Chapter 17. Weaver, 2/e. Mol Biol X107A.

Ch 17. The mechanism of translation. I. Initiation.

Schedule note: The three chapters of Part VI (Ch 17-19) are closely interrelated.

Reminder: The material on protein synthesis in Ch 17-19 depends on and builds on parts of Ch 3, especially the subsection on Translation.

Clark & Russell: Ch 7 (for Weaver Ch 17-19). You may also find it useful to look up specific topics in the index, which seems rather good. It is normal that material on protein synthesis is organized quite differently in different books.

A. Introduction

Protein synthesis is the most complex process in the operation of the central dogma. This is not surprising, since it involves a change (translation) from nucleotide language to amino acid language. Part VI of Weaver describes this process.

The big picture of protein synthesis (Ch 17-19, and recall introduction to translation in Ch 3):

- The players: messenger RNA, ribosomes, transfer RNA; initiation factors, elongation factors, termination (release) factors; aminoacyl-tRNA synthetases; ATP & GTP.

- The main steps: Initiation, elongation, and termination of peptide chain synthesis. Activation of amino acid precursors. Accuracy issues; proofreading. Protein folding and localization.

  Protein folding and localization are not X107A topics. (We alluded to some issues of protein folding in Ch 3. I have added a brief section, "The final steps: folding and transport -- and degradation", to the Ch 18 handout, to introduce these topics and give some references. I do not plan to cover that Sect in this course.)

Ch 17 highlights:

- Initiation of protein synthesis involves assembly of the translation apparatus.

- An important difference between prokaryotic and eukaryotic protein synthesis is how initiation sites are selected.

- ATP is the energy source for peptide bond formation. It is used via tRNA charging.

- GTP is involved as an energy source at several steps. Binding and hydrolysis of GTP drives cycles of protein conformational changes.

B. Overview of protein synthesis

The ribosome is the site of protein synthesis in all cells. The ribosome is composed of 2 subunits (Fig 3.16). Each subunit is a complex of 1-2 RNA molecules plus many proteins.

Proteins are made by translating a transient copy of the gene, the messenger RNA (mRNA).

Protein synthesis starts by forming an initiation complex between a message, the charged initiator tRNA, and the small ribosomal subunit. This initiation step includes finding (choosing?) the proper initiation site. The most important differences between prokaryotic and eukaryotic protein synthesis are at the initiation stage. (Sect D, below.)

After the initiation step, there is a succession of elongation-translocation cycles. Add an amino acid, then move along the message, etc. Amino acids are used attached to codon-specific tRNAs. The cycle concludes at a termination codon, where the new protein is released. Ultimately, the message is released and the ribosomal subunits dissociate. These steps are all developed in Ch 18.

A variety of protein "factors", some using GTP, are required at various steps. (Sect E & I, below; more in Ch 18.)

C. Ribosomes: overview

You should understand the general organization of ribosomes, e.g. Fig 3.16.

S values, used to describe ribosomes and their constituents, are a measure of size (p 49). (Technically, an S value is the sedimentation coefficient, in Svedbergs.) However, the relationship is neither linear nor additive. Further, terms such as "30S" have become generic names, regardless of the actual measured size.

It is a useful generality that there are two types of ribosomes: prokaryotic (including organelle) and eukaryotic. These correspond to the different initiation mechanisms in these organisms. Hybrid ribosomes made from two organisms of the same type are sometimes functional.

Ribosomes are a major cellular constituent; many copies of genes for ribosomal products are required. Nomura (1999) and Warner (1999) discuss issues of making such large amounts of ribosomes.

> Intriguingly, some ribosomal proteins have other functions (Wool, 1996); the significance of this finding is not clear.

D. Initiation of protein synthesis (prokaryotic and eukaryotic)

For protein synthesis, the initiation process involves assembling the apparatus and laying down the first amino acid. As a result, the ribosome cycle (association and dissociation of ribosomal subunits) is interconnected with initiation and termination.

Some features of initiation of bacterial protein synthesis:

- Key role of the small ribosomal subunit (30S in bacteria) in forming the initiation complex.

- Use of special initiator tRNA.

- Use of modified (formylated) amino acid.

  The formyl group and, sometimes, the initiating methionine are removed from the protein, p 535. (Liu et al, 1998, show that the enzyme that removes methionine is the target of an anti-cancer drug.) Interestingly, in yeast mitochondria the formylation is not "essential"; Li et al (2000).

- Key role of ribosome binding site ("rbs"; also called "Shine-Dalgarno" sequence), on message near beginning of each gene, in determining where translation starts. pp 536 ff.

In eukaryotes, only the first two of these still hold.

- A special initiator tRNA is used, but the amino acid is just plain methionine.

- The more important difference between bacterial and eukaryotic initiation is how a ribosome finds the start codon. In the major pathway for initiation in eukaryotes, the ribosome binds to the 5′ end of the message, then travels along the message to the first AUG (Fig 17.20). The end-binding requires a special "cap" structure on the message (Ch 15 Sect 1). There is some context requirement for the AUG to be an effective initiator codon, but most (~95%; p 545) eukaryotic messages are translated from the first AUG. This standard mode of eukaryotic initiation is known as ribosome scanning. (It is also called the Kozak model.)

A corollary of the standard initiation mechanisms is that internal initiations are allowed with bacterial mRNA, but not with eukaryotic mRNA.

Examples of special cases

- [not in book] Some bacterial genes that are downstream on a polycistronic mRNA lack a normal rbs. In such cases, a ribosome may progress directly from one gene to another without dissociating from the message. This requires that the two genes be very close together (sometimes even overlapping). This phenomenon is sometimes called ribosome coupling or translational coupling.

- [not in book] There are rare bacterial mRNAs that lack a leader, hence lack an rbs. Moll et al (2001) discuss, but certainly do not explain, how such mRNAs are translated.

- Some eukaryotic genes do have something analogous to an rbs, called an IRES (= internal ribosome entry site). It serves as an internal entry point for ribosomes on eukaryotic messages. (The direct effect of the IRES is to recruit initiation factors, not the ribosome itself.) It is likely that "scanning" proceeds from the IRES similarly to how it normally proceeds from the cap. More about internal initiation of eukaryotic messages in the hw.

  Sachs (2000) reviews recent work showing cell-cycle regulated IRES. Wilson et al (2000) discuss an unusual type of IRES-mediated translational event that bypasses not only the cap but also the initiator tRNA. Guo et al (2001) show another alternative initiation process, which also involves an mRNA loop.

Recent work now allows us to view the variety of eukaryotic initiation mechanisms, both standard and non-standard, as variations on a common theme. Fig 17.33 introduces this, but you should learn about initiation factors, especially eIF4, first (Weaver p 551; Sect E, below).

Liu et al (1997) discuss a case of regulation of use of multiple initiation codons for one gene in a eukaryote.

E. Initiation factors

Biochemical analysis shows a number of proteins that are required for a process. These are simply called "factors", and given cryptic names pending analysis of their real functions. We will not attempt any systematic analysis of protein synthesis factors, but will note some as they come up.

As general terminology for protein synthesis factors…

- IF = initiation factor
- EF = elongation factor (Ch 18)
- RF = release factor (termination) (Ch 18)
- The prefix e is often used for eukaryotic factors.

Fig 17.19 summarizes the prokaryotic translation initiation factors, and Fig 17.28 summarizes the eukaryotic initiation factors. These Figs are incomplete, and some functions are unclear. As you read these parts, try to emphasize the functions that occur, not memorizing lists of factors.

Two of the three bacterial IF are involved in making the small ribosomal subunit available for initiation. Fig 17.9 summarizes their roles.

Perhaps the most interesting initiation factor is IF2 (or eIF2), which "delivers" the aminoacyl tRNA to the ribosome. We will discuss this GTP-dependent factor in Sect I, below.

The most interesting distinctively eukaryotic factors are parts of eIF4:

- eIF4F (or more specifically, its 4E component), the cap-binding factor.

- eIF4A-B has RNA helicase activity; it helps unwind RNA secondary structure during scanning. (Fig 17.32.)

Marcotrigiano et al (1999) describe an example of a protein interfering with an initiation factor, thus serving to regulate translation.

Fortes et al (2000) discuss the exchange of nuclear proteins that bind to the mRNA with cytoplasmic proteins -- initiation factors, in this case.

With some appreciation, now, of the complexity of eIF4, look at Fig 17.33. This Fig, which was noted earlier, summarizes several variations of how eukaryotic initiation may occur, using various combinations of the cap, an IRES, and the poly(A) tail.

Wells et al (1998) observe the circularized initiation complexes predicted by the involvement of both ends of the mRNA. Marcotrigiano et al (2001) show how a single initiation factor may work in various ways. Khaleghpour et al (2001) show that interfering with the proper action of the poly(A) tail interferes with translational initiation. Paz & Choder (2001) implicate cap-independent translation (presumably IRES-dependent??) in survival during starvation, by analysis of the roles of the initiation factors.

F. tRNA charging: Terminology

The aminoacyl tRNA is also called the charged tRNA or activated amino acid.

The process of attaching an amino acid to its tRNA is called tRNA charging OR amino acid activation.

The enzymes that carry out that process are informally called activating enzymes OR more formally aminoacyl-tRNA synthetases.

G. tRNA charging

tRNA is involved in two recognition steps:

- First, it recognizes the appropriate "activating enzyme", to get its amino acid.
- Second, it recognizes the appropriate codon on the message.

In Ch 17 (p 529), Weaver briefly notes how tRNA is charged with its amino acid. Further aspects of tRNA function, including details of recognition, are left until Ch 19.

tRNA charging is a two step reaction:

1. $AA + ATP (= AMP\sim P\sim P) \rightleftarrows AA\sim AMP + P\sim P_i$

2. $AA\sim AMP + tRNA \rightleftarrows AA\sim tRNA + AMP$

In these equations, the ~ stands for a high energy bond. (The only difference between what I wrote here and what Weaver shows on p 529 is that I have explicitly noted the high energy bonds.)

Both reactions are catalyzed by one "activating enzyme". (There is one activating enzyme for each amino acid.)

The net result is that ATP is used to activate the amino acid, with the energy of ATP hydrolysis being stored in the ester bond between the amino acid and the tRNA.

> [As with nucleic acid synthesis, pyrophosphate, $P\sim P_i$, is released; its hydrolysis yields another ~7 kcal/mol, to provide a net driving force. Recall Ch 2 handout p 4.]
>
> Ambiguous term: Activated amino acid can refer to either AA~AMP or AA~tRNA. In most contexts, the latter is more relevant. However, if one is dissecting the mechanism of activation, then be careful with this term.

Wakasugi & Schimmel (1999) discuss a role of an aminoacyl-tRNA synthetase outside of protein synthesis. Kumar & Yarus (2001) have developed a ribozyme that can carry out amino acid activation; this has implications for the "RNA world" view of early life (Ch 3 handout Sect I).

H. The energy for peptide bond formation

The energy to drive the polymerization comes from ATP hydrolysis, although the mechanism is more complex than for nucleic acid synthesis. The immediate precursor for polymerization is the aminoacyl tRNA (Fig 17.2): tRNA carrying the amino acid in a high energy ester bond. The ATP was spent earlier; pyrophosphate was released. (Sect G)

The key point for now is to recognize the role of ATP -- and to contrast this (expected) role with the role of GTP, below.

I. The energy for protein synthesis; GTP and protein conformations

ATP provides the energy for peptide bond formation (above). In addition, GTP is needed for protein synthesis. The GTP is used quite differently than the ATP. The GTP is hydrolyzed to GDP + P_i (rather than to GMP + PP_i); coupling of this reaction to another chemical reaction is not readily apparent.

In Ch 17, we learn about the role of GTP with bacterial IF2. We will see other, similar roles for GTP in other steps of protein synthesis in Ch 18.

We now understand that the different molecular forms of GTP and the product GDP control a cycle. They affect the shape of the factor they bind to, thus affecting the biological activity of that factor. Actually, there are three forms of the factor: GTP-bound, GDP-bound, and free. GTP binds to the factor, is hydrolyzed to GDP while bound, and then the GDP leaves. After the GDP leaves, the factor is free, and ready to bind another GTP. The result is that the factor undergoes a cycle of conformational changes, driven by the GTP binding and hydrolysis. Additional proteins may modulate various steps in this cycle, including GTP hydrolysis and GDP departure. The GEF (= eIF2B), p 350, is an example.

For IF2, the form that binds to the ribosome is IF2-GTP. Hydrolysis of the GTP causes release of the IF2, thus recycling it for further use. Fig 17.19. Roll-Mecak et al (2000) observe the conformational changes of IF2 during its cycling.

> (The general role of IF2 is to "deliver" the initiator tRNA. In Ch 18, we will see a similar role for EF-Tu in delivering other tRNAs, during elongation. A GTP cycle is also involved in this case.)

A change in protein conformation at one site caused by the binding of a small molecule at a distant site is called allostery. The effect of GTP on the shape of a protein is a quite reasonable

example of allostery. What is different here is the control of the binding by GTP hydrolysis, thus promoting a cyclic reaction.

A similar use of GTP, to control protein conformation, occurs with the "G proteins", which are involved in hormone responses in eukaryotes (Ellis & Miles, 2001). Song et al (2000) discuss the role of the GTP cycle in targeting proteins for secretion. Lu et al (2000) provide evidence that a key bacterial cell division protein is a G protein.

Generalizing further, we now recognize many proteins that use some NTP-NDP cycle to control the conformation of the protein. For example, we will see proteins that use ATP-ADP cycles to deliver a protein to DNA. Vale & Milligan (2000; Ch 1 FR) discuss how this cycle is used for motor proteins, such as muscle myosin or microtubule kinesins.

It is often said that enzymes catalyze reactions involving covalent bonds. The GTPases discussed here, and related enzymes, seem to catalyze reactions involving weak bonds (hydrogen bonds, etc), and biochemists have been uncomfortable with this. Purich (2001) addresses this, and attempts to legitimize this class of enzyme.

J. Regulation of translation

(Ch 17 Sect 3; briefly)

Any process that involves interactions (between proteins, nucleic acids, or small molecules) can be affected by other factors that stimulate or inhibit those interactions. Thus, protein synthesis can be regulated.

In prokaryotes, one kind of regulation that is particularly interesting involves the role of mRNA secondary structure in affecting access to the initiation sites. Fig 17.12. More about this in the homework.

The more complex eukaryotic initiation complex is subject to more complex regulation. We will probably not discuss this.

Marcotrigiano et al (1999) discuss an example of translational control, by interference with an initiation factor; this paper raises the idea of molecular mimics, about which more in Ch 18. Carrera et al (2000) discuss an example of activation via an initiation factor. Harding et al (2001) implicate translational control in a disease. Ostareck et al (2001) and Khaleghpour et al (2001) are other examples of work on regulation of eukaryotic initiation.

K. Further reading

I G Wool, Extraribosomal functions of ribosomal proteins. TIBS 21:164, 5/96. One view of the ribosome is that it was originally an RNA molecule, and it later acquired proteins. Where did those proteins come from? Possible clues come from findings that some ribosomal

proteins have other activities, and in some cases other essential functions. Wool lists several known examples; all involve some aspect of nucleic acid metabolism.

Y Liu et al, Thermally regulated translational control of FRQ mediates aspects of temperature responses in the Neurospora circadian clock. Cell 89:477, 5/2/97. FRQ is a protein involved in circadian rhythms in Neurospora. The gene contains two in-frame initiation codons (actually three, but one seems irrelevant). The relative usage of the initiation codons varies with temperature, leading to two forms of the FRQ protein -- with somewhat different properties. The basis of the choice of initiation codon is unknown.

S E Wells et al, Circularization of mRNA by eukaryotic translation initiation factors. Mol Cell 2:135, 7/98. They directly observe the circular initiation complex with a eukaryotic mRNA. Why circular? Because of the involvement of the 3′-poly(A) tail.

S Liu et al, Structure of human methionine aminopeptidase-2 complexed with fumagillin. Science 282:1324, 11/13/98. Methionine aminopeptidase is the enzyme that removes the N-terminal methionine from many new proteins. Fumagillin is a fungal metabolite that may have potential as an anticancer drug; it inhibits the process of angiogenesis (formation of new blood vessels, needed for significant tumor formation). Turns out… fumagillin inhibits MAP.

K Wakasugi & P Schimmel, Two distinct cytokines released from a human aminoacyl-tRNA synthetase. Science 284:147, 4/2/99. (+ News, Weiner & Maizels, p 63.) We have discussed the role of the aminoacyl-tRNA synthetases in protein synthesis. From time to time, one of these enzymes pops up in some other role. This paper reports that a particular activating enzyme can be cleaved into two fragments with cytokine activity. They present some evidence that this is physiologically relevant, during apoptosis.

J Marcotrigiano et al, Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF-4G. Mol Cell 3:707, 6/99. An example of the complexity of eukaryotic initiation. A protein that is similar enough to interact in one way but different enough to not interact in another way can be a regulatory protein, by interference. We will see other examples of molecular mimicry in Ch 18.

M Nomura, Regulation of ribosome biosynthesis in Escherichia coli and Saccharomyces cerevisiae: diversity and common principles. J Bact 181:6857, 11/99. Commentary.

J R Warner, The economics of ribosome biosynthesis in yeast. TIBS 24:437, 11/99.

C Lu et al, Straight and curved conformations of FtsZ are regulated by GTP hydrolysis. J Bact 182:164, 1/00. FtsZ is a protein involved in bacterial cell division; it forms the framework for the cell division machinery, and may be an evolutionary precursor of tubulin. Here they show that it undergoes an interesting conformational change in vitro, based on the GTP cycle. Relevance to in vivo function remains to be determined.

P Carrera et al, VASA mediates translation through interaction with a Drosophila yIF2 homolog. Mol Cell 5:181, 1/00. Example of translational activation via interaction with an initiation factor. The VASA protein is an RNA-binding protein, probably an RNA helicase, that is essential for proper differentiation. It acts through an initiation factor. Details are not

clear, but the idea is that this regulatory protein somehow aids in assembly of an initiation complex.

W Song et al, Role of Sec61α in the regulated transfer of the ribosome-nascent chain complex from the signal recognition particle to the translocation channel. Cell 100:333, 2/4/00. For secreted proteins in eukaryotes, translation is coupled directly to the secretion apparatus. That process involves the SRP (signal recognition particle) and G proteins.

A B Sachs, Cell cycle-dependent translation initiation: IRES elements prevail. Cell 101(3):243, 4/28/00. Minireview. Few mRNAs are translated during the M phase of the eukaryotic cell cycle, apparently due to loss of functional cap-binding protein. Here, Sachs reviews new work showing that some of the few messages that are translated at that time are initiated at IRES. These IRES function preferentially at the G2-M boundary. Whether this is due entirely to loss of cap-binding activity or due to presence of some other IRES-stimulator activity is not known. As part of the discussion, he discusses how "standard" and IRES-mediated initiations can be considered variations on a theme.

Y Li et al, Initiation of protein synthesis in Saccharomyces cerevisiae mitochondria without formylation of the initiator tRNA. J Bacteriol 182:2886-2892, 5/00. Mutant yeast unable to formylate the mitochondrial initiator tRNA grow almost completely normally. Similar work in bacteria showed major growth reductions, though not a complete loss of viability. Note that no example has been found in which formylation is "naturally" absent in a prokaryote or organelle system; the work merely shows that the formylation is "non-essential".

P Fortes et al, The yeast nuclear cap binding complex can interact with translation factor eIF4G and mediate translation initiation. Molecular Cell 6(1):191, 7/00. They explore the process of exchange between the nuclear protein that binds the mRNA cap and the cytoplasmic protein that binds the cap. The first step may be the binding of (one subunit of) the cytoplasmic protein to the nuclear protein that is on the cap. They suggest that the first round of translation may actually involve this exchange -- and therefore be somewhat distinct from "normal" translation. (This is part of a larger story of how mRNA -- and other things -- are transported between the nucleus and cytoplasm, through the nuclear pores. This may be briefly noted in the Ch 14 handout, but is beyond this course.)

G J Belsham & N Sonenberg, Picornavirus RNA translation: roles for cellular proteins. Trends in Microbiology 8(7):330 7/00. Review.

J E Wilson et al, Initiation of protein synthesis from the A site of the ribosome. Cell 102(4):511, 8/18/00. They study an IRES-mediated initiation event in CPV (cricket paralysis virus). The basic story is that they seem to have uncovered a new class of initiation events that not only bypasses the cap but also bypasses the normal initiator tRNA role. (Also see U L RajBhandary, More surprises in translation: Initiation without the initiator tRNA. PNAS 97(4):1325–1327, 2/15/00. This "Commentary" discusses some other examples.)

A Roll-Mecak et al, X-ray structures of the universal translation initiation factor IF2/eIF5B: Conformational changes on GDP and GTP binding. Cell 103(5):781, 11/22/00. Direct observation of the conformational changes during the G-cycle for an initiation factor. Because

the factor is lever-shaped, the conformational change is transmitted and amplified over 90Å away from the GTP binding site.

D H Ostareck et al, Lipoxygenase mRNA silencing in erythroid differentiation: The 3′ UTR regulatory complex controls 60S ribosomal subunit joining. Cell 104(2):281-290, 1/26/01. This paper explores how a downstream site on the mRNA affects assembly of the initiation complex. The presence of a translational silencing protein at the regulatory site prevents joining of the large ribosomal subunit.

J Marcotrigiano et al, A conserved HEAT domain within eIF4G directs assembly of the translation initiation machinery. Molecular Cell 7(1):193-203, 1/01. They identify specific structural features of eIF4G that interact with eIF4A or with an IRES.

K Khaleghpour et al, Translational repression by a novel partner of human poly(A) binding protein, Paip2. Molecular Cell 7(1):205-216, 1/01. The protein Paip2 interferes with binding of the Poly(A)-binding protein (PABP) to the poly(A) tail of the mRNA -- and thus inhibits initiation of translation.

C M T Spahn et al, Hepatitis C virus IRES RNA-induced changes in the conformation of the 40S ribosomal subunit. Science 291:1959, 3/9/01. They show that binding of the mRNA, via a viral IRES, changes the ribosomal conformation. Specifically, it closes the mRNA binding cleft.

L Guo et al, Base-pairing between untranslated regions facilitates translation of uncapped, nonpolyadenylated viral RNA. Molecular Cell 7(5):1103-1109, 5/01. The emerging standard model of translational initiation for eukaryotes involves a looping between the two ends of the mRNA, mediated by proteins that bind to sequences near each end. Here, they show that an RNA that lacks the ability to make this protein-mediated loop still has a loop -- by direct base pairing between sequences near the two ends.

B E Ellis & G P Miles, Plant biology: One for all? Science 292:2022, 6/15/01. News. Compares the use of G-proteins for regulation in plants and animals. Interestingly, plants seem to have very few of these, yet they use them extensively.

R K Kumar & M Yarus, RNA-catalyzed amino acid activation. Biochemistry 40(24):6998-7004, 6/19/01. From their abstract: “In conjunction with previous RNA-catalyzed aminoacyl-RNA synthesis, peptide bond formation, and RNA-based coding, these amino acid-activating RNAs complete an experimental demonstration that the four fundamental reactions of protein biosynthesis can be RNA-mediated. The appearance of translation in an RNA world is therefore supported.”

I Moll et al, Evidence against an interaction between the mRNA downstream box and 16S rRNA in translation initiation. J Bacteriol 183:3499-3505, 6/01. An example of a special case of bacterial mRNA: there is no Shine-Dalgarno sequence, and the start codon is the first three nucleotides of the mRNA. How such leaderless mRNAs are translated remains unclear. A common proposal is that an mRNA sequence downstream of the start codon interacts with the rRNA. Here they show that such interaction is unlikely to have a general role.

H P Harding et al, Diabetes mellitus and exocrine pancreatic dysfunction in Perk-/- mice reveals a role for translational control in secretory cell survival. Molecular Cell 7(6):1153-1163, 6/01. (+ related article, Scheuner et al, p 1165.) They are studying a protein kinase, which reduces translation by phosphorylating an initiation factor in response to ER stress. Mice lacking this enzyme develop diabetes.

D L Purich, Opinion: Enzyme catalysis: a new definition accounting for noncovalent substrate- and product-like states. TIBS 26(7):417, 7/01. Abstract: "Biological catalysis frequently causes changes in noncovalent bonding. By building on Pauling's assertion that any long-lived, chemically distinct interaction is a chemical bond, this article redefines enzyme catalysis as the facilitated making and/or breaking of chemical bonds, not just of covalent bonds. It is also argued that nearly every ATPase or GTPase is misnamed as a hydrolase and actually belongs to a distinct class of enzymes, termed here 'energases'. By transducing covalent bond energy into mechanical work, energases mediate such fundamental processes as protein folding, self-assembly, G-protein interactions, DNA replication, chromatin remodeling and even active transport."

I Paz & M Choder, Eukaryotic translation initiation factor 4E-dependent translation is not essential for survival of starved yeast cells. J Bacteriol 183:4477-4483, 8/01. eIF4E is essential for normal cap-dependent translation. Continuing protein synthesis, at a low level, is essential for survival in stationary phase. But here they show that eIF4E is not essential in stationary phase. The logical conclusion is that the key protein synthesis required to survive in stationary phase is cap-independent.

L. Computer resources

Some of the sites listed in the Ch 3 handout are appropriate for the current chapters on protein synthesis. In particular, note the animations of protein synthesis at the Akron site. At the web site, you can link to these from Ch 3.

M. Homework

1. Two sites must be recognized in an mRNA in order to identify where to start making a protein. What are these two sites in bacteria? in eukaryotes?

2. Why are two sites used to define the beginning of protein synthesis (as discussed in the previous question)? Why not simply devote one codon to initiation, and have that codon be sufficient to define an initiation site.

3. Is there a correlation between the standard mechanism of translational initiation in prokaryotes and eukaryotes and the presence or absence of polycistronic messages? Explain. Is the correlation logical, or merely empirical?

4. The efficiency of translating a bacterial message is affected by the distance between the ribosome binding site (rbs) and the initiation codon. The optimum spacing is ~6 bases. The following sequence is from the beginning of a message. The rbs and initiation codon are underlined; assume that these are correct.

…UAAGGAGAGGGGCUUCGGCCCCUCUAAAUAUG…

This message is translated quite efficiently, despite the clearly excessive distance between the two critical sites. Why?

5. Secondary structure of the mRNA can prevent ribosome attachment. Consider two mRNAs with inhibitory secondary structures that differ in stability by 1.4 kcal/mol.

a. Given this energy difference, how much higher is the equilibrium constant for the more stable secondary structure?

> (The equilibrium constant is directly related to the free energy difference: $\Delta G_o = -RT \ln K_{eq}$. At room temperature, RT ~ 0.6 kcal/mol.)

b. How much more active will the one with a less stable structure be?

> Assume that both secondary structures are fairly stable, so that the fraction of the mRNA which is unfolded is low in both cases. In this case, the fraction of the molecules in the unfolded state is proportional to the K_{eq}. (If most molecules are unfolded, then the fraction of unfolded molecules is nearly constant -- at ~100%. In that case, the question here would be of little interest.)

6. It's common now to express genes from thermophiles in E. coli. It works, but sometimes not well; sometimes, genes from thermophiles give low expression levels in E. coli. Ishida & Oshima (J Bact 176:2767, 5/94) found that they could get high expression levels by removing a palindromic sequence very near the ribosome binding site. Why is this sequence a problem in Escherichia but not in Thermus? (Hint… the difference is not due to the ribosomes. And it is logically related to current X107 material.)

7. GTP binds to IF2; lactose binds to its repressor. In each case, how do you get the ligand (the small molecule) off of the protein? Why the difference, in terms of the physiological role?

8. A company looking for effective new antibiotics (anti-bacterials) has decided to screen for inhibitors of the deformylase (enzyme that removes formyl group from new proteins). Why is this a good idea? What assumptions are being made?

9. Some viruses use RNA as the genetic material. In some of these, the viral strand is the mRNA, and codes for several proteins. In bacterial viruses of this type there is a ribosome binding site near the beginning of each gene on the RNA. But for mammalian viruses of this type (such as poliovirus), there is a problem. What is the problem? Can you propose a solution?

10. Gene fusions, of various kinds, are popular tools in molecular biology. Consider the following situation… A "reporter" sequence is cloned randomly into eukaryotic transcriptional units. The "reporter" sequence in this case is -- precisely -- a sequence coding for a sequence of amino acids that will give a measurable enzyme activity. (Recall Ch 6 handout Sect P for an introduction to reporters.) For simplicity, let's assume that we know that the cloning location is within a sequence that is transcribed. The purpose of the fusion is to measure the level of transcription (perhaps under various conditions). (Another good purpose is simply to find transcriptional units -- genes.)

a. What is the probability that the reporter enzyme will be made? (Generally, high or low?) Why? Give specific reasons that tend to reduce the likelihood that the enzyme will be made.

Think about… Will the initiation codon of the reporter gene initiate?

b. What happens to that probability if your reporter construct also contains an "IRES" (internal ribosome entry site; see Sect D)? Explain.

++++ SPECIAL

Extra credit. Propose a question to be added to a homework set for any X107A chapter. Submit the question and the answer. If you intend that your question replace a current question, briefly explain why yours is an improvement. Questions will be accepted any time, not later than final exam night. I will give one point extra credit (added onto test total) for each question worthy of serious consideration; limit 2 pts.

N. Partial answers

1. Start codon (all organisms), plus rbs (Shine-Dalgarno) in bacteria or cap in eukaryotes.

2. As you try to develop an idea, ask yourself how that idea would apply to termination codons (though we can leave that part for Ch 18). For class discussion.

3. The mechanism of initiation in eukaryotes works from an end, and thus logically is limited to one initiation site per message. In contrast, bacterial initiation involves direct recognition of a rbs, and thus can occur anywhere. Thus, bacterial initiation allows (but does not require) polycistronic messages.

4. Hint… look at the runs of G and of C.

The sequence between the rbs and the initiation codon can fold into a hairpin, bringing the two sites only 6 bases apart. (This problem is based on a real case.)

5. a. The given ΔG difference of 1.4 kcal/mole corresponds to a 10-fold difference in K_{eq}.

b. Thus the mRNA with the less stable structure will have a 10-fold higher fraction of its molecules in the unfolded state -- and therefore will be 10-fold more active in translation.

The second half of the previous sentence makes an assumption -- that activity is proportional to the equilibrium fraction of unfolded molecules. What if ribosomes have some ability to unfold secondary structure during the binding and initiation steps? Then activity would be greater than "expected". de Smit & van Duin (Control of prokaryotic translational initiation by mRNA secondary structure. Prog Nucl Acid Res 38:1, 4/90) have analyzed a large collection of mutations in one mRNA secondary structure, and find that activity very closely follows the expected fraction of unfolded molecules. They conclude that ribosomes cannot unfold mRNA during binding. (This conclusion only applies during binding. Bound ribosomes are able to break secondary structure as they move along the message; helicases may assist. See Fig 17.12, which is for the mRNA discussed here; more on pp 556-7.)

> [Questions 4-5 provide an example of mRNA 2° structure stimulating translation and an example of it inhibiting translation.]

6. A palindromic DNA sequence may result in a hairpin structure in the mRNA transcript (Recall Ch 2 handout p 7). The hairpin could prevent a ribosome from finding the rbs. So why the different expression level in the two hosts? One reasonable possibility is that the hairpin is much less stable at the growth temperature of the thermophile, hence is less inhibitory.

7. GTP is removed from its protein by hydrolyzing it to GDP, which binds more weakly. Lactose is removed from its protein when the concentration of lactose becomes too low for effective binding.

8. The first key point is that the deformylation reaction is specific to bacteria.

Note that Liu et al (1998) deals with a quite different step.

9. Poliovirus is a single message for multiple proteins, in a (eukaryotic) world where only monocistronic messages are allowed.

The poliovirus solution is to translate the entire coding region in the RNA into a single "polyprotein", which is then cleaved to the individual viral proteins. Historically, this (then strange) finding was one of the first clues that eukaryotic cells did not deal with polycistronic messages.

> [Polyprotein processing is due to a viral protease. How can that be? Poliovirus does not carry protease with it. However, the uncleaved polyprotein has protease activity.]

Although poliovirus provided early evidence on the nature of eukaryotic mRNA, it turns out it is atypical (because it has an internal initiation site; see next question). Nevertheless, the story of polyproteins from monocistronic mRNA is true, both for poliovirus and eukaryotic cells.

Belsham and Sonenberg (2000) review poliovirus translation.

10. a. There are several factors that conspire to lower the probability.

1. The reporter code will be translated only if it is within the translated region of the transcript. [Why? Because the initiation codon will not be recognized as such. Eukaryotic mRNAs only (or usually) initiate at one codon, the AUG nearest the cap.] The magnitude of this effect can be estimated if the gene structure is known. (The reporter would also be translated if it were upstream of the normal initiation codon; this probably occurs at a low frequency.)

2. The reporter code will be translated properly only if the fusion is "in frame" with the native gene. Clearly, the odds of this are 1 in 3.

3. The reporter protein sequence must function properly, even though it is now contained within a larger protein. This is hard to predict in general. However, commonly used reporters are those that experience shows to be fairly robust.

Note that only the second point can be quantitatively specified in general. #1 can be in specific cases.

b. What the IRES does is to make the reporter an independent translation unit. This completely eliminates factor #2, and presumably also reduces #3. And it should help some with #1, although there is still a risk that the reporter insert might be spliced out, and therefore not have a chance to be translated.

> In one direct comparison reported some years ago, 10-fold more expressing clones were obtained by including an IRES in the reporter construct.