Biol 213 Genetics (Fall 2000)
RNA and Translation

It is one week before the first exam. Things may have been OK up to now, but you begin to feel the walls closing in. The first lab report is due this week. There's yet another problem set, worse than the last if that's possible. A strange sequence to worry about, not to mention strange software. And the eyes! The fly eyes all around!... I can't take it... I just can't take it anymore...

All right. Just sit down and relax. And I don't think you'll be needing that knife right now. Take it one thing at a time. First of all, you shouldn't feel that you're in this alone. While the ultimate goal of a liberal arts education is to make you an expert independent learner, it doesn't have to happen all at once. There's lots of ways to get help, and not just at problem sessions. TA's are on hand during their office hours to serve you. So are we. Take a look at the hours posted on the web. Then there's your groups. If you find problem sessions do you some good, try them outside of class. If you find they don't, try putting together another group.

Second, start the last problem set now. If you're going to need some help, let it be when you have time to get some help. There will be extra help sessions, including a major review session over the weekend (stay tuned).

Finally, keep in mind that you're here for your education. Exams come and go. If you focus on improving your mind, no one can take that away from you. Focus on the task at hand, never mind who's watching. Forget about those eyes, in their tiny heads, in their stomachs, red and hideous, vermilion, cinnabar, even chartreuse or green all trained on your every step, that

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I. RIBOSOMAL RNA AND MESSENGER RNA

I.A. Life history of RNA: pulse-chase experiment

You should now be able to take DNA and predict with some confidence what protein it may encode. But you have a brain. Hidden in our discussion thus far is the cellular intelligence that connects the passive genetic information of DNA with the active information within protein. Part of today's goal is to identify this intelligence.

Obviously, the missing link in our discussion is RNA. A central role for RNA was suspected early on. In eukaryotes, DNA is made in the nucleus and resides there, while protein is made and generally acts in the cytoplasm. RNA can be found in both compartments.

The geography may be right, but RNA can serve as an intermediary only if it moves from the nucleus to the cytoplasm. The experiment diagrammed in Figure 1 showed that this is the case. The type of experiment, pulse-chase, has proven its utility time and again in the elucidation of functional biological relationships. It bears a family resemblance to an experiment we've already examined, the experiment by Hershey-Chase (no relation).

In a pulse-chase experiment, a radioactive precursor is added to the cell for a period of time in order to label specifically the molecule of interest. This is called the pulse. Only RNA made after the addition of the precursor is labeled.

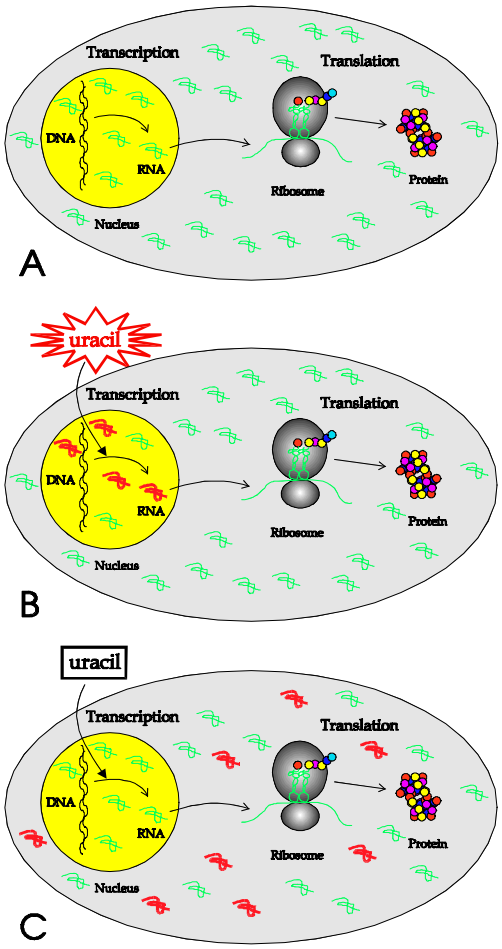

Figure 1. Pulse-chase experiment. (A) State of cell prior to beginning of experiment. RNA (green squiggles) is made in the nucleus and transported into the cytoplasm. **(B)** Radioactive uracil is taken up by the cell, thereby labeling newly synthesized RNA (the pulse). **(C)** Excess nonradioactive RNA is added to prevent further labeling of RNA, while previously labeled RNA is transported to the cytoplasm (the chase).

SQ1. What radioactively-labeled molecule could be added to cells to label RNA but not protein? Why?

SQ2. What radioactively-labeled molecule could be added to cells to label RNA but not DNA or protein? Why?

A huge excess of nonradioactive precursor is then added in order to dilute the radioactive precursor to insignificance. This is called the chase. RNA that is labeled is thus known to have been synthesized during the brief pulse of radioactivity. In this way, one can follow a population of RNA through its life history.

At different times in the experiment shown in Figure 1, uracil-labeled cells were cracked open and fractionated to separate the nuclei from the cytoplasm. From each fraction, large molecules were selectively precipitated to separate them from the radioactive precursor (recall that you've done something similar in Genetics lab, separating nucleic acid from small molecules by alcohol precipitation). When cells were

broken shortly after the pulse, the label was found mostly in the nuclear fraction. When cells were broken at a later period, label was found mostly in the cytoplasmic fraction.

SQ3. How can you reconstruct the life history of RNA from this pulse-chase experiment?

SQ4. Suppose that radioactive precursor were present continuously. What would be the distribution of radioactive label? How could you interpret this result?

SQ5. Suppose that RNA were synthesized on ribosomes and transferred into the nucleus. How would that have changed the results?

II.B. Ribosomes and ribosomal RNA *(pp.375-377)*

In the 1950's when such experiments were done, the RNA that moved from the nucleus to the cytoplasm carrying genetic information was presumed to be what we now call ribosomal RNA (rRNA) and what was then called just "RNA" (because no other class of RNA was known). The true role of rRNA was elucidated in the 1960's and is illustrated in Figure 14-6. Ribosomal RNA serves as a scaffold on which the many proteins comprising a ribosome are assembled into the mature structure. If you feel overwhelmed by the structural and functional possibilities of a single polypeptide chain, consider how complex must be ribosomes, composed of over 50 polypeptides!

SQ6. What are the squiggles and circles shown in Fig. 14-6? How do they relate to the large pieces of modern art shown below them?

All prokaryotes have ribosomes of the same type, while all eukaryotes have similar but larger ribosomes. We exploit the kinship amongst prokaryotic ribosomes when we use certain broad-spectrum antibiotics, e.g. tetracycline. They work because they bind specifically to prokaryotic ribosomes and not to eukaryotic ribosomes, killing bacteria or inhibiting their growth, without affecting us.

III.C. Messenger RNA

Because only rRNA was initially known, it was presumed that one gene made one ribosome that made one protein, i.e., each ribosome contained within it RNA that was an image a particular gene. Each ribosome, therefore, was specialized to produce a single protein (Figure 2). It was expected, then the infection of a bacterium by a phage should produce a wholesale turnover of ribosomes, since the phage directs the synthesis of a different complement of protein than the bacterium. The problem with this idea was that ribosomes, and presumably rRNA, were known to be stable, not given to transient rises and falls.

In 1961, Sydney Brenner, François Jacob, and Matthew Meselson demonstrated conclusively through an experiment combining a pulse-chase with the density labeling previously used in the Meselson-Stahl experiment that indeed rRNA remained stable during phage infection, but that a transiently labeled RNA nonetheless was associated with ribosomes. The RNA that carries genetic information must be a minor component, not rRNA. That minor component is now called messenger RNA (mRNA). Ribosomes

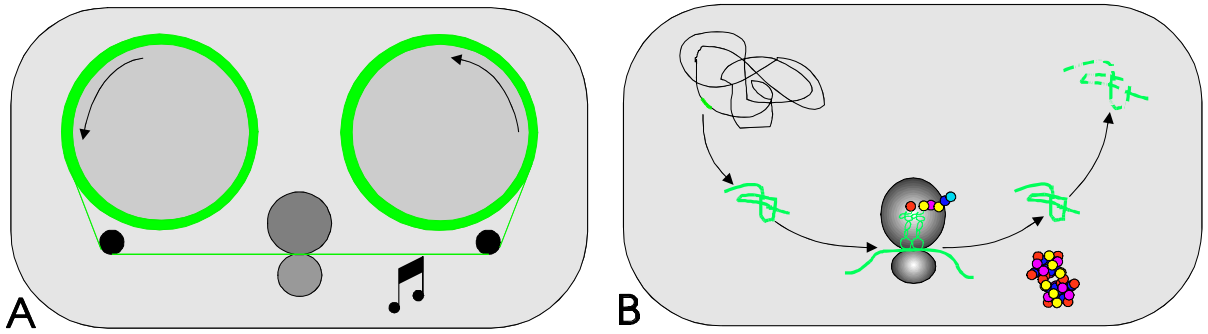

Figure 2. Ribosomes compared and contrasted with tape recorder. (A) A tape recorder reads a long message, the tape, through the play heads to make music. The tape must be huge to contain the entire message. **(B)** A ribosome reads a short message, mRNA, to make protein. The mRNA is transcribed only when its particular message is needed, and it is soon destroyed.

are therefore like tape recorders (Figure 2): they are the machine, not the message. Just as a single tape recorder can read the message of any number of tapes, so can a ribosome read many mRNAs, which determine the music. Cells are stuffed with ribosomes, each playing tiny messages that comprise only a small fraction of the total piece of music. In this way, most of the RNA in the cell is machinery; the mRNA is a minor component, but the one that carries the important message. Figure 14-16 shows a picture of many ribosomes translating the same mRNA even as it is being transcribed.

RNA is now known to fall into three major types (there are others) shown in Table 2: rRNA, mRNA, and transfer RNA (tRNA). rRNA and tRNA isolated from an organism have precise sizes, while mRNA comes in a large number of sizes. This is reasonable in the case of rRNA, since all ribosomes within a cell are identical and so are their RNA scaffolds. Likewise, tRNA's have the same function, as we'll see, and so have a limited range of sizes. There's far more rRNA and tRNA in a cell than mRNA.

The functions of the three RNA's can be seen in Fig. 14-9 (although you have to use your imagination to see the rRNA). The blue ribosome is built around (invisible) rRNA. It and the purple tRNA read the information within read mRNA.

SQ7. In retrospect, considering the stability, size, and heterogeneity of the three major classes of RNA, why does mRNA stand out as only good choice to mediate the transfer of genetic information from gene to protein?

Table 1. Comparison of classes of RNA in a typical bacterium (*E. coli*)

Class	Types	Fraction	Sizes	Stability	Function
Ribosomal RNA (rRNA)	3	80%	2904bp, 1542bp, 120bp	stable	Scaffold for ribosomal proteins, participates in translation
Transfer RNA (tRNA)	37	12%	76bp to 91 bp	stable	Connects codon to amino acid
Messenger RNA (mRNA)	1000's	5%	~300 bp to ~10,000 bp	unstable	Carries information on amino acid sequence of protein

III. TRANSFER RNA *(pp.367-373)*

III.A. Anatomy of tRNA

Figure 14-1 shows the essential role of tRNA in the process of translation. The three-base anticodon at one end of tRNA binds to the triplet codon on the mRNA, while the amino acid at the other end of tRNA is connected to the growing chain of amino acids.

SQ8. Notice the AAG anticodon of $tRNA^{phe}$ shown Fig. 14-1. Like any nucleic acid, it has direction. Considering the structure of double stranded nucleic acids, is the implied direction of the anticodon shown in the figure 5'-AAG-3' or 3'-AAG-5'?

Francis Crick predicted the existence of an adapter RNA molecule like tRNA well before it was discovered. He figured from what was already known about double-stranded DNA that a codon could most reasonably interpreted by an RNA able to basepair with mRNA. He postulated the existence of an adapter that had a triplet anticodon and a few more nucleotides to which the amino acid is attached, perhaps 10 nucleotides in total.

SQ9. Looking at Fig. 14-2, how far off was Crick in his estimate of the size of the adapter? What do you think might be the function of the extra nucleotides? (If you have no idea, stay tuned)

tRNA has the same basic structure in all living cells, its shape determined by internal base pairs (Fig. 4-2). The anticodon loop is spaced distant from the 3' end to which the amino acid is attached. We'll consider the middle part missed by Crick in a moment.

III.B. Who knows the code? Amino acid swap experiment *(pp.368-372)*

It looks like that tRNA is the brains of the outfit, doing what we do when we mentally translate a DNA sequence into protein. Is this true? Fritz Lippman's group put this question the question to the test, by seeing whether it is possible to fool the translation machinery with a disguised amino acid. tRNAs with the anticodons that recognize cysteine codons (called $tRNA^{Cys}$) were charged with radioactive cysteine, that is, cysteine was enzymatically attached to the tRNA, to form cys*-$tRNA^{Cys}$. This tRNA directs the insertion of cysteine wherever there are cysteine-specific codons. Now, the cysteine was chemically altered, changing the $-CH_2SH$ R group to $-CH_3$, the R group of alanine, to form ala*-$tRNA^{Cys}$. If the translation machinery has insight, it would recognize the switch and refuse to put alanine where cysteine ought to go, and radioactivity would not be detected in protein translated in vitro with the altered tRNA. A flow chart of the experiment is shown on pp.370-371.

SQ10. What was the source of the $tRNA^{Cys}$ used in the experiment? Did they use purified tRNA?

SQ11. How did they charge the $tRNA^{Cys}$ with radioactive cysteine? Would they have been able to do so if they had used purified tRNA rather than cell extract?

SQ12. How did they convert the cysteine on the charged $tRNA^{Cys}$ to alanine? Notice the structural difference between cysteine and alanine.

SQ13. Why was a poly(U,G=1:1) polymer used to direct translation? Would a poly(U,G,A=1:1:1) polymer have worked as well?

SQ14. Compare and contrast this experimental protocol to that used by Nirenberg and Matthaei to measure amino acid incorporation directed by artifical RNA polymers, discussed in the notes to Friday, September 15.

The results of the experiment (shown on p.371) show that more than 300-times more cysteine than alanine was incorporated into protein when unmodified cys-tRNA^{cys} was used, but somewhat more then 2-times more alanine than cysteine was incorporated when the extract was treated with Raney nickel.

SQ15. Why do you imagine the difference between alanine incorporation and cysteine incorporation was so small after treatment with Raney nickel? Why not 300-fold more alanine?

The fact that alanine was incorporated, presumably at cysteine codons, indicated that ribosomes are ignorant of the genetic code and rely on tRNA's to be charged properly.

SQ16. Draw a picture of the critical molecular moment in this experiment: a specific cysteine codon basepairing with a specific tRNA^{Cys} to which is attached radioactive alanine. Show the bases involved.

III.C. Attachment of amino acids to tRNA *(p.373)*

If tRNAs and ribosomes are brainless tools, then what knows the code? Clearly, the connection between nucleotide codon and amino acid is all but accomplished as soon as the proper amino acid is attached to the proper tRNA. This is done by a class of enzymes called aminoacyl-tRNA synthetases. Consider Figure 14-3, which shows the process by which a generic tRNA is charged with the appropriate amino acid, catalyzed by a tRNA synthetase. You should bear in mind that each tRNA has its own tRNA synthetase. Each one recognizes the shape of a specific tRNA and the shape of specific amino acid, positions the molecules close to one another, and then attaches them. These synthetases are the only entities within the cell that recognize both a specific amino acid and (indirectly) a specific codon. Each one knows one part of the genetic code. Collectively, they are the only things on earth, beside ourselves, that know the code.

SQ17. Reconsider SQ9.

IV. The process of translation *(pp.378-381; 384-386)*

Figure 14-9 shows a broad view of the process of translation. We will focus here on how the ribosome knows where to start and where to stop. Translation begins at a codon – usually AUG but sometimes GUG or UUG – that is recognized by a specialized tRNA, tRNA^{fMet}. It is charged by formyl-methionine, a derivative of the amino acid methionine in which the amino end has been blocked by amidification with formic acid. We thus have a situation we previously thought was intolerable: a single codon, AUG, that is recognized by different tRNA's carrying different amino acids, either methionine

or formyl-methionine. How does the ribosome know whether to insert f-methionine at the beginning of a protein, or methionine in the middle?

SQ18. Maybe AUG codons are sufficiently rare not to pose a problem. How many AUG codons would you expect in a typical gene? Consider that a typical protein is about 300 amino acids, so a typical gene is about 3x300. . . call it 1000 nucleotides.

Ribosomes don't get confused by AUG codons because they look beyond the three nucleotides. Initiation of translation requires not only a start codon but also a nearby Shine-Dalgarno sequence or Ribosome Binding Site. This sequence is complementary to one end of ribosomal RNA and in this way is recognized by ribosomes. If there is also a start codon a bit 3' to the site, then ribosomes accept fMet-tRNA^{fMet} and begin translation. In contrast, AUG codons that occur in the middle of the genes are not preceded by ribosome binding sites. At these codons, ribosomes accept Met-tRNA^{Met} and continue translation.

The 3' end of 16S rRNA in many bacteria is 3'-AUUCCUCC-5'. Ribosomes therefore recognize sites that have the sequence 5'-AAGGAGG-3'. Actually, even four bases (e.g., AGGA) that are complementary somewhere in this region of 16S rRNA is often sufficient to facilitate ribosomal binding to the beginning of a gene. Ribosome binding sites appear near the beginning of many but not all genes. We can therefore use it as a diagnostic (albeit an imperfect one) to determine where in a sequence translation begins. The degree to which a ribosome binding site matches the ideal sequence often determines how well the mRNA is translated and thus how much protein is made.

SQ19. Which would you expect to have a better ribosome binding site: (A) a gene encoding an enzyme that's needed to catalyze the degradation of an antibiotic in the growth medium, or (B) a gene encoding a protein designed to bind to a specific site on the DNA?

SQ20. Look at the sequence of pUR3 (Appendix I of Lab 2), focusing on the sequence just preceding *bla* (encoding an enzyme that degrades penicillins) and the sequence just preceding *lacI* (encoding a regulatory protein that binds to the Lac operator). What are the two ribosome binding sites preceding these genes? Which is better?

It is much easier to recognize the end of a gene than the beginning. Almost always, a gene ends with one of the three codons, UAG, UAA, or UAG, that do not encode amino acids. Readthrough past stop codons sometimes occurs, however, and presumably to guard against this, genes often end with multiple stop codons.

SQ21. Look at the *lacZ* gene in pUR3. What sequence appears at its end?