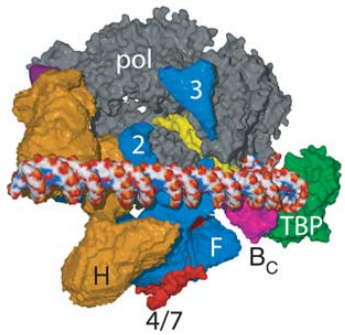
General Transcription Factors

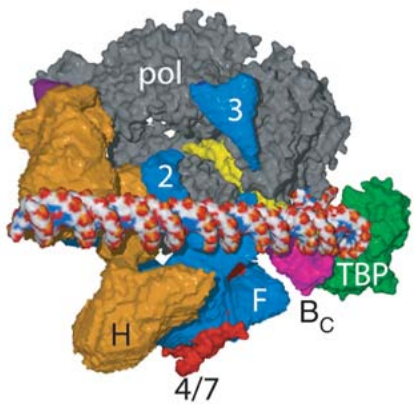
1. Assembly of Pre-initiation complex
2. TFIID components
3. TFIIB, F, H, E
4. Elongation Factors
5. GTF's for Pol I and Pol



RNA Pol II GTF's

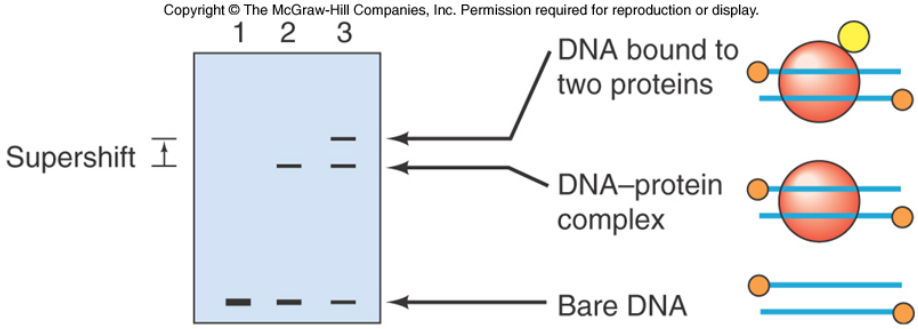
Minimum Factors for Site Specific Transcription

- RNA Polymerase II
- GTF's
  - TFIID
  - TFIIA
  - TFIIB
  - TFIIF
  - TFIIE
  - TFIIH

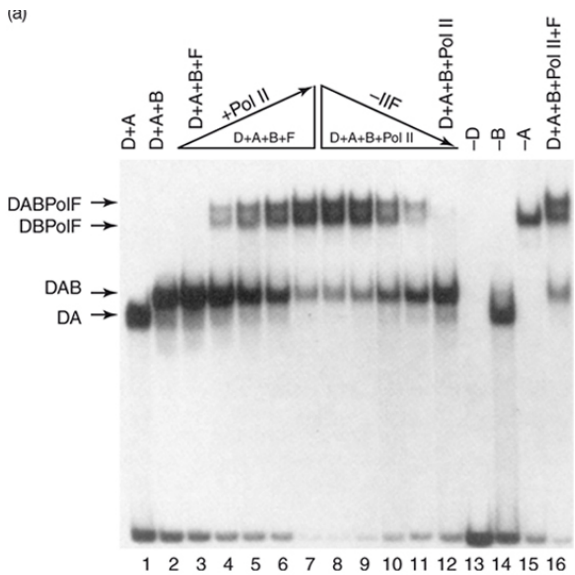


Mobility Shift Assay

Protein-DNA Interaction

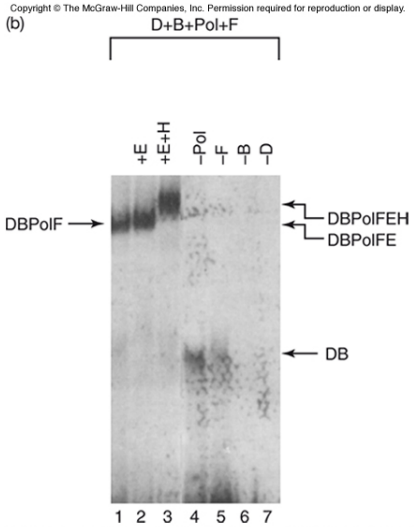


GTF Factors



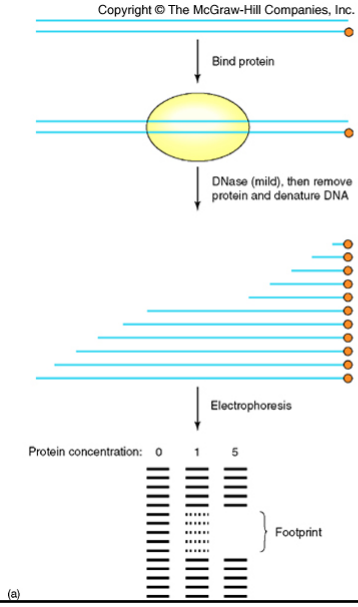
- Order of binding
- Role of TFIIF

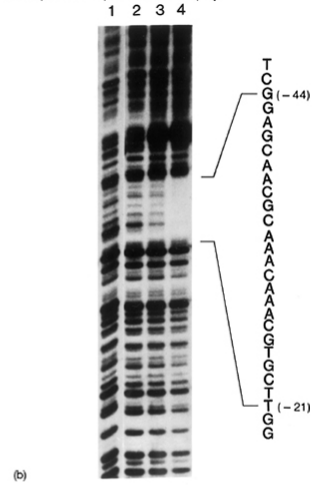
Binding of TFIIE/H



© Cortes, P., O. Flores, and D. Reinberg, 1992. Factors involved in specific transcription by mammalian RNA polymerase II: Purification and analysis of transcription factor iiA and identification of transcription factor IIJ. Molecular and Cellular

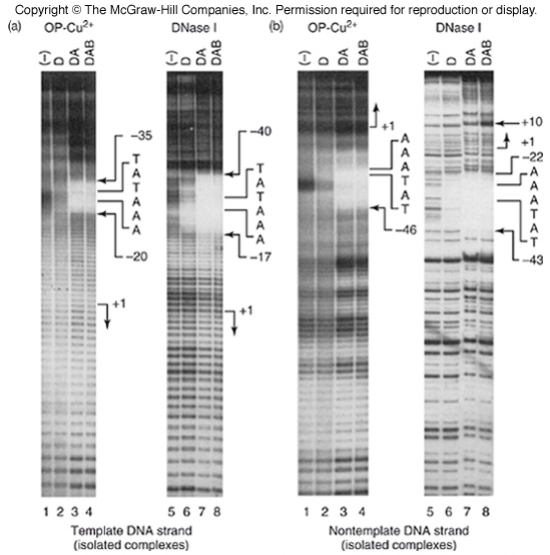
DNAse Protection Assay

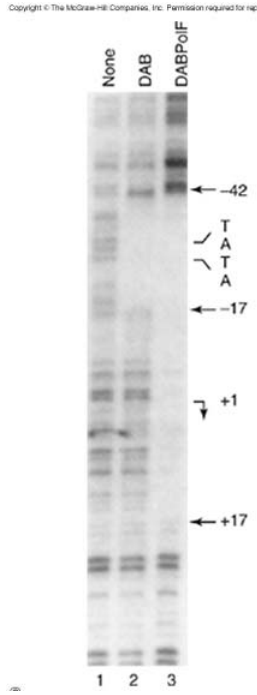




© Ho et al. Bacteriophage lambda protein "cII binds promoters on the opposite face of the DNA helix from RNA polymerase. "Nature" 304 (25 Aug 1983) p. 705, f. 3, © Macmillan Magazines Ltd.

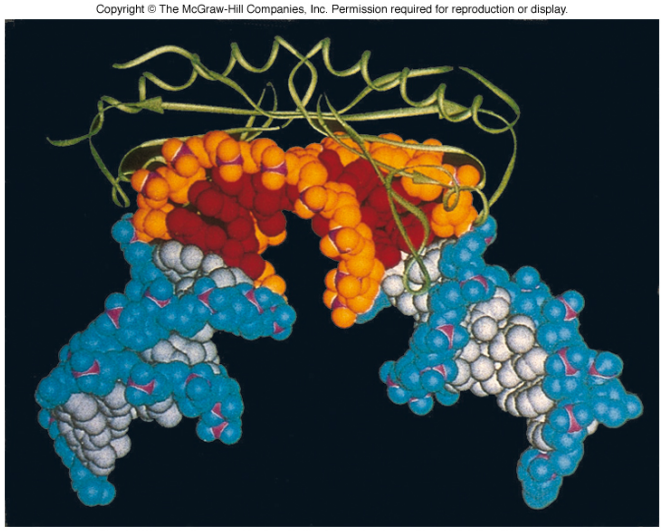
DAB DNAse Protection Assay



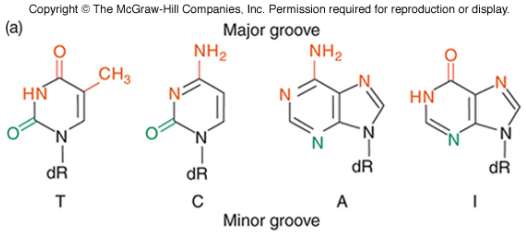


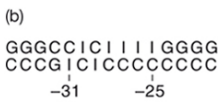
DABPolF Footprint

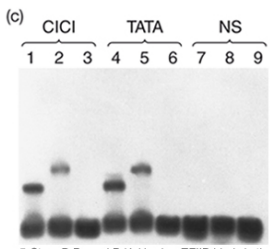
TBP



Evidence for Minor Groove

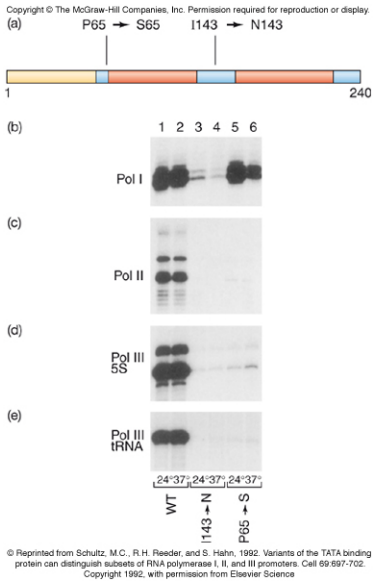






Lanes 1 and 4 yeast TBP
Lanes 2 and 5 Human TBP

TBP and three polymerases



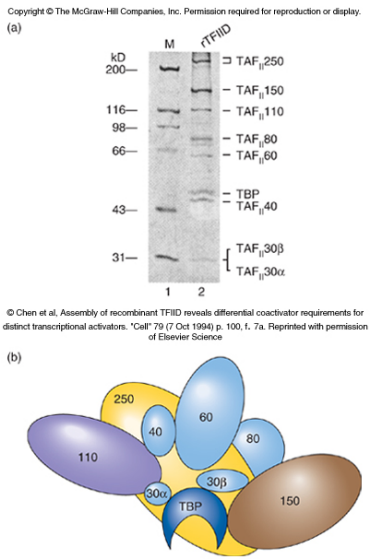
© Reprinted from Schultz, M.C., R.H. Reeder, and S. Hahn, 1992. Variants of the TATA binding protein can distinguish subsets of RNA polymerase I, II, and III promoters. Cell 69:697-702. Copyright 1992, with permission from Elsevier Science

Temperature Sensitive Mutants

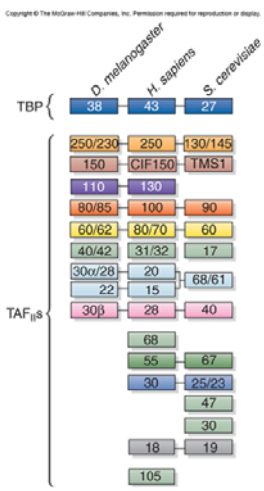
Cell extracts prepared from wildtype and mutants

Invitro transcription on several templates

TAF_{II}'s

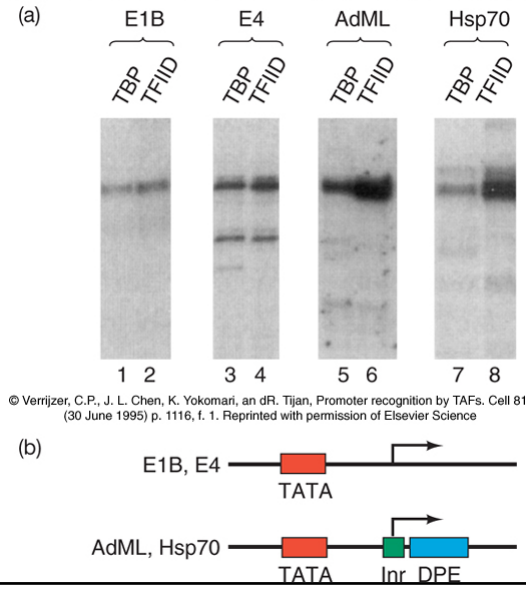


© Chen et al, Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. "Cell" 79 (7 Oct 1994) p. 100, f. 7a. Reprinted with permission of Elsevier Science



TBP vs TFIID

Role of Taf's in Recognition of core elements



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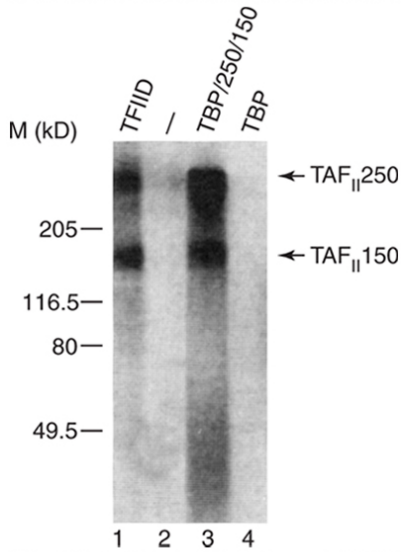
TAF interactions with DNA
Crosslinking to DNA



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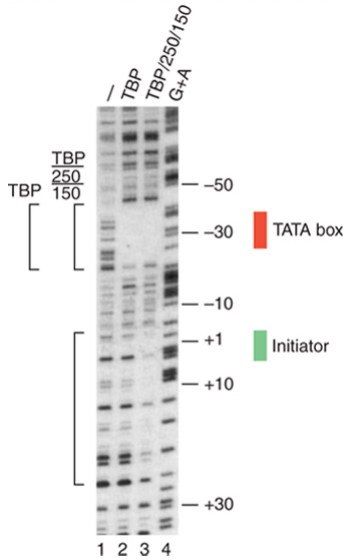
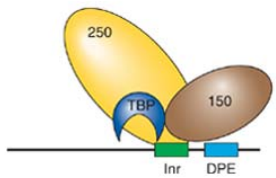
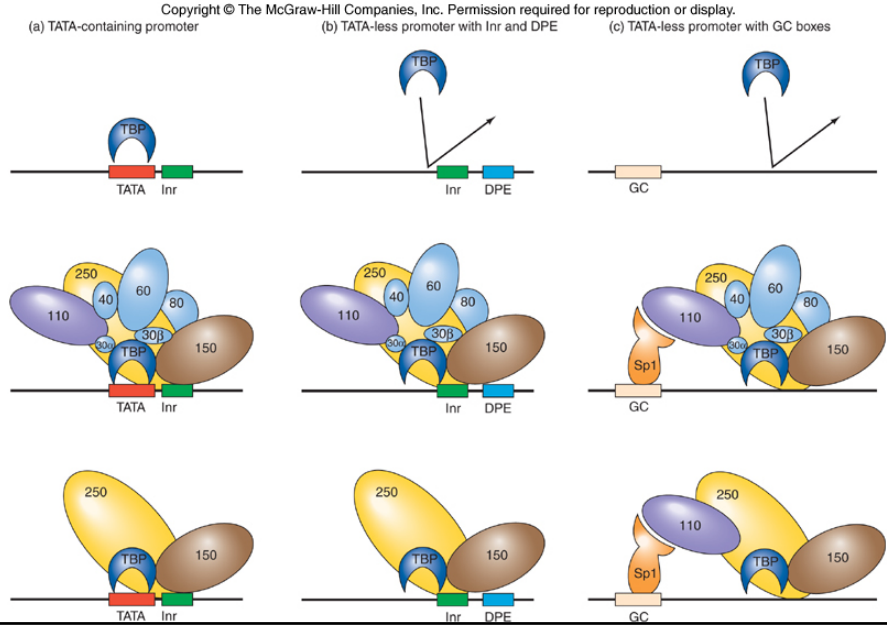
DNase Footprinting TAFS

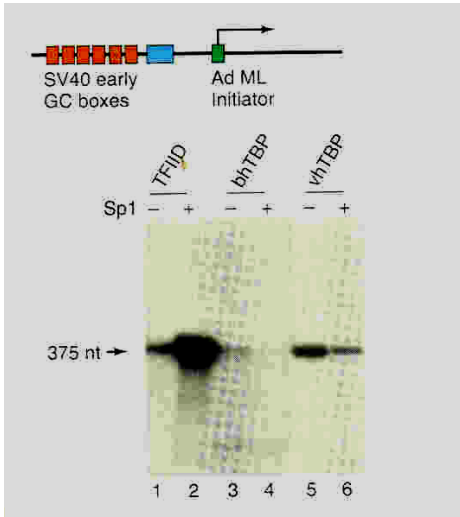




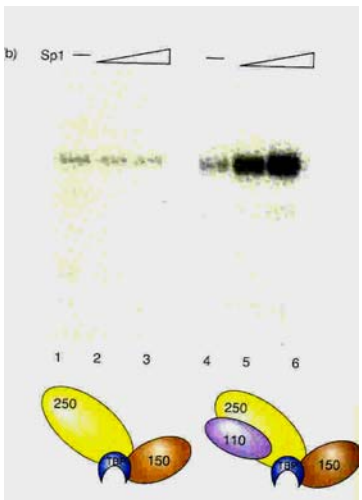
TAF's and Promoter Recognition

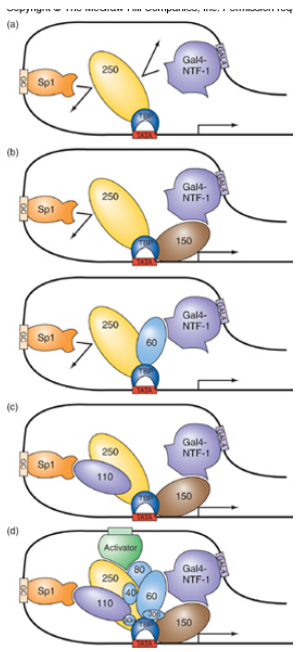


TAF dependent Activation



TAF Specific Activation



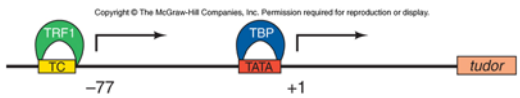


Requirement for TAF'S

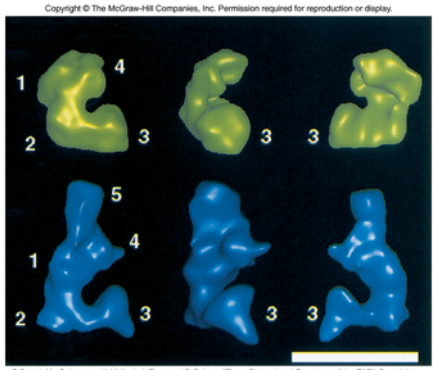
Table 11.1 Whole Genome Analysis of Transcription Requirements in Yeast

General Transcription Factor (Subunit)	Fraction of Genes Dependent on Subunit Function (%)
TFIID ($TAF_{II}145$)	16
TFIID ($TAF_{II}17$)	67
TFIIE (Tfa1)	54
TFIIH (Kin28)	87

Alternatives to TBP

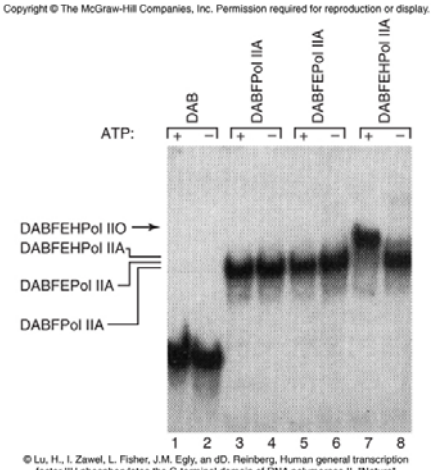


Neuron specific TBP alternative



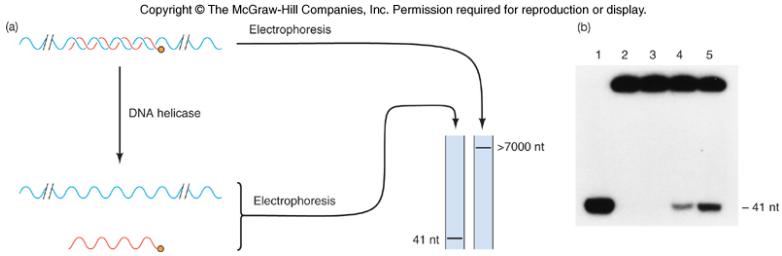
Kinase Activity TFIIH

- Kinase
- Helicase



© Lu, H., I. Zawel, L. Fisher, J.M. Egly, an dD. Reinberg, Human general transcription factor IIH phosphorylates the C-terminal domain of RNA polymerase II. "Nature" 358 (20 Aug 1992) p. 642, f.1. Copyright © Macmillan Magazines Ltd.

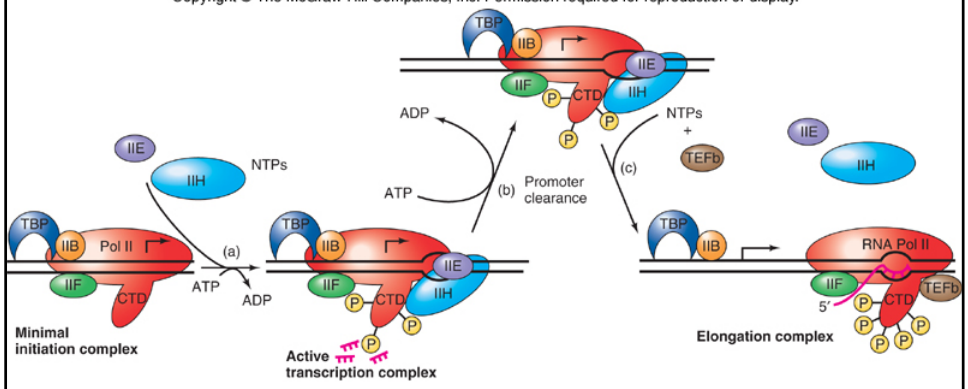
TFIIH-Helicase

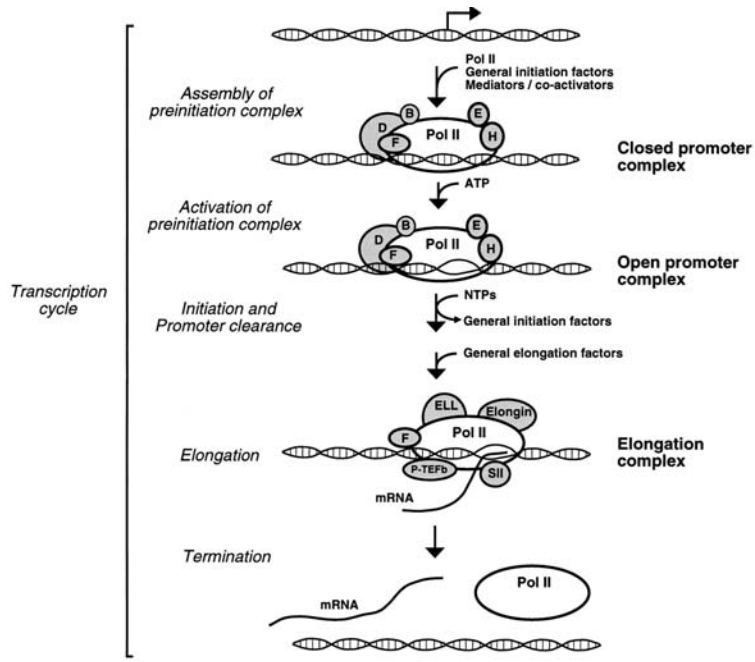


Lane
1 No Protein
2 10ng TFIIH
3 20ng TFIIH
4 10ng TFIIH +ATP
5 20ng TFIIH + ATP

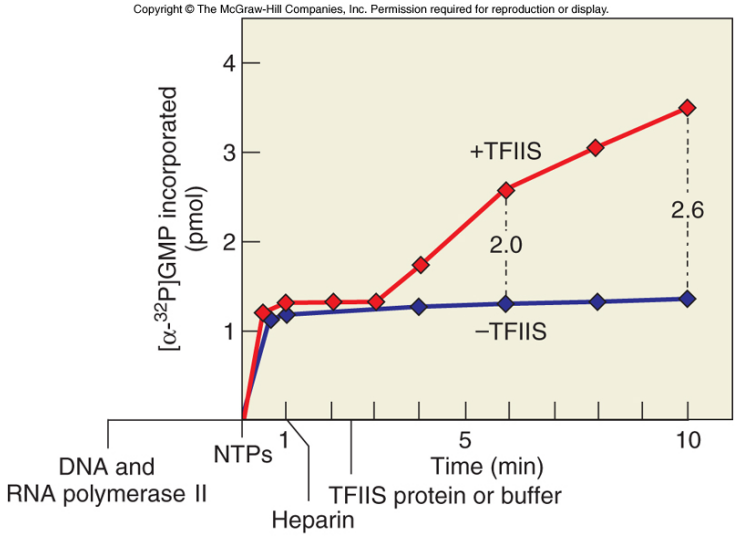
© Gudzer, S.N., P. Sung, V. Bailly, L. Prakash, and S. Prakash, RAD25 is a DNA helicase required for DNA repair and RNA polymerase II transcription. "Nature" 369 (16 June 1994) p. 579, f. 2c. Copyright © Macmillan Magazines Ltd.

Promoter Clearing

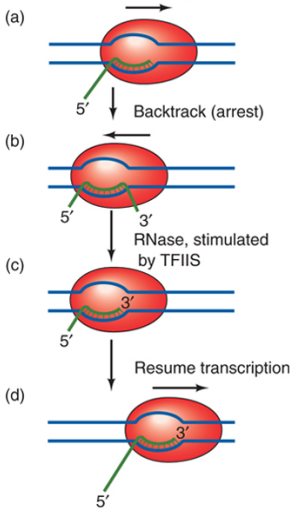


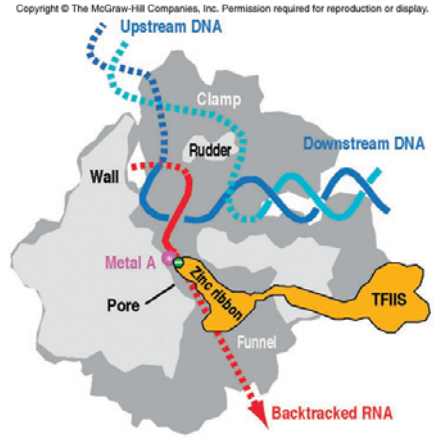


TFIIS



TFIIS Mechanism

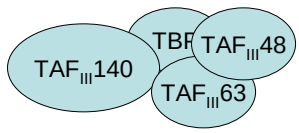




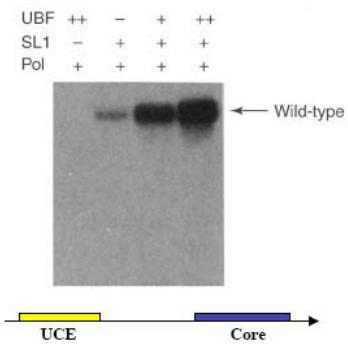
Pausing and Proofreading

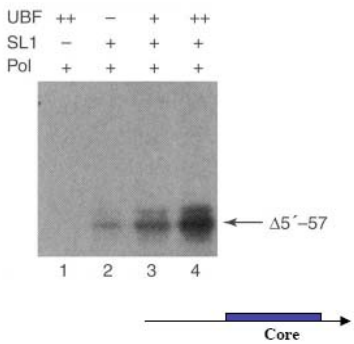
UBF

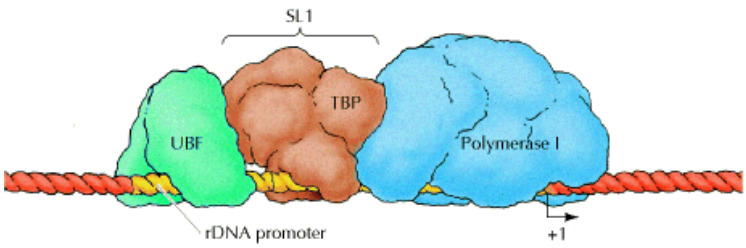
TIF-IB
(SLI)



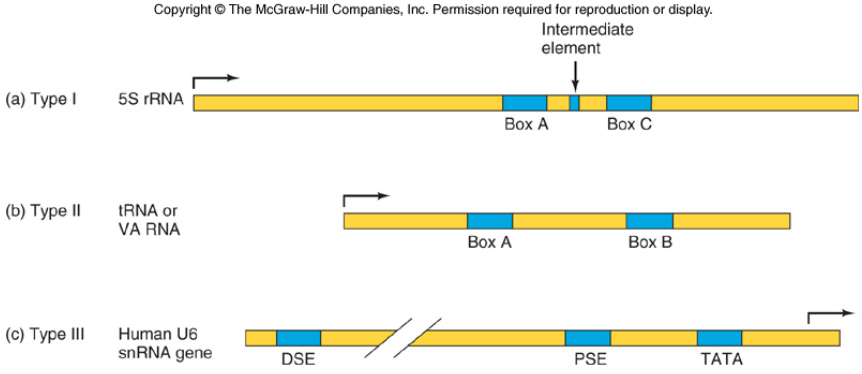
Role of SL1/UBF



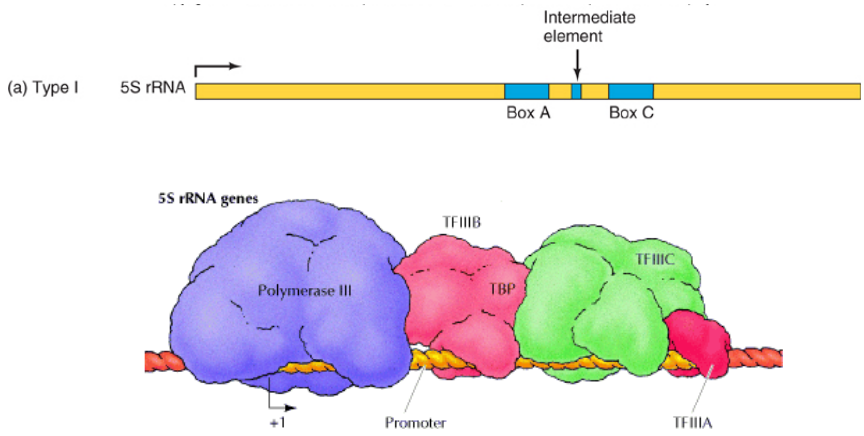




Pol III Promoters

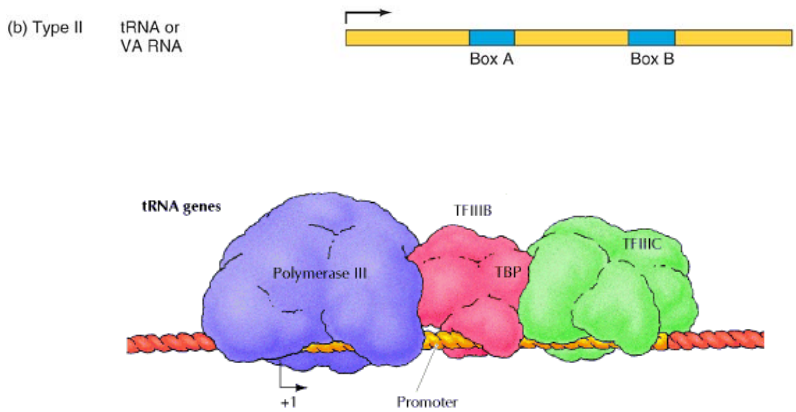


Type I

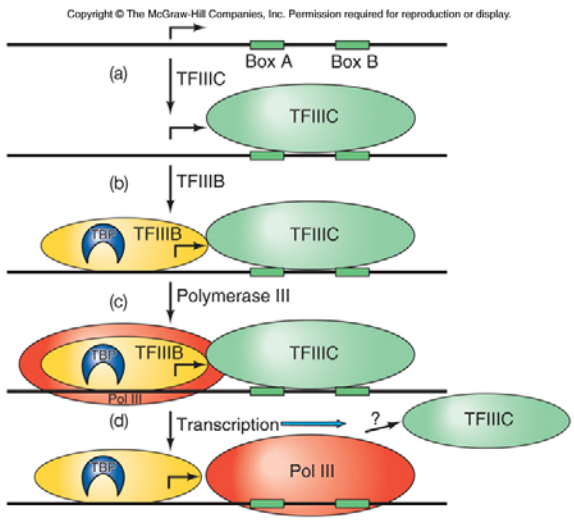


Cooper, The Cell A Molecular Approach

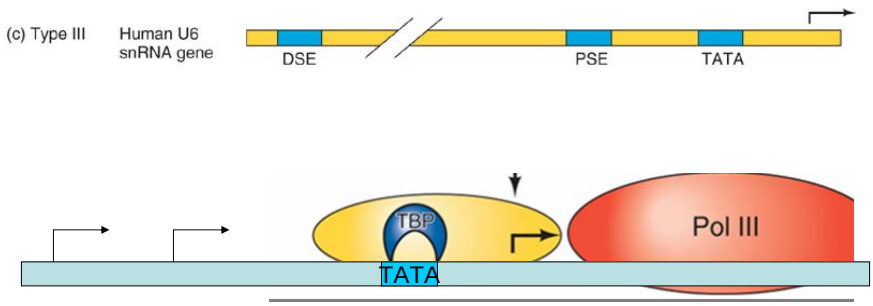
Type II



Assembly of Complex



Type III



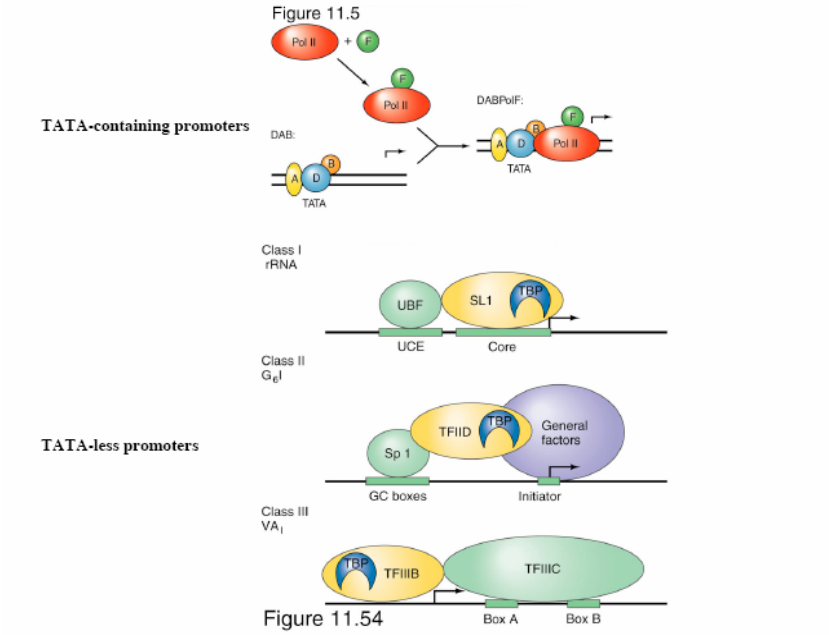


Figure 11.5
TATA-containing promoters
Pol II
F
DAB:
A
D
B
TATA
DABPolF:
Pol II
TATA
Class I
rRNA
UBF
SL1
TBP
UCE
Core
Class II
G_6I
TATA-less promoters
Sp 1
TFIID
TBP
General
factors
GC boxes
Initiator
Class III
VA_I
TBP
TFIIIB
TFIIIC
Box A
Box B
Figure 11.54