

Regulation of Protein Translation

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This Teaching Resource provides a summary and slides derived from a lecture on protein translation and is part of the course “Cell Signaling Systems: A Course for Graduate Students.” The lecture begins with a discussion of the various components that perform the translation process and then proceeds to describe the initiation, scanning, and ribosomal entry processes. The lecture concludes with the signaling mechanisms underlying translation regulation.

Lecture Notes

Translation of messenger RNA (mRNA) requires elaborate translational machinery. The mRNA is “read” by ribosomes, which are composed of two subunits, a large one and a small one. The ribosome contains a number of ribosomal RNA molecules (rRNA) with three rRNA molecules in the large ribosomal subunit and one in the small subunit. In addition, the ribosome comprises 78 different proteins. The precursors of the ribosome are synthesized in the nucleolus and in the cytoplasm in a highly coordinated process (1). However, the final assembly of the ribosome occurs as part of the translational process.

Assembly of the 43S pre-initiation complex

The assembly and activity of the ribosome are controlled by initiation and elongation factors. There are at least 11 initiation factors, some comprising multiple subunits, and three elongation factors. These factors are activated either by phosphorylation or by exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP), with the help of a guanine nucleotide exchange factor (GEF). Phosphorylation may also inhibit the activity of a translation factor, as is the case with eukaryotic translation initiation factor 2 (eIF2) and elongation factor 2 (eEF2) (2).

Assembly of ribosomes requires the participation of activated initiation factors (eukaryotic translation initiation factors, eIFs). Building of the ribosome begins with the binding of eIF1, eIF1A, and eIF3 to the small ribosomal subunit (40S), which can then associate with eIF2. GTP bound to methionyl-tRNA (met-tRNA) is called initiator tRNA, because it recognizes the initiation codon (AUG) of the mRNA and will eventually start the process of translation (3). At this point, the resulting complex is called 43S pre-initiation complex and is ready to bind mRNA.

mRNA contributes to initiation

Specific information that is important for initiation is contained in the 5′ and 3′ parts of the mRNA. At the 5′ terminus, mRNA has a 7-methylguanosine (m7G) cap, which binds a complex of initiation factors. The initiation factor eIF4E binds to the cap and controls the rate of initiation. eIF4E then binds eIF4G, which acts as a scaffold and brings together additional

initiation factors (eIF4A, eIF4B, and eIF4H), which function as a helicase that melts the secondary structure of mRNA close to the cap. Once this secondary structure is eliminated, eIF4G binds eIF3, thus joining the cap structure to the 43S pre-initiation complex, to form the 48S initiation complex, which also includes eIF5 (2).

The 3′ untranslated region (UTR) of mRNAs possesses elements that allow extension of the poly(A) (polyadenylate) tail to the 3′ end of the mRNA. Generally, these elements include the hexanucleotide AAUAAA, which enhances polyadenylation by binding a factor named cleavage and polyadenylation specificity factor (CPSF). CPSF then interacts with polyadenylation polymerase (PAP) to induce the elongation of the poly(A) tail. The poly(A) tail binds polyadenylate binding protein (PABP), which then interacts with eIF4G that is part of the 48S complex and increases its affinity for eIF4E and the cap itself. This cyclizing of the transcript increases the rate of translation by allowing rapid recycling ribosomes to the 5′ end when the ribosomes reach the end of the mRNA.

Some transcripts include the cytoplasmic polyadenylation element (CPE: UUUUUAU), to which CPEB specifically binds. When CPEB becomes phosphorylated [by either the kinase Aurora or by calcium/calmodulin-dependent protein kinase II (CaMKII)], it interacts with CPSF and further enhances polyadenylation. In addition, CPEB binds maskin, which binds to and inhibits the action of eIF4E. The elongated poly(A) tail liberates eIF4E from maskin, which allows translation to commence (4). In contrast to the general mechanism that accelerates translation, CPEB phosphorylation [or conformational change (5)] may control the translation of selected mRNAs. Finally, the interactions of the poly(A) tail with the cap structure contribute to circularization of the mRNA, which increases its stability.

Once fully formed, the initiation complex moves along the mRNA, away from the cap region toward the 3′ end. This movement continues until the Met-tRNA anticodon (CAU) recognizes an initiation codon (AUG), where translation starts. The movement of the initiation complex is powered by the helicase action of eIF4A, which requires the hydrolysis of adenosine triphosphate (ATP). Once the codon-anticodon match occurs, the initiation factors dissociate from the initiation complex, and the now 40S small ribosomal subunit binds the 60S large ribosomal subunit to form the 80S mature ribosome. The action of eIF5B, which hydrolyzes GTP, is required for this union (6).

Internal ribosomal entry site (IRES)

Some mRNAs are translated without utilizing the cap mechanism, but rather begin translation at a site within the 5′ UTR. Such elements are found in viral RNA and also in eukaryotic mRNAs. Many of these transcripts encode proteins that protect cells from stress. IRES elements are long (dozens to hundreds of nucleotides) and have a complex secondary structure. The translation process requires participation of the 43S pre-initiation complex and the eventual joining of the 60S ribosomal subunit (7).

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The elongation process

The mature ribosome possesses three sequential tRNA-binding sites, named A, P, and E. Site A is the aminoacyl site, site P is the peptidyl site, and site E is the exit site. Both subunits of the ribosome contribute to all three sites. The first tRNA to bind to the A site is the Met-tRNA, which is then translocated to the P site. A new aminoacyl-tRNA (AA-tRNA) now binds to the A site. Next, the methionine on the P site dissociates from its tRNA and forms a peptide bond with the amino acid of the new AA-tRNA, located on the A site. The new AA-tRNA now carries a di-peptide, whereas the tRNA on the P site has become deacylated. Finally, the deacylated tRNA moves from the P site to the E site, the tRNA (now carrying a di-peptide) moves from the A site to the P site, and a new AA-tRNA binds to the A site. In the next cycle, the deacylated tRNA will be shed off the E site. As a result of this cycle, the mRNA has moved one codon along the ribosome, and the nascent peptide has acquired a new amino acid. The cycle repeats many times, until the mRNA is fully decoded and the protein fully translated (8). The binding of AA-tRNA to the A site is mediated by elongation factors 1A and 1B (eEF1A, eEF1B). The active form of eEF1A is the GTP-bound form, and eEF1B is its GEF. The transfer of the nascent peptide from the P site to the A site is the function of eEF2, a peptidyl transferase. Again, the active form is the GTP-bound eEF2, but this elongation factor does not require a GEF (9). As the mRNA is threaded through the ribosome, the cap advances and can initiate the formation of another ribosome. As the process repeats, the mRNA becomes attached to a number of ribosomes, forming a polyribosome (polysome). The nascent peptides fold as they are formed (10).

Termination of translation

Translation stops when one of three stop codons (UAA, UAG, or UGA) enters the A site. This results in the binding of a releasing factor (RF1) to the stop codon and the hydrolysis of the aminoacyl bond between the nascent peptide chain and the tRNA located at the P site, thus terminating translation. RF1 binds RF3 that then attaches to PABP. PABP in turn binds eIF4G and eIF4E to start assembling another cap binding complex. The bridge that PABP forms between the poly(A) tail and 5' cap allows the ribosomal subunits to recycle from the stop codon back to the 5' end of the same mRNA and begin another round of translation. At some point, additional factors intervene to remove the poly(A) tail and the RFs, as well as the cap structures, from the mRNA and allow its degradation (11, 12). The number of ribosomes on a polysome depends on the rate of initiation and the rate of elongation. The faster the initiation and the slower the elongation, the longer the polysome (the more ribosomes on a given transcript). Measured rates of translation indicate that substantial amounts of proteins can be made in a matter of minutes.

Regulation of translation

The control of translation offers the cell the means to control its composition rapidly, with flexibility, fine control, and spatial discrimination. Generally, initiation controls the rate of translation, although control through elongation is also known. The major signaling pathway that regulates translation in response to extracellular signals and the availability of amino acids includes a cascade of kinases—phosphoinositide 3-kinase (PI3K), phosphoinositide-dependent protein kinase 1 (PDK1), protein kinase B (PKB, also known as Akt), and the mammalian target

of rapamycin (mTOR)—with some additional factors (13). mTOR phosphorylates the inhibitory eIF4E binding proteins, thus releasing eIF4E from inhibition and allowing initiation to proceed. In addition, mTOR facilitates translation of transcripts with terminal oligopyrimidine tracts (TOP mRNAs). These transcripts encode translational proteins, such as eEF1A, eEF2, PABP, and S6, to increase translational capacity (14). The mitogen-activated protein kinase (MAPK) cascade influences the PI3K-dependent pathway (15) and vice versa (16). Another type of translational regulation involves microRNAs, which repress gene translation (17).

The slides rely on the citations listed in the lecture notes and these additional references (18–20).

Related Resources*Teaching Resources*

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Review

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Connections Maps

- L. C. Cantley, PI3K pathway. *Sci. STKE* (Connections Map, as seen December 2005) http://stke.sciencemag.org/cgi/cm/stkecm;CMP_6557.
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