SEM1 CC2

Translation

Translation is the third stage of protein biosynthesis. In translation, mRNA produced by transcription is decoded by the ribosome to produce a specific amino acid chain, or polypeptide that will later fold into an active protein. Translation occurs in the cell's cytoplasm, where the large and small subunits of the ribosome are located, and bind to the mRNA. The ribosome facilitates decoding by inducing the binding of tRNAs with complementary anticodon sequences to that of the mRNA. The tRNAs carry specific amino acids that are chained together into a polypeptide as the mRNA passes through and is "read" by the ribosome.

In many instances, the entire ribosome/mRNA complex will bind to the outer membrane of the rough endoplasmic reticulum and release the nascent protein polypeptide inside for later vesicle transport and secretion outside of the cell. Many types of transcribed RNA, such as t RNA, r RNA, and snRNA, do not undergo translation into proteins.

Translation proceeds in four phases: activation, initiation, elongation and termination. Amino acids are brought to ribosomes and assembled into proteins. In activation, the correct amino acid is covalently bonded to the correct tRNA. The amino acid is joined by its carboxyl group to the 3' OH of the tRNA by a ester bond. When the tRNA has an amino acid linked to it, it is termed "charged". Initiation involves the small subunit of the ribosome binding to 5' end of mRNA with the help of initiation factors (IF). Termination of the polypeptide happens when the A site of the ribosome faces a stop codon (UAA, UAG, or UGA). No tRNA can recognize or bind to this codon. Instead, the stop codon induces the binding of a release factor protein that prompts the disassembly of the entire ribosome/mRNA complex.

A number of antibiotics act by inhibiting translation; these include anisomycin, cycloheximide, chloramphenicol, tetracycline, streptomycin, erythromycin, and puromycin, among others. Prokaryotic ribosomes have a different structure from that of eukaryotic ribosomes, and thus antibiotics can specifically target bacterial infections without any detriment to a eukaryotic host's cells.

Ribosome Structure and Assembly

Ribosomes are compact ribonucleoprotein particles found in the cytosol of all cells, as well as in the matrix of mitochondria and the stroma of chloroplasts. Ribosomes are mechano-chemical systems that

move along mRNA templates, orchestrating the interactions between successive codons and the corresponding anticodons presented by aminoacyl-tRNAs. As they align successive amino acids via codon-anticodon recognition, ribosomes also catalyze the formation of peptide bonds between adjacent amino acid residues.

The Composition of Prokaryotic Ribosomes

Escherichia coli ribosomes are representative of the structural organization of the prokaryotic versions of these supramolecular protein-synthesizing machines. The E. *coli* ribosome is a roughly globular particle with a diameter of 25 nm, a sedimentation coefficient of 70S, and a mass of about 2520 kD. It consists of two unequal subunits that dissociate from each other at Mg^{2+} concentrations below 1 mM. The smaller, or 30S, subunit has a mass of 930 kD and is composed of 21 different proteins and a so-called 16S ribosomal RNA (rRNA) molecule 1542 nucleotides long. The larger 50S subunit has a mass of 1590 kD and consists of 31 different polypeptides (L1 to L34) and two rRNAs, a 2904-nucleotide 23S rRNA and a 120-nucleotide 5S rRNA.

Ribosomal Proteins

There is one copy of each ribosomal protein per 70S ribosome, excepting protein L7/L12 (L7 and L12 have identical amino acid sequences and differ only in the degree of N-terminal acetylation). Only one protein is common to both the small and large subunit: S20 = L26. The amino acid sequences of all 52 E. *coli* ribosomal proteins are known. The largest is S1 (557 residues, 61.2 kD); the smallest is L34 (46 residues, 5.4 kD). The sequences of ribosomal proteins share little similarity. These proteins are typically rich in the cationic amino acids *Lys* and *Arg* and have few aromatic amino acid residues, properties appropriate to proteins intended to interact strongly with polyanionic RNAs. Crystal structures obtained thus far for ribosomal proteins reveal a variety of structures. Because RNA adopts a greater range of structures than DNA, RNA-protein recognition motifs are expected to be diverse.

rRNAs

The three E. *coli* rRNA molecules—23S, 16S, and 5S—are derived from a single 30S rRNA precursor transcript that also includes several tRNAs. Ribosomal RNAs show extensive potential for intrachain hydrogen bonding and assume secondary structures reminiscent of tRNAs, although substantially more complex. Almost half the bases in 16S rRNA are base paired. Relatively small double-helical regions are

punctuated by short, single-stranded stretches, generating hairpin configurations that dominate the molecule; four distinct domains (I through IV) can be discerned in the secondary structure. Models of the three-dimensional structure of E. *coli* rRNAs show that, to a good approximation, these rRNA structures conform to the general shapes of the ribosomal subunits

Self-Assembly of Ribosomes

Ribosomal subunit self-assembly is one of the paradigms for the spontaneous formation of supramolecular complexes from their macromolecular components. If the individual proteins and rRNAs composing ribosomal subunits are mixed together in vitro under appropriate conditions of pH and ionic strength, spontaneous self-assembly into functionally competent subunits takes place without the intervention of any additional factors or chaperones. Apparently, the rRNA acts as a scaffold upon which the various ribosomal proteins convene. Ribosomal proteins bind in a specified order.

Assembly of 30S subunits begins even as the rRNA precursor is being transcribed. The first part of the 16S rRNA to be transcribed, the 5'-region, possesses a cluster of the strongest protein-binding sites.

Ribosomal Architecture

Ribosomal subunits have a characteristic three-dimensional architecture that has been revealed by image reconstructions from cryoelectron microscopy and X-ray and neutron solution scattering. The small subunit features a “head” and a “base” from which a “platform” projects. A “cleft” is defined by the spatial relationship between the head, base, and platform. The large subunit is a globular structure with three distinctive projections: a “central protuberance,” the “stalk,” and a winglike ridge. The two subunits associate with each other so that the side of the small subunit nestles into the cleft of the large subunit, with the platform of the small subunit oriented toward the “wing” of the large subunit. An extensive cavity between the two ribosomal subunits is large enough to hold two or more tRNAs, as well as several protein factors involved in protein synthesis. The small subunit has a channel leading into the cleft, through which the mRNA passes. The cleft is aligned somewhat with a branched tunnel in the large subunit, and it is supposed that the growing peptidyl chain is threaded through this tunnel as protein synthesis proceeds. Even though the ribosomal proteins are arranged peripherally around the rRNAs in ribosomes, rRNA occupies 30 to 40% of the ribosomal subunit surface areas.

Eukaryotic Ribosomes

Eukaryotic cells have ribosomes in their mitochondria (and chloroplasts) as well as in the cytosol. The mitochondrial and chloroplastic ribosomes resemble prokaryotic ribosomes in size, overall organization, structure, and function, a fact reflecting the prokaryotic origins of these organelles. Whereas eukaryotic cytosolic ribosomes retain many of the structural and functional properties of their prokaryotic counterparts, they are larger and considerably more complex. Further, higher eukaryotes have more complex ribosomes than lower eukaryotes. For example, the yeast cytosolic ribosomes have major rRNAs of 3392 (large subunit) and 1799 nucleotides (small subunit); the major rRNAs of mammalian cytosolic ribosomes are 4718 and 1874 nucleotides, respectively. Their mass is almost 1.7 times the mass of E. *coli* ribosomes, and proteins contribute a relatively greater proportion of this mass. Small (40S) subunits have 33 different proteins and large (60S) subunits have 49. Large subunits have three characteristic rRNAs: 28S, 5.8S, and 5S. The sequence of the 5.8S rRNA shows homology to the 5'-end of prokaryotic 23S rRNA, suggesting it may be an evolutionary derivative of it. This 5.8S rRNA forms a secondary structure with 28S rRNA through complementary base pairing. Comparison of base sequences and secondary structures of rRNAs from different organisms suggests that evolution has worked to conserve the secondary structure of these molecules, although not necessarily the nucleotide sequences creating such structure. That is, the retention of a base pair at a particular location seems more important than whether the base pair is G:C or A:U. The morphology of eukaryotic cytosolic ribosomes resembles that of prokaryotic ribosomes.

The Mechanics of Protein Synthesis

Peptide Chain Initiation in Prokaryotes

The components required for peptide chain initiation include (a) mRNA, (b) 30S and 50S ribosomal subunits, (c) a set of proteins known as initiation factors**,** (d) GTP, and (e) a specific charged tRNA, f-Met-$tRNA_f^{Met}$. A discussion of the properties of these components and their interaction follows.

Initiator tRNA

$tRNA_f^{Met}$ is a particular tRNA for reading an AUG (or GUG, or even UUG) codon that signals the start site, or N-terminus, of a polypeptide chain. This $tRNA_f^{Met}$ does not read internal AUG codons, so it does not participate in chain elongation. Instead, that role is filled by another methionine-specific tRNA, referred to as $tRNA_m^{Met}$, which cannot replace $tRNA_f^{Met}$ in peptide chain initiation. (However, both of these tRNAs are loaded with Met by the same methionyl-tRNA synthetase.) The structure of E. *coli* $tRNA_f^{Met}$ has several distinguishing features. Unlike the case with all other tRNAs, the 5'-terminal base is not matched with a complementary base in the $tRNA_f^{Met}$ acceptor stem, and thus no base pair forms here. $tRNA_f^{Met}$ also has a unique CCU sequence in its D loop and an exclusive set of three G:C base pairs in its anticodon stem. Collectively, these features identify this tRNA as essential to initiation and inappropriate for chain elongation.

The synthesis of all E. *coli* polypeptides begins with the incorporation of a modified methionine residue, N-formyl-Met, as N-terminal amino acid. However, in about half of the E. *coli* proteins, this Met residue is removed once the growing polypeptide is 10 or so residues long; as a consequence, many mature proteins in E. *coli* lack N-terminal Met. The methionine contributed in peptide chain initiation by $tRNA_f^{Met}$ is unique in that its amino group has been formylated. This reaction is catalyzed by a specific enzyme, **methionyl-$tRNA_f^{Met}$ formyl transferase**. Note that the addition of the formyl group to the Met-NH_2 creates an N-terminal block resembling a peptidyl grouping. That is, the initiating Met is transformed into a minimal analog of a peptidyl chain.

mRNA Recognition and Alignment

In order for the mRNA to be translated accurately, its sequence of codons must be brought into proper register with the translational apparatus. Recognition of translation initiation sequences on mRNAs involves the 16S rRNA component of the 30S ribosomal subunit. Base pairing between a pyrimidine-rich sequence at the 3'-end of 16S rRNA and complementary purine-rich tracts at the 5'-end of prokaryotic mRNAs positions the 30S ribosomal subunit in proper alignment with an initiation codon on the mRNA. The purine-rich mRNA sequence, the **ribosome-binding site,** is often called the **Shine-Dalgarno sequence** in honor of its discoverers. Figure 33.9 shows various Shine-Dalgarno sequences found in prokaryotic mRNAs, along with the complementary 3'-tract on E. *coli* 16S rRNA. The 3'-end of 16S rRNA resides in the “head” region of the 30S small subunit.

Note:-These recognition events are verified by studies of prokaryotic ribosomes treated with the bacteriocidal protein colicin E3**.** This protein is a phospho-diesterase that specifically cleaves the bond

after position 1493 in 16S rRNA, removing the 3'-terminal 49 nucleotides of 16S rRNA that include the pyrimidine-rich Shine-Dalgarno-binding sequence. Colicin E3-treated ribosomes are competent in the elongation of polypeptide chains whose synthesis has already been initiated, but these ribosomes can no longer initiate mRNA translation.

Initiation Factors

Initiation involves interaction of the **initiation factors (IFs)** with GTP, N-formyl-Met-$tRNA_f^{Met}$, mRNA, and the 30S subunit to give a **30S initiation complex** to which the 50S subunit then adds to form a **70S initiation complex.** The initiation factors are soluble proteins required for assembly of proper initiation complexes. Initiation begins when a 30S subunit:(IF-3:IF-1) complex binds mRNA and a complex of IF-2, GTP, and f-Met-$tRNA_f^{Met}$.. Although IF-3 is absolutely essential for mRNA binding by the 30S subunit, it is not involved in locating the proper translation initiation site on the message. The presence of IF-3 on 30S subunits also prevents them from reassociating with 50S subunits. IF-3 must dissociate before the 50S subunit will associate with the mRNA: 30S subunit complex. IF-2 delivers the initiator f-Met-$tRNA_f^{Met}$ in a GTP-dependent process. Apparently, the 30S subunit is aligned with the mRNA such that the initiation codon is situated within the “30S part” of the P site. Upon binding, f-Met-$tRNA_f^{Met}$ enters this 30S portion of the P site. The GTP analog, GMPPCP, can replace GTP in promoting IF-2-mediated binding of initiator tRNA to mRNA and the 30S subunit. However, GTP hydrolysis is necessary to form an active 70S ribosome. GTP hydrolysis is accompanied by IF-1 and IF-2 release and probably occurs when the 50S subunit joins. It is likely that GTP hydrolysis is catalyzed by a ribosomal protein, not IF-2. In any event, GTP hydrolysis is believed to drive a conformational alteration that renders the 70S ribosome competent in chain elongation. The A site of the 70S initiation complex is poised to accept an incoming aminoacyl-tRNA.

Peptide Chain Elongation

The requirements for peptide chain elongation are (a) an mRNA: 70S ribosome:peptidyl-tRNA complex (peptidyl-tRNA in the P site), (b) aminoacyl-tRNAs, (c) a set of proteins known as **elongation factors,** and (d) GTP. Chain elongation can be divided into three principal steps:

1. Codon-directed binding of the incoming aminoacyl-tRNA at the A site.

2. Peptide bond formation: transfer of the peptidyl chain from the tRNA bearing it to the -NH_2 group of the new amino acid.

3. Translocation of the “one-residue-longer” peptidyl-tRNA to the P site to make room for the next aminoacyl-tRNA at the A site. These shifts are coupled with movement of the ribosome one codon further along the mRNA.

The Elongation Cycle

Elongation factors are present in large quantities, reflecting the great importance of protein synthesis to cell vitality. For example, elongation factor Tu (EF-Tu) is the most abundant protein in E. *coli*, accounting for 5% of total cellular protein.

Aminoacyl-tRNA Binding

EF-Tu binds aminoacyl-tRNA and GTP. There is only one EF-Tu species serving all the different aminoacyl-tRNAs, and aminoacyl-tRNAs will bind to the A site of active 70S ribosomes only in the form of aminoacyl-tRNA:EF-Tu:GTP complexes. Once the aminoacyl-tRNA is situated in the A site, the GTP is hydrolyzed to GDP and P_i, and the EF-Tu molecules are released as EF-Tu:GDP complexes EF-Tu does not interact with f-Met-$tRNA_f^{Met}$. **Elongation factor Ts (EF-Ts)** promotes the recycling of EF-Tu by mediating the displacement of GDP from EF-Tu and its replacement by GTP. EF-Ts accomplishes its job through entry into a transient complex with EF-Tu. GTP then displaces EF-Ts from EF-Tu .

Peptidyl Transfer

Peptidyl transfer, or **transpeptidation,** is the central reaction of protein synthesis, the actual peptide bond-forming step. No energy input is needed; the ester bond linking the peptidyl moiety to tRNA is intrinsically reactive. Peptidyl transferase, the activity catalyzing peptide bond formation, is associated with the 50S ribosomal subunit. , E. *coli* 50S ribosomal subunits from which virtually all ribosomal proteins have been removed retain significant peptidyl transferase activity. Experiments, carried out by Harry Noller and his colleagues, imply that *the peptidyltransferase enzyme is the 23S rRNA*. Nucleotide sequences in this region of 23S rRNA are among the most highly conserved in all biology.

Translocation

Three things remain to be accomplished in order to return the active 70S ribosome:mRNA complex to the starting point in the elongation cycle:

1. The deacylated tRNA must be removed from the P site.

2. The peptidyl-tRNA must be moved (translocated) from the A site to the P site.

3. The ribosome must move one codon down the mRNA so that the next codon is positioned in the A site.

The precise events in translocation are still being resolved, but several distinct steps are clear. Within the 70S ribosome, the anticodon ends of both the A-site and P-site tRNAs interact with the 30S subunit within an area referred to as the **decoding center;** it is here that mRNA codon:tRNA anticodon recognition takes place. In contrast, the acceptor ends (the aminoacylated ends) of both A-site and P-site tRNAs interact with the 50S subunit. In the first step, the acceptor ends of the A- and P-site tRNAs move with respect to the 50S subunit concomitantly with peptidyl transfer. Nucleophilic attack of the a-amino group of the aminoacyl-tRNA in the A site on the C-terminal carbonyl carbon of the peptidyl-tRNA results in peptide bond formation and transfer of the peptidyl chain to the A-site tRNA. Because the growing peptidyl chain does not move during peptidyl transfer, this reaction brings the acceptor end of the A-site tRNA into the P site as it picks up the peptidyl chain. The acceptor end of the P-site tRNA is shunted into the E site. These movements result in two hybrid states of tRNA binding: the *E/P state* and the *P/A state* (Figure 33.15d). Thus, the two ends of a tRNA are associated with different sites on the two ribosomal subunits (for example, in the P/A state, the peptidyl chain is linked to a tRNA having its acceptor end in the 50S P site and its anticodon end in the 30S A site). In the second step, the tRNAs and mRNA move together with respect to the 30S subunit so that the mRNA is passively dragged one codon along as the anticodon end of the peptidyl-tRNA is transferred from the A site of the 30S subunit to its P site (Figure 32.15e). Concomitantly, the deacylated tRNA is moved into the E site. These movements are catalyzed by the translocation protein **elongation factor G (EF-G),** which couples the energy of GTP hydrolysis to movement. Translocation of the mRNA relative to the 30S subunit delivers the next codon to the 30S A site. EF-G binds to the ribosome as an EF-G:GTP complex. GTP hydrolysis is essential not only for translocation but also for subsequent EF-G dissociation. Because EF-G and EF-Tu compete for a common binding site on the ribosome, EF-G release is a prerequisite for return of the 70S ribosome:mRNA to the beginning point in the elongation cycle. This basic two-step model for

translocation identifies six different states of tRNA binding: **P/P** (peptidyl-tRNA in the P site), **A/T** (an aminoacyl-tRNA entering the A site), **A/A** (an aminoacyl-tRNA in the A site), **P/A** (the peptide chain has been transferred to the aminoacyl-tRNA in the A site), **E/P** (the deacylated tRNA exiting the P site, and **E** (the deacylated tRNA in the E site). The A/T state is the first state in tRNA selection at the A site and involves interaction of the ribosome with an aminoacyl-tRNA:EF-Tu:GTP ternary complex. Codon-anticodon recognition and consequent translational fidelity must be determined at this stage. Figure 33.16 illustrates the relative positions of the two tRNA molecules within the 30S:50S intersubunit cavity during these different states. In the simple model of peptidyl transfer and translocation, the opposite ends of both tRNAs move relative to the two ribosomal subunits in two discrete steps, the acceptor ends moving first and then the anticodon ends. Further, the readjustments needed to reposition the ribosomal subunits relative to the mRNA and to one another imply that the 30S and 50S subunits must move relative to one another. *In proposing that the small and large subunits move relative to each other, as opposed to moving as a unit, this model provides a convincing explanation for why ribosomes are universally organized into a two-subunit structure.*

Peptide Chain Termination

The elongation cycle of polypeptide synthesis continues until the 70S ribosome encounters a “stop” codon. At this point, polypeptidyl-tRNA occupies the P site and the arrival of a “stop” or nonsense codon in the A site signals that the end of the polypeptide chain has been reached. These nonsense codons are not “read” by any “terminator tRNAs” but instead are recognized by specific proteins known as **release factors,** so named because they promote polypeptide release from the ribosome. The release factors bind at the A site. **RF-1** (36 kD) recognizes UAA and UAG, while **RF-2** (41 kD) recognizes UAA and UGA. There is about one molecule each of RF-1 and RF-2 per 50 ribosomes. Ribosomal binding of RF-1 or RF-2 is competitive with EF-G. The binding of RF-1 or RF-2 is promoted by a third release factor, **RF-3** (46 kD). RF-3 function requires GTP. The presence of release factors with a nonsense codon in the A site creates a **70S ribosome: RF-1** (or **RF-2**)**: RF-3-GTP: termination signal** complex that transforms the ribosomal peptidyl transferase into a hydrolase. That is, instead of catalyzing the transfer of the polypeptidyl chain from a polypeptidyl-tRNA to an acceptor aminoacyl-tRNA, the peptidyl transferase hydrolyzes the ester bond linking the polypeptidyl chain to its tRNA carrier. In actuality, peptidyl transferase transfers the polypeptidyl chain to a water molecule instead of an aminoacyl-tRNA. GTP hydrolysis now drives conformational events leading to the dissociation of the uncharged tRNA and expulsion of the release factors from the ribosome .

The Ribosome Life Cycle

Ribosomal subunits cycle rapidly through protein synthesis. In actively growing bacteria, 80% of the ribosomes are engaged in protein synthesis at any instant. Once a polypeptide chain is synthesized and the nascent polypeptide chain is released, the 70S ribosome dissociates from the mRNA and separates into free 30S and 50S subunits. Intact 70S ribosomes are inactive in protein synthesis because only free 30S subunits can interact with the initiation factors. Binding of initiation factor IF-3 by 30S subunits and interaction of 30S subunits with 50S subunits are mutually exclusive. 30S subunits with bound initiation factors associate with mRNA, but 50S subunit addition requires IF-3 release from the 30S subunit.

Polyribosomes Are the Active Structures of Protein Synthesis

Active protein-synthesizing units consist of an mRNA with several ribosomes attached to it. Such structures are **polyribosomes,** or, simply, **polysomes** . All protein synthesis occurs on polysomes. In the polysome, each ribosome is traversing the mRNA and independently translating it into polypeptide. The further a ribosome has moved along the mRNA, the greater the length of its associated polypeptide product. In prokaryotes, as many as 10 ribosomes may be found in a polysome. Ultimately, as many as 300 ribosomes may translate an mRNA, so as many as 300 enzyme molecules may be produced from a single transcript. Eukaryotic polysomes typically contain fewer than 10 ribosomes.

The Relationship Between Transcription and Translation in Prokaryotes

In prokaryotes, ribosomes attach to mRNA even before transcription of the mRNA is completed, and as a consequence, polysomes can be found in association with DNA. Biochemical evidence for this relationship between transcription and translation comes from a study on the expression of the enzymes for tryptophan biosynthesis in E. *coli*. These enzymes are encoded in the *trp* operon, a set of five contiguous genes. Transcription of the *trp* operon occurs only in the absence of tryptophan, and transcription of the entire operon takes more than 5 minutes. The first two genes, E and D, encode the subunits of the enzyme, anthranilate synthase. Within 2 1/2 minutes of the start of *trp* operon transcription, anthranilate synthase activity is detectable, demonstrating that translation has begun before transcription is completed. That is, the mRNA is translated while the operon is still being transcribed. Oscar Miller, Barbara Hamkalo, and Charles Thomas provided visual evidence for

concomitant transcription and translation, as shown in Figure 33.21. The leading ribosome may actually make physical contact with RNA polymerase.

Protein Synthesis in Eukaryotic Cells

Eukaryotic mRNAs are characterized by two post-transcriptional modifications: the **5'-^{7}methyl-GTP cap**and the **poly(A) tail** (Figure 33.22). The ^{7}methyl-GTP cap is essential for ribosomal binding of mRNAs in eukaryotes and also enhances the stability of these mRNAs by preventing their degradation by 5'-exonucleases. The poly(A) tail enhances both the stability and translational efficiency of eukaryotic mRNAs. The Shine-Dalgarno sequences found at the 5'-end of prokaryotic mRNAs are absent in eukaryotic mRNAs.

Peptide Chain Initiation in Eukaryotes

The eukaryotic initiator tRNA is a unique tRNA functioning only in initiation. Like the prokaryotic initiator tRNA, the eukaryotic version carries only Met. However, unlike prokaryotic f-Met-$tRNA_f^{Met}$, the Met on this tRNA is not formylated. Hence, the eukaryotic initiator tRNA is usually designated **$tRNA_i^{Met}$,** with the "i" indicating "initiation." Eukaryotic initiation can be divided into three fundamental steps. *Step 1:* Association of Met-$tRNA_i^{Met}$ and initiation factors eIF2, eIF1A, and eIF3 with the 40S ribosomal subunit to form the **43S preinitiation complex**. Met-$tRNA_i^{Met}$ is delivered to the 40S subunit as an eIF2:GTP:Met-$tRNA_i^{Met}$ ternary complex. Binding of eIF4A and eIF3 to 40S subunits generates a 43S ribosomal subunit:initiation factor complex; eIF4A catalyzes the association of eIF2:GTP:Met-$tRNA_i^{Met}$ with the 43S complex to yield the 43S preinitiation complex. Unlike in prokaryotes, binding of Met-$tRNA_i^{Met}$ by eukaryotic ribosomes occurs in the absence of mRNA, so Met-$tRNA_i^{Met}$ binding is not codon-directed. *Step 2:* Binding of the 43S preinitiation complex to mRNA and migration of the 40S ribosomal subunit to the correct AUG initiation codon. The 43S preinitiation complex binds mRNA at its 5'-terminal ^{7}methyl-GTP cap. eIF4E, the mRNA cap-binding protein, represents a key regulatory element in eukaryotic translation. For eIF4E to be active, it must associate with eIF4G to form a complex designated **eIF4F.** eIF4F also contains eIF4A; eIF4F binding to the cap structure is a prerequisite for association of eIF4B and formation of the 48S preinitiation complex. Translation is inhibited when eIF4E binds with **4E-BP** (the eIF4E binding protein). Growth factors stimulate protein synthesis by causing the phosphorylation of 4E-BP, which prevents its binding to eIF4E. The 59-terminal ^{7}methyl-GTP cap and the 39-poly(A) tail act synergistically to increase translational effi-ciency. **Pab1p,** the **p**oly(**A**)- **b**inding **p**rotein, has two

binding sites, one for binding to the poly(A) tract on mRNAs and a second for interaction with eIF4G. Thus, eIF4G serves as a bridge between the cap-binding eIF4E, the poly(A) tail, and the 40S subunit via eIF3. These interactions initiate scanning of the 40S subunit in search of an AUG codon. *Step 3:* Addition of the 60S ribosomal subunit to the 48S preinitiation complex, forming the **80S initiation complex,** whereupon translation commences (Figure 33.23, stage 3). When the 43S preinitiation complex stops at an AUG codon, GTP hydrolysis in the eIF2:Met-$tRNA_i^{Met}$ ternary complex causes ejection of the initiation factors bound to the 40S ribosomal subunit. Release of these factors allows 60S subunit association. eIF2:GDP is recycled to eIF2:GTP by eIF2B; eIF2B is a *guanine nucleotide exchange factor.*

Regulation of Eukaryotic Peptide Chain Initiation

Regulation of gene expression can be exerted post-transcriptionally through control of mRNA translation. Phosphorylation/dephosphorylation of translational components is a dominant mechanism for control of protein synthesis. Peptide chain initiation, the initial phase of the synthetic process, is the optimal place for such control. Phosphorylation of 40S ribosomal protein S6 facilitates initiation of protein synthesis, resulting in a shift of the ribosomal population from inactive ribosomes to actively translating polysomes. S6 phosphorylation is stimulated by serum growth factors . The action of eIF4F, the mRNA cap-binding complex, is promoted by phosphorylation. On the other hand, phosphorylation of other translational components inhibits protein synthesis. For example, the a-subunit of eIF2 can be reversibly phosphorylated at a specific Ser residue by an eIF2a kinase/phosphatase system (Figure 33.25). Phosphorylation of eIF2a ultimately inhibits peptide chain initiation, not because eIF2a-P is ineffectual, but because phosphorylated eIF2 binds eIF2B much more tightly than does eIF2. All of the eIF2B, which is present at only 20 to 30% of eIF2 levels, becomes sequestered in eIF2:eIF2B complexes, and eIF2 cannot be regenerated from eIF2:GDP for further cycles of initiation. Reversible phosphorylation of eIF2 is an important control governing globin synthesis in reticulocytes. If heme for hemoglobin synthesis becomes limiting in these cells, eIF2a is phosphorylated so globin mRNA is not translated and chains are not synthesized. Availability of heme reverses the inhibition through phosphatase-mediated removal of the phosphate group from the Ser residue.

Peptide Chain Elongation in Eukaryotes

Eukaryotic peptide elongation occurs in very similar fashion to the process in prokaryotes. An incoming aminoacyl-tRNA enters the ribosomal A site while peptidyl-tRNA occupies the P site. Peptidyl transfer

then occurs, followed by translocation of the ribosome one codon further along the mRNA. Two elongation factors, EF1 and EF2, mediate the elongation steps. EF1 consists of two components: EF1A, a 50-kD protein, and EF1B, a complex of 31-kD (b) and 50-kD (g) protein subunits, EF1A is the eukaryotic counterpart of EF-Tu; it serves as the aminoacyl-tRNA binding factor and requires GTP. EF1B is the eukaryotic equivalent of prokaryotic EF-Ts; it catalyzes the exchange of bound GDP on EF1:GDP for GTP so active EF1:GTP can be regenerated. EF2, a 100-kD polypeptide, is the eukaryotic translocation factor. Like its prokaryotic kin, EF-G, EF2 binds GTP, and GTP hydrolysis accompanies translocation.

Eukaryotic Peptide Chain Termination

Whereas prokaryotic termination involves three different release factors (RFs), just one RF is sufficient for eukaryotic termination. Eukaryotic RF (110 kD) is an a_2 dimer of 55-kD subunits. Eukaryotic RF binding to the ribosomal A site is GTP-dependent, and RF:GTP binds at this site when it is occupied by a termination codon. Then, hydrolysis of the peptidyl-tRNA ester bond, hydrolysis of GTP, release of nascent polypeptide and deacylated tRNA, and ribosome dissociation from mRNA ensue.

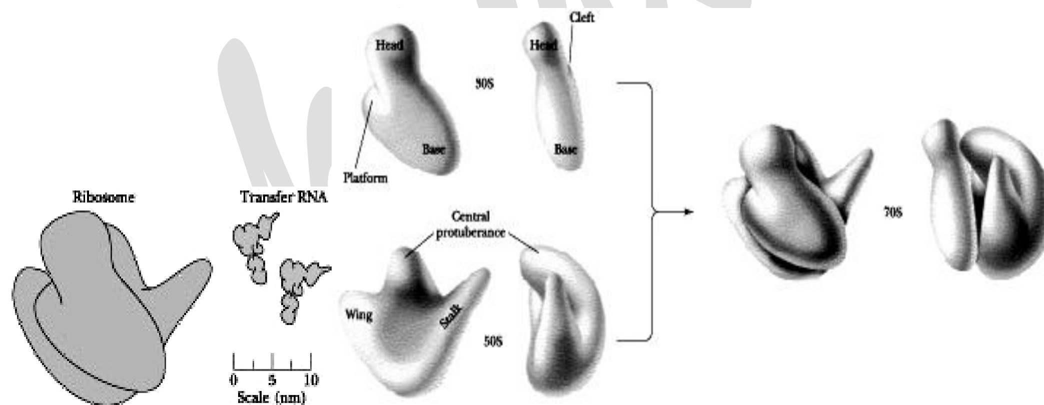

Fig:-Three-dimensional model for the E. *coli* ribosome as deduced by image reconstruction. The 30S, 50S, and 70S structures are presented in two views that are rotated 90° relative to each other. The small and large ribosomal subunits each have characteristic morphological features, such as protuberances and clefts. The 30S subunit is somewhat elongated and asymmetric and has the dimensions 5.5 x 22 x 22 nm. The 50S subunit is predominantly spheroidal but has three projections; its dimensions are 15 x 20 x 20 nm. A 2.5-nm tunnel passes through the large subunit in the central “valley” region between the subunit’s three protrusions.

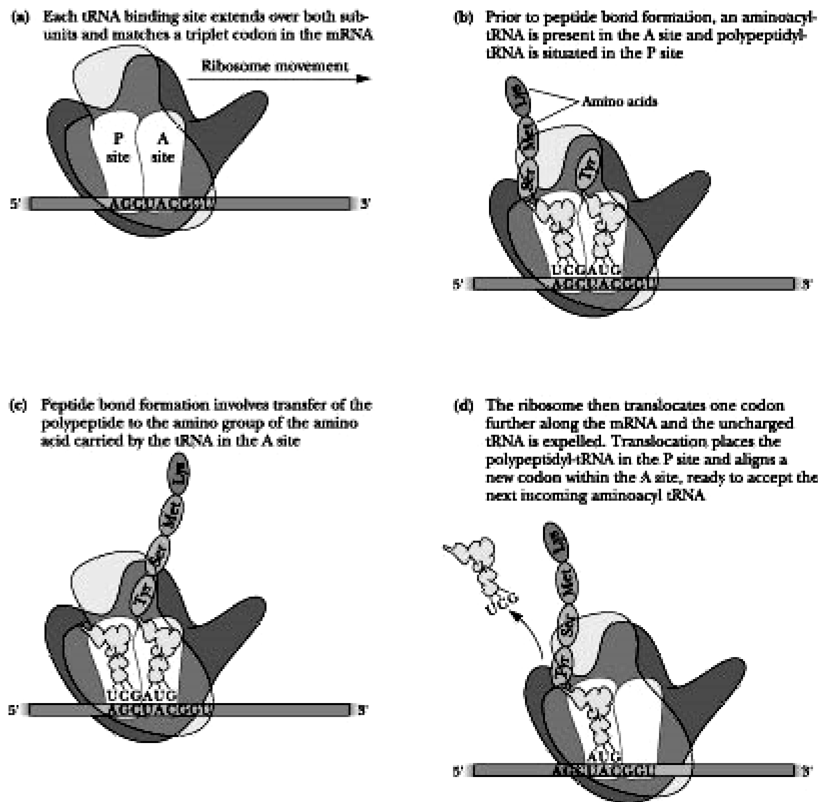

Fig:-The basic steps in protein synthesis. Note that the ribosome has two principal sites for binding tRNA: the A, or acceptor, site and the P, or peptidyl, site.

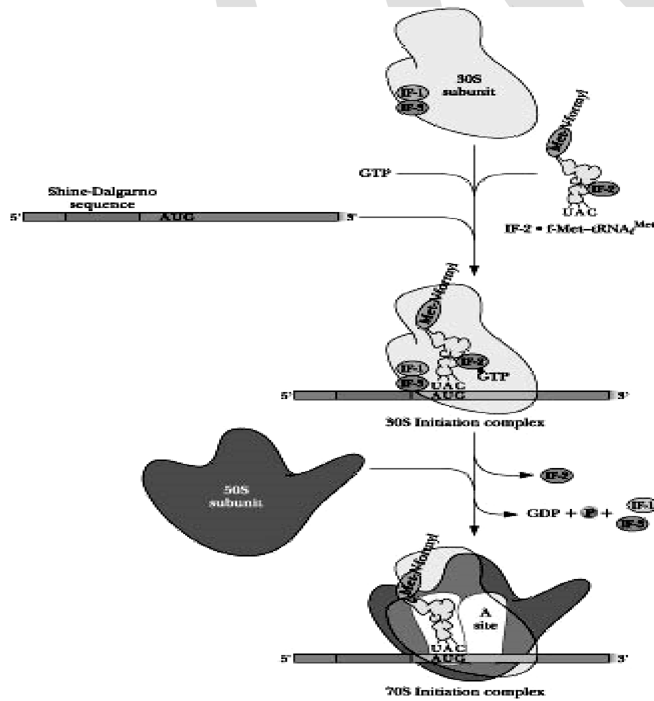

Fig: The sequence of events in peptide chain initiation

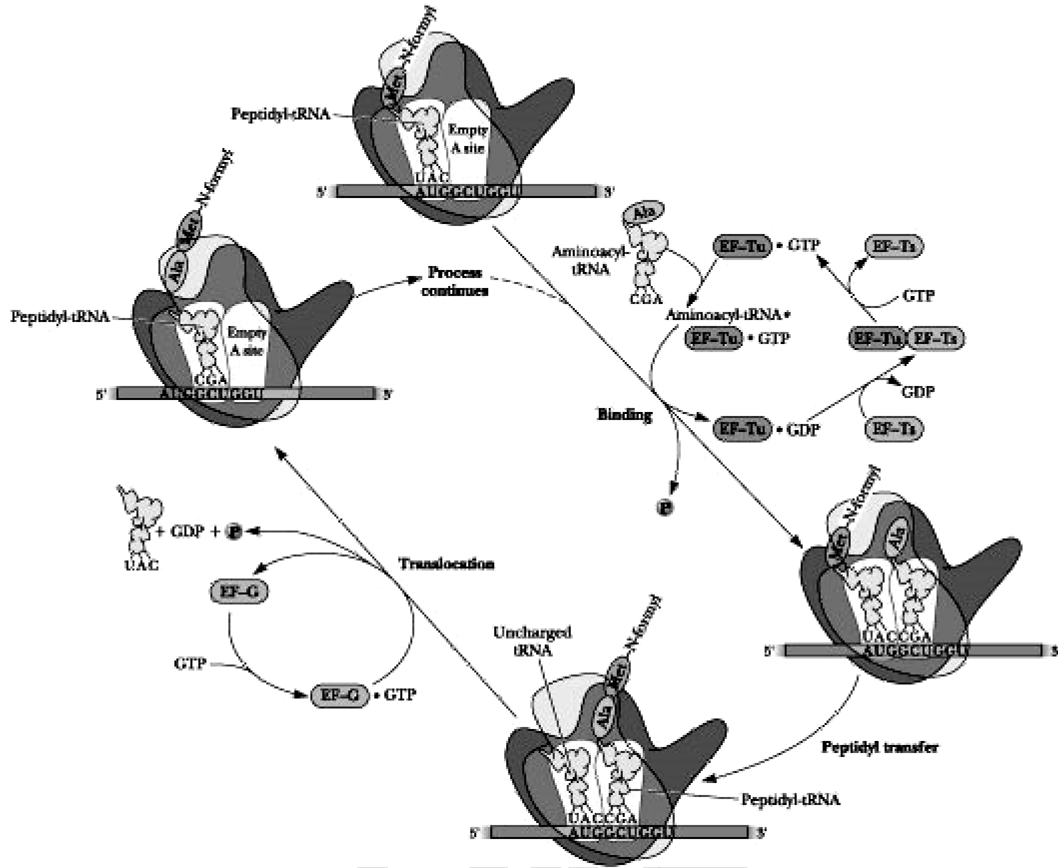

Fig:-The cycle of events in peptide chain elongation on *E. coli* ribosome.

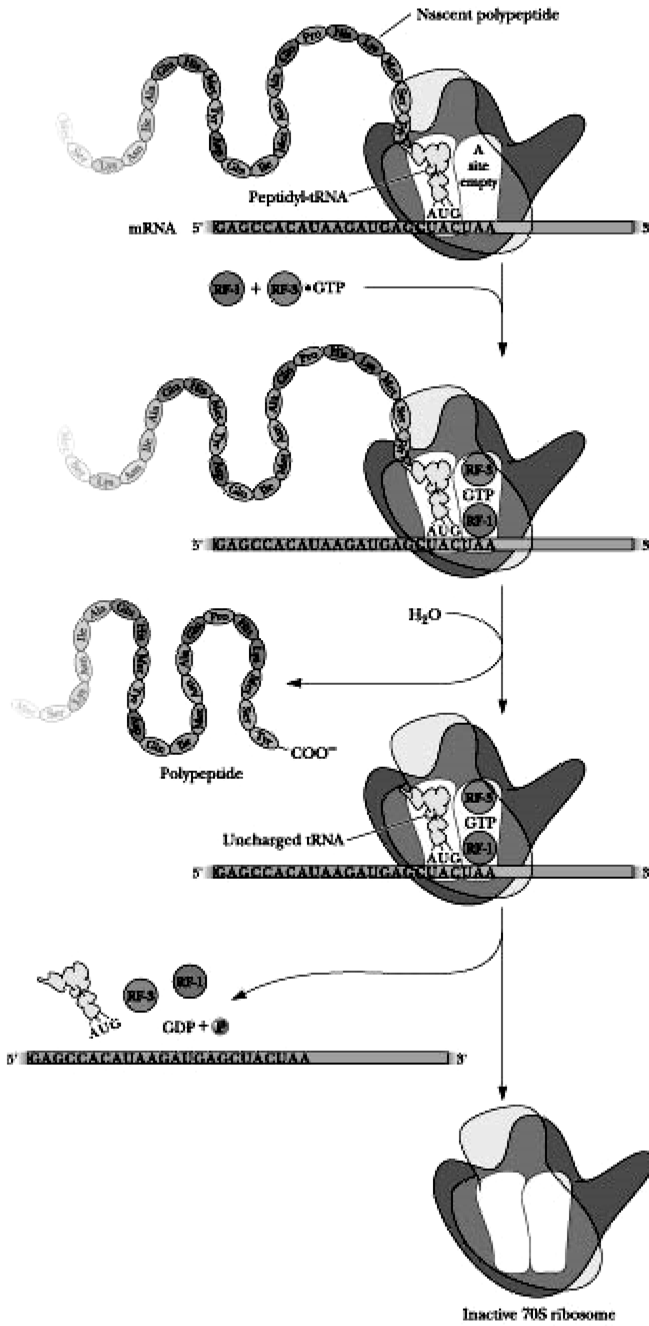

Fig:-The events in peptide chain termination