

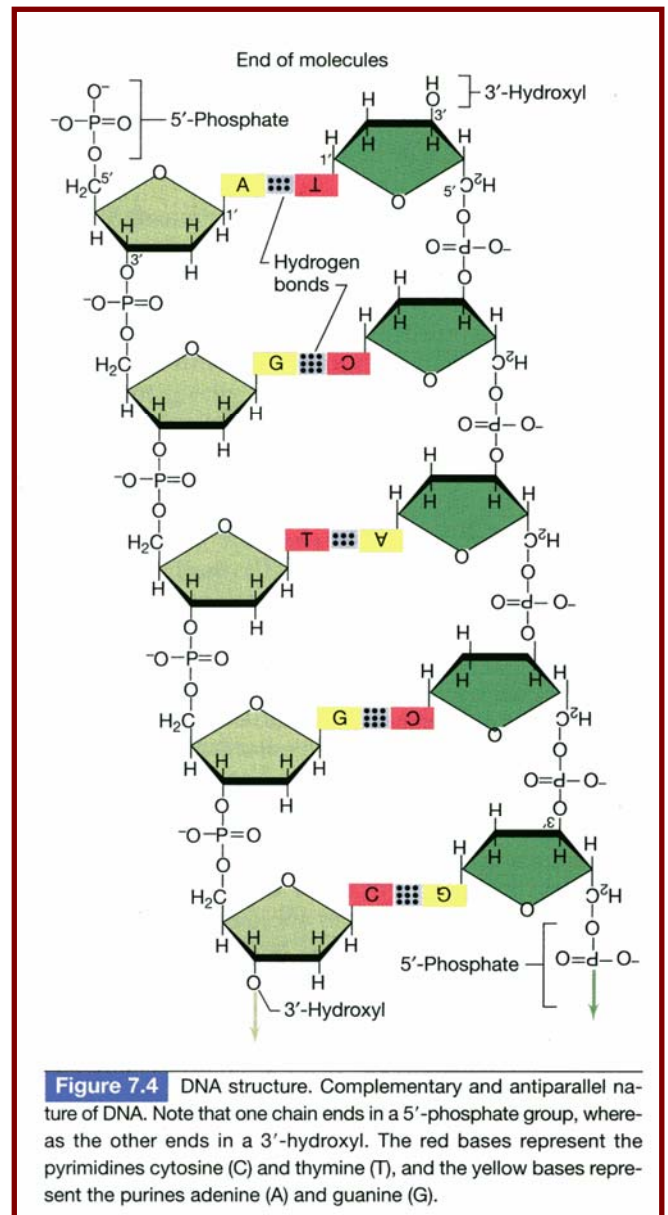
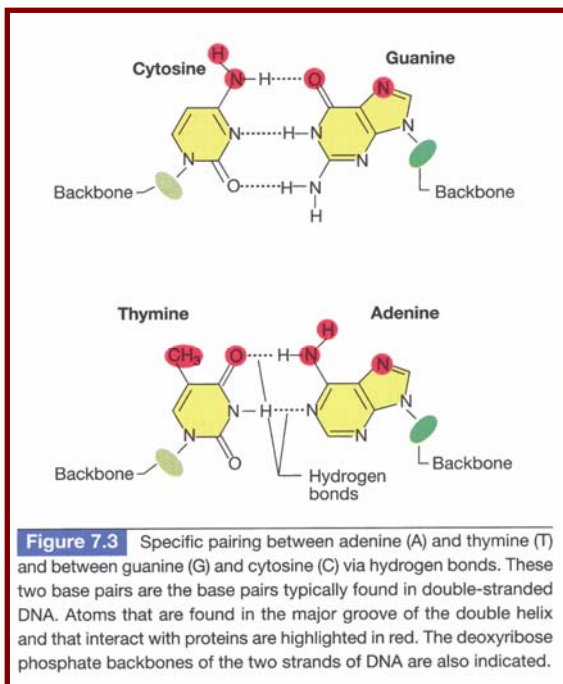
Molecular Biology: Some foundations
(Text pages 166-204)

Ultimately, the question we wish to address is: How is it that prokaryotes gain new genetic ability? The cells are haploid and reproduce by fission...so how does and genetic novelty arise?

- Macromolecules and genetic information
 - DNA
 - RNA
 - Protein
- Step in information flow
 - Replication
 - Duplication of the DNA
 - Transcription
 - Transfer of information of DNA to RNA (creation of mRNA)
 - Decoding of that information by rRNA and tRNAs to create proteins

Some simple review (source of Figures 7.3 and 7.4: Madigan et al 2002).

- Chromosome is circular in 99% of bacteria
- Chromosome is centrally located
- Base composition
 - Pyrimidines: Cytosine and Thymine
 - Purines: Adenine and Guanosine
 - Sugar: 2-deoxyribose
- Bases are covalently linked to one-another 5'-P to 3'-OH to create a long polymer
- Two polymers hydrogen bond to form anti-parallel strands



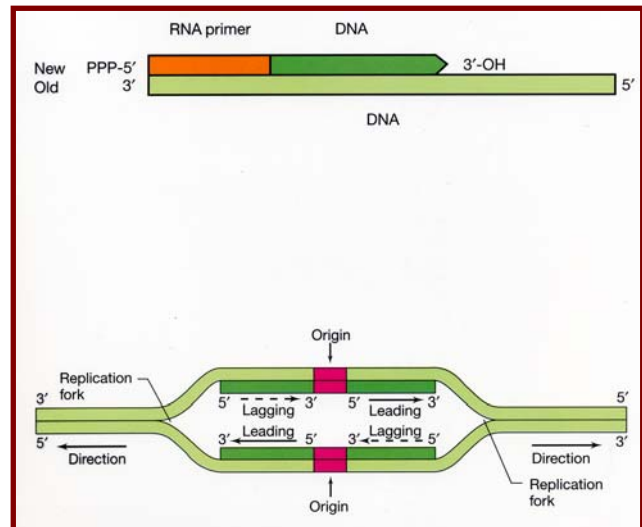
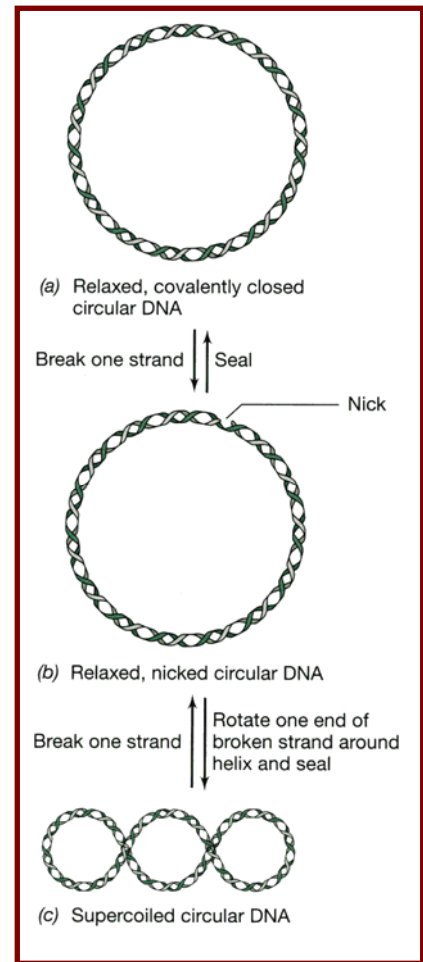
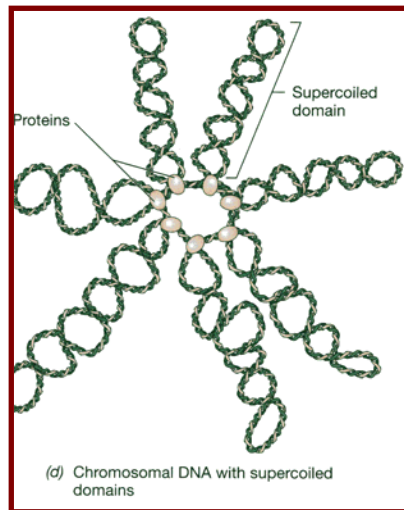
How does DNA exist in cells? (Source of Figures: Madigan et al 2002).

- A highly compacted structure that comprises 3-5% of cellular dry mass.
- In an actively growing cell, there are the equivalent of 3 genomes
- There are about 10 bases per turn of the helix and the helix is in-turn supercoiled (coiled upon itself).
- In addition, supercoiled loops create Domains where each domain is a topological unit defined by sites on the DNA molecule that bind anchoring proteins.

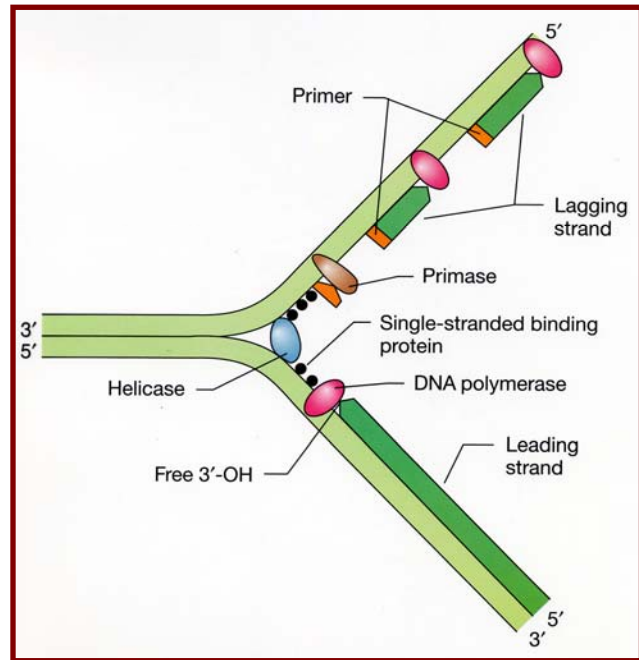
Replication in Bacteria

Overview

- Over 30 different proteins are involved
- Replication begins at a unique area of the chromosome called the origin. This area is about 245 base pairs and called *oriC*. A number of proteins bind to this site to begin replication. Replication proceeds outward from this site in two directions.
- Review topoisomerase function and location
- Review the primosome (loading of helicase (unwinds the helix), SSB (single strand binding proteins), and primase (RNA synthesis))
- Review 'leading' and 'lagging' strands and the symmetry of the replication bubble.
 - Bases must be added to the free 3' end of a chain
- Review the concept of the Okazaki fragment (1000-2000 base fragments)
- Review DNA ligase



Events occurring at the replication forks.
(Figure from Madigan et al. 2002)



RNA synthesis and processing

Products of transcription

- rRNA
- mRNA
- tRNA

Overview:

- A gene is required.
- The gene must be transcribed and mRNA produced.
- The mRNA must be translated into a protein.

Synthesis of RNA

- RNA synthesized by DNA dependent-RNA polymerase
- The polymerase is a large multi-subunit enzyme complex. In *E. coli* the complex has five sub-units α α β β' σ .
- Bases are added to the 3' end of a chain of nucleotides and no 'primer' is required.

Initiation

- The polymerase must bind to DNA. This occurs at a specific site called the Promoter site. (Many such sites exist).
- Promoters are 20-200 bases long and there are two important areas in the promoter sequence.
- 5-10 bases upstream (-10 position) of the gene 'start' site is a sequence of bases called the 'Pribnow Box'
- a sequence about -35 bases away from the transcription start site.

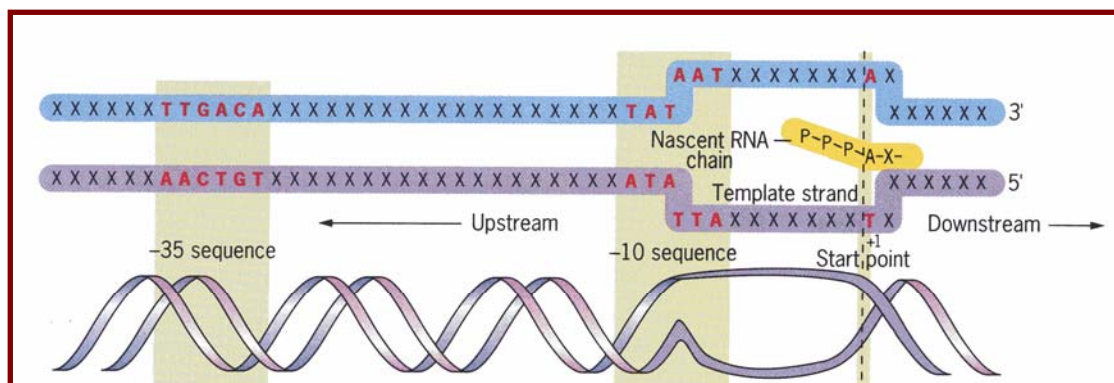


Figure 11.7 The basic elements of a promoter region in the DNA of the bacterium *E. coli*. The key regulatory sequences required for initiation of transcription are found in regions located at -35 and -10 base pairs from the site at which transcription is initiated. The initiation site marks the boundary between the + and - sides of the gene.

Source of Figure 11.7: Karp 2002

- The core enzyme of RNA polymerase has all components except sigma. This enzyme is capable of synthesizing RNA essentially anywhere along the DNA strand but the RNA produced is non-sense. Sigma is the unit that is essential to starting the synthesis at the right place on the DNA. Sigma appears to target the -35 and Pribnow sites and align the polymerase onto the promoter.

Elongation

- After the next 8-12 bases have been added, sigma is abruptly released and is recycled to a different polymerase.
- Elongation by the polymerase continue

Termination

- This is the cessation of elongation, release of the transcript, and dissociation of the polymerase from the template.

Translation (protein synthesis)

The basic premise of protein synthesis

- Triplet code contained in mRNA
- Translation of the code means matching the proper codon with the proper amino acid
- The role of the ribosome and the tRNA in doing the reading and codon matching
- Processive reading of the code with the subsequent production of a protein

Two major problems to be solve when synthesizing proteins

- Selection of the proper amino acid
- Fitting the monomers into a polymer

These problems are solved by the interaction of RNAs and groups of proteins

Ribosomes

- Ribosomes in prokaryotes are 70s and composed of two distinct subunits; 50s and 30s
- When the ribosome is completely assembled, it has sites within the structure that function to bind the mRNA, tRNAs carrying the appropriate amino acids, and provide the conditions that will catalyze the formation of polymers. Review the P and A sites (Source of Figure: Karp 2002.

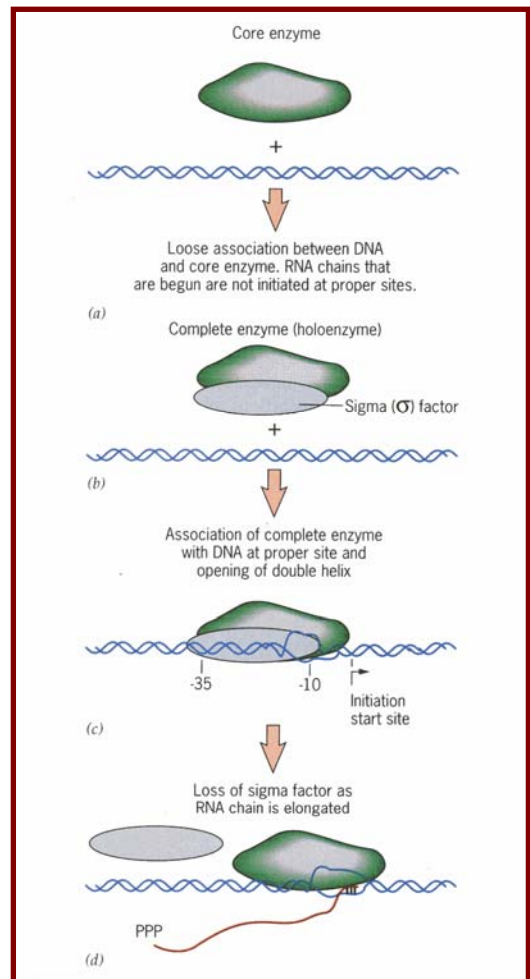
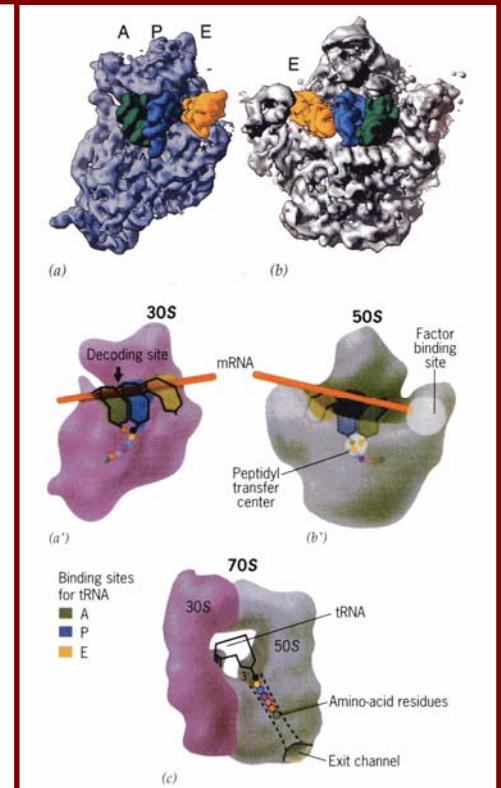


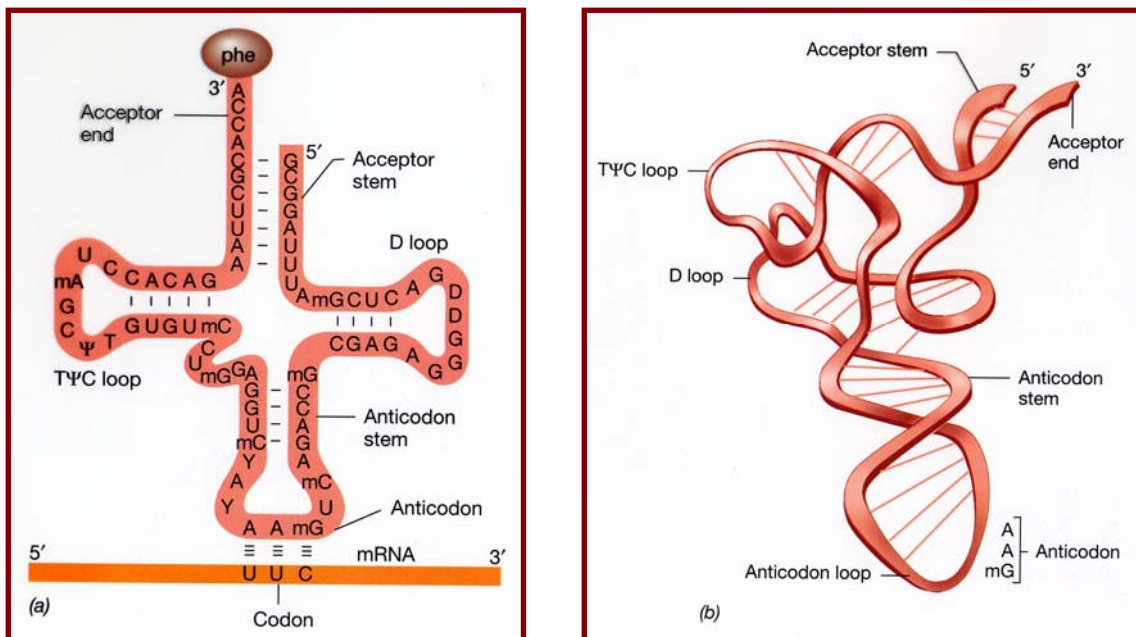
Figure 11.6 Initiation of transcription in prokaryotes. (a) In the absence of the σ factor, the core enzyme cannot interact with the DNA at specific initiation sites. (b–d) When the core enzyme is associated with the σ factor, the complete enzyme is able to recognize and bind to the promoter region of the DNA, separate the strands of the DNA double helix, and initiate transcription at the proper start site (see Figure 11.7). The σ factor then dissociates from the core enzyme, which is capable of transcription elongation.

Source of Figures: Karp 2002



Transfer RNA

- This is a single stranded RNA that folds onto itself to form 3 hairpin loops that in the eyes of some, resemble a cloverleaf (Source of Figures: Madigan et al. 2002).
- There are two decided important regions of the molecule
 - The 'amino-end' of the structure is where the amino acid is esterified (COOH – 3'OH) to the ribose of adenylic acid
 - The anticodon loop holds the triplet of bases that hybridize to the mRNA template



Steps in protein synthesis (Figure from Madigan et al. 2002)

- Charging the tRNA with the proper amino acid takes place in two steps carried out by the same enzyme. There is one version of this enzyme (aminoacyl-tRNA synthetase) for each amino acid in the cell)
- Activation (linking of an amino acid to AMP)
- Transfer to tRNA acceptor

Initiating synthesis

- Essentially all proteins in prokaryotes have as the first amino acid formyl-methionine. This unique amino acid marks the place where the triplet code is set so that each three nucleotides following are in the proper reading frame.

Binding of the ribosome to the mRNA

- Takes place sequentially.
- There are several protein factors that influence the binding of the ribosome subunits to the mRNA

Elongation

- There are several protein factors involved with elongation

Peptide bond formation

- A peptide bond forms when the free amino group on the amino acid bound to the tRNA at the A site displaces the tRNA at the P site.

Translocation

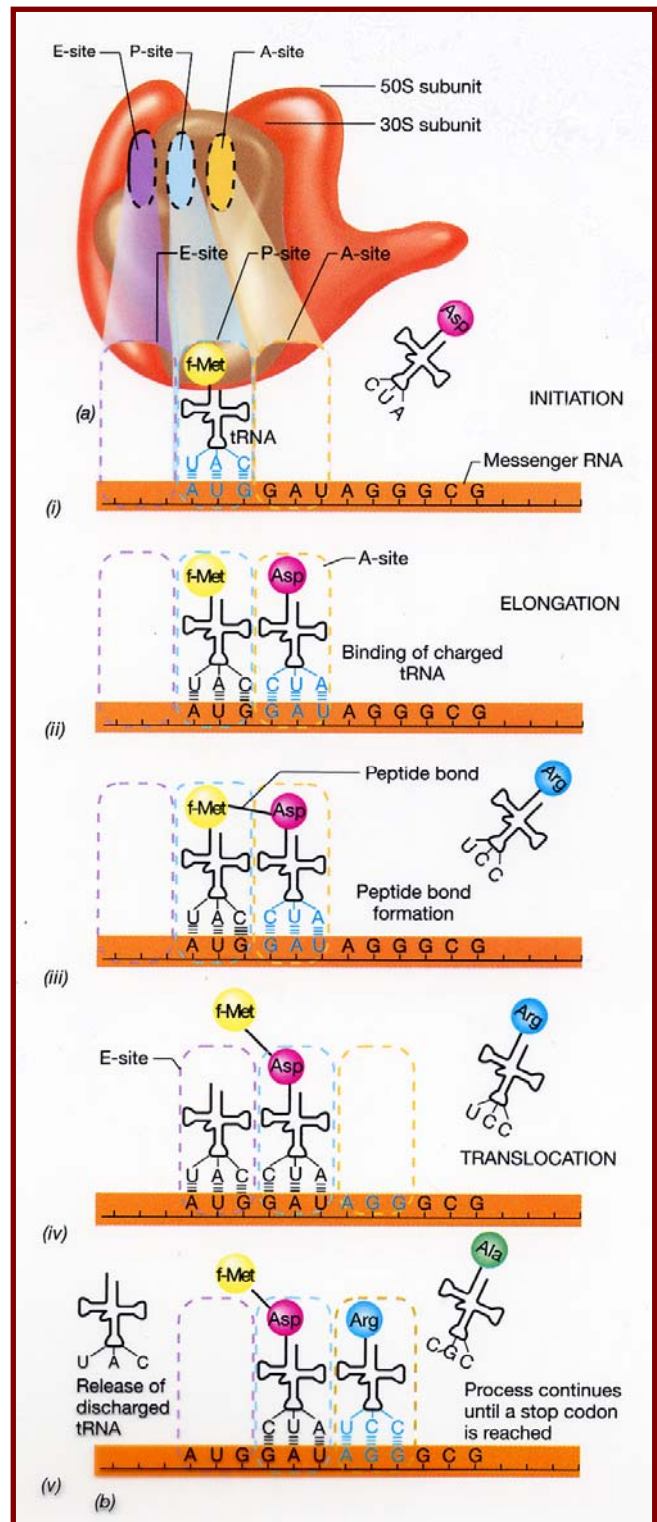
- The uncharged tRNA is ejected from the P site and the ribosome move one codon upstream to the peptidyl-tRNA moves from the A site to what becomes the new P site.
- The process repeats over and over and over until termination

Termination

- Three protein factors involved with release
 - RF1
 - RF2
 - RF3
- The protein is released and the ribosome disassociates.

Polysomes

- mRNA is translate by several ribosomes in series (~50).
- This increases the amount of protein created from a single message and delays degradation
- Translation and transcription are coupled events. The ribosomes attach to the mRNA as soon as it is produced.
- Often, the mRNA is being read even before synthesis has been completed.



References:

Karp, G. 2002. Cell and molecular biology: Concepts and Experiments. John Wiley and Sons, Inc., New York.

Madigan, M. T., J.M. Martinko, and J. Parker. 2002. Brock Biology of Microorganisms 10th ed. Prentice Hall.