S1.Describe the important events that occur during the three stages of gene transcription in bacteria. What proteins play critical roles in the three stages?

Answer: The three stages are initiation, elongation, and termination.

Initiation: RNA polymerase holoenzyme scans along the DNA until sigma factor recognizes the sequence of a promoter. Sigma factor binds tightly to this sequence, forming a closed complex. The DNA is then denatured to form a bubblelike structure known as the open complex.

Elongation: RNA polymerase core enzyme slides along the DNA, synthesizing RNA as it goes. The a subunits of RNA polymerase keep the enzyme bound to the DNA, while the *ß* subunits are responsible for the catalytic synthesis of RNA. During elongation, RNA is made according to the AU/GC rule, with nucleotides being added in the 5′ to 3′ direction.

Termination: RNA polymerase eventually reaches a sequence at the end of the gene that signals the cessation of transcription. In *p*-independent termination, the properties of the termination sequences in the DNA are sufficient to cause termination. In *p*-dependent termination, the *p* protein recognizes a sequence within the RNA, binds there, and travels toward RNA polymerase. When the formation of a stem-loop structure causes RNA polymerase to pause, *p* catches up and knocks it off the DNA.

S2. What is the difference between a structural gene and a nonstructural gene?

Answer: Structural genes encode mRNA that is translated into a polypeptide sequence. Nonstructural genes encode RNAs that are never translated. Examples of nonstructural genes include tRNA and rRNA, which function during translation; 7S RNA, which is part of SRP; the RNA of RNaseP; snoRNA, which is involved in rRNA trimming; and snRNA, which is part of spliceosomes. In many cases, the RNA from nonstructural genes becomes part of a complex composed of RNA molecules and protein subunits.

S3. When RNA polymerase transcribes DNA, only one of the two DNA strands is used as a template. Take a look at figure 12.3 and explain how RNA polymerase determines which DNA strand is the template strand.

Answer: The binding of sigma factor and RNA polymerase depends on the sequence of the promoter. RNA polymerase binds to the promoter in such a way that the –35 sequence TTGACA and the –10 sequence TATAAT are within the coding strand, while the –35 sequence AACTGT and the –10 sequence ATATTA are within the template strand.

S4. The process of transcriptional termination is not as well understood in eukaryotes as it is in bacteria. Nevertheless, current evidence suggests that there are several different mechanisms for termination. Like bacteria, the termination of certain genes appears to occur via intrinsic terminators (i.e., like *p*-independent termination) while the termination of other genes may involve RNA-binding proteins (i.e., like *p*-dependent termination). A third type of mechanism is also found for the termination of rRNA genes by RNA polymerase I. In this case, a protein known as TTFI (transcription termination factor I) binds to the DNA downstream from the termination site. Discuss how the binding of a protein downstream from the termination site could promote transcriptional termination.

Answer: There are several possibilities. First, the binding of TTFI could act as a roadblock to the movement of RNA polymerase I. Second, TTFI could promote the dissociation of the RNA transcript and RNA polymerase I from the DNA; it may act like a helicase. Or third, it could cause a change in the structure of the DNA that prevents RNA polymerase from moving past the termination site. Though multiple effects are possible, the third effect seems the most likely since TTFI is known to cause a bend in the DNA when it binds to the termination sequence.