inversion (cap it and turn it upside down a few times).
2. Grind a strawberry and a kiwi by hand in a plastic bag, or using a mortar and pestle, or with a metal bowl and the end of a blunt instrument. Grind for at least two minutes per fruit.
3. Add 10 mL of the DNA extraction buffer to each fruit, and mix well for at least one minute.
4. Remove cellular debris by filtering each fruit mixture through cheesecloth or porous cloth and into a funnel placed in a test tube or an appropriate container.
5. Pour ice-cold ethanol or isopropanol (rubbing alcohol) into the test tube. You should observe white, precipitated DNA.
6. Gather the DNA from each fruit by winding it around separate glass rods.

Record your observations: Because you are not quantitatively measuring DNA volume, you can record for each trial whether the two fruits produced the same or different amounts of DNA as observed by eye. If one or the other fruit produced noticeably more DNA, record this as well. Determine whether your observations are consistent with several pieces of each fruit.

Analyze your data: Did you notice an obvious difference in the amount of DNA produced by each fruit? Were your results reproducible?

Draw a conclusion: Given what you know about the number of chromosomes in each fruit, can you conclude that chromosome number necessarily correlates to DNA amount? Can you identify any drawbacks to this procedure? If you had access to a laboratory, how could you standardize your comparison and make it more quantitative?

15.2 Prokaryotic Transcription

By the end of this section, you will be able to do the following:

- List the different steps in prokaryotic transcription
- Discuss the role of promoters in prokaryotic transcription
- Describe how and when transcription is terminated

The prokaryotes, which include Bacteria and Archaea, are mostly single-celled organisms that, by definition, lack membrane-bound nuclei and other organelles. A bacterial chromosome is a closed circle that, unlike eukaryotic chromosomes, is not organized around histone proteins. The central region of the cell in which prokaryotic DNA resides is called the nucleoid region. In addition, prokaryotes often have abundant **plasmids**, which are shorter, circular DNA molecules that may only contain one or a few genes. Plasmids can be transferred independently of the bacterial chromosome during cell division and often carry traits such as those involved with antibiotic resistance.

Transcription in prokaryotes (and in eukaryotes) requires the DNA double helix to partially unwind in the region of mRNA synthesis. The region of unwinding is called a **transcription bubble.** Transcription always proceeds from the same DNA strand for each gene, which is called the **template strand**. The mRNA product is complementary to the template strand and is almost identical to the other DNA strand, called the **nontemplate strand**, or the coding strand. The only nucleotide difference is that in mRNA, all of the T nucleotides are replaced with U nucleotides (Figure 15.7). In an RNA double helix, A can bind U via two hydrogen bonds, just as in A–T pairing in a DNA double helix.

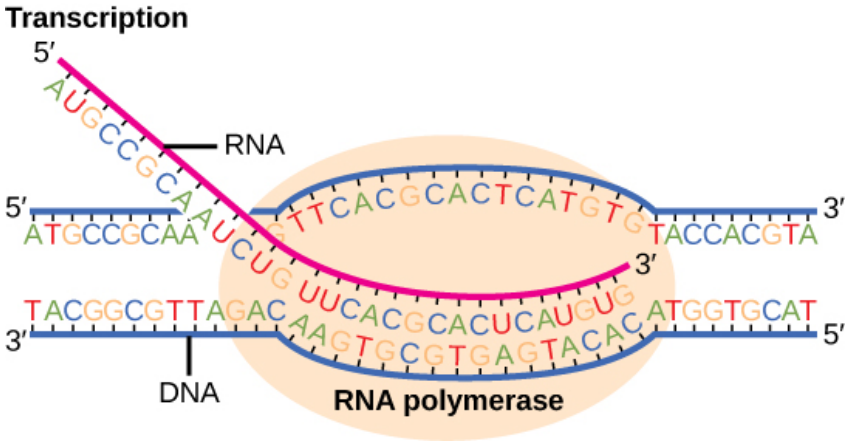

Figure 15.7 Messenger RNA is a copy of protein-coding information in the coding strand of DNA, with the substitution of U in the RNA for T

in the coding sequence. However, new RNA nucleotides base pair with the nucleotides of the template strand. RNA is synthesized in its 5'-3' direction, using the enzyme RNA polymerase. As the template is read, the DNA unwinds ahead of the polymerase and then rewinds behind it.

The nucleotide pair in the DNA double helix that corresponds to the site from which the first 5' mRNA nucleotide is transcribed is called the +1 site, or the **initiation site**. Nucleotides preceding the initiation site are denoted with a "-" and are designated *upstream nucleotides*. Conversely, nucleotides following the initiation site are denoted with "+" numbering and are called *downstream nucleotides*.

Initiation of Transcription in Prokaryotes

Prokaryotes do not have membrane-enclosed nuclei. Therefore, the processes of transcription, translation, and mRNA degradation can all occur simultaneously. The intracellular level of a bacterial protein can quickly be amplified by multiple transcription and translation events that occur concurrently on the same DNA template. Prokaryotic genomes are very compact, and prokaryotic transcripts often cover more than one gene or cistron (a coding sequence for a single protein). Polycistronic mRNAs are then translated to produce more than one kind of protein.

Our discussion here will exemplify transcription by describing this process in *Escherichia coli*, a well-studied eubacterial species. Although some differences exist between transcription in *E. coli* and transcription in archaea, an understanding of *E. coli* transcription can be applied to virtually all bacterial species.

Prokaryotic RNA Polymerase

Prokaryotes use the same RNA polymerase to transcribe all of their genes. In *E. coli*, the polymerase is composed of five polypeptide subunits, two of which are identical. Four of these subunits, denoted α, α, β, and β', comprise the polymerase **core enzyme**. These subunits assemble every time a gene is transcribed, and they disassemble once transcription is complete. Each subunit has a unique role; the two α-subunits are necessary to assemble the polymerase on the DNA; the β-subunit binds to the ribonucleoside triphosphate that will become part of the nascent mRNA molecule; and the β' subunit binds the DNA template strand. The fifth subunit, σ, is involved only in transcription initiation. It confers transcriptional specificity such that the polymerase begins to synthesize mRNA from an appropriate initiation site. Without σ, the core enzyme would transcribe from random sites and would produce mRNA molecules that specified protein gibberish. The polymerase comprised of all five subunits is called the **holoenzyme**.

Prokaryotic Promoters

A **promoter** is a DNA sequence onto which the transcription machinery, including RNA polymerase, binds and initiates transcription. In most cases, promoters exist upstream of the genes they regulate. The specific sequence of a promoter is very important because it determines whether the corresponding gene is transcribed all the time, some of the time, or infrequently. Although promoters vary among prokaryotic genomes, a few elements are evolutionarily conserved in many species. At the -10 and -35 regions upstream of the initiation site, there are two *promoter consensus sequences*, or regions that are similar across all promoters and across various bacterial species (Figure 15.8). The -10 sequence, called the -10 region, has the consensus sequence TATAAT. The -35 sequence has the consensus sequence TTGACA. These consensus sequences are recognized and bound by σ. Once this interaction is made, the subunits of the core enzyme bind to the site. The A–T-rich -10 region facilitates unwinding of the DNA template, and several phosphodiester bonds are made. The transcription initiation phase ends with the production of abortive transcripts, which are polymers of approximately 10 nucleotides that are made and released.

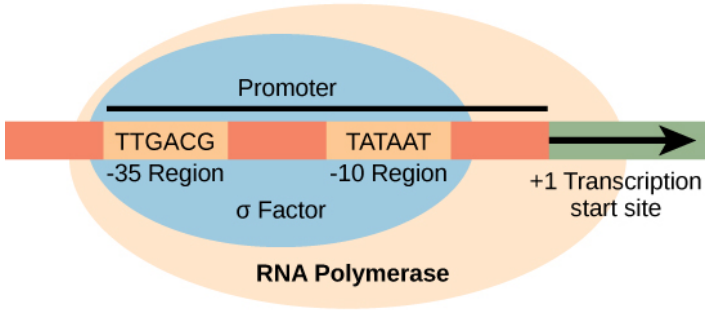

Figure 15.8 The σ subunit of prokaryotic RNA polymerase recognizes consensus sequences found in the promoter region upstream of the transcription start site. The σ subunit dissociates from the polymerase after transcription has been initiated.

LINK TO LEARNING

View this MolecularMovies animation (http://openstax.org/l/transcription) to see the first part of transcription and the base sequence repetition of the TATA box.

Elongation and Termination in Prokaryotes

The transcription elongation phase begins with the release of the σ subunit from the polymerase. The dissociation of σ allows the core enzyme to proceed along the DNA template, synthesizing mRNA in the 5′ to 3′ direction at a rate of approximately 40 nucleotides per second. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it. The base pairing between DNA and RNA is not stable enough to maintain the stability of the mRNA synthesis components. Instead, the RNA polymerase acts as a stable linker between the DNA template and the nascent RNA strands to ensure that elongation is not interrupted prematurely.

Prokaryotic Termination Signals

Once a gene is transcribed, the prokaryotic polymerase needs to be instructed to dissociate from the DNA template and liberate the newly made mRNA. Depending on the gene being transcribed, there are two kinds of termination signals. One is protein-based and the other is RNA-based. **Rho-dependent termination** is controlled by the rho protein, which tracks along behind the polymerase on the growing mRNA chain. Near the end of the gene, the polymerase encounters a run of G nucleotides on the DNA template and it stalls. As a result, the rho protein collides with the polymerase. The interaction with rho releases the mRNA from the transcription bubble.

Rho-independent termination is controlled by specific sequences in the DNA template strand. As the polymerase nears the end of the gene being transcribed, it encounters a region rich in C–G nucleotides. The mRNA folds back on itself, and the complementary C–G nucleotides bind together. The result is a **stable hairpin** that causes the polymerase to stall as soon as it begins to transcribe a region rich in A–T nucleotides. The complementary U–A region of the mRNA transcript forms only a weak interaction with the template DNA. This, coupled with the stalled polymerase, induces enough instability for the core enzyme to break away and liberate the new mRNA transcript.

Upon termination, the process of transcription is complete. By the time termination occurs, the prokaryotic transcript would already have been used to begin synthesis of numerous copies of the encoded protein because these processes can occur concurrently. The unification of transcription, translation, and even mRNA degradation is possible because all of these processes occur in the same 5′ to 3′ direction, and because there is no membranous compartmentalization in the prokaryotic cell (Figure 15.9). In contrast, the presence of a nucleus in eukaryotic cells precludes simultaneous transcription and translation.

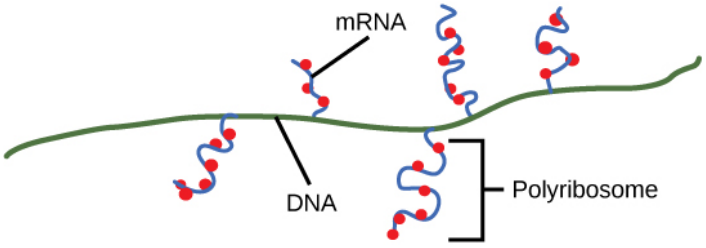

Figure 15.9 Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

LINK TO LEARNING

Visit this BioStudio animation (http://openstax.org/l/transcription2) to see the process of prokaryotic transcription.