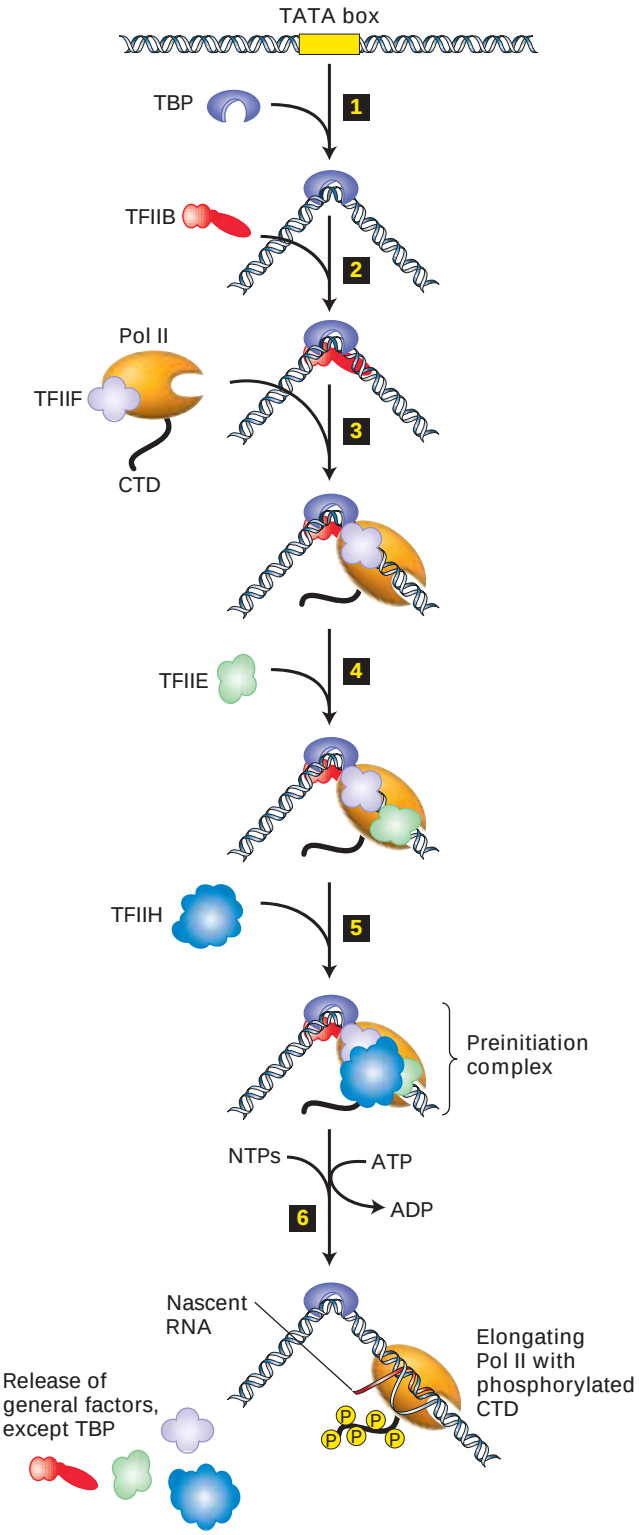
polymerase transcribes away from the promoter region, another subunit of TFIIH phosphorylates the Pol II CTD at multiple sites (see Figure 11-27). In the minimal in vitro transcription assay containing only these general transcription factors and purified RNA polymerase II, TBP remains bound to the TATA box as the polymerase transcribes away from the promoter region, but the other general transcription factors dissociate.



MEDICINE

The first subunits of TFIIH to be cloned from humans were identified because mutations in the genes encoding them cause defects in the repair of damaged DNA. In normal individuals, when a transcribing RNA polymerase becomes stalled at a region of damaged template DNA, a subcomplex of TFIIH is thought to recognize the stalled polymerase and then recruit other proteins that function with TFIIH in repairing the damaged DNA region. In the absence of functional TFIIH, such repair of damaged DNA in transcriptionally active genes is impaired. As a result, affected individuals have extreme skin sensitivity to sunlight (a common cause of DNA damage) and exhibit a high incidence of cancer. Depending on the severity of the defect in TFIIH function, these individuals may suffer from diseases such as xeroderma pigmentosum and Cockayne's syndrome (Chapter 23).

In Vivo Transcription Initiation by Pol II Requires Additional Proteins

Although the general transcription factors discussed above allow Pol II to initiate transcription in vitro, another general transcription factor, TFIIA, is required for initiation by Pol II in vivo. Purified TFIIA forms a complex with TBP and TATA-box DNA. X-ray crystallography of this complex shows that TFIIA interacts with the side of TBP that is upstream from the direction of transcription. Biochemical experiments suggest that in cells of higher eukaryotes TFIIA and TFIID, with its multiple TAF subunits, bind first to TATA-box DNA and then the other general transcription factors subsequently bind as indicated in Figure 11-27.

The TAF subunits of TFIID appear to play a role in initiating transcription from promoters that lack a TATA box. For instance, some TAF subunits contact the initiator element in promoters where it occurs, probably explaining how such sequences can replace a TATA box. Additional TFIID TAF subunits can bind to a consensus sequence A/G-G-A/T-C-G-T-G centered ≈30 base pairs downstream from the tran-

◀ FIGURE 11-27 **In vitro assembly of RNA polymerase II preinitiation complex.** The indicated general transcription factors and purified RNA polymerase II (Pol II) bind sequentially to TATA-box DNA to form a preinitiation complex. ATP hydrolysis then provides the energy for unwinding of DNA at the start site by a TFIIH subunit. As Pol II initiates transcription in the resulting open complex, the polymerase moves away from the promoter and its CTD becomes phosphorylated. In vitro, the general transcription factors (except for TBP) dissociate from the TBP-promoter complex, but it is not yet known which factors remain associated with promoter regions following each round of transcription initiation in vivo.