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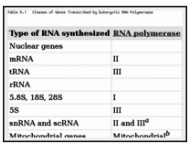
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Eukaryotic RNA Polymerases and General Transcription Factors

Although transcription proceeds by the same fundamental mechanisms in all cells, it is considerably more complex in eukaryotic cells than in bacteria. This is reflected in two distinct differences between the prokaryotic and eukaryotic systems. First, whereas all genes are transcribed by a single RNA polymerase in bacteria, eukaryotic cells contain multiple different RNA polymerases that transcribe distinct classes of genes. Second, rather than binding directly to promoter sequences, eukaryotic RNA polymerases need to interact with a variety of additional proteins to specifically initiate transcription. This increased complexity of eukaryotic transcription presumably facilitates the sophisticated regulation of gene expression needed to direct the activities of the many different cell types of multicellular organisms.

Eukaryotic RNA Polymerases

Eukaryotic cells contain three distinct nuclear RNA polymerases that transcribe different classes of genes (Table 6.1). Protein-coding genes are transcribed by RNA polymerase II to yield mRNAs; ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) are transcribed by RNA polymerases I and III. RNA polymerase I is specifically devoted to transcription of the three largest species of rRNAs, which are designated 28S, 18S, and 5.8S according to their rates of sedimentation during velocity centrifugation. RNA polymerase III transcribes the genes for tRNAs and for the smallest species of ribosomal RNA (5S rRNA). Some of the small RNAs involved in splicing and protein transport (snRNAs and scRNAs) are also transcribed by RNA polymerase III, while others are polymerase II transcripts. In addition, separate RNA polymerases (which are similar to bacterial RNA polymerases) are found in chloroplasts and mitochondria, where they specifically transcribe the DNAs of those organelles.



Type of RNA synthesized	RNA polymerase
Nuclear genes	
mRNA	II
tRNA	III
rRNA	
5.8S, 18S, 28S	I
5S	III
snRNA and scRNA	II and III[a]
Mitochondrial genes	Mitochondrial[b]

Table 6.1

Classes of Genes Transcribed by Eukaryotic RNA Polymerases.

All three of the nuclear RNA polymerases are complex enzymes, consisting of 8 to 14 different subunits each. Although they recognize different promoters and transcribe distinct classes of genes, they share several common features. The two largest subunits of all three eukaryotic RNA polymerases are related to the β and β′subunits of the single *E. coli* RNA polymerase. In addition, five subunits of the eukaryotic RNA polymerases are common to all three different enzymes. Consistent with these structural similarities, the different eukaryotic polymerases share several functional properties, including the need to interact with other proteins to appropriately initiate transcription.

General Transcription Factors and Initiation of Transcription by RNA Polymerase II

Because RNA polymerase II is responsible for the synthesis of mRNA from protein-coding genes, it has been the focus of most studies of transcription in eukaryotes. Early attempts at studying this enzyme indicated that its activity is different from that of prokaryotic RNA

polymerase. The accurate transcription of bacterial genes that can be accomplished *in vitro* simply by the addition of purified RNA polymerase to DNA containing a promoter is not possible in eukaryotic systems. The basis of this difference was elucidated in 1979, when Robert Roeder and his colleagues discovered that RNA polymerase II is able to initiate transcription only if additional proteins are added to the reaction. Thus, transcription in the eukaryotic system appeared to require distinct initiation factors that (in contrast to bacterial σ factors) were not associated with the polymerase.

Biochemical fractionation of nuclear extracts has now led to the identification of specific proteins (called transcription factors) that are required for RNA polymerase II to initiate transcription. Indeed, the identification and characterization of these factors represents a major part of ongoing efforts to understand transcription in eukaryotic cells. Two general types of transcription factors have been defined. General transcription factors are involved in transcription from all polymerase II promoters and therefore constitute part of the basic transcription machinery. Additional transcription factors (discussed later in the chapter) bind to DNA sequences that control the expression of individual genes and are thus responsible for regulating gene expression.

Five general transcription factors are required for initiation of transcription by RNA polymerase II in reconstituted *in vitro* systems (Figure 6.12). The promoters of many genes transcribed by polymerase II contain a sequence similar to TATAA 25 to 30 nucleotides upstream of the transcription start site. This sequence (called the TATA box) resembles the -10 sequence element of bacterial promoters, and the results of introducing mutations into TATAA sequences have demonstrated their role in the initiation of transcription. The first step in formation of a transcription complex is the binding of a general transcription factor called TFIID to the TATA box (*TF* indicates *t*ranscription *f*actor; *II* indicates polymerase *II*). TFIID is itself composed of multiple subunits, including the TATA-binding protein (**TBP**), which binds specifically to the TATAA consensus sequence, and 10-12 other polypeptides, called **TBP-associated factors** (**TAFs**). TBP then binds a second general transcription factor (TFIIB) forming a TBP-TFIIB complex at the promoter (Figure 6.13). TFIIB in turn serves as a bridge to RNA polymerase, which binds to the TBP-TFIIB complex in association with a third factor, TFIIF.



Figure 6.12

Formation of a polymerase II transcription complex. Many polymerase II promoters have a TATA box (consensus sequence TATAA) 25 to 30 nucleotides upstream of the transcription start site. This sequence is recognized by transcription factor TFIID, which (more...)

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Figure 6.13

Model of the TBP-TFIIB complex bound to DNA. The DNA is shown as a stick figure consisting of yellow and green strands, with the site of transcription initiation designated +1. TBP consists of two repeats, colored light blue and dark blue. TFIIB repeats (more...)

Following recruitment of RNA polymerase II to the promoter, the binding of two additional factors (TFIIE and TFIIH) is required for initiation of transcription. TFIIH is a multisubunit factor that appears to play at least two important roles. First, two subunits of TFIIH are helicases, which may unwind DNA around the initiation site. (These subunits of TFIIH are also required for nucleotide excision repair, as discussed in Chapter 5.) Another subunit of TFIIH is a protein kinase that phosphorylates repeated sequences present in the C-terminal domain of the largest subunit of RNA polymerase II. Phosphorylation of these sequences is thought to release the polymerase from its association with the initiation complex, allowing it to proceed along the template as it elongates the growing RNA chain.

In addition to a TATA box, the promoters of many genes transcribed by RNA polymerase II contain a second important sequence element (an initiator, or Inr, sequence) that spans the transcription start site. Moreover, some RNA polymerase II promoters contain only an Inr element, with no TATA box. Initiation at these promoters still requires TFIID (and TBP), even though TBP obviously does not recognize these promoters by binding directly to the TATA sequence. Instead, other subunits of TFIID (TAFs) appear to bind to the Inr sequences. This binding recruits TBP to the promoter, and TFIIB, polymerase II, and additional transcription factors then assemble as already described. TBP thus plays a central role in initiating polymerase II transcription, even on promoters that lack a TATA box.

Despite the development of *in vitro* systems and the characterization of several general transcription factors, much remains to be learned concerning the mechanism of polymerase II transcription in eukaryotic cells. The sequential recruitment of transcription factors described here represents the minimal system required for transcription *in vitro*; additional factors may be needed within the cell. Furthermore, RNA polymerase II appears to be able to associate with some transcription factors *in vivo* prior to the assembly of a transcription complex on DNA. In particular, preformed complexes of RNA polymerase II with TFIIB, TFIIE, TFIIF, TFIIH, and other transcriptional regulatory proteins have been detected in both yeast and mammalian cells. These large complexes (called polymerase II holoenzymes) can be recruited to a promoter via direct interaction with TFIID (Figure 6.14). The relative contributions of stepwise assembly of individual factors versus recruitment of the RNA polymerase II holoenzyme to promoters within the cell thus remain to be determined.

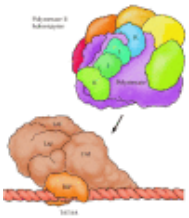


Figure 6.14

RNA polymerase II holoenzyme. The holoenzyme consists of a preformed complex of RNA polymerase II, the general transcription factors TFIIB, TFIIE, TFIIF, and TFIIH, and several other proteins that activate transcription. This complex can be recruited (more...)

Transcription by RNA Polymerases I and III

As previously discussed, distinct RNA polymerases are responsible for the transcription of genes encoding ribosomal and transfer RNAs in eukaryotic cells. All three RNA polymerases, however, require additional transcription factors to associate with appropriate promoter sequences. Furthermore, although the three different polymerases in eukaryotic cells recognize distinct types of promoters, a common transcription factor—the TATA-binding protein (TBP)—appears to be required for initiation of transcription by all three enzymes.

RNA polymerase I is devoted solely to the transcription of ribosomal RNA genes, which are present in tandem repeats. Transcription of these genes yields a large 45S pre-rRNA, which is then processed to yield the 28S, 18S, and 5.8S rRNAs (Figure 6.15). The promoter of ribosomal RNA genes spans about 150 base pairs just upstream of the transcription initiation site. These promoter sequences are recognized by two transcription factors, UBF (upstream binding factor) and SL1 (selectivity factor 1), which bind cooperatively to the promoter and then recruit polymerase I to form an initiation complex (Figure 6.16). The SL1 transcription factor is composed of four protein subunits, one of which, surprisingly, is TBP. The role of TBP has been demonstrated directly by the finding that yeasts carrying mutations in TBP are defective not only for transcription by polymerase II, but also for transcription by polymerases I and III. Thus, TBP is a common transcription factor required by all three classes of eukaryotic RNA polymerases. Since the promoter for ribosomal RNA genes does not contain a TATA box, TBP does not bind to specific promoter sequences. Instead, the association of TBP with ribosomal RNA genes is mediated by the binding of other proteins in the SL1 complex to the promoter, a situation similar to the association of TBP with the Inr sequences of polymerase II genes that lack TATA boxes.

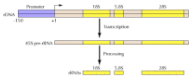


Figure 6.15

The ribosomal RNA gene. The ribosomal DNA (rDNA) is transcribed to yield a large RNA molecule (45S pre-rRNA), which is then cleaved into 28S, 18S, and 5.8S rRNAs.

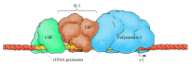


Figure 6.16

Initiation of rDNA transcription. Two transcription factors, UBF and SL1, bind cooperatively to the rDNA promoter and recruit RNA polymerase I to form an initiation complex. One subunit of SL1 is the TATA-binding protein (TBP).

The genes for tRNAs, 5S rRNA, and some of the small RNAs involved in splicing and protein transport are transcribed by polymerase III. These genes are characterized by promoters that lie within, rather than upstream of, the transcribed sequence (Figure 6.17). The most thoroughly studied of the genes transcribed by polymerase III are the 5S rRNA genes of *Xenopus*. TFIIIA (which is the first transcription factor to have been purified) initiates assembly of a transcription complex by binding to specific DNA sequences in the 5S rRNA promoter. This binding is followed by the sequential binding of TFIIIC, TFIIIB, and the polymerase. The promoters for the tRNA genes differ from the 5S rRNA promoter in that they do not contain the DNA sequence recognized by TFIIIA. Instead, TFIIIC binds directly to the promoter of tRNA genes, serving to recruit TFIIIB and polymerase to form a transcription complex. TFIIIB is composed of multiple subunits, one of which (once again) is the TATA-binding protein, TBP. Thus, although the three RNA polymerases of eukaryotic cells recognize different promoters, TBP appears to be a common element that links promoter recognition with polymerase recruitment to the transcription complex.

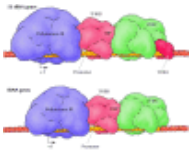


Figure 6.17

Transcription of polymerase III genes. The promoters of 5S rRNA and tRNA genes are downstream of the transcrip-tion initiation site. Transcription of the 5S rRNA gene is initiated

by the binding of TFIIIA, followed by the binding of TFIIIC, TFIIIB, and (more...)

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