Chapter 19. Weaver, 2/e. Mol Biol X107A.

Ch 19. Ribosomes and transfer RNA.

Reading note. Skip Sect 1 (Ribosomes), as explained below.

Clark & Russell. See Ch 17 handout.

A. Introduction

There are two major sections in Ch 19, one each on ribosomes and tRNA. As discussed below, we will spend little time on Sect 1 (Ribosomes), but will emphasize the tRNA. The discussion of tRNA will include more about the activating enzymes. We will also discuss some issues of accuracy in protein synthesis.

Highlights

- The shape of tRNA, secondary and tertiary.
- tRNA base modifications.
- Recognizing and attaching the amino acid; activating enzymes.
- Accuracy of translation
  - Proofreading of amino acid-tRNA interaction.
  - Proofreading of tRNA-codon interaction; role of GTP.

B. Ribosomes: review

It is sufficient that you understand ribosomes at the level discussed in previous chapters.

We have looked generally at the functional features of the ribosome in previous chapters. These include the two major tRNA binding sites (A and P), and the peptidyl transferase center, in the large rRNA.

C. Ribosomes: new

In Ch 19 Weaver discusses structure and function issues for the ribosome in some detail. I choose to skip this, partly for reasons of time.

The ribosome is extremely complex, and determination of structure has been a major experimental challenge. Much of the methodology is interesting. Nevertheless, there is little in the details presented here that we need for our basic story.

Some work on ribosomal structure was presented earlier, mainly in the context of identifying and characterizing the peptidyl transferase center in the 50S subunit (Ch 18 handout Sect H, and FR therein). Weaver presents much of the latest information in Figs 19.6-8, summarizes it in Fig 19.9, and discusses the implications for aspects of ribosome function throughout the section. For example, on pp 622-3, he discusses the possible role of a particular rRNA base in the catalysis of peptide bond formation; this particular point is in dispute, I think.

Dahlberg (2001) updates the ribosomal structure story.

Interestingly, the 23S rRNA is fragmented in many bacteria (Evguenieva-Hackenberg & Klug, 2000). Izutsu et al (2001) discuss an ambiguity of ribosomal composition.

The ability of ribosomes to self-assemble is interesting (p 613).

You should be aware of the term polysome (short for polyribosome), meaning a group of ribosomes on the same mRNA (p 625).

rRNA is extremely useful for taxonomic purposes. López-García et al (2001) show how it is useful in analyzing natural communities.

D. Transfer RNA: the adaptor; general structure

The problem is how to translate from one language to another.

tRNA mediates the translation; tRNA is the adaptor. tRNA is indeed a nucleic acid, and it does recognize codons in the message by base pairing. However, tRNA is an unusual RNA molecule: it is very small, and has a very specific 3-D shape. Usually we think that the key property of nucleic acids is base sequence, with shape of lesser importance. “Shape” is

generally a property of proteins. But tRNA is part "nucleic acid-like" and part "protein-like"; it does part of its job by specific base pairing and part by specific shape interactions.

There is one or more specific tRNA species for each amino acid. There is a specific "activating enzyme" for each amino acid, which recognizes both the amino acid and the aa-specific tRNA(s). This enzyme activates the aa, and then attaches the aa to the tRNA. The tRNAs for all 20 amino acids are available. The correct charged tRNA binds to the ribosome -- non-specifically -- and to the codon on the mRNA -- specifically, thus bringing the amino acid it carries into position to be attached to the growing peptide chain.

tRNA is often drawn in the "cloverleaf" form, Fig 19.31. This shows the secondary structure: the local base pairing. But the real 3-D structure is much more folded up than this, e.g., Figs 19.33-34. The additional, tertiary, structure is also due to weak bonds, such as hydrogen bonds and stacking forces; many of these are shown in Fig 19.35. However, these are not limited to the classical Watson-Crick base pairs, because the overall structure is not constrained to be a double helix.

Interestingly, the anticodon of the tRNA is constrained in a conformation that is very suitable for pairing. Fig 19.36 shows part of the evidence, but needs the discussion on p 630 to make the point that such pairing does not occur with free trinucleotides.

E. tRNA (and rRNA) processing; base modifications

Both tRNA and rRNA are processed extensively from the original transcripts. This processing is introduced in Ch 16, Sect 1-2 -- which we do not cover in this course. Ciammaruconi & Londei (2001) explore rRNA processing.

A dramatic aspect of tRNA processing is an extensive set of base modifications.

tRNA contains unusual bases not normally found in nucleic acids (e.g., Fig 19.32). Many of the modifications are characteristic of tRNA. Pre-tRNA (the primary transcript and its cleavage products) contains the normal bases. It is then modified by enzymes -- specific enzymes that modify selected bases in tRNA. As Weaver notes, the effects of individual modifications are typically subtle, though there is no question that the modifications taken together are essential. Durand et al (1997) and Persson et al (1998) are examples of work on specific modifications. Also recall the key role of the modified anticodon base inosine in the wobble phenomenon; Ch 18 handout Sect E; Gerber & Keller (1999) discuss how this base is made.

> rRNA also carries a substantial number of modified bases. Recent work has uncovered an interesting story about how eukaryotic rRNA base modifications are determined; Smith & Steitz (1997). Noon et al (1998) discuss the situation in prokaryotes.

The CCA tail. All tRNAs have CCA at the 3′ end. This sequence is sometimes encoded in the tRNA gene, sometimes not. In any event, this tail is maintained by addition of CCA to tRNAs that lack it.

F. Activating enzymes; tRNA recognition

The particular structural features of some tRNAs that the activating enzymes recognize have been uncovered. For $tRNA^{Ala}$, the key recognition feature is a specific base pair in the acceptor stem; for $tRNA^{Met}$, the key recognition feature is the anticodon itself. The general view is that activating enzymes bind in the bend of the L; Figs 19.39 & 40. The enzymes make specific recognition of various features along the tRNA, with different enzymes emphasizing different features.

Simos et al (1998) develop the idea that activating enzymes are organized into complexes. Fàbrega et al (2001) show that the story of two classes of activating enzymes is an oversimplification.

G. Accuracy of protein synthesis

We have noted that tRNA is involved in two recognition steps. The accuracy of these two recognition steps determines the overall accuracy of protein synthesis.

The accuracy of amino acid activation actually involves two parts, the tRNA-enzyme interaction and the amino acid-enzyme interaction.

Since both the tRNA and the enzyme are fairly large, this first recognition is not a big problem: the correct tRNA binds effectively whereas incorrect ones do not. We briefly noted the basis for this in Sect F. (This discrimination is not complete, but is good enough for us now.)

However, some of the amino acids are very similar, and more complex proofreading is required to ensure the correct amino acid. A general feature of proofreading reactions is competition. The correct interaction favors one pathway; the incorrect interaction favors another pathway. In this case, a process known as double sieve editing is responsible for ensuring accuracy of amino acid activation. Weaver discusses the example of isoleucine tRNA, Fig 19.41. Fukai et al (2000) and Dock-Bregeon et al (2000) discuss other examples; these show the generality of double sieve editing, but also that the discrimination is not entirely based on size.

The second accuracy step is codon-anticodon recognition. This recognition occurs by base pairing. However, it is clear that there is not enough energy difference between right and wrong pairings to reach the desired accuracy. The problem is worse when you realize that the concentration of incorrect tRNA is always much higher than that of correct tRNA. Somehow, this recognition process, too, must be proofread.

We do not clearly understand how the proofreading occurs. However, we do recognize that the true biological process of codon-anticodon recognition must be more complex than simple

codon-anticodon base pairing in isolation. There must be events that magnify the difference between right and wrong pairings. There may be conformational changes or competing reactions. And there may well be energy consumption.

One good model for codon-anticodon proofreading is the EF-Tu clock. In the simple version of the elongation story, EF-Tu "delivers" the tRNA to the ribosome. Why do we need a delivery system? [Recall that EF-Tu is one of the most abundant proteins in the cell, and that GTP hydrolysis is required; this is an expensive system.] The EF-Tu clock model proposes that EF-Tu departure determines the timing of peptide bond formation. If the tRNA is still bound to the message, then a peptide bond is made. However, poorly bound tRNAs are likely to have diffused away before EF-Tu departure. In this case, "nothing happens", except for the EF-Tu cycle.

Thus, the series of steps (EF-Tu delivery - GTP hydrolysis - EF-Tu departure - peptide bond formation) becomes a functionally interesting series, with the final three steps all coupled. By this model, the key role of EF-Tu is not delivery per se, but timing.

As Weaver noted back in Ch 18 (pp 585-6), there is evidence that accuracy is inversely proportional to the rate of peptide bond formation. That is, if EF-Tu hydrolyzes its GTP faster and therefore leaves faster, peptide bond formation will be faster. However, there is less time for incorrect tRNAs to leave, so the accuracy will be lower.

Of course, the general concept of a slow clock allowing incorrect tRNAs to diffuse away does not mean that this is the complete story of the clock. For example it is possible that proper codon-anticodon pairing triggers GTP hydrolysis. This would enhance the result already discussed. On p 619 Weaver discusses the action of the antibiotic paromomycin, in the context of ribosome structure. He discusses a conformational change of two rRNA bases near the decoding site. This may be part of the accuracy mechanism. Yoshizawa et al (1999) explore how this may happen -- how the ribosome detects proper codon-anticodon pairing. Their Fig 3 is attached, p 9.

Similar roles for GTPases have been proposed in other processes, such as protein targeting and splicing events.

Ibba & Söll (1999) broadly review translational accuracy.

The discovery that limited protein synthesis may actually occur in the nucleus may help to explain why it seems that both mRNA and tRNA are checked to see that they are ok before being transported to the cytoplasm. Recall Iborra et al (2001; Ch 18 FR).

H. Further reading

C M Smith & J A Steitz, Sno storm in the nucleolus: New roles for myriad small RNPs. Cell 89:669, 5/30/97. Minireview. Eukaryotic rRNAs carry a low level of well-defined modifications, particularly ribose methylation and pseudouridines. We now know that the position of these modifications is guided by small RNA molecules that are complementary to specific parts of the rRNA. These antisense "guide" RNAs, which have been known generally

as “small nucleolar RNAs” (snoRNAs), target the modifying enzymes to the rRNA. (This strikes me as somewhat reminiscent of guide RNAs for RNA “editing”, Fig 16.22. In both cases, an “antisense” RNA directs an RNA modification. The editing event is much more complex.) Other snoRNAs target cleavage enzymes involved in processing. Intriguingly, some of the snoRNAs are encoded in introns of other genes. (And in one case, 8 introns of one message become snoRNAs, while the exons are apparently destroyed without doing anything.)

J M B Durand et al, The modified nucleoside 2-methylthio-N^6-isopentenyladenosine in tRNA of Shigella flexneri is required for expression of virulence genes. J Bact 179:5777, 9/97. This work shows a clear phenotype associated with lack of a particular tRNA modification. They show that the level of a particular virulence protein is drastically reduced. There is also some effect on growth rate. This particular base modification is known to have substantial (though not particularly well defined) effects on translation in in vitro systems. Also see Persson et al (1998).

G Simos et al, A conserved domain within Arc1p delivers tRNA to aminoacyl-tRNA synthetases. Mol Cell 1:235, 1/98. In eukaryotes, some of the activating enzymes are found in multi-enzyme complexes. The Arc1p protein of yeast is part of a complex of two activating enzymes, and it stimulates their activity.

K R Noon et al, Posttranscriptional modifications in 16S and 23S rRNAs of the archaeal hyperthermophile Sulfolobus solfataricus. J Bact 180:2883, 6/98. Discussion of rRNA base modifications in eubacteria and archaea. In particular, they discuss the possibility that higher levels of RNA methylation are related to thermotolerance.

B C Persson et al, The ms^2io^6A37 modification of tRNA in Salmonella typhimurium regulates growth on citric acid cycle intermediates. J Bact 180:3144, 6/98. In general, the effects of tRNA modifications are subtle, even elusive. Here they show that that a particular modification is necessary for the function of certain genes. Intriguingly, they are not at all sure that the effect is mediated through the translational role of the tRNA -- suggesting that this tRNA may play some other role.

B T Porse & R A Garrett, Ribosomal mechanics, antibiotics, and GTP hydrolysis. Cell 97:423, 5/14/99. Minireview. Discusses recent work that seems to reveal details of a conformational change that occurs during the competition between EF-Tu and EF-G. As they note, the story is not consistent with all available data.

S Yoshizawa et al, Recognition of the codon-anticodon helix by ribosomal RNA. Science 285:1722, 9/10/99. They show that the rRNA can sense proper codon-anticodon pairing of the mRNA and tRNA. Proper pairing positions the mRNA to make specific H-bonds with the 16S rRNA. Work with mutant rRNAs suggests that this sensing is functionally important. Their Fig 3 is attached, p 9.

A P Gerber & W Keller, An adenosine deaminase that generates inosine at the wobble position of tRNAs. Science 286:1146, 11/5/99. They relate the tRNA modifying enzyme to enzymes involved in the rather exotic process of RNA editing (Ch 16 Sect 4, but Weaver does not discuss adenosine deaminase editing).

M Ibba & D Söll, Quality control mechanisms during translation. Science 286:1893, 12/3/99. Review. Discusses production of proper tRNA and mRNA, tRNA charging, and accuracy interactions on the ribosome. An article from the feature section “Frontiers in Cell Biology: Quality Control”.

E Evguenieva-Hackenberg & G Klug, RNase III processing of intervening sequences found in helix 9 of 23S rRNA in the alpha subclass of Proteobacteria. J Bacteriol 182:4719-4729, 9/00. In many bacteria, the RNA of the large ribosomal subunit is fragmented, in specific ways. Little is known about the significance of this. (Weaver introduces RNase III in its more common role in rRNA processing in Ch 16 Sect 1. Both this enzyme and RNase E, discussed there, are also involved in mRNA decay. Weaver discusses mRNA decay in Ch 16 Sect 5, but does not include examples from bacteria.)

S Fukai et al, Structural basis for double-sieve discrimination of L-valine from L-isoleucine and L-threonine by the complex of tRNAVal and Valyl-tRNA synthetase. Cell 103(5):793, 11/22/00. In this case, the aminoacylation step precludes Ile, but includes Thr. Thr is removed at another active site, which discriminates against hydrophobic amino acids.

A-C Dock-Bregeon et al, Transfer RNA-mediated editing in threonyl tRNA synthetase: The class II solution to the double discrimination problem. Cell 103:877, 12/8/00. In this case, valine is excluded at activation. However, serine is included, and later hydrolyzed. They show that the floppiness of the CCA end of the tRNA, to which the amino acid is attached, is important; this lets the amino acid move between sites on the activating enzyme.

1) P López-García et al, Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. Nature 409:603, 2/1/01. 2) S Y Moon-Van Der Staay et al, Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. Nature 409:607, 2/1/01. Two papers using rRNA analysis from natural communities to explore organism diversity. Traditionally, microbial diversity was evaluated by what could be grown in the lab. Now, the emphasis is on analyzing the molecules that are found in the natural population. rRNA is one of the most popular; it is amplified by PCR. Turns out that most organisms in natural populations cannot be cultivated in the lab.

C Fàbrega et al, An aminoacyl tRNA synthetase whose sequence fits into neither of the two known classes. Nature 411:110, 5/3/01. Genome analysis seems to show no activating enzyme for Cys in some Archaea. One proposal was that an enzyme identified as a Pro activating enzyme would fill in and also activate Cys. But here they show there really is a dedicated Cys activating enzyme, but a novel type not recognized by pattern analysis.

A E Dahlberg, Ribosome structure: The ribosome in action. Science 292:868, 5/4/01. News, to accompany two articles in this issue, with the latest on ribosome structure. Probably a good place to start.

K Izutsu et al, Escherichia coli ribosome-associated protein SRA, whose copy number increases during stationary phase. J Bacteriol 183:2765-2773, 5/01. In Ch 6 we noted that the composition of “RNA polymerase” varies; the σ cycle is an aspect of this. Also true for ribosomes. Here they explore a ribosomal protein that is present at about 0.1 copy per ribosome during exponential growth but increases to 0.4 per ribosome during stationary phase.

The function is unknown, and deletion of the gene for this protein leads to no phenotype observed so far. So, this might all be an artifact, but it might also have some subtle function not yet determined.

A Ciammaruconi & P Londei, In vitro processing of the 16S rRNA of the thermophilic archaeon Sulfolobus solfataricus. J Bacteriol 183:3866-3874, 7/01. An exploration of some of the steps between making a primary transcript of the rRNA genes and getting an actual rRNA molecule.

I. Homework

This homework set contains some questions that might be considered general protein synthesis questions, covering multiple chapters.

1. What are the constant and variable features of tRNAs? Consider structure and function.

2. Would you expect that a bacterial tRNA would function properly in yeast? Explain.

3. The overall error rate in protein synthesis is about $5x10^{-4}$ per codon. If you make a protein chain of 1000 amino acids, what is the (approximate) probability of making the entire chain correctly?

4. Consider the following experimental situation. You have a yeast strain that lacks the met-tRNA that is normally used in elongation. Of course, that would be a lethal defect. So, to allow the strain to grow, you include a plasmid that contains the missing gene. You now treat the strain to remove the plasmid, and find a low frequency of survivors. What mutation could cause a survivor? (Assume that the plasmid is indeed entirely lost, as intended. The question is intended to deal with met-tRNA. There are many technical issues beyond that, but we don't need them for now.)

5. Consider the unusual amino acid norleucine. Its side chain is $CH_3(CH_2)_3$-. (The "nor" is the organic chemistry normal, meaning straight chain.) Unfortunately, E. coli can both make Nor and incorporate it into proteins. In both cases, it's because the unusual amino acid (or a biosynthetic precursor) is "accidentally" accepted by an enzyme intended for another amino acid. In fact, in this story Nor behaves as an analog of two, quite unrelated amino acids.

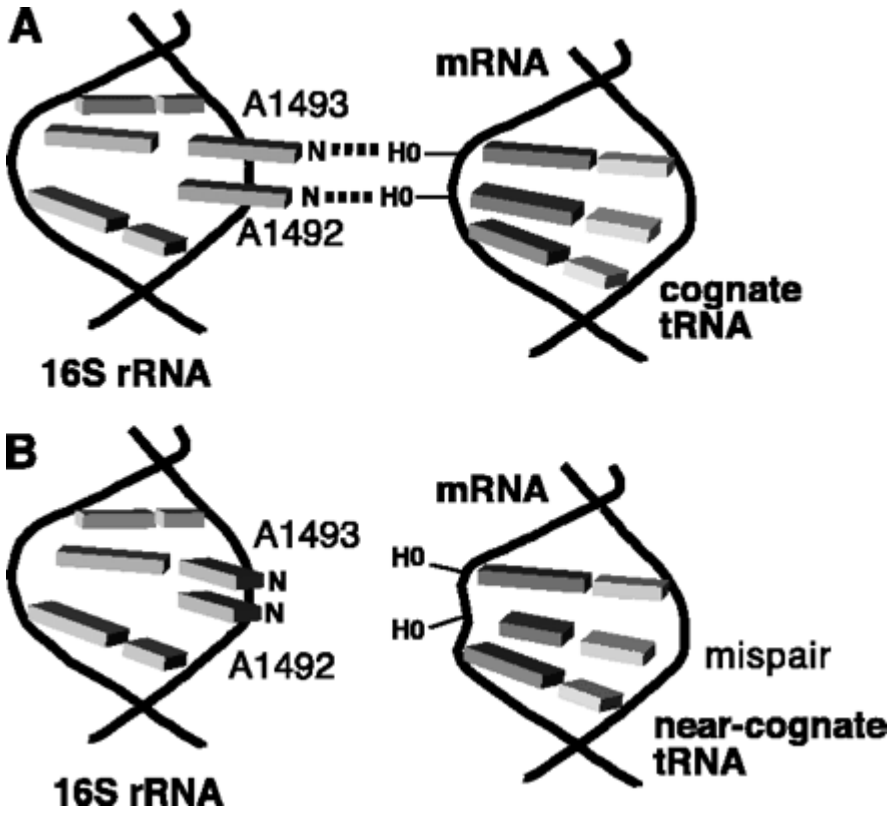
a. Which two? (Look at Fig 3.2 for some guidance. You might also want to look at the biosynthetic pathways, if you have a biochem book handy. There is no particular reason that you should figure this out completely. However, it's an interesting exercise to give it an honest try. Deal with part a, and its answer, before going on to part b.)

b. What would you do about it? (It? The misincorporation of Nor into your important protein product in place of the correct amino acid.) There are several possible solutions. A good goal for now is two simple solutions. (Changing the specificity of a protein -- e.g. of a protein doing the misrecognizing -- is not simple.) For class discussion.

6. Give an example of a type of RNA that is normally found free in the cell (i.e., that is normally not bound to protein).

7. Look over your notes for Ch 17-19 as well as the Chapter summaries. Comments? Suggestions? (Feel free to slip me notes about this, especially comments that I should consider in organizing this material for next year.)

"Fig 3" from Yoshizawa et al (1999), re Sect G.



[The next section is on p 11.]

J. Partial Answers

1. At a simple functional level, all tRNAs interact with the same ribosomes and elongation factors. However, they interact with different codons (in a similar context), and with different amino acid activating enzymes. [Of course, there are special cases, such as the initiator tRNA and the tRNA for selenocysteine.]

One can then look for structural correlates of these functional aspects.

2. As a practical matter, this question should lead to a listing of the steps involved in tRNA function. Predictions about what you would expect certainly are welcomed, but emphasize the steps in the process. For class discussion. (Weaver has some information on p 632.)

3. Simple calculation: 1000 codons x $5x10^{-4}$ errors per codon = 0.5 errors per chain. That is, there is about a 50% chance of getting the entire chain right.

In fact, very few peptide chains are that long. The problem of accurately making a long peptide chain is presumably one reason.

> [If you have had some statistics, you might do a "more rigorous" calculation: the probability of an error-free chain is $(1 - 5x10^{-4})^{1000} = 60\%$. However, the difference is not of consequence here. Further, the error rate given is approximate, and undoubtedly varies for different codons.]

4. The original strain lacks elongator met-tRNA. A survivor must have somehow acquired this activity. How could that happen? Let's discuss your list of possibilities. (The question is based on Åström & Byström, Rit1, a tRNA backbone-modifying enzyme that mediates initiator and elongator tRNA discrimination. Cell 79:535, 11/4/94.)

5. a. Leu and Met. Nor is the straight chain analog of Leu. And it looks very much like Met, with a $-CH_2-$ group instead of an -S-. Turns out that Nor is made by the biosynthetic pathway for Leu, simply by starting with the wrong precursor. Then, Nor is recognized by the activating enzyme for Met, thus gets incorporated into proteins in place of Met.

b (or general). Why are we discussing this? It has turned out to be a significant practical problem for biotech companies making high levels of pharmaceutical grade proteins in E. coli. G Bogosian et al (Biosynthesis and incorporation into protein of norleucine by Escherichia coli. J Biol Chem 264:531, 1/5/89; from Monsanto) discuss an example, and their solutions.

6. Probably none. And it is likely that naked RNA is rapidly degraded.