TRANSFER OF GENETIC INFORMATION

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Reference texts: Berg et al, Chapter 4
Weaver 5th ed, Chapters 2,3,18

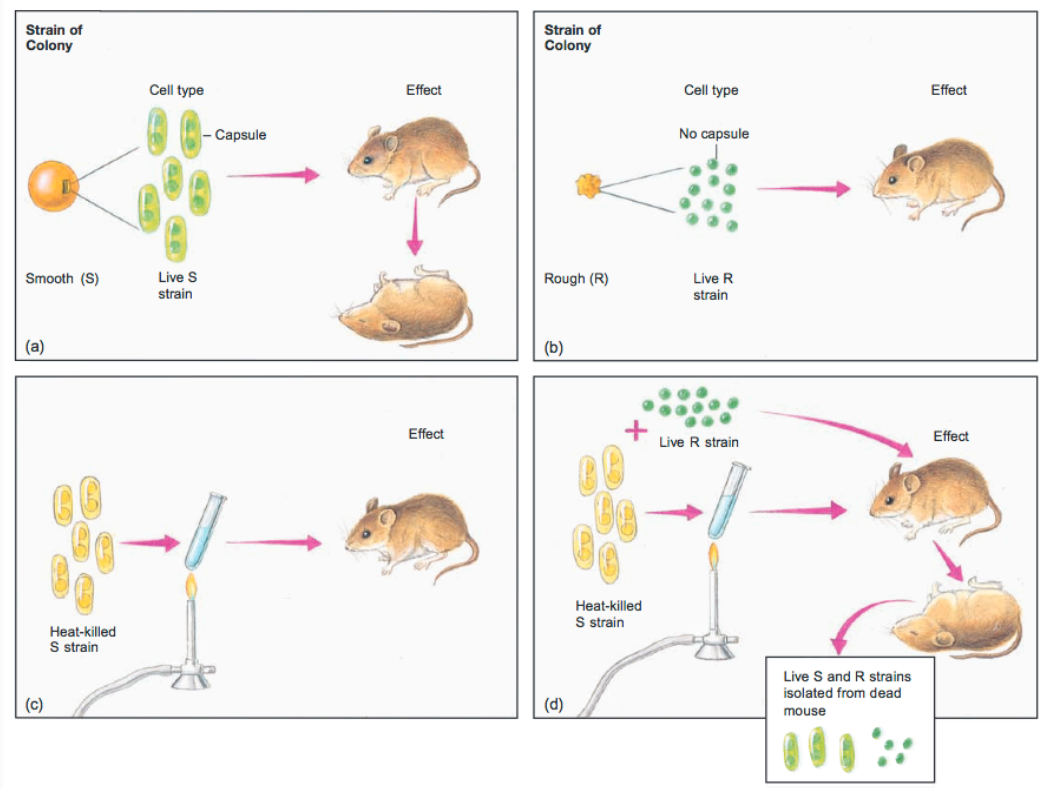


Figure 2.2 Griffith's transformation experiments. **(a)** Virulent strain S *S. pneumoniae* bacteria kill their host; **(b)** avirulent strain R bacteria cannot infect successfully, so the mouse survives; **(c)** strain S bacteria that are heat-killed can no longer infect; **(d)** a mixture of strain R and heat-killed strain S bacteria kills the mouse. The killed virulent (S) bacteria have transformed the avirulent (R) bacteria to virulent (S).

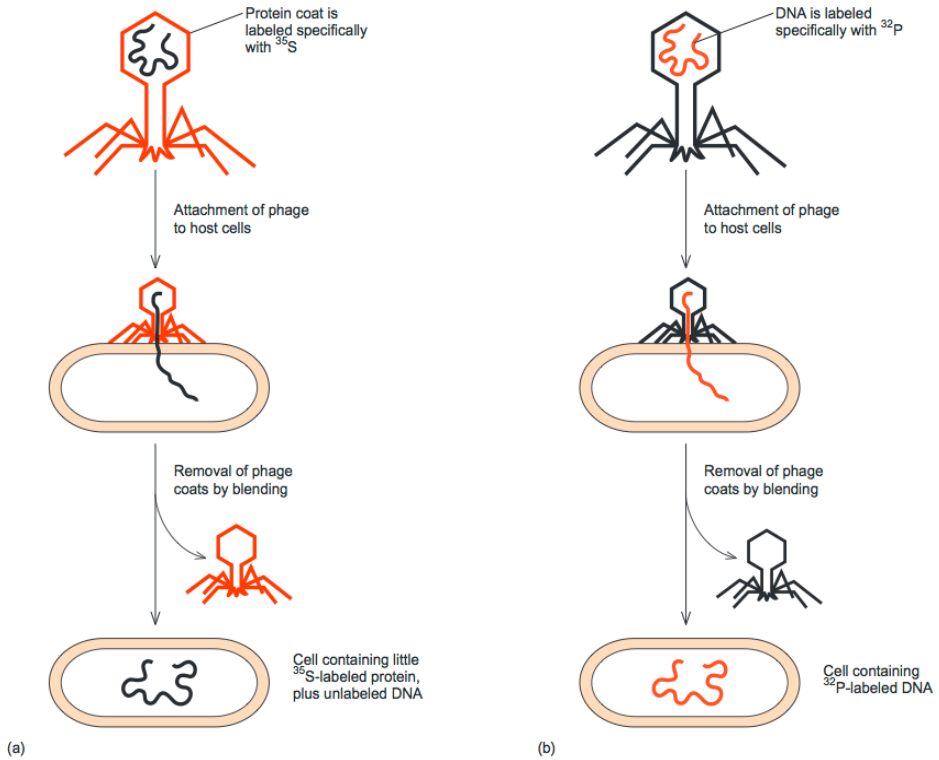


Figure 2.4 The Hershey—Chase experiment. Phage T2 contains genes that allow it to replicate in *E. coli.* Because the phage is composed of DNA and protein only, its genes must be made of one of these substances. To discover which, Hershey and Chase performed a two-part experiment. In the first part **(a),** they labeled the phage protein with ^{35}S (red), leaving the DNA unlabeled (black). In the second part **(b),** they labeled the phage DNA with ^{32}P (red), leaving the protein unlabeled (black). Since the phage genes must enter the cell, the experimenters reasoned that the type of label found in the infected cells would indicate the nature of the genes. Most of the labeled protein remained on the outside and was stripped off the cells by use of a blender (a), whereas most of the labeled DNA entered the infected cells (b). The conclusion was that the genes of this phage are made of DNA.

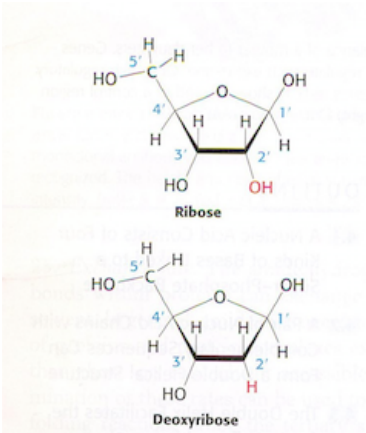


Figure 4.2 Ribose and deoxyribose. Atoms in sugar units are numbered with primes to distinguish them from atoms in bases (see Figure 4.4).

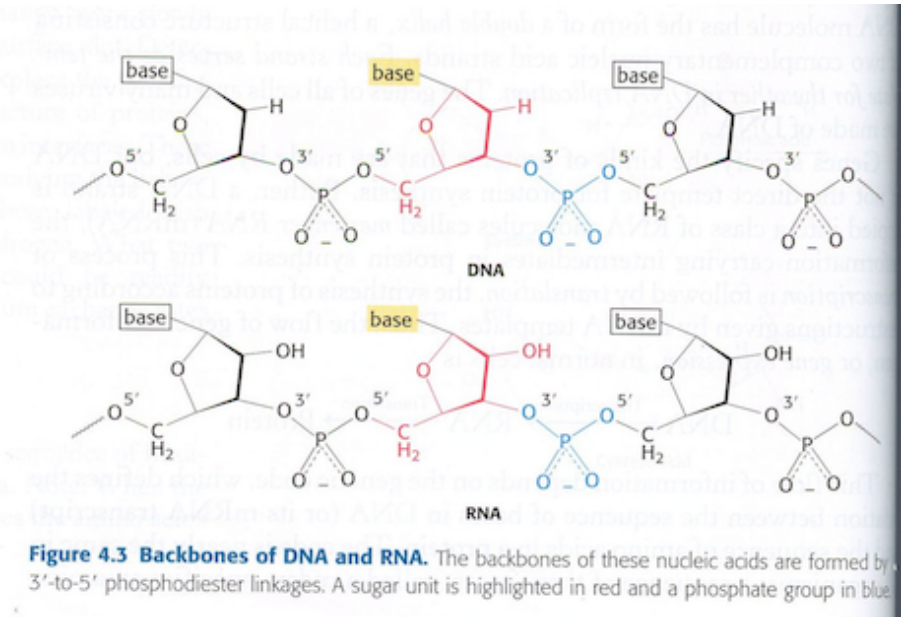
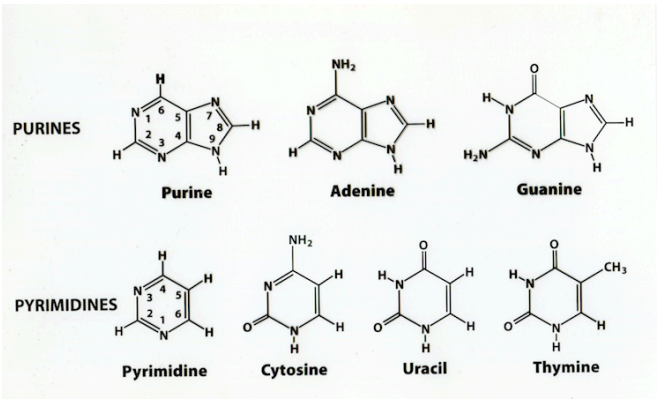


Figure 4.3 Backbones of DNA and RNA. The backbones of these nucleic acids are formed by 3′-to-5′ phosphodiester linkages. A sugar unit is highlighted in red and a phosphate group in blue.



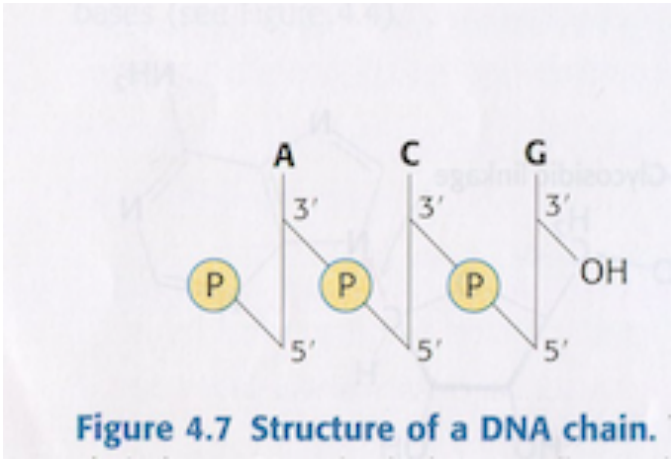


Figure 4.7 Structure of a DNA chain.

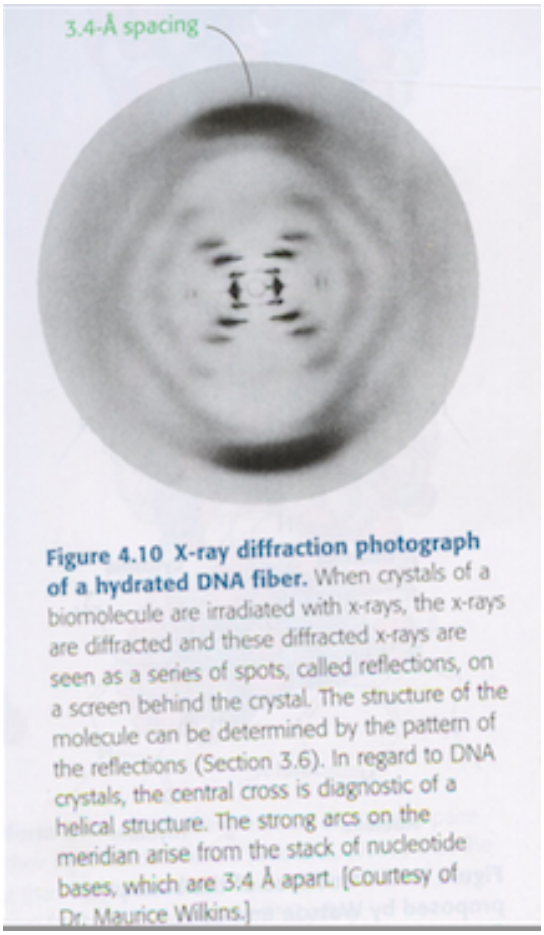


Figure 4.10 X-ray diffraction photograph of a hydrated DNA fiber. When crystals of a biomolecule are irradiated with x-rays, the x-rays are diffracted and these diffracted x-rays are seen as a series of spots, called reflections, on a screen behind the crystal. The structure of the molecule can be determined by the pattern of the reflections (Section 3.6). In regard to DNA crystals, the central cross is diagnostic of a helical structure. The strong arcs on the meridian arise from the stack of nucleotide bases, which are 3.4 Å apart. [Courtesy of Dr. Maurice Wilkins.]

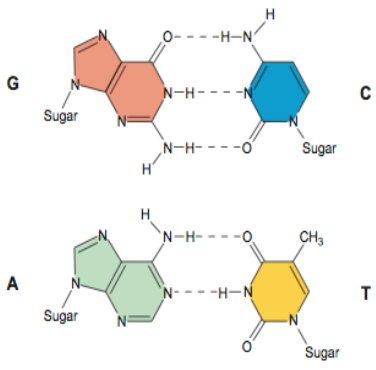


Figure 2.13 The base pairs of DNA. A guanine–cytosine pair (G–C), held together by three hydrogen bonds (dashed lines), has almost exactly the same shape as an adenine–thymine pair (A–T), held together by two hydrogen bonds.

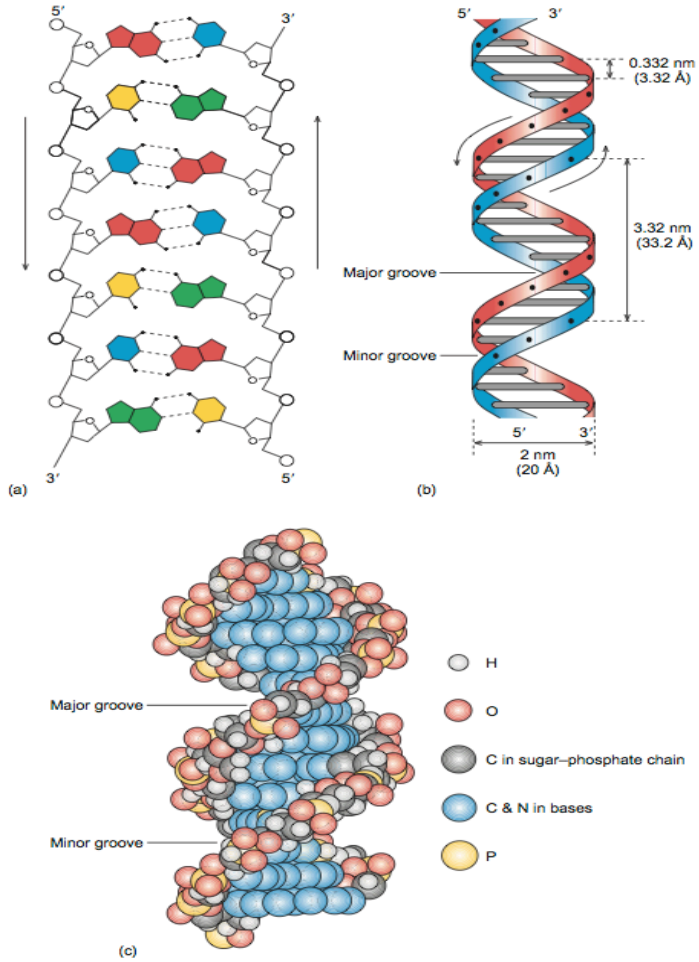


Figure 2.14 Three models of DNA structure. (a) The helix is straightened out to show the base pairing in the middle. Each type of base is represented by a different color, with the sugar–phosphate backbones in black. Note the three hydrogen bonds in the G–C pairs and the two in the A–T pairs. The vertical arrows beside each strand point in the 5′→3′ direction and indicate the antiparallel nature of the two DNA strands. The left strand runs 5′→3′, top to bottom; the right strand runs 5′→3′, bottom to top. The deoxyribose rings (white pentagons with O representing oxygen) also show that the two strands have opposite orientations: The rings in the right strand are inverted relative to those in the left strand. **(b)** The DNA doul helix is presented as a twisted ladder whose sides represent sugar–phosphate backbones of the two strands and whose represent base pairs. The curved arrows beside the two stra indicate the 5′→3′ orientation of each strand, further illustra the two strands are antiparallel. **(c)** A space-filling model. Th sugar–phosphate backbones appear as strings of dark gray, gray, and yellow spheres, whereas the base pairs are render horizontal flat plates composed of blue spheres. Note the m minor grooves in the helices depicted in parts (b) and (c).

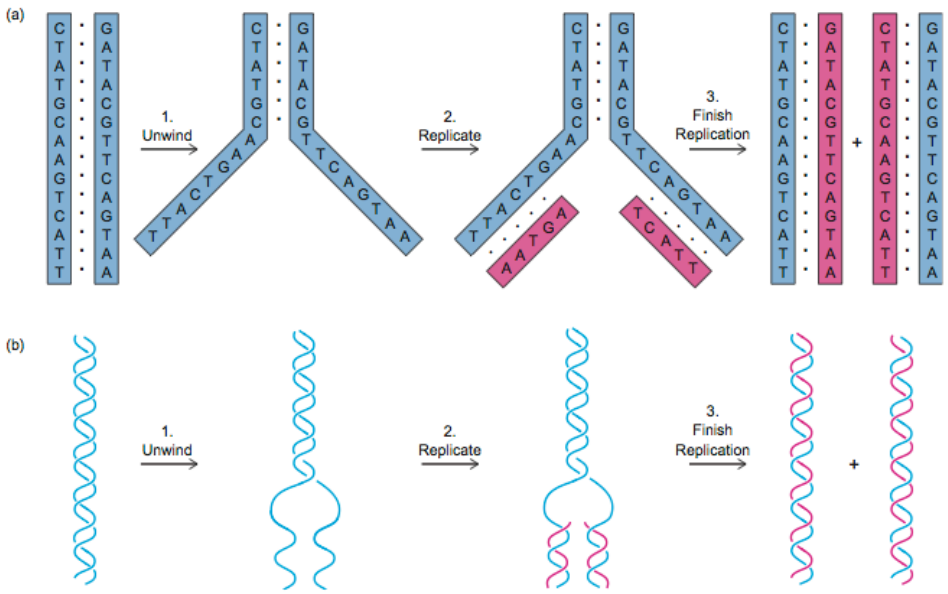


Figure 2.15 Replication of DNA. (a) For simplicity, the two parental DNA strands (blue) are represented as parallel lines. Step 1: During replication these parental strands separate, or unwind. Step 2: New strands (pink) are built with bases complementary to those of the separated parental strands. Step 3: Replication is finished, with the parental strands totally separated and the new strands completed. The end result is two double-stranded DNA duplexes identical to the original. Therefore, each daughter duplex gets one parental strand (blue) and one new strand (pink). Because only one parental strand is conserved in each of the daughter duplexes, this mechanism of replication is called semiconservative. **(b)** A more realistic portrayal of the same process. Here the strands are shown in a double helix instead of as parallel lines. Notice again that two daughter duplexes are generated, each with one parental strand (blue) and one new strand (pink).

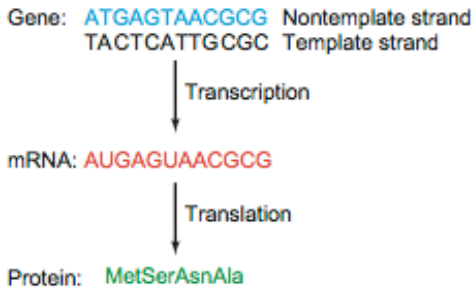


Figure 3.1 Outline of gene expression. In the first step, transcription, the template strand (black) is transcribed into mRNA. Note that the nontemplate strand (blue) of the DNA has the same sequence (except for the T–U change) as the mRNA (red). In the second step, the mRNA is translated into protein (green). This little "gene" is only 12 bp long and codes for only four amino acids (a tetrapeptide). Real genes are much larger.

Table 4.3 RNA molecules in *E. coli*

Type	Relative amount (%)	Sedimentation coefficient (*s*)	Mass (kd)	Number of nucleotides
Ribosomal RNA (rRNA)	80	23	1.2×10^3	3700
		16	0.55×10^3	1700
		5	3.6×10^1	120
Transfer RNA (tRNA)	15	4	2.5×10^1	75
Messenger RNA (mRNA)	5		Heterogeneous	

Table 4.3
Biochemistry, Seventh Edition

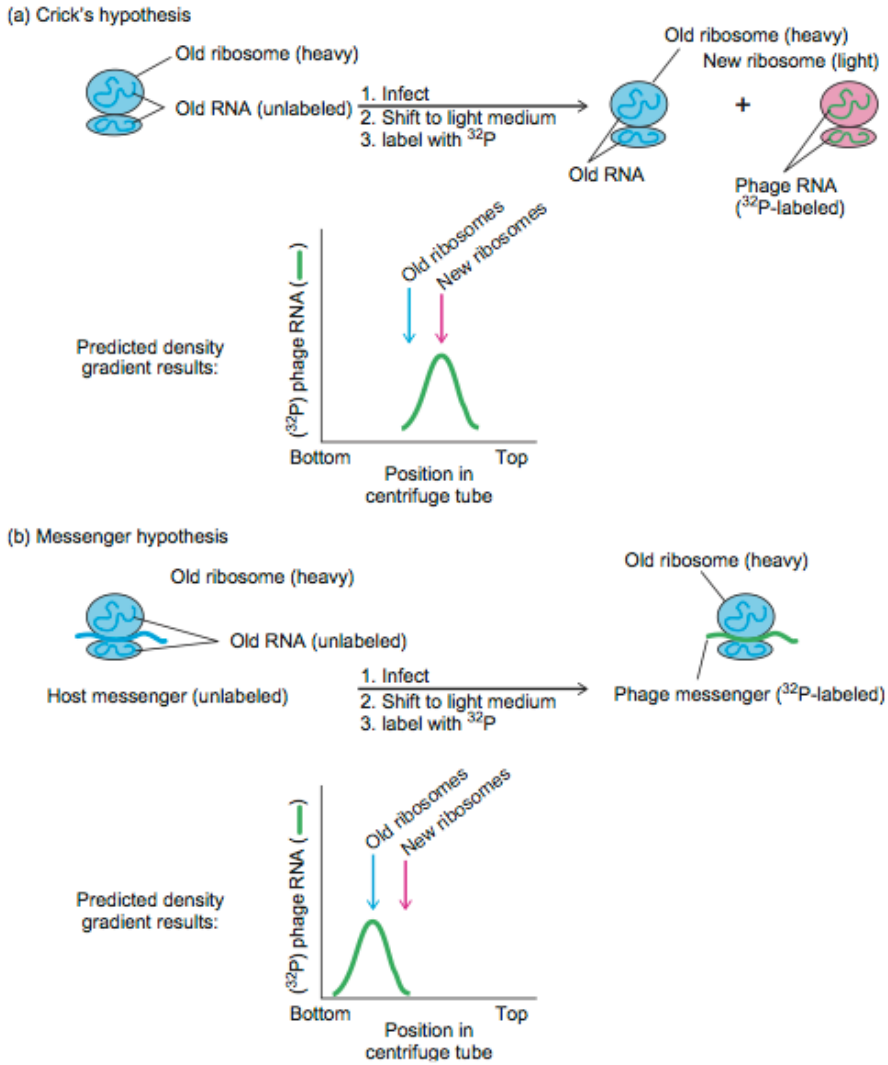


Figure 3.12 Experimental test of the messenger hypothesis. Heavy *E. coli* ribosomes were made by labeling the bacterial cells with heavy isotopes of carbon and nitrogen. The bacteria were then infected with phage T2 and simultaneously shifted to "light" medium containing the normal isotopes of carbon and nitrogen, plus some ^{32}P to make the phage RNA radioactive. **(a)** Crick had proposed that ribosomal RNA carried the message for making proteins. If this were so, then whole new ribosomes with phage-specific ribosomal RNA would have been made after phage infection. In that case, the new ^{32}P-labeled RNA (green) should have moved together with the new, light ribosomes (pink). **(b)** Jacob and colleagues had proposed that a messenger RNA carried genetic information to the ribosomes. According to this hypothesis, phage infection would cause the synthesis of new, phage-specific messenger RNAs that would be ^{32}P-labeled (green). These would associate with old, heavy ribosomes (blue). The radioactive label would therefore move together with the old, heavy ribosomes in the density gradient. This was indeed what happened.

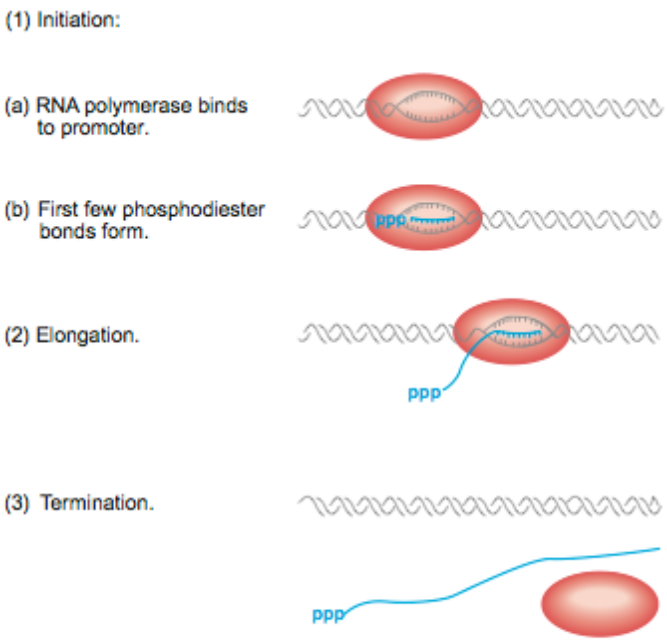


Figure 3.14 Transcription. **(1a)** In the first stage of initiation, RNA polymerase (red) binds tightly to the promoter and "melts" a short stretch of DNA. **(1b)** In the second stage of initiation, the polymerase joins the first few nucleotides of the nascent RNA (blue) through phosphodiester bonds. The first nucleotide retains its triphosphate group (ppp). **(2)** During elongation, the melted bubble of DNA moves with the polymerase, allowing the enzyme to "read" the bases of the DNA template strand and make complementary RNA. **(3)** Termination occurs when the polymerase reaches a termination signal, causing the RNA and the polymerase to fall off the DNA template.

The Adaptor Hypothesis

In 1958, Francis Crick wrote:

"RNA represents mainly a sequence of sites where hydrogen bonding could occur. One would expect, therefore, that whatever went into the template in a ***specific*** way did so by forming hydrogen bonds. It is therefore a natural hypothesis that the amino acid is carried to the template by an adaptor molecule, and that the adaptor is the part that actually fits into the RNA. In its simplest form one would require twenty adaptors, one for each amino acid."

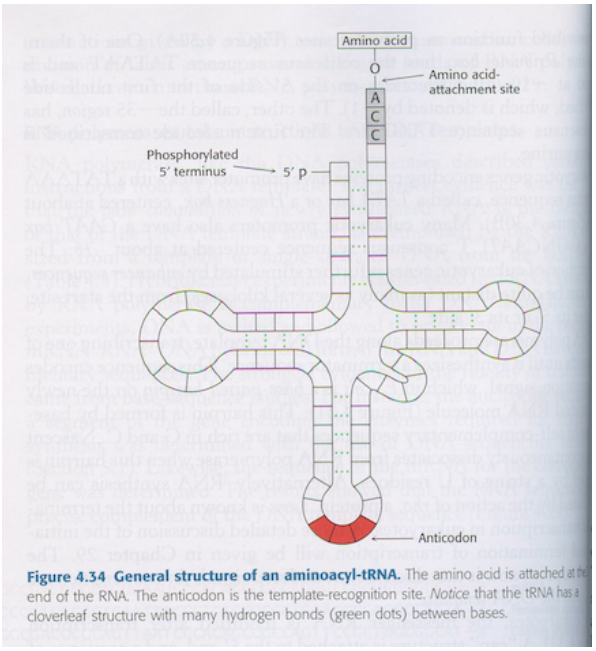


Figure 4.34 General structure of an aminoacyl-tRNA. The amino acid is attached at the end of the RNA. The anticodon is the template-recognition site. *Notice* that the tRNA has a cloverleaf structure with many hydrogen bonds (green dots) between bases.

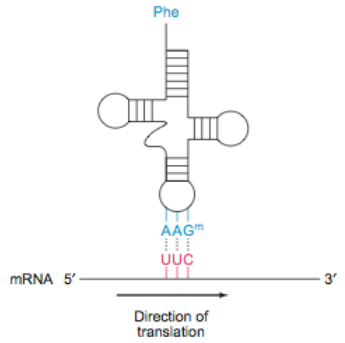


Figure 3.18 Codon–anticodon recognition. The recognition between a codon in an mRNA and a corresponding anticodon in a tRNA obeys essentially the same Watson–Crick rules as apply to other polynucelotides. Here, a 3′AAG^{m}5′ anticodon (blue) on a tRNA^{Phe} is recognizing a 5′UUC3′ codon (red) for phenylalanine in an mRNA. The G^{m} denotes a methylated G, which base-pairs like an ordinary G. Notice that the tRNA is pictured backwards (3′→5′) relative to normal convention, which is 5′→3′, left to right. That was done to put its anticodon in the proper orientation (3′→5′, left to right) to base-pair with the codon, shown conventionally reading 5′→3′, left to right. Remember that the two strands of DNA are antiparallel; this applies to any double-stranded polynucleotide, including one as small as the 3-bp codon–anticodon pair.

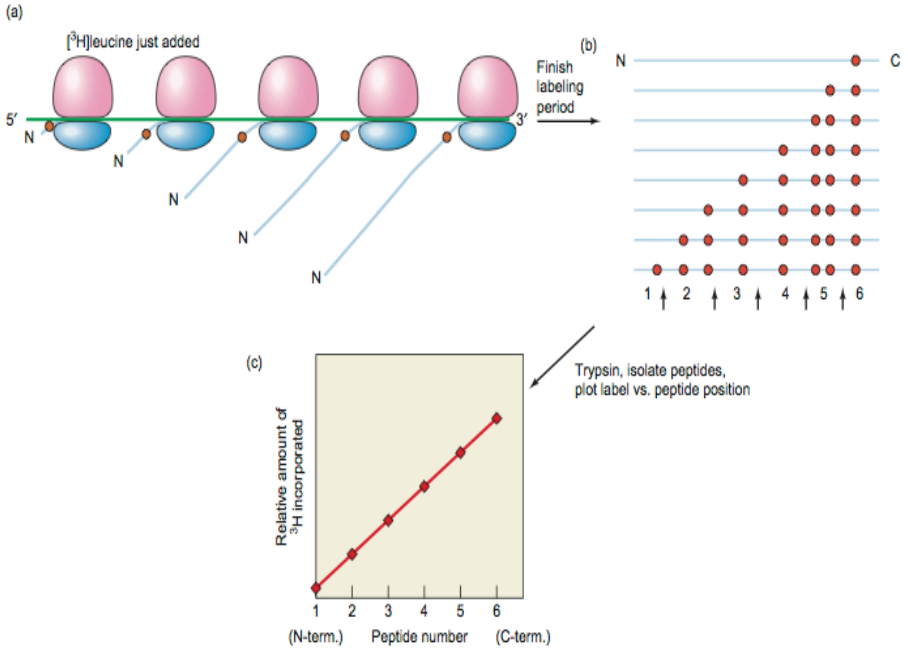


Figure 18.1 Experimental strategy to determine the direction of translation. **(a)** Labeling the protein. Consider an mRNA (green) being translated by several ribosomes (pink and blue), assuming that the mRNA is translated in the 5′→3′ direction and the proteins are made in the amino (N) to carboxyl (C) direction. A labeled amino acid ([^{3}H]leucine) has just been added to the system, so it has begun to be incorporated into the growing protein chains (blue), as indicated by the red dots. It is incorporated near the N-terminus in the polypeptides on the left, where protein synthesis has just begun, but only near the C-terminus in the polypeptides on the right, which are almost completed. **(b)** Distribution of label in completed proteins after a moderate labeling period. The proteins near the top, with label only near the C-terminus correspond to the nearly completed proteins near the right in panel (a). Those near the bottom, with label distributed toward the N-terminus, correspond to the growing proteins near the left in panel (a). These have had time to incorporate label throughout a greater length of the protein. Cutting sites for trypsin within the protein are indicated by arrows at bottom, and the resulting peptides are numbered 1–6 according to their positions in the protein. **(c)** Model experimental results. One plots the relative amount of ^{3}H labeling in each of the peptides, 1–6, and finds that the C-terminal peptides are the most highly labeled. This is what we expect if translation started at the N-terminus. If it had started at the C-terminus (opposite to the picture in panel [a]), then the N-terminal peptides would be the most highly labeled.

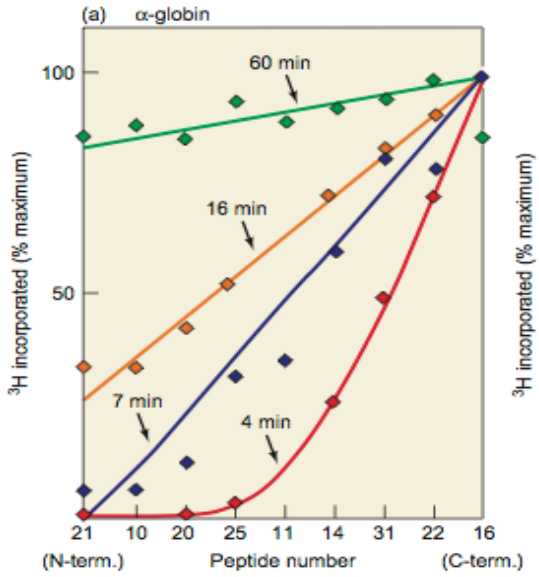


Figure 18.2 Determining the direction of translation. Dintzis carried out the experimental plan outlined in Figure 18.1 with rabbit reticulocytes, which make almost nothing but α- and β-globins. He labeled the reticulocytes with [^{3}H]leucine for various lengths of time, then separated the α- and β-globins, cut each protein into peptides with trypsin, and determined the label in each peptide. He plotted the relative amount of ^{3}H label against the peptide number, with the N-terminal peptide on the left, and the C-terminal peptide on the right. The curves for α- and β-globin showed the most label in the C-terminal peptides, especially after short labeling times. (Only the α-globin results are shown here.) This is what we expect if translation starts at the N-terminus of a protein. Note that the peptide numbers are not related to their position in the protein, as they are in the example in Figure 18.1. (Source: Adapted from Dintzis,

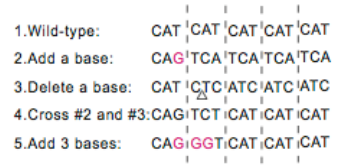


Figure 18.3 Frameshift mutations. Line 1: An imaginary gene has the same codon, CAT, repeated over and over. The vertical dashed lines show the reading frame, starting from the beginning. Line 2: Adding a base, G (pink), in the third position changes the first codon to CAG and shifts the reading frame one base to the left so that every subsequent codon reads TCA. Line 3: Deleting the fifth base, A (marked by the triangle), from the wild-type gene changes the second codon to CTC and shifts the reading frame one base to the right so that every subsequent codon reads ATC. Line 4: Crossing the mutants in lines 2 and 3 occasionally gives a recombined "pseudo-wild-type" revertant with an insertion and a deletion close together. The end result is a DNA with its first two codons altered, but all the other ones put back into the correct reading frame. Line 5: Adding three bases, GGG (pink), after the first two bases disrupts the first two codons, but leaves the reading frame unchanged. The same would be true of deleting three bases.

Figure 18.4 Coding properties of several synthetic mRNAs. **(a)** Poly(UC) contains two alternating codons, UCU and CUC, which code for serine (Ser) and leucine (Leu), respectively. Thus, the product is poly(Ser-Leu). **(b)** Poly(UUC) contains three codons, UUC, UCU, and CUU, which code for phenylalanine (Phe), serine (Ser), and leucine (Leu), respectively. The product is therefore poly(Phe), or poly(Ser), or poly(Leu), depending on which of the three reading frames the ribosome uses. **(c)** Poly(UAUC) contains four codons in a repeating sequence: UAU, CUA, UCU, and AUC, which code for tyrosine (Tyr), leucine (Leu), serine (Ser), and isoleucine (Ile), respectively. The product is therefore poly(Tyr-Leu-Ser-Ile).

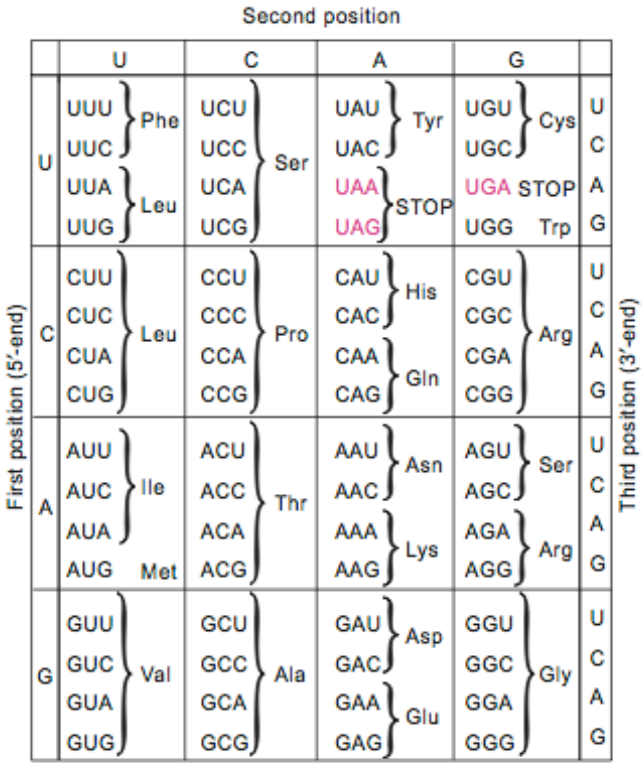


Figure 18.6 The genetic code. All 64 codons are listed, along with the amino acid for which each codes. To find a given codon—ACU, for example—we start with the wide horizontal row labeled with the name of the first base of the codon (A) on the left border. Then we move across to the vertical column corresponding to the second base (C). This brings us to a box containing all four codons beginning with AC. It is now a simple matter to find the one among these four we are seeking, ACU. We see that this triplet codes for threonine (Thr), as do all the other codons in the box: ACC, ACA, and ACG. This is an example of the degeneracy of the code. Notice that three codons (pink) do not code for amino acids; instead, they are stop signals.

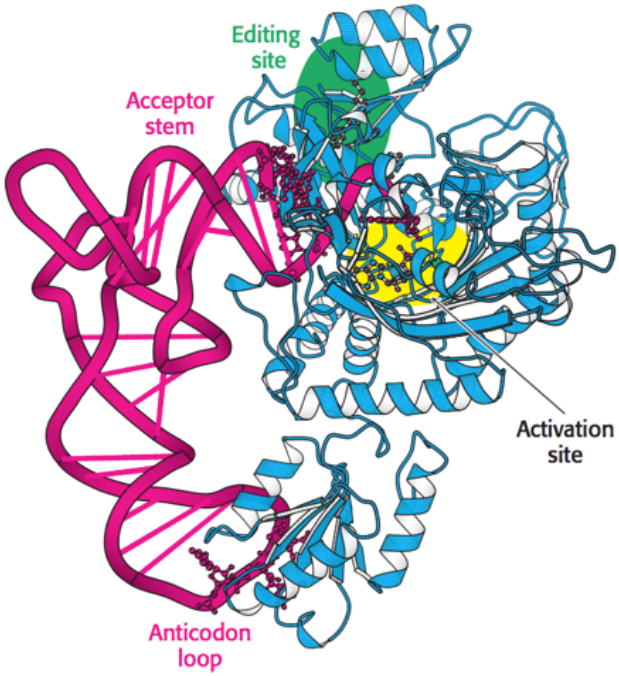


Figure 30.11 Threonyl-tRNA synthetase complex. The structure shows the complex between threonyl-tRNA synthetase and $tRNA^{Thr}$. *Notice* that the synthetase binds to both the acceptor stem and the anticodon loop.

mRNA has start and stop signals