14}C or ^{3}H)	Phe-tRNA (^{3}H)
None	7.6	0.4	0.1
+ T + GTP	3.0	4.5	2.8
+ T + GMP-PCP	7.0	0.5	4.8

Functions of the Elongation Factors T and G

In the meantime, Lucas-Lenard and Haenni (*34*) had begun a study of the functions of GTP in conjunction with factors T and G using polymerization of phenylalanine from Phe-tRNA on polyuridylic acid. They found it most advantageous to charge the polyuridylic acid ribosomes with *N*-acetyl-Phe-tRNA, essentially a peptidyl analog, as initiator. The ribosome complex thus marked with initiator on the donor site was isolated and used for binding of differently marked Phe-tRNA to the acceptor site. Table 2 indicates that the T_u and T_s subfractions of factor T and GTP act jointly (*56*).

In such experiments, a large part of the bound Phe-tRNA was found immediately to engage *N*-acetyl-Phe-tRNA to form the dipeptide *N*-acetyl-Phe-Phe-tRNA (Table 3, line 2), where the reaction stopped. In the absence of factor G, no tripeptide formed, although an excess of Phe-tRNA was added. A rather complex function of GTP in transpeptidation is indicated by the following. An analog of GTP, 5′-guanylyl methylenediphosphonate (*57*) (the oxygen linking the β- and γ-phosphoryls is replaced by CH_2) has proved an interesting tool; it can replace (*49*) GTP in the F_1-F_2-linked binding of fMet-tRNA to *E. coli* ribosomes. It can also replace GTP in factor T-linked

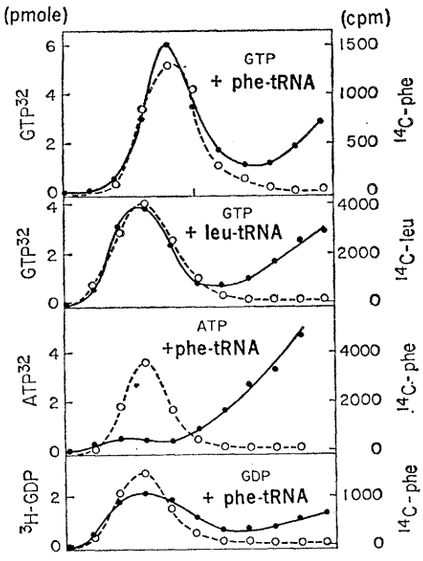


Fig. 12. Complex formation between factor T, GTP, and aminoacyl-tRNA assayed by Sephadex G-50 filtration (*51*).

binding of Phe-tRNA (*56, 58*), although, unlike GTP, it does not cause a peptide to form (Table 3, line 3). Yet, Monro *et al.* (*37*) had shown that peptidyl transferase activity is not dependent on supernatant or GTP. Therefore, if GTP but not the analog permits peptide bond formation with Phe-tRNA, the function of GTP must relate specifically to the alignment of Phe-tRNA connecting with mRNA on the whole ribosome (*56*). That an analog of GTP which cannot furnish a high-energy phosphate bond is sufficient for binding but not for peptide synthesis, has been interpreted to mean that the GTP is split in the latter reaction. However, since transpeptidation as such goes without GTP, I prefer to defer interpretation until the interrelation between binding, transpeptidation, and translocation is better understood. The best available comparison of P_i release with overall polymerization seemed to indicate a 1:1 ratio (*28*). However, in these experiments, the background P_i release was rather high, and we are attempting a recheck.

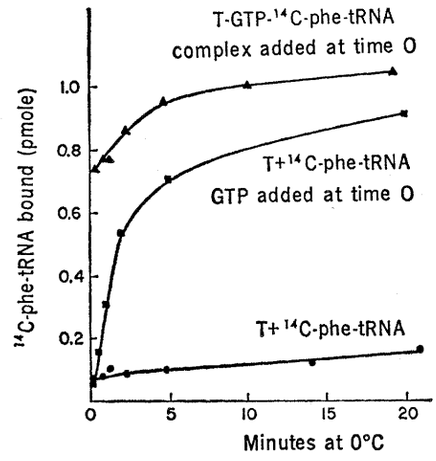


Fig. 13. Nearly instantaneous binding of Phe-tRNA from T-GTP-Phe-tRNA complex (*34*).

Function of Factor G

In the preceding experiments, using factor T and GTP alone, the elongation process was found to stop after a single addition of phenylalanine. The addition of puromycin to *N*-acetyl-Phe-Phe-tRNA did not cause release of the dipeptide (Table 4) (*59*), although prebound initiator *N*-acetyl-Phe-tRNA was easily released as *N*-acetyl-Phe-puromycin (Fig. 11). The dipeptidyl-tRNA is brought into reaction only after contact with the G factor and GTP. Furthermore, Table 4 shows the inability of the analog to replace GTP in the factor G-linked reaction. The analog inhibits translocation if added together with GTP; it also inhibits amino acid polymerization.

After exposure to factor T and GTP alone [Fig. 10 (*iii*)], the transpeptidation has left the ribosome with a free tRNA on the donor-site and newly elongated peptidyl-tRNA on the acceptor site. Recently, Lucas-Lenard and Haenni (*34*) have shown that the removal of free tRNA from the donor site is tightly coupled with the factor G and GTP-promoted translocation of peptidyl-tRNA from the acceptor to the donor site. For this purpose, tritiated tRNA was isolated from uracil-dependent *E. coli* grown with ^{3}H-uracil. From this tRNA, *N*-acetyl-^{14}C-Phe-^{3}H-tRNA was prepared and ribosomes carrying N-acetyl-^{14}C-Phe-^{3}H-tRNA were incubated for transpeptidation to ^{12}C-Phe-tRNA with factor T and GTP as described in Table 3. The isolated ribosomes were found to carry both ^{3}H-tRNA and N-acetyl-^{14}C-Phe-^{12}C-Phe-tRNA, the latter being nonreactive with puromycin. The incubation of these charged ribosomes with factor G and GTP was shown then to release simultaneously ^{3}H-tRNA and ^{14}C-dipeptidyl puromycin in stoichiometric amounts.

These results support the proposition that factor G and GTP function in translocation whereby the free tRNA is pushed off the ribosomes. The inability of *N*-acetyl-Phe-Phe-tRNA to react with puromycin after peptide formation is interpreted to mean that dipeptidyl-tRNA at this stage is in the acceptor site where the dipeptide was formed when Phe-tRNA accepted *N*-acetyl-Phe from its tRNA (Fig. 10). Factor G and GTP are needed, then, to transfer the dipeptidyl-tRNA to the donor site, where it becomes properly situated to engage the ribosomal peptidyl transferase and connect with the amino group of puromycin or of aminoacyl-tRNA. To prove the latter, the continuation of elongation to tripeptidyl-tRNA was tested (*56*). The data in Table 5 confirm that peptidyl can transfer to a new aminoacyl-tRNA only after reaction with factor G and GTP. The experiment shows, furthermore, that the thus modified ribosomal

Table 4. Puromycin release of *N*-acetyl-Phe-Phe. Ribosomes carrying 5.4 pmole of *N*-acetyl-^{14}C-Phe-^{3}H-Phe-tRNA were isolated by centrifugation and incubated under the above conditions. The puromycin products were extracted by ethyl acetate (*59*).

Additions	*N*-Acetyl-Phe-Phe-puromycin released (pmole) (^{14}C or ^{3}H)
Puromycin	1.8
G + GTP + puromycin	5.4
G + GTP + fusidic acid + puromycin	2.0
G + GMP-PCP + puromycin	1.8
G + GTP + GMP-PCP + puromycin	4.1

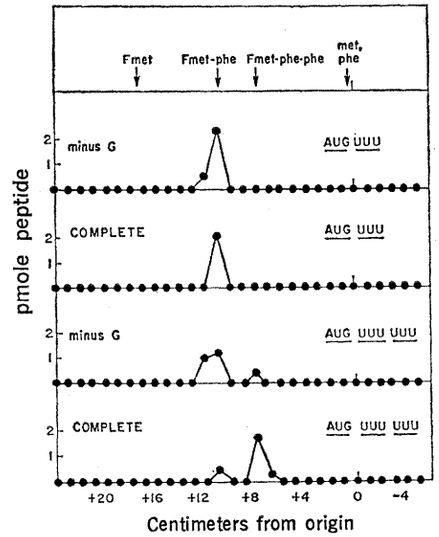


Fig. 14. Electrophoretic analysis of peptide products formed on ribosome-bound AUG(U)$_3$ and AUG(U)$_6$ from a mixture of fMet-tRNA, Phe-tRNA, factor T, and GTP, with and without addition of factor G. [Reproduced with permission of Erbe and Leder (*60*)]

complex may be isolated before addition of Phe-tRNA-T-GTP.

A similar conclusion was reached by Erbe and Leder (*60*). They synthesized the two polynucleotides $AUG(U)_3$ and $AUG(U)_6$; both start with the fMet codon, followed by either one or two U_3 sequences, thus permitting, respectively, one or two phenylalanine additions. As shown in Fig. 14 [Erbe and Leder (*60*)], with only $AUG(U)_3$ added a dipeptide fMet-Phe forms on addition of factor T and GTP, with or without factor G. With $AUG(U)_6$, likewise, a dipeptide, with a trace only of tripeptide, forms with factor T and GTP alone. When factor G is added, however, most of the dipeptide converts to the tripeptide fMet-Phe-Phe. This experiment elegantly shows that a translocation by factor G is needed to expose the second U_3-triplet in $AUG(U)_6$ for attachment and connection of the second Phe-tRNA.

To identify the factor G-linked reaction, the antibiotic fusidic acid isolated and characterized as a steroid by Godtfredsen (*61*), has been most helpful. Its specificity for factor G was first observed by Tanaka *et al.* (*62*), who found that fusidic acid inhibits factor G-linked guanosine triphosphatase as well as polymerization. Haenni and Lucas-Lenard (*56*) have used fusidic acid to confirm the role of factor G in translocation (Tables 4 and 5).

Table 5. Formation of *N*-acetyl-Phe-Phe-Phe-tRNA by addition of Phe-tRNA to ribosomes carrying *N*-acetyl-Phe-Phe-tRNA. After the treatments listed, the ribosomes were centrifuged and incubated with factor T, GTP, and ^{3}H-Phe-tRNA.

Treatment of ribosomes	*N*-Acetyl-Phe-Phe-Phe-tRNA formed (^{3}H) (pmole)
None	0.5
G + GTP	3.8
G + GTP + fusidic acid	1.3

Conclusion

The identification of the elongation factors T (T_u and T_s) and G made it possible systematically to analyze their function. In gross outline, at least, we are beginning to understand in the bacterial system the mechanism by which peptide chains are elongated. The process in the eukaryotic cell seems to be essentially similar since, functionally, the two factors TF-1 and TF-2 of Arlinghaus *et al.* (*39*) appear to parallel factors T and G (*41, 63*).

Implicit in the outlined mechanism of elongation is its transaction on the same ribosome. By the repetition of binding a new aminoacyl-tRNA to mRNA, of transpeptidation, and of translocation of a newly elongated peptidyl-tRNA, the peptide chain grows to completion while the ribosome moves along on mRNA, to be released eventually at the termination signal. The tRNA's cycle through the system; they are discharged by transpeptidation after elongation has taken place; they are recharged with amino acids; and then they form a complex with factor T and GTP, which carries them back to the ribosome-bound mRNA. A successive addition of new ribosomes to mRNA forming a polysome is not an intrinsic link in the process of production, but rather serves as a means of simultaneously producing several copies. The ribosomes travel along the same template more or less independently, progressively completing the protein copy that was started on them individually.

References and Notes

1. Abbreviations are: A, adenosine; C, cytidine; G, guanosine; U, uridine; *G, 2′-*O*-methylguanosine; GDP, guanosine diphosphate; GMP-PCP, 5′-guanylyl methylene-diphosphonate; RT, ribothymidine; DHU, 4,5-dihydrouridine; DMG, N^2-dimethylguanosine; 1-MG, 1-methylguanosine; I, inosine; 1-MI, 1-methylinosine; PSU, pseudouridine; DEAE, diethylaminoethyl; Ala, alanine; Gly, glycine; Leu, leucine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Tyr, tyrosine; Val, valine.
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