MESSENGER RNA

2.5.1 Introduction

Key Terms

Messenger RNA (mRNA) is the intermediate that represents one strand of a gene coding for protein. Its coding region is related to the protein sequence by the triplet genetic code.

Transfer RNA (tRNA) is the intermediate in protein synthesis that interprets the genetic code. Each tRNA can be linked to an amino acid. The tRNA has an anticodon sequence that complementary to a triplet codon representing the amino acid.

Ribosomal RNA (rRNA) is a major component of the ribosome. Each of the two subunits of the ribosome has a major rRNA as well as many proteins.

RNA is a central player in gene expression. It was first characterized as an intermediate in protein synthesis, but since then many other RNAs have been discovered that play structural or functional roles at other stages of gene expression. The involvement of RNA in many functions concerned with gene expression supports the general view that the entire process may have evolved in an "RNA world" in which RNA was originally the active component in maintaining and expressing genetic information. Many of these functions were subsequently assisted or taken over by proteins, with a consequent increase in versatility and probably efficiency.

As summarized in **Figure 5.1**, three major classes of RNA are directly involved in the production of proteins:

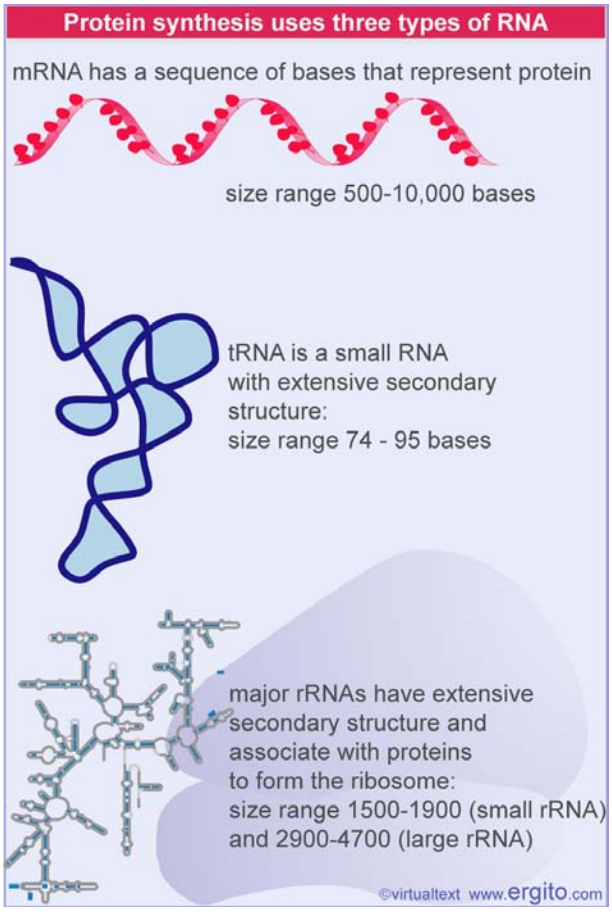

Figure 5.1 The three types of RNA universally required for gene expression are mRNA (carries the coding sequence), tRNA (provides the amino acid corresponding to each codon), and rRNA (a major component of the ribosome that provides the environment for protein synthesis).

- Messenger RNA (**mRNA**) provides an intermediate that carries the copy of a DNA sequence that represents protein.
- Transfer RNAs (**tRNA**) are small RNAs that are used to provide amino acids corresponding to each particular codon in mRNA.
- Ribosomal RNAs (**rRNA**) are components of the ribosome, a large ribonucleoprotein complex that contains many proteins as well as its RNA components, and which provides the apparatus for actually polymerizing amino acids into a polypeptide chain.

The type of role that RNA plays in each of these cases is distinct. For messenger RNA, its sequence is the important feature: each nucleotide triplet within the coding region of the mRNA represents an amino acid in the corresponding protein. However, the structure of the mRNA, in particular the sequences on either side of the coding region, can play an important role in controlling its activity, and therefore the amount of protein that is produced from it.

In tRNA, we see two of the common themes governing the use of RNA: its three dimensional structure is important; and it has the ability to base pair with another RNA (mRNA). The three dimensional structure is recognized first by an enzyme as providing a target that is appropriate for linkage to a specific amino acid. The linkage creates an aminoacyl-tRNA, which is recognized as the structure that is used for protein synthesis. The specificity with which an aminoacyl-tRNA is used is controlled by base pairing, when a short triplet sequence (the anticodon) pairs with the nucleotide triplet representing its amino acid.

With rRNA, we see another type of activity. One role of RNA is structural, in providing a framework to which ribosomal proteins attach. But it also participates directly in the activities of the ribosome. One of the crucial activities of the ribosome is the ability to catalyze the formation of a peptide bond by which an amino acid is incorporated into protein. This activity resides in one of the rRNAs.

The important thing about this background is that, as we consider the role of RNA in protein synthesis, we have to view it as a component that plays an active role and that can be a target for regulation by either proteins or by other RNAs, and we should remember that the RNAs may have been the basis for the original apparatus. The theme that runs through all of the activities of RNA, in both protein synthesis and elsewhere, is that its functions depend critically upon base pairing, both to form its secondary structure, and to interact specifically with other RNA molecules. The coding function of mRNA is unique, but tRNA and rRNA are examples of a much broader class of noncoding RNAs with a variety of functions in gene expression.

Last updated on 7-30-2002

MESSENGER RNA

2.5.2 mRNA is produced by transcription and is translated

Key Terms

Transcription describes synthesis of RNA on a DNA template.

Translation is synthesis of protein on the mRNA template.

A **coding region** is a part of the gene that represents a protein sequence.

The **antisense strand (Template strand)** of DNA is complementary to the sense strand, and is the one that acts as the template for synthesis of mRNA.

The **coding strand (Sense strand)** of DNA has the same sequence as the mRNA and is related by the genetic code to the protein sequence that it represents.

Key Concepts

- Only one of the two strands of DNA is transcribed into RNA.

Gene expression occurs by a two-stage process.

- **Transcription** generates a single-stranded RNA identical in sequence with one of the strands of the duplex DNA.
- **Translation** converts the nucleotide sequence of mRNA into the sequence of amino acids comprising a protein. The entire length of an mRNA is not translated, but each mRNA contains at least one **coding region** that is related to a protein sequence by the genetic code: each nucleotide triplet (codon) of the coding region represents one amino acid.

Only one strand of a DNA duplex is transcribed into a messenger RNA. We distinguish the two strands of DNA as depicted in **Figure 5.2**:

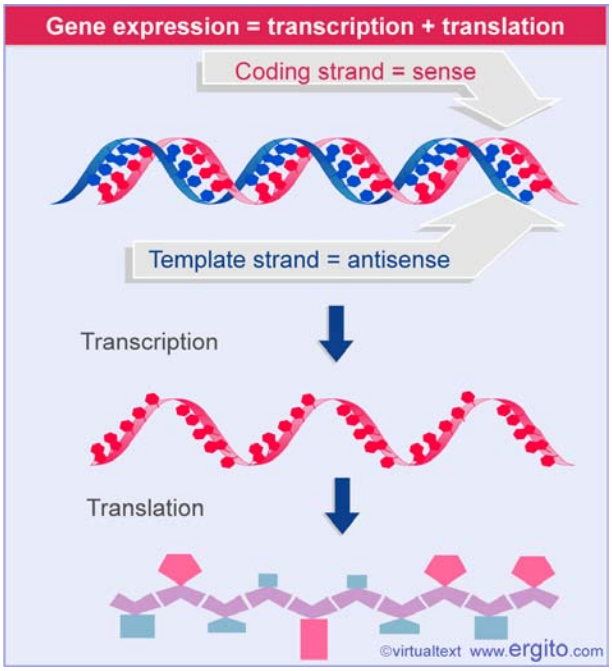

Figure 5.2 Transcription generates an RNA which is complementary to the DNA template strand and has the same sequence as the DNA coding strand. Translation reads each triplet of bases into one amino acid. Three turns of the DNA double helix contain 30 bp, which code for 10 amino acids.

- The strand of DNA that directs synthesis of the mRNA via complementary base pairing is called the **template strand** or **antisense strand**. (*Antisense* is used as a general term to describe a sequence of DNA or RNA that is complementary to mRNA.)
- The other DNA strand bears the *same* sequence as the mRNA (except for possessing T instead of U), and is called the **coding strand** or **sense strand**.

In this chapter we discuss mRNA and its use as a template for protein synthesis. In *Molecular Biology 2.6 Protein synthesis* we discuss the process by which a protein is synthesized. In *Molecular Biology 2.7 Using the genetic code* we discuss the way the genetic code is used to interpret the meaning of a sequence of mRNA. And in *Molecular Biology 2.8 Protein localization* we turn to the question of how a protein finds its proper location in the cell when or after it is synthesized.

MESSENGER RNA

2.5.3 Transfer RNA forms a cloverleaf

Key Terms

Transfer RNA (tRNA) is the intermediate in protein synthesis that interprets the genetic code. Each tRNA can be linked to an amino acid. The tRNA has an anticodon sequence that complementary to a triplet codon representing the amino acid.

The **anticodon** is a trinucleotide sequence in tRNA which is complementary to the codon in mRNA and enables the tRNA to place the appropriate amino acid in response to the codon.

The **cloverleaf** describes the structure of tRNA drawn in two dimensions, forming four distinct arm-loops.

A **stem** is the base-paired segment of a hairpin structure in RNA.

A **loop** is a single-stranded region at the end of a hairpin in RNA (or single-stranded DNA); it corresponds to the sequence between inverted repeats in duplex DNA.

An **arm** of tRNA is one of the four (or in some cases five) stem-loop structures that make up the secondary structure.

The **acceptor arm** of tRNA is a short duplex that terminates in the CCA sequence to which an amino acid is linked.

The **anticodon arm** of tRNA is a stem loop structure that exposes the anticodon triplet at one end.

The **D arm** of tRNA has a high content of the base dihydrouridine.

The **extra arm** of tRNA lies between the T ψC and anticodon arms. It is the most variable in length in tRNA, from 3-21 bases. tRNAs are called class 1 if they lack it, and class 2 if they have it.

Invariant base positions in tRNA have the same nucleotide in virtually all (>95%) of tRNAs.

Conserved positions are defined when many examples of a particular nucleic acid or protein are compared and the same individual bases or amino acids are always found at particular locations.

A **semiconserved (Semiinvariant)** position is one where comparison of many individual sequences finds the same type of base (pyrimidine or purine) always present.

An **aminoacyl-tRNA** is a tRNA linked to an amino acid. The COOH group of the amino acid is linked to the 3′- or 2′-OH group of the terminal base of the tRNA.

Aminoacyl-tRNA synthetases are enzymes responsible for covalently linking amino acids to the 2′- or 3′-OH position of tRNA.

Key Concepts

- A tRNA has a sequence of 74-95 bases that folds into a clover-leaf secondary

structure with four constant arms (and an additional arm in the longer tRNAs).

- tRNA is charged to form aminoacyl-tRNA by forming an ester link from the 2′ or 3′ OH group of the adenylic acid at the end of the acceptor arm to the COOH group of the amino acid.
- The sequence of the anticodon is solely responsible for the specificity of the aminoacyl-tRNA.

Messenger RNA can be distinguished from the apparatus responsible for its translation by the use of *in vitro* cell-free systems to synthesize proteins. *A protein-synthesizing system from one cell type can translate the mRNA from another, demonstrating that both the genetic code and the translation apparatus are universal.*

Each nucleotide triplet in the mRNA represents an amino acid. The incongruity of structure between trinucleotide and amino acid immediately raises the question of how each codon is matched to its particular amino acid. The "adapter" is **transfer RNA** (**tRNA**). A tRNA has two crucial properties:

- It represents a single amino acid, to which it is *covalently linked.*
- It contains a trinucleotide sequence, the **anticodon**, which is *complementary to the codon representing its amino acid.* The anticodon enables the tRNA to recognize the codon via complementary base pairing (417; 446).

All tRNAs have common secondary and tertiary structures. The tRNA secondary structure can be written in the form of a **cloverleaf**, illustrated in **Figure 5.3**, in which complementary base pairing forms **stems** for single-stranded **loops**. The stem-loop structures are called the **arms** of tRNA. Their sequences include "unusual" bases that are generated by modification of the 4 standard bases after synthesis of the polynucleotide chain (for review see 42).

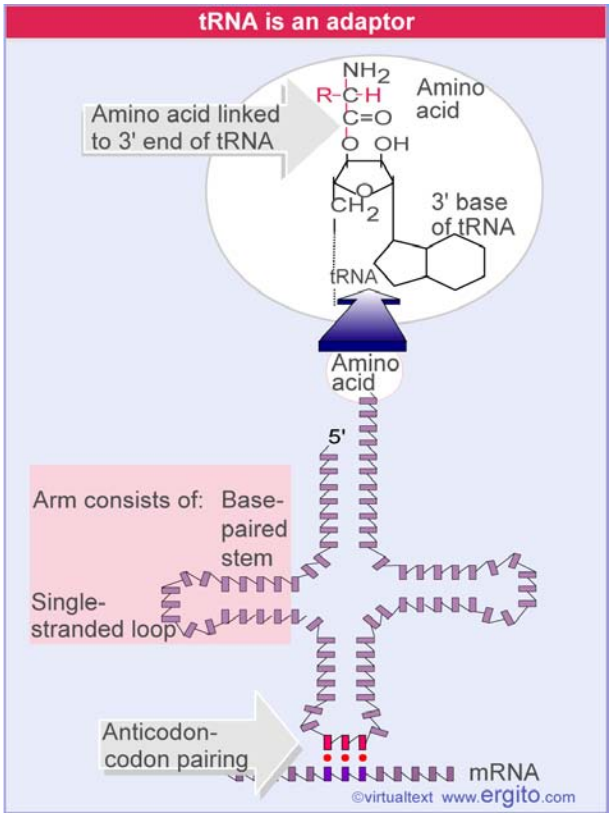

Figure 5.3 A tRNA has the dual properties of an adaptor that recognizes both the amino acid and codon. The 3′ adenosine is covalently linked to an amino acid. The anticodon base pairs with the codon on mRNA.

The construction of the cloverleaf is illustrated in more detail in **Figure 5.4**. The four major arms are named for their structure or function:

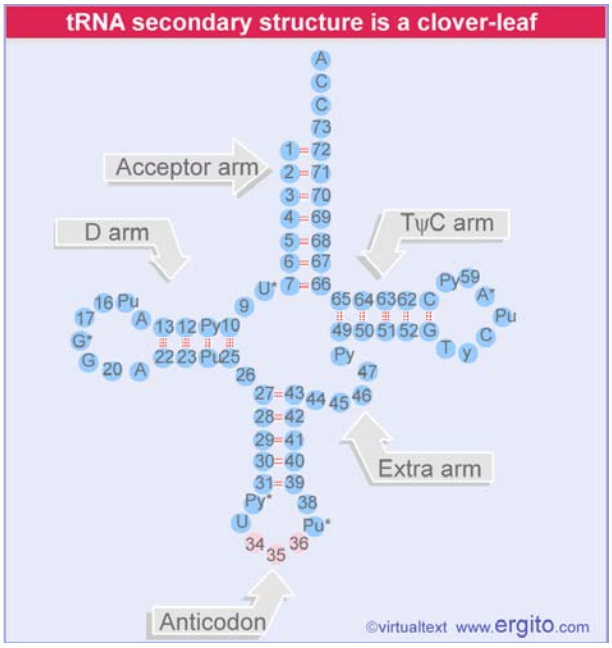

Figure 5.4 The tRNA cloverleaf has invariant and semi-invariant bases, and a conserved set of base pairing interactions.

- The **acceptor arm** consists of a base-paired stem that ends in an unpaired sequence whose free 2′– or 3′–OH group can be linked to an amino acid.
- The *T ψC arm* is named for the presence of this triplet sequence. (ψ stands for pseudouridine, a modified base.)
- The **anticodon arm** always contains the anticodon triplet in the center of the loop.
- The **D arm** is named for its content of the base dihydrouridine (another of the modified bases in tRNA).
- The **extra arm** lies between the T ψC and anticodon arms and varies from 3-21 bases.

The numbering system for tRNA illustrates the constancy of the structure. Positions are numbered from 5′ to 3′ according to the most common tRNA structure, which has 76 residues. The overall range of tRNA lengths is 74-95 bases. The variation in length is caused by differences in the D arm and extra arm (for more details see *Molecular Biology Supplement 32.8 tRNA sequences*).

The base pairing that maintains the secondary structure is shown in **Figure 5.4**. Within a given tRNA, most of the base pairings are conventional partnerships of A·U and G·C, but occasional G·U, G·ψ, or A· ψ pairs are found. The additional types of base pairs are less stable than the regular pairs, but still allow a double-helical structure to form in RNA.

When the sequences of tRNAs are compared, the bases found at some positions are **invariant** (or **conserved**); almost always a particular base is found at the position (see *Molecular Biology Supplement 32.8 tRNA sequences*). Some positions are described as **semiinvariant** (or **semiconserved**) because they are restricted to one type of base (purine versus pyrimidine), but either base of that type may be present.

When a tRNA is *charged* with the amino acid corresponding to its anticodon, it is called **aminoacyl-tRNA**. The amino acid is linked by an ester bond from its carboxyl group to the 2′ or 3′ hydroxyl group of the ribose of the 3′ terminal base of the tRNA (which is always adenine). The process of charging a tRNA is catalyzed by a specific enzyme, **aminoacyl-tRNA synthetase**. There are (at least) 20 aminoacyl-tRNA synthetases. Each recognizes a single amino acid and all the tRNAs on to which it can legitimately be placed.

There is at least one tRNA (but usually more) for each amino acid. A tRNA is named by using the three letter abbreviation for the amino acid as a superscript. If there is more than one tRNA for the same amino acid, subscript numerals are used to distinguish them. So two tRNAs for tyrosine would be described as $tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$. A tRNA carrying an amino acid – that is, an aminoacyl-tRNA – is indicated by a prefix that identifies the amino acid. Ala-tRNA describes $tRNA^{Ala}$ carrying its amino acid.

Does the anticodon sequence alone allow aminoacyl-tRNA to recognize the correct codon? A classic experiment to test this question is illustrated in **Figure 5.5**. Reductive desulfuration converts the amino acid of cysteinyl-tRNA into alanine, generating alanyl-$tRNA^{Cys}$. The tRNA has an anticodon that responds to the codon UGU. Modification of the amino acid does not influence the specificity of the anticodon-codon interaction, so the alanine residue is incorporated into protein in place of cysteine. *Once a tRNA has been charged, the amino acid plays no further role in its specificity, which is determined exclusively by the anticodon* (421).

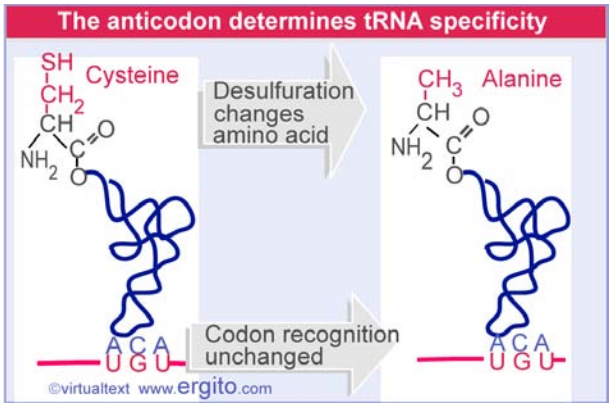

Figure 5.5 The meaning of tRNA is determined by its anticodon and not by its amino acid.

Reviews

42. Soll, D. and RajBhandary, U. L. (1995). . tRNA Structure, Biosynthesis, and Function.

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421. Chapeville, F. et al. (1962). *On the role of soluble RNA in coding for amino acids*. Proc. Natl. Acad. Sci. USA 48, 1086-1092.

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MESSENGER RNA

2.5.4 The acceptor stem and anticodon are at ends of the tertiary structure

Key Concepts

- The clover-leaf forms an L-shaped tertiary structure with the acceptor arm at one end and the anticodon arm at the other end.

The secondary structure of each tRNA folds into a compact L-shaped tertiary structure in which the 3′ end that binds the amino acid is distant from the anticodon that binds the mRNA. All tRNAs have the same general tertiary structure, although they are distinguished by individual variations.

The base paired double-helical stems of the secondary structure are maintained in the tertiary structure, but their arrangement in three dimensions essentially creates two double helices at right angles to each other, as illustrated in **Figure 5.6**. The acceptor stem and the T ψC stem form one continuous double helix with a single gap; the D stem and anticodon stem form another continuous double helix, also with a gap. The region between the double helices, where the turn in the L-shape is made, contains the T ψC loop and the D loop. So the amino acid resides at the extremity of one arm of the L-shape, and the anticodon loop forms the other end.

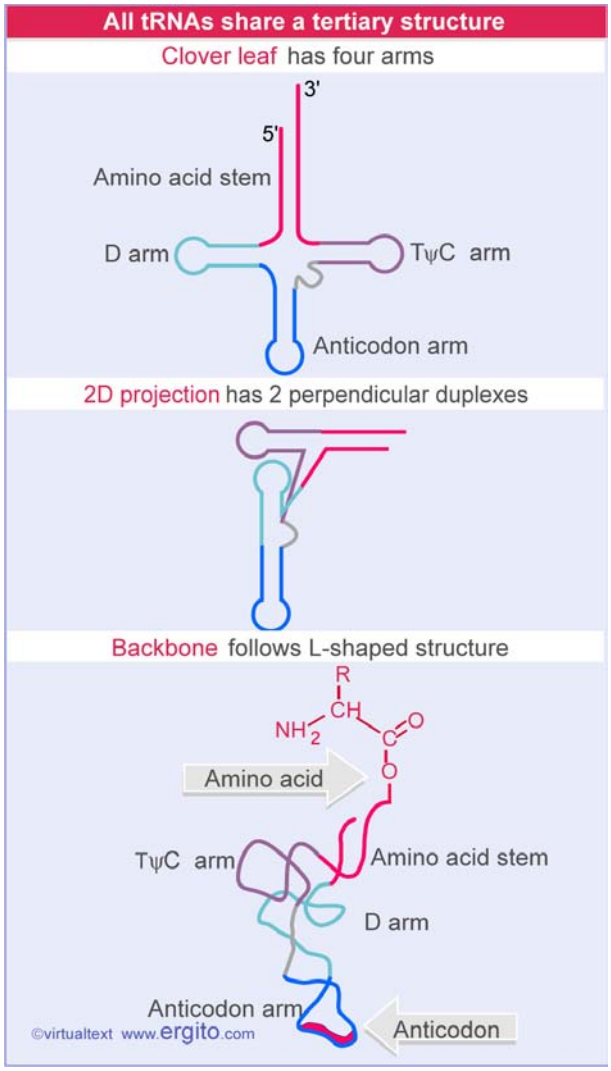

Figure 5.6 Transfer RNA folds into a compact L-shaped tertiary structure with the amino acid at one end and the anticodon at the other end.

The tertiary structure is created by hydrogen bonding, mostly involving bases that are unpaired in the secondary structure. Many of the invariant and semiinvariant bases are involved in these H-bonds, which explains their conservation. Not every one of these interactions is universal, but probably they identify the *general* pattern for establishing tRNA structure.

A molecular model of the structure of yeast $tRNA^{Phe}$ is shown in **Figure 5.7**. The left view corresponds with the bottom panel in **Figure 5.6**. Differences in the structure are found in other tRNAs, thus accommodating the dilemma that all tRNAs must have a similar shape, yet it must be possible to recognize differences between them. For example, in $tRNA^{Asp}$, the angle between the two axes is slightly greater, so the molecule has a slightly more open conformation.

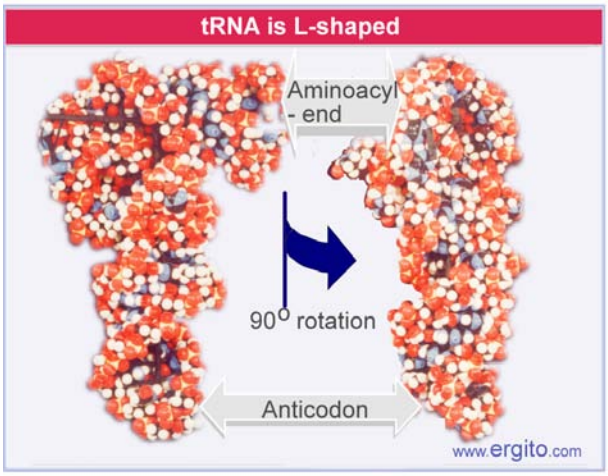

Figure 5.7 A space-filling model shows that $tRNA^{Phe}$ tertiary structure is compact. The two views of tRNA are rotated by 90°. Photograph kindly provided by S. H. Kim.

The structure suggests a general conclusion about the function of tRNA. *Its sites for exercising particular functions are maximally separated.* The amino acid is as far distant from the anticodon as possible, which is consistent with their roles in protein synthesis.

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MESSENGER RNA

2.5.5 Messenger RNA is translated by ribosomes

Key Terms

The **ribosome** is a large assembly of RNA and proteins that synthesizes proteins under direction from an mRNA template. Bacterial ribosomes sediment at 70S, eukaryotic ribosomes at 80S. A ribosome can be dissociated into two subunits.

A **ribonucleoprotein** is a complex of RNA with proteins.

The **large subunit** of the ribosome (50S in bacteria, 60S in eukaryotes) has the peptidyl transferase active site that synthesizes the peptide bond.

The **small subunit** of the ribosome (30S in bacteria, 40S in eukaryotes) binds the mRNA.

Key Concepts

- Ribosomes are characterized by their rate of sedimentation (70S for bacterial ribosomes and 80S for eukaryotic ribosomes).
- A ribosome consists of a large subunit (50S or 60S for bacteria and eukaryotes) and a small subunit (30S or 40S).
- The ribosome provides the environment in which aminoacyl-tRNAs add amino acids to the growing polypeptide chain in response to the corresponding triplet codons.
- A ribosome moves along an mRNA from 5′ to 3′.

Translation of an mRNA into a polypeptide chain is catalyzed by the **ribosome**. Ribosomes are traditionally described in terms of their (approximate) rate of sedimentation (measured in Svedbergs, in which a higher S value indicates a greater rate of sedimentation and a larger mass). Bacterial ribosomes generally sediment at ~70S. The ribosomes of the cytoplasm of higher eukaryotic cells are larger, usually sedimenting at ~80S.

The ribosome is a compact **ribonucleoprotein** particle consisting of two subunits. Each subunit has an RNA component, including one very large RNA molecule, and many proteins. The relationship between a ribosome and its subunits is depicted in **Figure 5.8**. The two subunits dissociate *in vitro* when the concentration of Mg^{2+} ions is reduced. In each case, the **large subunit** is about twice the mass of the **small subunit**. Bacterial (70S) ribosomes have subunits that sediment at 50S and 30S. The subunits of eukaryotic cytoplasmic (80S) ribosomes sediment at 60S and 40S. The two subunits work together as part of the complete ribosome, but each undertakes distinct reactions in protein synthesis.

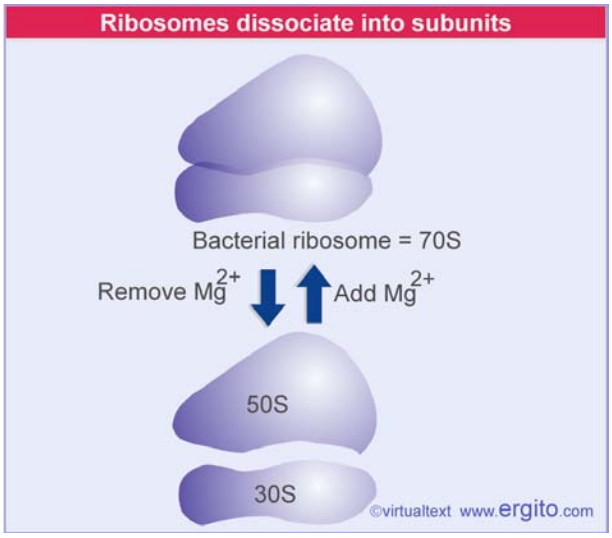

Figure 5.8 A ribosome consists of two subunits.

All the ribosomes of a given cell compartment are identical. *They undertake the synthesis of different proteins by associating with the different mRNAs that provide the actual coding sequences.*

The ribosome provides the environment that controls the recognition between a codon of mRNA and the anticodon of tRNA. Reading the genetic code as a series of adjacent triplets, protein synthesis proceeds from the start of a coding region to the end. *A protein is assembled by the sequential addition of amino acids in the direction from the N-terminus to the C-terminus as a ribosome moves along the mRNA* (419).

A ribosome begins translation at the 5′ end of a coding region; it translates each triplet codon into an amino acid as it proceeds towards the 3′ end. At each codon, the appropriate aminoacyl-tRNA associates with the ribosome, donating its amino acid to the polypeptide chain. At any given moment, the ribosome can accommodate the two aminoacyl-tRNAs corresponding to successive codons, making it possible for a peptide bond to form between the two corresponding amino acids. At each step, the growing polypeptide chain becomes longer by one amino acid.

References

419. Dintzis, H. M. (1961). *Assembly of the peptide chain of hemoglobin*. Proc. Natl. Acad. Sci. USA 47, 247-261.

MESSENGER RNA

2.5.6 Many ribosomes bind to one mRNA

Key Terms

A **polyribosome (Polysome)** is an mRNA that is simultaneously being translated by several ribosomes.

A **nascent protein** has not yet completed its synthesis; the polypeptide chain is still attached to the ribosome via a tRNA.

Key Concepts

- An mRNA is simultaneously translated by several ribosomes. Each ribosome is at a different stage of progression along the mRNA.

When active ribosomes are isolated in the form of the fraction associated with newly synthesized proteins, they are found in the form of a complex consisting of an mRNA associated with several ribosomes. This is the **polyribosome** or **polysome**. The 30S subunit of each ribosome is associated with the mRNA, and the 50S subunit carries the newly synthesized protein. The tRNA spans both subunits.

Each ribosome in the polysome independently synthesizes a single polypeptide during its traverse of the messenger sequence. Essentially the mRNA is pulled through the ribosome, and each triplet nucleotide is translated into an amino acid. So the mRNA has a series of ribosomes that carry increasing lengths of the protein product, moving from the 5′ to the 3′ end, as illustrated in **Figure 5.9**. A polypeptide chain in the process of synthesis is sometimes called a **nascent protein**.

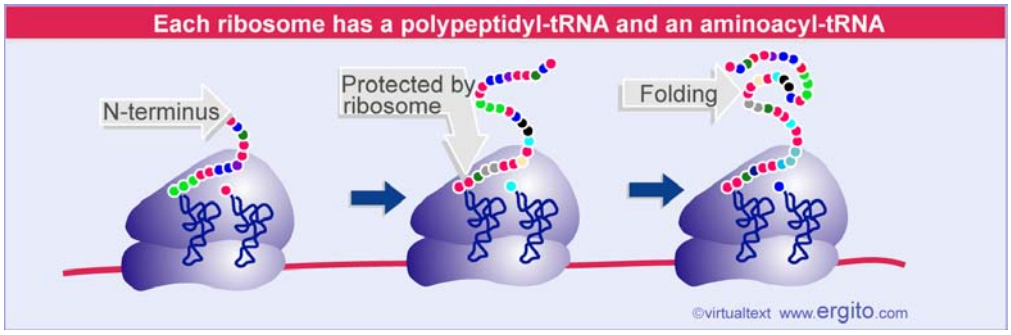

Figure 5.9 A polyribosome consists of an mRNA being translated simultaneously by several ribosomes moving in the direction from 5′-3′. Each ribosome has two tRNA molecules, one carrying the nascent protein, the second carrying the next amino acid to be added.

Roughly the most recent 30-35 amino acids added to a growing polypeptide chain are protected from the environment by the structure of the ribosome. Probably all of the preceding part of the polypeptide protrudes and is free to start folding into its proper conformation. So proteins can display parts of the mature conformation even before synthesis has been completed.

A classic characterization of polysomes is shown in the electron micrograph of **Figure 5.10**. Globin protein is synthesized by a set of 5 ribosomes attached to each mRNA (pentasomes). The ribosomes appear as squashed spherical objects of ~7 nm (70 Å) in diameter, connected by a thread of mRNA. The ribosomes are located at various positions along the messenger. Those at one end have just started protein synthesis; those at the other end are about to complete production of a polypeptide chain (422).

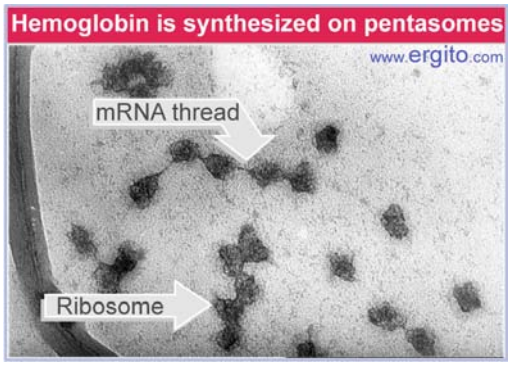

Figure 5.10 Protein synthesis occurs on polysomes. Photograph kindly provided by Alex Rich.

The size of the polysome depends on several variables. In bacteria, it is very large, with tens of ribosomes simultaneously engaged in translation. Partly the size is due to the length of the mRNA (which usually codes for several proteins); partly it is due to the high efficiency with which the ribosomes attach to the mRNA.

Polysomes in the cytoplasm of a eukaryotic cell are likely to be smaller than those in bacteria; again, their size is a function both of the length of the mRNA (usually representing only a single protein in eukaryotes) and of the characteristic frequency with which ribosomes attach. An average eukaryotic mRNA probably has ~8 ribosomes attached at any one time.

Figure 5.11 illustrates the life cycle of the ribosome. Ribosomes are drawn from a pool (actually the pool consists of ribosomal subunits), used to translate an mRNA, and then return to the pool for further cycles. The number of ribosomes on each mRNA molecule synthesizing a particular protein is not precisely determined, in either bacteria or eukaryotes, but is a matter of statistical fluctuation, determined by the variables of mRNA size and efficiency.

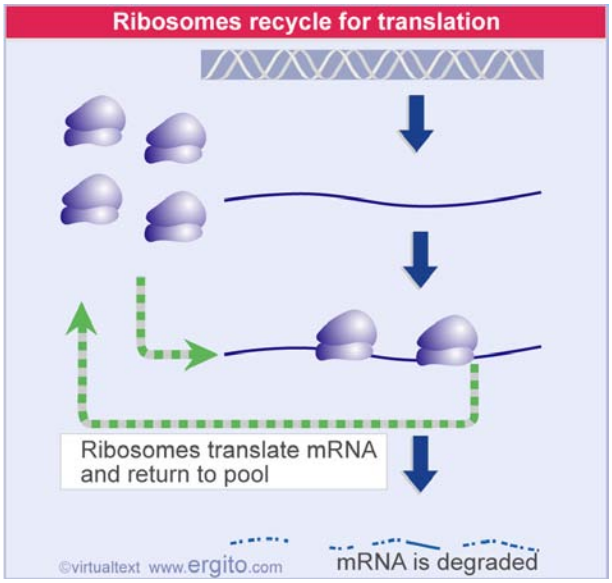

Figure 5.11 Messenger RNA is translated by ribosomes that cycle through a pool.

An overall view of the attention devoted to protein synthesis in the intact bacterium is given in **Figure 5.12**. The 20,000 or so ribosomes account for a quarter of the cell mass. There are >3000 copies of each tRNA, and altogether, the tRNA molecules outnumber the ribosomes by almost tenfold; most of them are present as aminoacyl-tRNAs, that is, ready to be used at once in protein synthesis. Because of their instability, it is difficult to calculate the number of mRNA molecules, but a reasonable guess would be ~1500, in varying states of synthesis and decomposition. There are ~600 different types of mRNA in a bacterium. This suggests that there are usually only 2-3 copies of each mRNA per bacterium. On average, each probably codes for ~3 proteins. If there are 1850 different soluble proteins, there must on average be >1000 copies of each protein in a bacterium.

25% of bacterial dry mass is concerned with gene expression

Component	Dry Cell Mass (%)	Molecules /cell	Different types	Copies of each type
Wall	10	1	1	1
Membrane	10	2	2	1
DNA	1.5	1	1	1
mRNA	1	1,500	600	2-3
tRNA	3	200,000	60	>3,000
rRNA	16	38,000	2	19,000
Ribosomal proteins	9	10^6	52	19,000
Soluble proteins	46	2.0×10^6	1,850	>1,000
Small molecules	3	7.5×10^6	800	

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Figure 5.12 Considering *E. coli* in terms of its macromolecular components.

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MESSENGER RNA

2.5.7 The life cycle of bacterial messenger RNA

Key Terms

Nascent RNA is a ribonucleotide chain that is still being synthesized, so that its 3′ end is paired with DNA where RNA polymerase is elongating.

Monocistronic mRNA codes for one protein.

Polycistronic mRNA includes coding regions representing more than one gene.

A **coding region** is a part of the gene that represents a protein sequence.

The **leader** of a protein is a short N-terminal sequence responsible for initiating passage into or through a membrane.

The **leader (5′ UTR)** of an mRNA is the nontranslated sequence at the 5′ end that precedes the initiation codon.

A **trailer (3′ UTR)** is a nontranslated sequence at the 3′ end of an mRNA following the termination codon.

The **intercistronic region** is the distance between the termination codon of one gene and the initiation codon of the next gene.

Key Concepts

- Transcription and translation occur simultaneously in bacteria, as ribosomes begin translating an mRNA before its synthesis has been completed.
- Bacterial mRNA is unstable and has a half-life of only a few minutes.
- A bacterial mRNA may be polycistronic in having several coding regions that represent different genes.

Messenger RNA has the same function in all cells, but there are important differences in the details of the synthesis and structure of prokaryotic and eukaryotic mRNA.

A major difference in the production of mRNA depends on the locations where transcription and translation occur:

- In bacteria, mRNA is transcribed and translated in the single cellular compartment; and the two processes are so closely linked that they occur simultaneously. Since ribosomes attach to bacterial mRNA even before its transcription has been completed, the polysome is likely still to be attached to DNA. Bacterial mRNA usually is unstable, and is therefore translated into proteins for only a few minutes.
- In a eukaryotic cell, synthesis and maturation of mRNA occur exclusively in the nucleus. Only after these events are completed is the mRNA exported to the

cytoplasm, where it is translated by ribosomes. Eukaryotic mRNA is relatively stable and continues to be translated for several hours.

Figure 5.13 shows that transcription and translation are intimately related in bacteria. Transcription begins when the enzyme RNA polymerase binds to DNA and then moves along making a copy of one strand. As soon as transcription begins, ribosomes attach to the 5′ end of the mRNA and start translation, even before the rest of the message has been synthesized. A bunch of ribosomes moves along the mRNA while it is being synthesized. The 3′ end of the mRNA is generated when transcription terminates. Ribosomes continue to translate the mRNA while it survives, but it is degraded in the overall 5′ → 3′ direction quite rapidly. The mRNA is synthesized, translated by the ribosomes, and degraded, all in rapid succession. An individual molecule of mRNA survives for only a matter of minutes or even less.

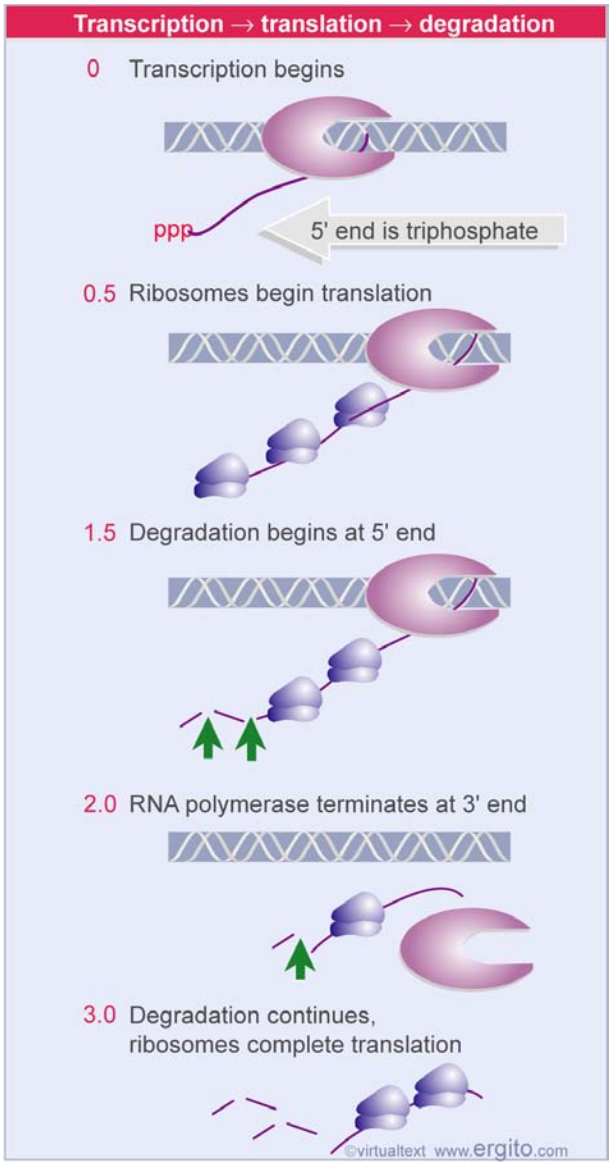

Figure 5.13 Overview: mRNA is transcribed, translated, and degraded simultaneously in bacteria.

Bacterial transcription and translation take place at similar rates. At 37°C, transcription of mRNA occurs at ~40 nucleotides/second. This is very close to the rate of protein synthesis, roughly 15 amino acids/second. It therefore takes ~2 minutes to transcribe and translate an mRNA of 5000 bp, corresponding to 180 kD of protein. When expression of a new gene is initiated, its mRNA typically will appear in the cell within ~2.5 minutes. The corresponding protein will appear within perhaps another 0.5 minute.

Bacterial translation is very efficient, and most mRNAs are translated by a large number of tightly packed ribosomes. In one example (*trp* mRNA), about 15 initiations of transcription occur every minute, and each of the 15 mRNAs probably is translated by ~30 ribosomes in the interval between its transcription and degradation.

The instability of most bacterial mRNAs is striking. Degradation of mRNA closely follows its translation. Probably it begins within 1 minute of the start of transcription. The 5′ end of the mRNA starts to decay before the 3′ end has been synthesized or translated. Degradation seems to follow the last ribosome of the convoy along the mRNA. But degradation proceeds more slowly, probably at about half the speed of transcription or translation.

The stability of mRNA has a major influence on the amount of protein that is produced. It is usually expressed in terms of the half-life. The mRNA representing any particular gene has a characteristic half-life, but the average is ~2 minutes in bacteria.

This series of events is only possible, of course, because transcription, translation, and degradation all occur in the same direction. The dynamics of gene expression have been caught *in flagrante delicto* in the electron micrograph of **Figure 5.14**. In these (unknown) transcription units, several mRNAs are under synthesis simultaneously; and each carries many ribosomes engaged in translation. (This corresponds to the stage shown in the second panel in **Figure 5.13**.) An RNA whose synthesis has not yet been completed is often called a **nascent RNA** (420).

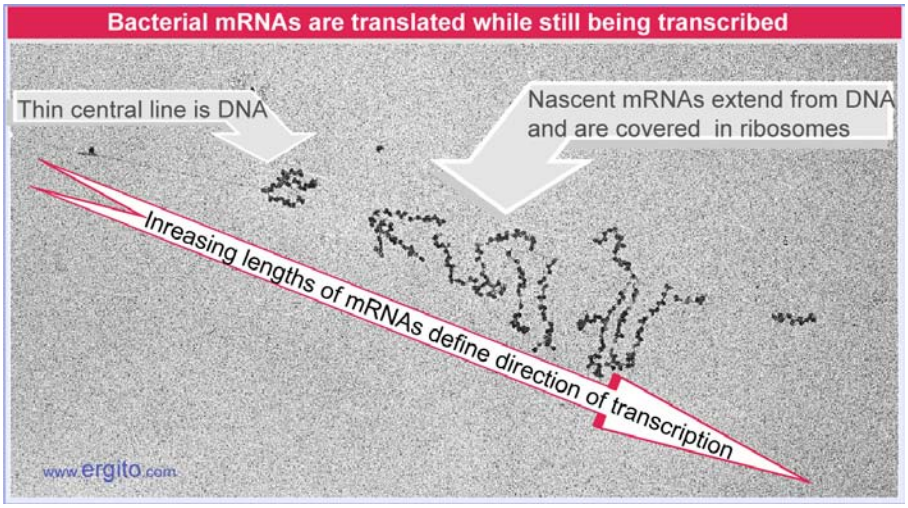

Figure 5.14 Transcription units can be visualized in bacteria. Photograph kindly provided by Oscar Miller.

Bacterial mRNAs vary greatly in the number of proteins for which they code. Some mRNAs represent only a single gene: they are **monocistronic**. Others (the majority) carry sequences coding for several proteins: they are **polycistronic**. In these cases, a single mRNA is transcribed from a group of adjacent genes. (Such a cluster of genes constitutes an operon that is controlled as a single genetic unit; see *Molecular Biology 3.10 The operon*.)

All mRNAs contain two types of region. The **coding region** consists of a series of codons representing the amino acid sequence of the protein, starting (usually) with AUG and ending with a termination codon. But the mRNA is always longer than the coding region, extra regions are present at both ends. An additional sequence at the 5′ end, preceding the start of the coding region, is described as the **leader** or **5′ UTR** (untranslated region). An additional sequence following the termination signal,

forming the 3′ end, is called the **trailer** or **3′ UTR**. Although part of the transcription unit, these sequences are not used to code for protein.

A polycistronic mRNA also contains **intercistronic regions**, as illustrated in **Figure 5.15**. They vary greatly in size. They may be as long as 30 nucleotides in bacterial mRNAs (and even longer in phage RNAs), but they can also be very short, with as few as 1 or 2 nucleotides separating the termination codon for one protein from the initiation codon for the next. In an extreme case, two genes actually overlap, so that the last base of one coding region is also the first base of the next coding region.

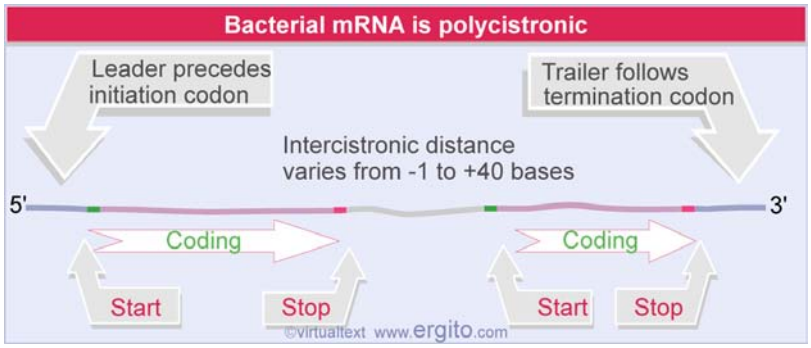

Figure 5.15 Bacterial mRNA includes non-translated as well as translated regions. Each coding region has its own initiation and termination signals. A typical mRNA may have several coding regions.

The number of ribosomes engaged in translating a particular cistron depends on the efficiency of its initiation site. The initiation site for the first cistron becomes available as soon as the 5′ end of the mRNA is synthesized. How are subsequent cistrons translated? Are the several coding regions in a polycistronic mRNA translated independently or is their expression connected? Is the mechanism of initiation the same for all cistrons, or is it different for the first cistron and the internal cistrons?

Translation of a bacterial mRNA proceeds sequentially through its cistrons. At the time when ribosomes attach to the first coding region, the subsequent coding regions have not yet even been transcribed. By the time the second ribosome site is available, translation is well under way through the first cistron. Usually ribosomes terminate translation at the end of the first cistron (and dissociate into subunits), and a new ribosome assembles independently at the start of the next coding region. (We discuss the processes of initiation and termination in *Molecular Biology 2.6 Protein synthesis*.)

References

420. Brenner, S. Jacob, F., and Meselson, M. (1961). *An unstable intermediate carrying information from genes to ribosomes for protein synthesis*. Nature 190, 576-581.

MESSENGER RNA

2.5.8 Eukaryotic mRNA is modified during or after its transcription

Key Terms

Poly(A) is a stretch of ~200 bases of adenylic acid that is added to the 3′ end of mRNA following its synthesis.

Key Concepts

- A eukaryotic mRNA transcript is modified in the nucleus during or shortly after transcription.
- The modifications include the addition of a methylated cap at the 5′ end and a sequence of poly(A) at the 3′ end.
- The mRNA is exported from the nucleus to the cytoplasm only after all modifications have been completed.

The production of eukaryotic mRNA involves additional stages after transcription. Transcription occurs in the usual way, initiating a transcript with a 5′ triphosphate end. However, the 3′ end is generated by cleaving the transcript, rather than by terminating transcription at a fixed site. Those RNAs that are derived from interrupted genes require splicing to remove the introns, generating a smaller mRNA that contains an intact coding sequence.

Figure 5.16 shows that both ends of the transcript are modified by additions of further nucleotides (involving additional enzyme systems). The 5′ end of the RNA is modified by addition of a "cap" virtually as soon as it appears. This replaces the triphosphate of the initial transcript with a nucleotide in reverse (3′ → 5′) orientation, thus "sealing" the end. The 3′ end is modified by addition of a series of adenylic acid nucleotides [polyadenylic acid or **poly(A)**] immediately after its cleavage. Only after the completion of all modification and processing events can the mRNA be exported from the nucleus to the cytoplasm. The average delay in leaving for the cytoplasm is ~20 minutes. Once the mRNA has entered the cytoplasm, it is recognized by ribosomes and translated.

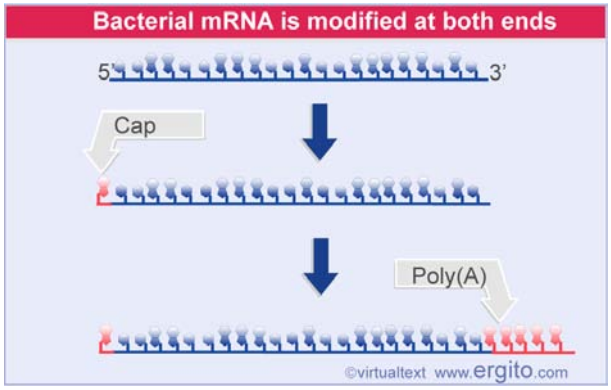

Figure 5.16 Eukaryotic mRNA is modified by addition of a cap to the 5′ end and poly(A) to the 3′ end.

Figure 5.17 shows that the life cycle of eukaryotic mRNA is more protracted than that of bacterial mRNA. Transcription in animal cells occurs at about the same speed as in bacteria, ~40 nucleotides per second. Many eukaryotic genes are large; a gene of 10,000 bp takes ~5 minutes to transcribe. Transcription of mRNA is not terminated by the release of enzyme from the DNA; instead the enzyme continues past the end of the gene. A coordinated series of events generates the 3′ end of the mRNA by cleavage, and adds a length of poly(A) to the newly generated 3′ end.

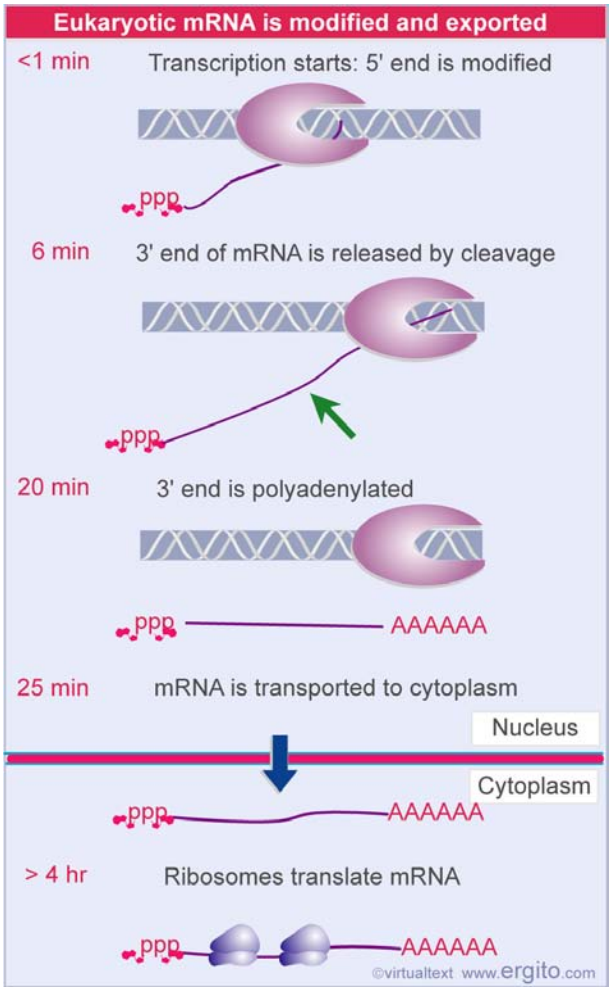

Figure 5.17 Overview: expression of mRNA in animal cells requires transcription, modification, processing, nucleocytoplasmic transport, and translation.

Eukaryotic mRNA constitutes only a small proportion of the total cellular RNA (~3% of the mass). Half-lives are relatively short in yeast, ranging from 1-60 minutes. There is a substantial increase in stability in higher eukaryotes; animal cell mRNA is relatively stable, with half-lives ranging from 4-24 hours.

Eukaryotic polysomes are reasonably stable. The modifications at both ends of the mRNA contribute to the stability.

This content is available online at http://www.ergito.com/main.jsp?bcs=MBIO.2.5.8

MESSENGER RNA

2.5.9 The 5′ end of eukaryotic mRNA is capped

Key Terms

A **cap** is the structure at the 5′ end of eukaryotic mRNA, introduced after transcription by linking the terminal phosphate of 5′ GTP to the terminal base of the mRNA. The added G (and sometimes some other bases) are methylated, giving a structure of the form ^{7}MeG5′ ppp5′ Np . . .

A **cap 0** at the 5′ end of mRNA has only a methyl group on 7-guanine.

A **cap 1** at the 5′ end of mRNA has methyl groups on the terminal 7-guanine and the 2′-O position of the next base.

A **cap 2** has three methyl groups (7-guanine, 2′-O position of next base, and N^6 adenine) at the 5′ end of mRNA.

Key Concepts

- A 5′ cap is formed by adding a G to the terminal base of the transcript via a 5′–5′ link. 1-3 methyl groups are added to the base or ribose of the new terminal guanosine.

Transcription starts with a nucleoside triphosphate (usually a purine, A or G). The first nucleotide retains its 5′ triphosphate group and makes the usual phosphodiester bond from its 3′ position to the 5′ position of the next nucleotide. The initial sequence of the transcript can be represented as:

5′ ppp$^{A}/_{G}$pNpNpNp...

But when the mature mRNA is treated *in vitro* with enzymes that should degrade it into individual nucleotides, the 5′ end does not give rise to the expected nucleoside triphosphate. Instead it contains two nucleotides, connected by a 5′–5′ triphosphate linkage and also bearing methyl groups. The terminal base is always a guanine that is added to the original RNA molecule after transcription.

Addition of the 5′ terminal G is catalyzed by a nuclear enzyme, guanylyl transferase. The reaction occurs so soon after transcription has started that it is not possible to detect more than trace amounts of the original 5′ triphosphate end in the nuclear RNA. The overall reaction can be represented as a condensation between GTP and the original 5′ triphosphate terminus of the RNA. Thus

5′ 5′

Gppp + pppApNpNp...

↓

5′—5′

GpppApNpNp... + pp + p

The new G residue added to the end of the RNA is in the reverse orientation from all the other nucleotides.

This structure is called a **cap**. It is a substrate for several methylation events. **Figure 5.18** shows the full structure of a cap after all possible methyl groups have been added. Types of caps are distinguished by how many of these methylations have occurred:

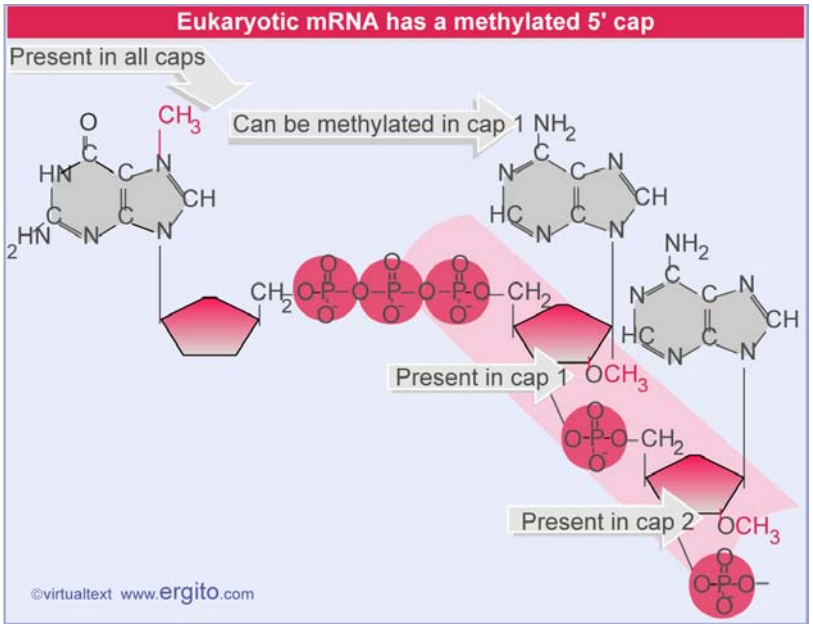

Figure 5.18 The cap blocks the 5′ end of mRNA and may be methylated at several positions.

- The first methylation occurs in all eukaryotes, and consists of the addition of a methyl group to the 7 position of the terminal guanine. A cap that possesses this single methyl group is known as a **cap 0**. This is as far as the reaction proceeds in unicellular eukaryotes. The enzyme responsible for this modification is called guanine-7-methyltransferase.

- The next step is to add another methyl group, to the 2′–O position of the penultimate base (which was actually the original first base of the transcript before any modifications were made). This reaction is catalyzed by another enzyme (2′–O-methyl-transferase). A cap with the two methyl groups is called **cap 1**. This is the predominant type of cap in all eukaryotes except unicellular organisms.

- In a small minority of cases in higher eukaryotes, another methyl group is added to the second base. This happens only when the position is occupied by adenine; the reaction involves addition of a methyl group at the N^6 position. The enzyme

responsible acts only on an adenosine substrate that already has the methyl group in the 2′–O position.

- In some species, a methyl group is added to the third base of the capped mRNA. The substrate for this reaction is the cap 1 mRNA that already possesses two methyl groups. The third-base modification is always a 2′–O ribose methylation. This creates the **cap 2** type. This cap usually represents less than 10-15% of the total capped population.

In a population of eukaryotic mRNAs, every molecule is capped. The proportions of the different types of cap are characteristic for a particular organism. We do not know whether the structure of a particular mRNA is invariant or can have more than one type of cap.

In addition to the methylation involved in capping, a low frequency of internal methylation occurs in the mRNA only of higher eukaryotes. This is accomplished by the generation of N^6 methyladenine residues at a frequency of about one modification per 1000 bases. There are 1-2 methyladenines in a typical higher eukaryotic mRNA, although their presence is not obligatory, since some mRNAs do not have any (for review see 25).

Reviews

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MESSENGER RNA

2.5.10 The 3′ terminus is polyadenylated

Key Terms

poly(A)$^+$ mRNA is mRNA that has a 3′ terminal stretch of poly(A).

Poly(A) polymerase is the enzyme that adds the stretch of polyadenylic acid to the 3′ of eukaryotic mRNA. It does not use a template.

Poly(A)-binding protein (PABP) is the protein that binds to the 3′ stretch of poly(A) on a eukaryotic mRNA.

cDNA is a single-stranded DNA complementary to an RNA, synthesized from it by reverse transcription *in vitro*.

poly(A)$^-$ mRNA is mRNA that has does not have a 3′ terminal stretch of poly(A).

Key Concepts

- A length of poly(A) ~200 nucleotides long is added to a nuclear transcript after transcription.
- The poly(A) is bound by a specific protein (PABP).
- The poly(A) stabilizes the mRNA against degradation.

The 3′ terminal stretch of A residues is often described as the poly(A) tail; and mRNA with this feature is denoted **poly(A)$^+$**.

The poly(A) sequence is not coded in the DNA, but is added to the RNA in the nucleus after transcription. The addition of poly(A) is catalyzed by the enzyme **poly(A) polymerase**, which adds ~200 A residues to the free 3′-OH end of the mRNA. The poly(A) tract of both nuclear RNA and mRNA is associated with a protein, the **poly(A)-binding protein** (**PABP**). Related forms of this protein are found in many eukaryotes. One PABP monomer of ~70 kD is bound every 10-20 bases of the poly(A) tail. So a common feature in many or most eukaryotes is that the 3′ end of the mRNA consists of a stretch of poly(A) bound to a large mass of protein. Addition of poly(A) occurs as part of a reaction in which the 3′ end of the mRNA is generated and modified by a complex of enzymes (see *Molecular Biology 5.24.19 The 3′ ends of mRNAs are generated by cleavage and polyadenylation*) (424).

Binding of the PABP to the initiation factor eIF4G generates a closed loop, in which the 5′ and 3′ ends of the mRNA find themselves held in the same protein complex (see **Figure 6.20** in *Molecular Biology 2.6.9 Eukaryotes use a complex of many initiation factors*). The formation of this complex may be responsible for some of the effects of poly(A) on the properties of mRNA. Poly(A) usually stabilizes mRNA. The ability of the poly(A) to protect mRNA against degradation requires binding of the PABP.

Removal of poly(A) inhibits the initiation of translation *in vitro*, and depletion of PABP has the same effect in yeast *in vivo*. These effects could depend on the binding of PABP to the initiation complex at the 5′ end of mRNA. There are many examples in early embryonic development where polyadenylation of a particular mRNA is correlated with its translation. In some cases, mRNAs are stored in a nonpolyadenylated form, and poly(A) is added when their translation is required; in other cases, poly(A)$^+$ mRNAs are de-adenylated, and their translation is reduced.

The presence of poly(A) has an important practical consequence. The poly(A) region of mRNA can base pair with oligo(U) or oligo(dT); and this reaction can be used to isolate poly(A)$^+$ mRNA. The most convenient technique is to immobilize the oligo(U or dT) on a solid support material. Then when an RNA population is applied to the column, as illustrated in **Figure 5.19**, only the poly(A)$^+$ RNA is retained. It can be retrieved by treating the column with a solution that breaks the bonding to release the RNA.

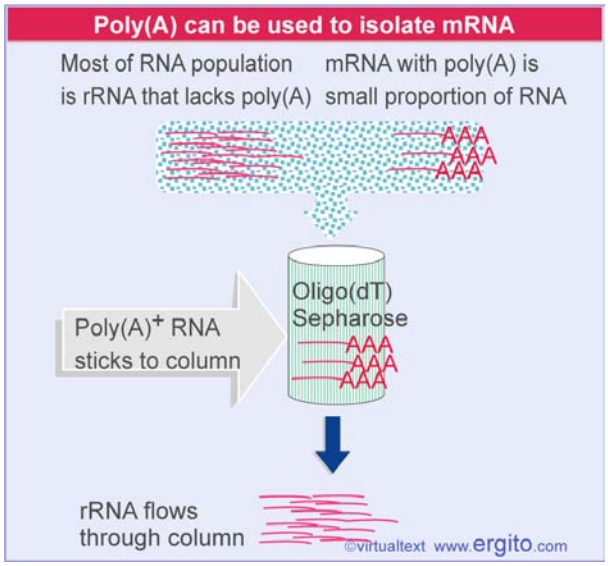

Figure 5.19 Poly(A)$^+$ RNA can be separated from other RNAs by fractionation on Sepharose-oligo(dT).

The only drawback to this procedure is that it isolates all the RNA that contains poly(A). If RNA of the whole cell is used, for example, both nuclear and cytoplasmic poly(A)$^+$ RNA will be retained. If preparations of polysomes are used (a common procedure), most of the isolated poly(A)$^+$ RNA will be active mRNA. However, in addition to mRNA in polysomes, there are also ribonucleoprotein particles in the cytosol that contain poly(A)$^+$ mRNA, but which are not translated. This RNA may be "stored" for use at some other time. Isolation of total poly(A)$^+$ mRNA therefore does not correspond exactly with the active mRNA population.

The "cloning" approach for purifying mRNA uses a procedure in which the mRNA is copied to make a complementary DNA strand (known as **cDNA**). Then the cDNA can be used as a template to synthesize a DNA strand that is identical with the original mRNA sequence. The product of these reactions is a double-stranded DNA corresponding to the sequence of the mRNA. This DNA can be reproduced in large amounts.

The availability of a cloned DNA makes it easy to isolate the corresponding mRNA by hybridization techniques. Even mRNAs that are present in only very few copies per cell can be isolated by this approach. Indeed, only mRNAs that are present in relatively large amounts can be isolated directly without using a cloning step.

Almost all cellular mRNAs possess poly(A). A significant exception is provided by the mRNAs that code for the histone proteins (a major structural component of chromosomal material). These mRNAs comprise most or all of the **poly(A)$^-$** fraction. The significance of the absence of poly(A) from histone mRNAs is not clear, and there is no particular aspect of their function for which this appears to be necessary (for review see 26).

Last updated on 1-22-2002

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MESSENGER RNA

2.5.11 Bacterial mRNA degradation involves multiple enzymes

Key Terms

The **degradosome** is a complex of bacterial enzymes, including RNAase and helicase activities, which may be involved in degrading mRNA.

Key Concepts

- The overall direction of degradation of bacterial mRNA is 5′–3′.
- Degradation results from the combination of exonucleolytic cleavages followed by endonucleolytic degradation of the fragment from 3′–5′.

Bacterial mRNA is constantly degraded by a combination of endonucleases and exonucleases (for review see 972). Endonucleases cleave an RNA at an internal site. Exonucleases are involved in trimming reactions in which the extra residues are whittled away, base by base from the end. Bacterial exonucleases that act on single-stranded RNA proceed along the nucleic acid chain from the 3′ end.

The way the two types of enzymes work together to degrade an mRNA is shown in **Figure 5.20**. Degradation of a bacterial mRNA is initiated by an endonucleolytic attack. Several 3′ ends may be generated by endonucleolytic cleavages within the mRNA. The overall direction of degradation (as measured by loss of ability to synthesize proteins) is 5′–3′. This probably results from a succession of endonucleolytic cleavages following the last ribosome. Degradation of the released fragments of mRNA into nucleotides then proceeds by exonucleolytic attack from the free 3′–OH end toward the 5′ terminus (that is, in the opposite direction from transcription). Endonucleolytic attack releases fragments that may have different susceptibilities to exonucleases. A region of secondary structure within the mRNA may provide an obstacle to the exonuclease, thus protecting the regions on its 5′ side. The stability of each mRNA is therefore determined by the susceptibility of its particular sequence to both endo- and exonucleolytic cleavages.

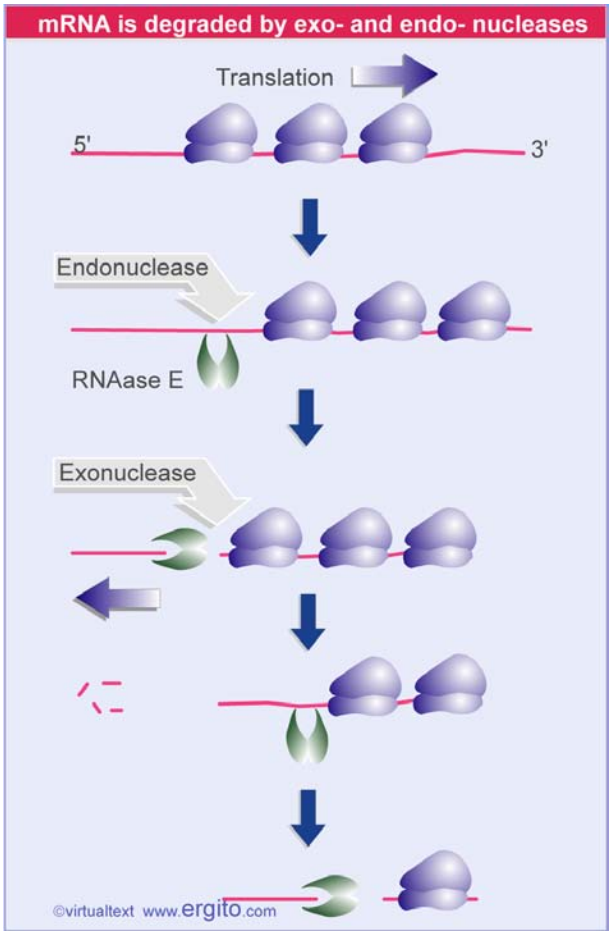

Figure 5.20 Degradation of bacterial mRNA is a two-stage process. Endonucleolytic cleavages proceed 5′-3′ behind the ribosomes. The released fragments are degraded by exonucleases that move 3′-5′.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.5.11 to view properly.

There are ~12 ribonucleases in *E. coli*. Mutants in the endoribonucleases (except ribonuclease I, which is without effect) accumulate unprocessed precursors to rRNA and tRNA, but are viable. Mutants in the exonucleases often have apparently unaltered phenotypes, which suggests that one enzyme can substitute for the absence of another. Mutants lacking multiple enzymes sometimes are inviable (for review see 29).

RNAase E is the key enzyme in initiating cleavage of mRNA. It may be the enzyme that makes the first cleavage for many mRNAs. Bacterial mutants that have a defective ribonuclease E have increased stability (2-3 fold) of mRNA. However, this is not its only function. RNAase E was originally discovered as the enzyme that is responsible for processing 5′ rRNA from the primary transcript by a specific endonucleolytic processing event.

The process of degradation may be catalyzed by a multienzyme complex (sometimes

called the **degradosome**) that includes ribonuclease E, PNPase, and a helicase (970). RNAase E plays dual roles. Its N-terminal domain provides an endonuclease activity. The C-terminal domain provides a scaffold that holds together the other components (971). The helicase unwinds the substrate RNA to make it available to PNPase. According to this model, RNAase E makes the initial cut and then passes the fragments to the other components of the complex for processing.

Polyadenylation may play a role in initiating degradation of some mRNAs in bacteria. Poly(A) polymerase is associated with ribosomes in *E. coli*, and short (10-40 nucleotide) stretches of poly(A) are added to at least some mRNAs. Triple mutations that remove poly(A) polymerase, ribonuclease E, and polynucleotide phosphorylase (PNPase is a 3′–5′ exonuclease) have a strong effect on stability. (Mutations in individual genes or pairs of genes have only a weak effect.) Poly(A) polymerase may create a poly(A) tail that acts as a binding site for the nucleases. The role of poly(A) in bacteria would therefore be different from that in eukaryotic cells (425).

Reviews

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MESSENGER RNA

2.5.12 mRNA stability depends on its structure and sequence

Key Concepts

- The modifications at both ends of mRNA protect it against degradation by exonucleases.
- Specific sequences within an mRNA may have stabilizing or destabilizing effects.
- Destabilization may be triggered by loss of poly(A)

The major features of mRNA that affect its stability are summarized in **Figure 5.21**. Both structure and sequence are important. The 5′ and 3′ terminal structures protect against degradation, and specific sequences within the mRNA may either serve as targets to trigger degradation or may protect against degradation:

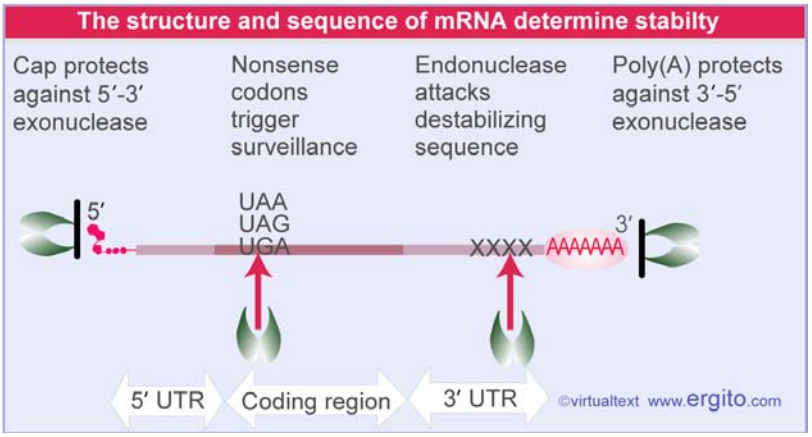

Figure 5.21 The terminal modifications of mRNA protect it against degradation. Internal sequences may activate degradation systems.

- The modifications at the 5′ and 3′ ends of mRNA play an important role in preventing exonuclease attack. The cap prevents 5′–3′ exonucleases from attacking the 5′ end, and the poly(A) prevents 3′–5′ exonucleases from attacking the 3′ end.
- Specific sequence elements within the mRNA may stabilize or destabilize it. The most common location for destabilizing elements is within the 3′ untranslated region. The presence of such an element shortens the lifetime of the mRNA.
- Within the coding region, mutations that create termination codons trigger a surveillance system that degrades the mRNA (see *Molecular Biology 2.5.14 Nonsense mutations trigger a surveillance system*).

Destabilizing elements have been found in several yeast mRNAs, although as yet we do not see any common sequences or know how they destabilize the mRNA. They do not necessarily act directly (by providing targets for endonucleases), but may function indirectly, perhaps by encouraging deadenylation. The criterion for defining a destabilizing sequence element is that its introduction into a new mRNA may cause it to be degraded. The removal of an element from an mRNA does not necessarily stabilize it, suggesting that an individual mRNA can have more than one destabilizing element.

A common feature in some unstable mRNAs is the presence of an AU-rich sequence of ~50 bases (called the ARE) that is found in the 3′ trailer region. The consensus sequence in the ARE is the pentanucleotide AUUUA, repeated several times. **Figure 5.22** shows that the ARE triggers destabilization by a two stage process: first the mRNA is deadenylated; then it decays. The deadenylation is probably needed because it causes loss of the poly(A)-binding protein, whose presence stabilizes the 3′ region (see *Molecular Biology 2.5.13 mRNA degradation involves multiple activities*).

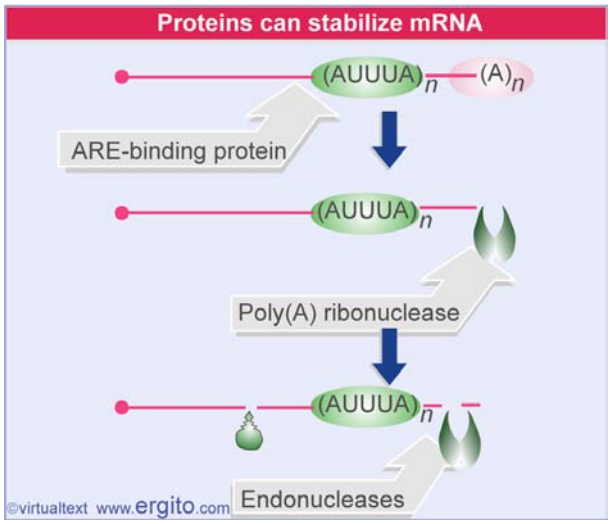

Figure 5.22 An ARE in a 3′ nontranslated region initiates degradation of mRNA.

In some cases, an mRNA can be stabilized by specifically inhibiting the function of a destabilizing element. Transferrin mRNA contains a sequence called the IRE, which controls the response of the mRNA to changes in iron concentration. The IRE is located in the 3′ nontranslated region, and contains stem-loop structures that bind a protein whose affinity for the mRNA is controlled by iron. **Figure 5.23** shows that binding of the protein to the IRE stabilizes the mRNA by inhibiting the function of (unidentified) destabilizing sequences in the vicinity. This is a general model for the stabilization of mRNA, that is, stability is conferred by inhibiting the function of destabilizing sequences (for review see 27; 28).

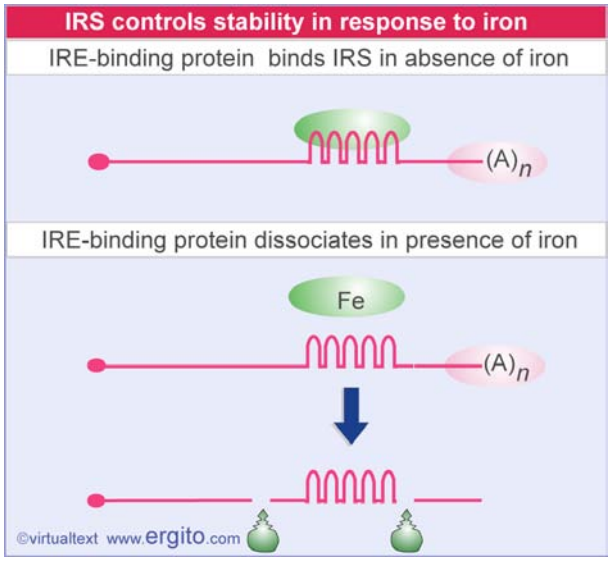

Figure 5.23 An IRE in a 3′ nontranslated region controls mRNA stability.

Last updated on 8-1-2001

Reviews

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MESSENGER RNA

2.5.13 mRNA degradation involves multiple activities

Key Concepts

- Degradation of yeast mRNA requires removal of the 5′ cap and the 3′ poly(A).
- One yeast pathway involves exonucleolytic degradation from 5′–3′.
- Another yeast pathway uses a complex of several exonucleases that work in the 3′–5′ direction.
- The deadenylase of animal cells may bind directly to the 5′ cap.

We know most about the degradation of mRNA in yeast. There are basically two pathways. Both start with removal of the poly(A) tail (for review see 1934). This is catalyzed by a specific deadenylase which probably functions as part of a large protein complex (1933). (The catalytic subunit is the exonuclease Ccr4 in yeast, and is the exonuclease PARN in vertebrates, which is related to RNAase D.) The enzyme action is processive—once it has started to degrade a particular mRNA substrate, it continues to whittle away that mRNA, base by base.

The major degradation pathway is summarized in **Figure 5.24**. Deadenylation at the 3′ end triggers decapping at the 5′ end. The basis for this relationship is that the presence of the PABP (poly(A)-binding protein) on the poly(A) prevents the decapping enzyme from binding to the 5′ end. PABP is released when the length of poly(A) falls below 10-15 residues. The decapping reaction occurs by cleavage 1-2 bases from the 5′ end.

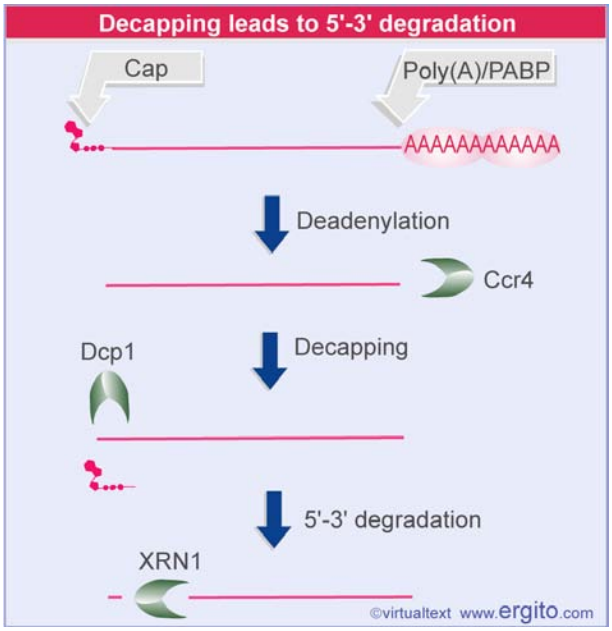

Figure 5.24 Deadenylation allows decapping to occur, which leads to endonucleolytic cleavage from the 5′ end.
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.5.13 to view properly.

Each end of the mRNA influences events that occur at the other end. This is explained by the fact that the two ends of the mRNA are held together by the factors involved in protein synthesis (see *Molecular Biology 2.6.9 Eukaryotes use a complex of many initiation factors*). The effect of PABP on decapping allows the 3′ end to have an effect in stabilizing the 5′ end. There is also a connection between the structure at the 5′ end and degradation at the 3′ end. The deadenylase directly binds to the 5′ cap, and this interaction is in fact needed for its exonucleolytic attack on the poly(A) (1435).

What is the rationale for the connection between events occurring at both ends of an mRNA? Perhaps it is necessary to ensure that the mRNA is not left in a state (having the structure of one end but not the other) that might compete with active mRNA for the proteins that bind to the ends.

Removal of the cap triggers the 5′–3′ degradation pathway in which the mRNA is degraded rapidly from the 5′ end, by the 5′–3′ exonuclease XRN1 (1935). The decapping enzyme is concentrated in discrete cytoplasmic foci, which may be "processing bodies" where the mRNA is deadenylated and then degraded after it has been decapped (3999).

In the second pathway, deadenylated yeast mRNAs can be degraded by the 3′–5′ exonuclease activity of the exosome , a complex of >9 exonucleases (426, 1936). The exosome is also involved in processing precursors for rRNAs. The aggregation of the individual exonucleases into the exosome complex may enable 3′–5′ exonucleolytic activities to be coordinately controlled. The exosome may also degrade fragments of mRNA released by endonucleolytic cleavage. **Figure 5.25**

shows that the 3′–5′ degradation pathway may actually involve combinations of endonucleolytic and exonucleolytic action. The exosome is also found in the nucleus, where it degrades unspliced precursors to mRNA (2190).

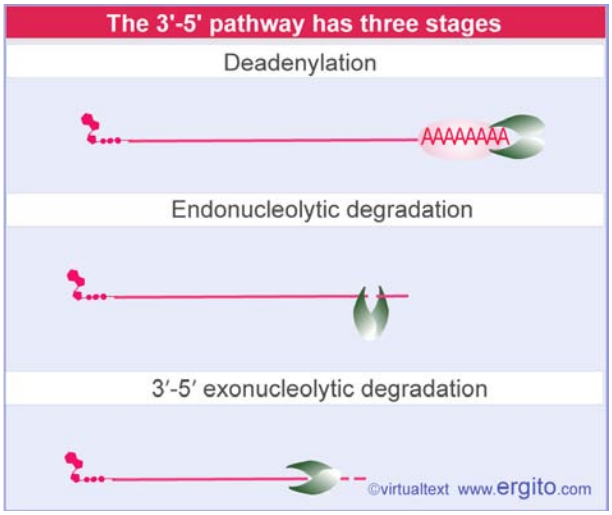

Figure 5.25 Deadenylation may lead directly to exonucleolytic cleavage and endonucleolytic cleavage from 3′ end(s).
This is a static version of an interactive figure; see http://www.ergito.com/main.jsp?bcs=MBIO.2.5.13 to view properly.

Yeast mutants lacking either exonucleolytic pathway degrade their mRNAs more slowly, but the loss of both pathways is lethal (426; for review see 30).

Last updated on 7-21-2003

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MESSENGER RNA

2.5.14 Nonsense mutations trigger a surveillance system

Key Terms

Nonsense-mediated mRNA decay is a pathway that degrades an mRNA that has a nonsense mutation prior to the last exon.

Surveillance systems check nucleic acids for errors. The term is used in several different contexts. One example is the system that degrades mRNAs that have nonsense mutations. Another is the set of systems that react to damage in the double helix. The common feature is that the system recognizes an invalid sequence or structure and triggers a response.

Key Concepts

- Nonsense mutations cause mRNA to be degraded.
- Genes coding for the degradation system have been found in yeast and worm.

Another pathway for degradation is identified by **nonsense-mediated mRNA decay**. **Figure 5.26** shows that the introduction of a nonsense mutation often leads to increased degradation of the mRNA. As may be expected from dependence on a termination codon, the degradation occurs in the cytoplasm. It may represent a quality control or **surveillance** system for removing nonfunctional mRNAs (for review see 973).

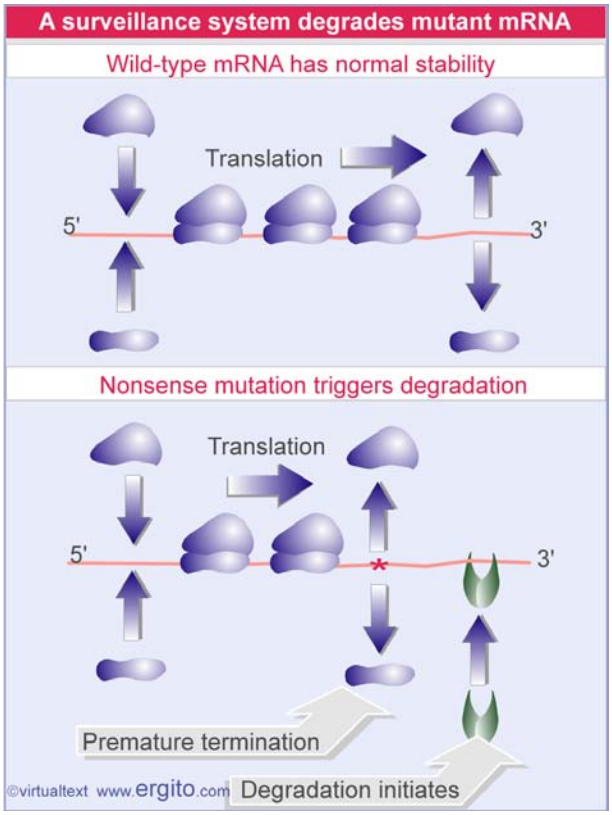

Figure 5.26 Nonsense mutations may cause mRNA to be degraded.

The surveillance system has been studied best in yeast and *C. elegans*, but may also be important in animal cells. For example, during the formation of immunoglobulins and T cell receptors in cells of the immune system, genes are modified by somatic recombination and mutation (see *Molecular Biology 5.25 Immune diversity*). This generates a significant number of nonfunctional genes, whose RNA products are disposed of by a surveillance system.

In yeast, the degradation requires sequence elements (called *DSE*) that are downstream of the nonsense mutation (974; 975). The simplest possibility would be that these are destabilizing elements, and that translation suppresses their use. However, when translation is blocked, the mRNA is stabilized. This suggests that the process of degradation is linked to translation of the mRNA, or to the termination event in some direct way.

Genes that are required for the process have been identified in *S. cerevisiae* (*upf* loci) and *C. elegans* (*smg* loci) by identifying suppressors of nonsense-mediated degradation (976; 977). Mutations in these genes stabilize aberrant mRNAs, but do not affect the stability of most wild-type transcripts. One of these genes is conserved in eukaryotes (*upf1/smg2*). It codes for an ATP-dependent helicase (an enzyme that unwinds double-stranded nucleic acids into single strands). This implies that recognition of the mRNA as an appropriate target for degradation requires a change in its structure (978; 979).

Upf1 interacts with the release factors (eRF1 and eRF3) that catalyze termination, which is probably how it recognizes the termination event (2232). It may then "scan" the mRNA by moving toward the 3′ end to look for the downstream sequence elements.

In mammalian cells, the surveillance system appears to work only on mutations located prior to the last exon – in other words, there must be an intron after the site of mutation. This suggests that the system requires some event to occur in the nucleus, before the introns are removed by splicing. One possibility is that proteins attach to the mRNA in the nucleus at the exon-exon boundary when a splicing event occurs (1906). **Figure 5.27** shows a general model for the operation of such a system. This is similar to the way in which an mRNA may be marked for export from the nucleus (see *Molecular Biology 5.24.10 Splicing is connected to export of mRNA*). Attachment of a protein to the exon-exon junction creates a mark of the event that persists into the cytoplasm. Human homologues of the yeast Upf2,3 proteins may be involved in such a system (1907). They bind specifically to mRNA that has been spliced.

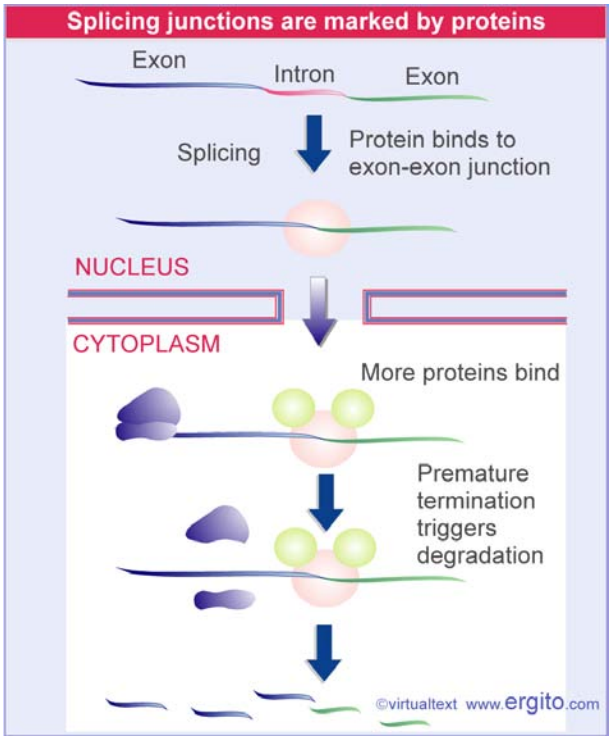

Figure 5.27 A surveillance system could have two types of components. Protein(s) must bind in the nucleus to mark the result of a splicing event. Other proteins could bind to the mark either in the nucleus or cytoplasm. They are triggered to act to degrade the mRNA when ribosomes terminate prematurely.

Last updated on 12-18-2001

Reviews

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MESSENGER RNA

2.5.15 Eukaryotic RNAs are transported

Key Concepts

- RNA is transported through a membrane as a ribonucleoprotein particle.
- All eukaryotic RNAs that function in the cytoplasm must be exported from the nucleus.
- tRNAs and the RNA component of a ribonuclease are imported into mitochondria.
- mRNAs can travel long distances between plant cells.

A bacterium consists of only a single compartment, so all the RNAs function in the same environment in which they are synthesized. This is most striking in the case of mRNA, where translation occurs simultaneously with transcription (see *Molecular Biology 2.5.7 The life cycle of bacterial messenger RNA*).

RNA is transported through membranes in the variety of instances summarized in **Figure 5.28**. It poses a significant thermodynamic problem to transport a highly negative RNA through a hydrophobic membrane, and the solution is to transport the RNA packaged with proteins.

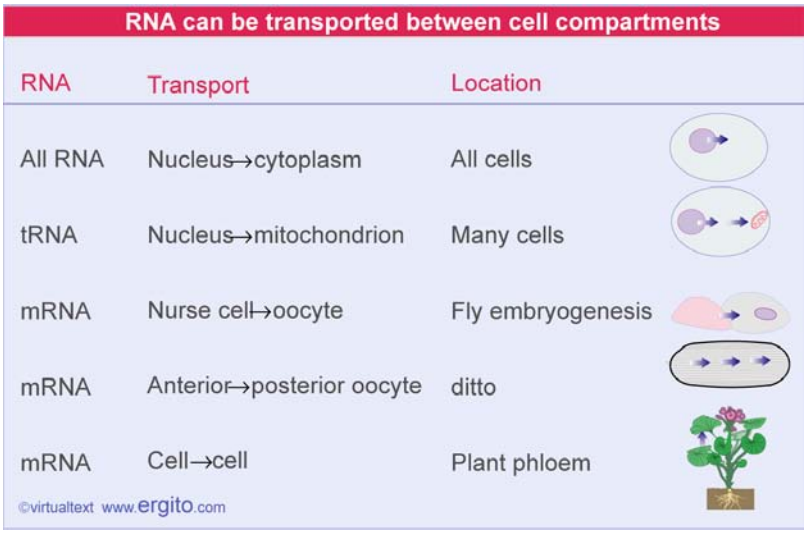

Figure 5.28 RNAs are transported through membranes in a variety of systems.

In eukaryotic cells, RNAs are transcribed in the nucleus, but translation occurs in the cytoplasm. Each type of RNA must be transported into the cytoplasm to assemble the apparatus for translation. The rRNA assembles with ribosomal proteins into immature ribosome subunits that are the substrates for the transport system. tRNA is transported by a specific protein system (see *Molecular Biology 2.8.28 Transport*

receptors carry cargo proteins through the pore). mRNA is transported as a ribonucleoprotein, which forms on the RNA transcript in the nucleus (see *Molecular Biology 5.24 RNA splicing and processing*). These processes are common to all eukaryotic cells. Many mRNAs are translated in the cytosol, but some are localized within the cell, by means of attachment to a cytoskeletal element. One situation in which localization occurs is when it is important for a protein product to be produced near to the site of its incorporation into some macromolecular structure (1921).

Some RNAs are made in the nucleus, exported to the cytosol, and then imported into mitochondria. The mitochondria of some organisms do not code for all of the tRNAs that are required for protein synthesis (see *Molecular Biology 1.3.20 Organelle genomes are circular DNAs that code for organelle proteins*). In these cases, the additional tRNAs must be imported from the cytosol. The enzyme ribonuclease P, which contains both RNA and protein subunits, is coded by nuclear genes, but is found in mitochondria as well as the nucleus (1915). This means that the RNA must be imported into the mitochondria.

We know of some situations in which mRNA is even transported between cells. During development of the oocyte in Drosophila, certain mRNAs are transported into the egg from the nurse cells that surround it. The nurse cells have specialized junctions with the oocyte that allow passage of material needed for early development. This material includes certain mRNAs. Once in the egg, these mRNAs take up specific locations. Some simply diffuse from the anterior end where they enter, but others are transported the full length of the egg to the posterior end by a motor attached to microtubules (see *Molecular Biology 6.31.7 How are mRNAs and proteins transported and localized?*).

The most striking case of transport of mRNA has been found in plants. Movement of individual nucleic acids over long distances was first discovered in plants, where viral movement proteins help propagate the viral infection by transporting an RNA virus genome through the plasmodesmata (connections between cells) (see 1919; 1920). Plants also have a defense system, that causes cells to silence an infecting virus, and this too may involve the spread of components including RNA over long distance between cells (see 1918). Now it has turned out that similar systems may transport mRNAs between plant cells. Although the existence of the systems has been known for some time, it is only recently that their functional importance has been demonstrated (1916). This was shown by grafting wild-type tomato plants onto plants that had the dominant mutation *Me* (which causes a change in the shape of the leaf). mRNA from the mutant stock was transported into the leaves of the wild-type graft, where it changed their shape.

Last updated on 7-23-2001

Reviews

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MESSENGER RNA

2.5.16 mRNA can be specifically localized

Key Concepts

- Yeast Ash1 mRNA forms a ribonucleoprotein that binds to a myosin motor.
- A motor transports it along actin filaments into the daughter bud.
- It is anchored and translated in the bud, so that the protein is found only in the bud.

An mRNA is synthesized in the nucleus but translated in the cytoplasm of a eukaryotic cell. It passes into the cytoplasm in the form of a ribonucleoprotein particle that is transported through the nuclear pore. Once in the cytosol, it may associate with ribosomes and be translated. The cytosol is a crowded place, occupied by a high concentration of proteins. It is not clear how freely a polysome can diffuse within the cytosol, and most mRNAs are probably translated in random locations, determined by their point of entry into the cytosol, and the distance that they may have moved away from it. However, some mRNAs are translated at specific sites. This may be accomplished by several mechanisms (for review see 2304; 2423):

- An mRNA may be specifically transported to a site where it is translated.
- It may be universally distributed but degraded at all sites except the site of translation.
- It may be freely diffusible but become trapped at the site of translation.

One of the best characterized cases of localization within a cell is that of Ash1 in yeast (for review see 2302). Ash1 represses expression of the HO endonuclease in the budding daughter cell, with the result that HO is expressed only in the mother cell. The consequence is that mating type is changed only in the mother cell (see *Molecular Biology 4.18.9 Regulation of HO expression controls switching*). The cause of the restriction to the daughter cell is that all the Ash1 mRNA is transported from the mother cell, where it is made, into the budding daughter cell (2348).

Mutations in any one of 5 genes, called *SHE1-5*, prevent the specific localization and cause Ash1 mRNA to be symmetrically distributed in both mother and daughter compartments. The proteins She1,2,3 bind Ash1 mRNA into a ribonucleoprotein particle that transports the mRNA into the daughter cell. **Figure 5.29** shows the functions of the proteins. She1p is a myosin (previously identified as Myo4), and She3 and She2 are proteins that connect the myosin to the mRNA. The myosin is a motor that moves the mRNA along actin filaments (2349).

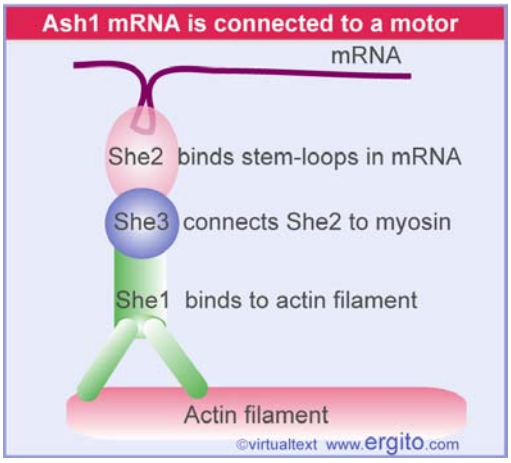

Figure 5.29 Ash1 mRNA forms a ribonucleoprotein containing a myosin motor that moves it along an actin filament.

Figure 5.30 summarizes the overall process. Ash1 mRNA is exported from the nucleus in the form of a ribonucleoprotein. In the cytoplasm it is first bound by She2, which recognizes some stem-loop secondary structures within the mRNA. Then She3 binds to She2, after which the myosin She1 binds. Then the particle hooks on to an actin filament and moves to the bud. When Ash1 mRNA reaches the bud, it is anchored there, probably by proteins that bind specifically to the mRNA.

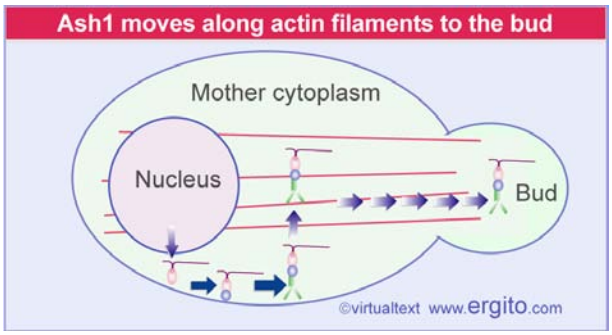

Figure 5.30 Ash1 mRNA is exported from the nucleus into the cytoplasm where it is assembled into a complex with the She proteins. The complex transports it along actin filaments to the bud.

Similar principles govern other cases where mRNAs are transported to specific sites. The mRNA is recognized by means of *cis*-acting sequences, which usually are regions of secondary structure in the 3′ untranslated region. (Ash1 mRNA is unusual in that the *cis*-acting regions are in the coding frame.) The mRNA is packaged into a ribonucleoprotein particle. In some cases, the transported mRNA can be visualized in very large particles, called mRNA granules. The particles are large enough (several times the size of a ribosome) to contain many protein and RNA components (for review see 1921).

A transported mRNP must be connected to a motor that moves it along a system of tracks. The tracks can be either actin filaments or microtubules. Whereas Ash1 uses a

myosin motor on actin tracks, *oscar* mRNA in the *Drosophila* egg uses a kinesin motor to move along microtubules (see *Molecular Biology 6.31.7 How are mRNAs and proteins transported and localized?*). Once the mRNA reaches its destination, it needs to be anchored in order to prevent it from diffusing away. Less is known about this, but the process appears to be independent of transport. An mRNA that is transported along microtubules may anchored to actin filaments at its destination.

Last updated on 1-30-2003

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MESSENGER RNA

2.5.17 Summary

Genetic information carried by DNA is expressed in two stages: transcription of DNA into mRNA; and translation of the mRNA into protein. Messenger RNA is transcribed from one strand of DNA and is complementary to this (noncoding) strand and identical with the other (coding) strand. The sequence of mRNA, in triplet codons 5′–3′, is related to the amino acid sequence of protein, N- to C-terminal.

The adaptor that interprets the meaning of a codon is transfer RNA, which has a compact L-shaped tertiary structure; one end of the tRNA has an anticodon that is complementary to the codon, and the other end can be covalently linked to the specific amino acid that corresponds to the target codon. A tRNA carrying an amino acid is called an aminoacyl-tRNA.

The ribosome provides the apparatus that allows aminoacyl-tRNAs to bind to their codons on mRNA. The small subunit of the ribosome is bound to mRNA; the large subunit carries the nascent polypeptide. A ribosome moves along mRNA from an initiation site in the 5′ region to a termination site in the 3′ region, and the appropriate aminoacyl-tRNAs respond to their codons, unloading their amino acids, so that the growing polypeptide chain extends by one residue for each codon traversed.

The translational apparatus is not specific for tissue or organism; an mRNA from one source can be translated by the ribosomes and tRNAs from another source. The number of times any mRNA is translated is a function of the affinity of its initiation site(s) for ribosomes and its stability. There are some cases in which translation of groups of mRNA or individual mRNAs is specifically prevented: this is called translational control.

A typical mRNA contains both a nontranslated 5′ leader and 3′ trailer as well as coding region(s). Bacterial mRNA is usually polycistronic, with nontranslated regions between the cistrons. Each cistron is represented by a coding region that starts with a specific initiation site and ends with a termination site. Ribosome subunits associate at the initiation site and dissociate at the termination site of each coding region.

A growing *E. coli* bacterium has ~20,000 ribosomes and ~200,000 tRNAs, mostly in the form of aminoacyl-tRNA. There are ~1500 mRNA molecules, representing 2-3 copies of each of 600 different messengers.

A single mRNA can be translated by many ribosomes simultaneously, generating a polyribosome (or polysome). Bacterial polysomes are large, typically with tens of ribosomes bound to a single mRNA. Eukaryotic polysomes are smaller, typically with fewer than 10 ribosomes; each mRNA carries only a single coding sequence.

Bacterial mRNA has an extremely short half-life, only a few minutes. The 5′ end starts translation even while the downstream sequences are being transcribed.

Degradation is initiated by endonucleases that cut at discrete sites, following the ribosomes in the 5′–3′ direction, after which exonucleases reduce the fragments to nucleotides by degrading them from the released 3′ end toward the 5′ end. Individual sequences may promote or retard degradation in bacterial mRNAs.

Eukaryotic mRNA must be processed in the nucleus before it is transported to the cytoplasm for translation. A methylated cap is added to the 5′ end. It consists of a nucleotide added to the original end by a 5′–5′ bond, after which methyl groups are added. Most eukaryotic mRNA has an ~200 base sequence of poly(A) added to its 3′ terminus in the nucleus after transcription, but $poly(A)^-$ mRNAs appear to be translated and degraded with the same kinetics as $poly(A)^+$ mRNAs. Eukaryotic mRNA exists as a ribonucleoprotein particle; in some cases mRNPs are stored that fail to be translated. Eukaryotic mRNAs are usually stable for several hours. They may have multiple sequences that initiate degradation; examples are known in which the process is regulated.

Yeast mRNA is degraded by (at least) two pathways. Both start with removal of poly(A) from the 3′ end, causing loss of poly(A)-binding protein, which in turn leads to removal of the methylated cap from the 5′ end. One pathway degrades the mRNA from the 5′ end by an exonuclease. Another pathway degrades from the 3′ end by the exosome, a complex containing several exonucleases.

Nonsense-mediated degradation leads to the destruction of mRNAs that have a termination (nonsense) codon prior to the last exon. The *upf* loci in yeast and the *smg* loci in worms are required for the process. They includes a helicase activity to unwind mRNA and a protein that interacts with the factors that terminate protein synthesis. The features of the process in mammalian cells suggest that some of the proteins attach to the mRNA in the nucleus when RNA splicing occurs to remove introns.

mRNAs can be transported to specific locations within a cell (especially in embryonic development). In the Ash1 system in yeast, mRNA is transported from the mother cell into the daughter cell by a myosin motor that moves on actin filaments. In plants, mRNAs can be transported long distances between cells.