

This brief discussion summarizes the essentials of how one DNA molecule is made from another and thus preserves and propagates the genetic information in the original molecule. Now we turn our attention to how this genetic information can be transferred.

4.4. TRANSCRIPTION: SENDING THE MESSAGE

The primary products of transcription are the three major types of RNA we introduced in Chapter 2: *m*-RNA, *t*-RNA, and *r*-RNA. Their rates of synthesis determine the cell's capacity to make proteins. RNA synthesis from DNA is mediated by the enzyme, *RNA polymerase*. To be functional, RNA polymerase must have two major subcomponents: the *core* enzyme and the *sigma factor*. The core enzyme contains the catalytic site, while the sigma factor is a protein essential to locating the appropriate beginning for the message. The core enzyme plus the sigma factor constitutes the *holoenzyme*.

The student may wonder which of the two strands of DNA is actually transcribed. It turns out that either strand can be read. RNA polymerase always reads in the 3'- to 5'-direction, so the direction of reading will be opposite on each strand. On one part of the chromosome, one strand of DNA may serve as the template or *sense strand*, and on another portion of the chromosome, the other strand may serve as a template.

The processes of initiation, elongation, and termination are summarized in Fig. 4.4. The sigma factor is involved only in initiation. The sigma factor recognizes a specific sequence of nucleotides on a DNA strand. This sequence is the *promoter region*. Promoters can vary somewhat, and this alters the affinity of the sigma factor (and consequently the holoenzyme) for a particular promoter. A *strong promoter* is one with a high affinity for the holoenzyme. The rate of formation of transcripts is determined primarily by the frequency of initiation of transcription, which is directly related to promoter strength. This will be important in our discussions of genetic engineering. Cells usually have one dominant sigma factor that is required to recognize the vast majority of promoters in the cell. However, other sigma factors can play important roles under different growth conditions (particularly stress) and are used to initiate transcription from promoters that encode proteins important to the cell for coping with unusual growth conditions or stress.

After the initiation site is recognized, elongation of the transcript begins. As soon as elongation is established, the sigma factor is released so it can be reused. The synthesis of the growing RNA molecule is energy requiring, so activated triphosphate monomers of the ribonucleotides are required.

The transcript is made until the RNA polymerase encounters a stop signal, or *transcription terminator*. At this point, the RNA polymerase disassociates from the DNA template and the RNA transcript is released. In some cases an additional protein, the *rho* protein, is required for termination. Terminators can be strong or weak. If a weak terminator is coupled with a strong promoter, some of the RNA polymerase will *read through* the terminator, creating an artificially long transcript and possibly disrupting subsequent control regions on that DNA strand. We must consider terminator regions and their strength when constructing recombinant DNA systems.

The transcripts that are formed may be roughly lumped as either stable or unstable RNA species. The stable RNA species are *r*-RNA and *t*-RNA. Messenger-RNA is highly unstable (about a 1-min half-life for a typical *E. coli* *m*-RNA, although *m*-RNA may be