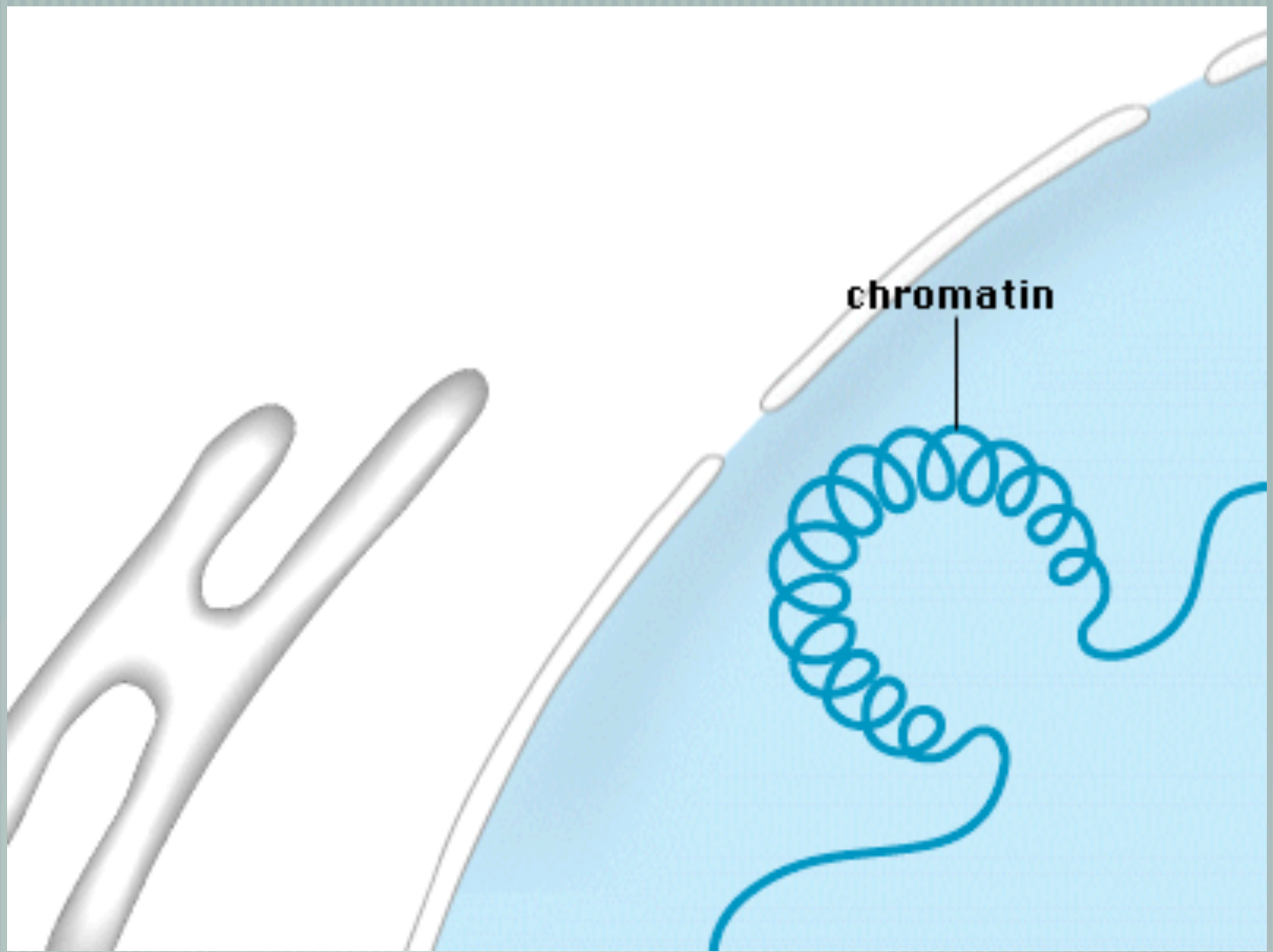
Polymerases & their promoters

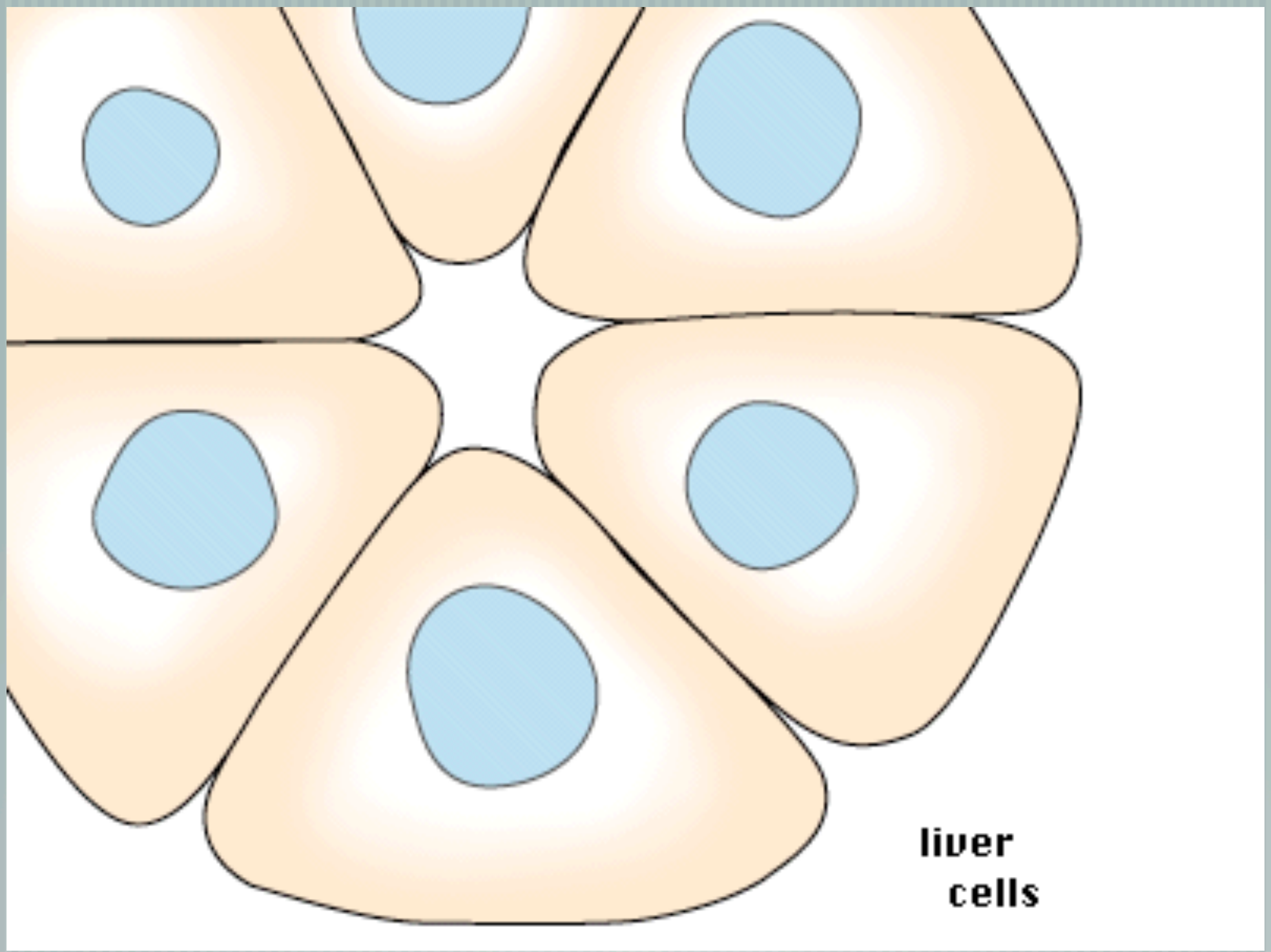
Chapter 10 & 11

Destroying Angel –
Angel of Death – Amanita bisporigera
Death Cap – Amanita phalloides
alpha-amanitan

Expectations - fact based learning

- You will be able to describe a core promoter.
- You will be able to describe the difference between a promoter and a core promoter.
- You will know how promoters are recognized.
  You will also understand what I mean by this jargonized/colloquial use of the word recognized.
- You will be able to describe what TFIID does. You will be able to decribe its components and their function.


chromatin


liver
cells

Eukaryotic polymerases

Polymerase	Genes transcribed	Subcellular localization	sensitivity to α-amanitin
RNA polymerase I	**rRNA genes** **this is actually the bulk of cellular transcription**	**nucleolus**	**insensitive**
RNA polymerase II	**protein encoding genes are transcribed to produce mRNA**	**nucleoplasm**	**usually very sensitive**
RNA polymerase III	**tRNAs, 5S RNA and other small nuclear RNAs**	**nucleoplasm**	**Sensitive but to a much larger dose -** **Species dependent**

Destroying Angel –
Angel of Death – Amanita bisporigera
Death Cap – Amanita phalloides

Eukaryotic RNA polymerases

- Biochemical purification is difficult because it is a fragile multi-protein complex. Falls apart easily
- Purify from as one unit from yeast, confirm that mutations in each protein interferes with transcription
  - 12 proteins
  - 3 are evolutionarily related to prokaryotic
  - Five subunits are common to all nuclear polymerases

Epitope tagging to confirm

- What is it?
- How is it good for?
- Why bother?
- What controls should one do?

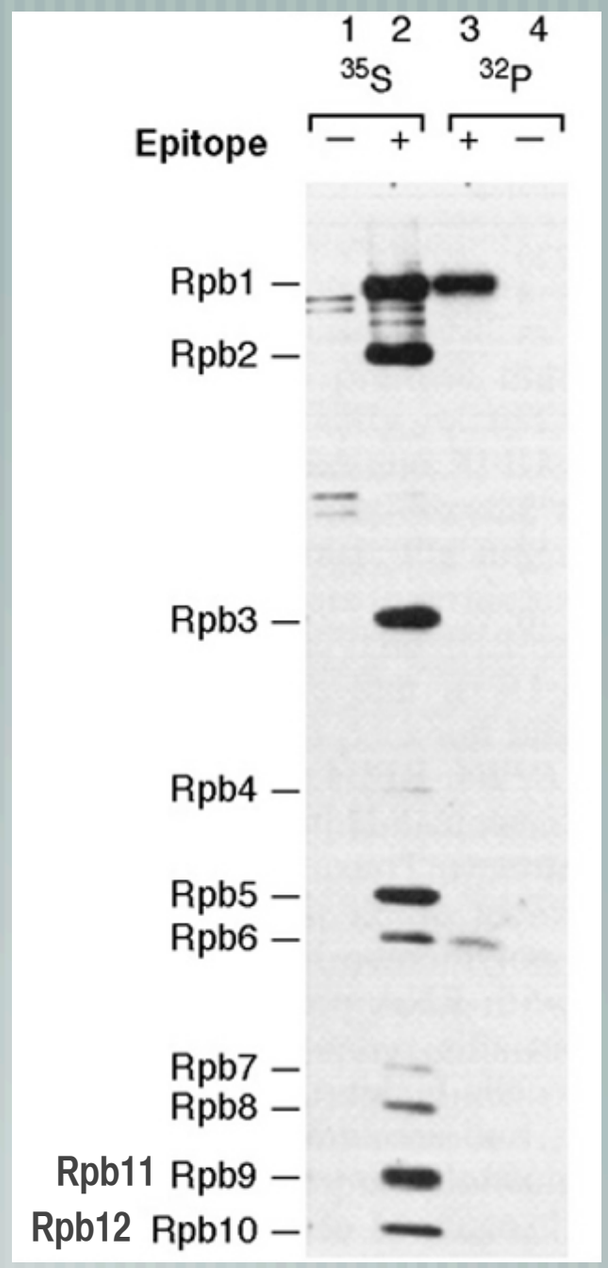
1 step purification
Do a cartoon.
Fig 10.6 Weaver 4th ed.

Epitope tagging

- To understand this slide you must understand what antibodies are and how they are made.
- Purpose: Antibodies are great tools for tracking and identifying a protein. A problem is that an antibody must be made against the protein that you are interested in studying. This can be pretty difficult. Furthermore, it requires a bit of luck since the antibody must be specific for your protein and not cross react with other proteins.
- If you don't have a way to obtain the protein of interest in pure form then it is just about impossible to make an antibody against it.
- A solution is epitope tagging.
  - An epitope is what a single antibody recognizes.
  - In proteins, epitopes are usually about 7 amino acids long.
  - An antibody is made against an epitope that is not common in the organism that you are studying.
  - The DNA sequence encoding the epitope is inserted into the gene that you are studying. It is inserted such that it is in the same reading frame as the gene.
  - If the modified gene is inserted into the organism and expressed the antibody will now recognize the protein because it contains the epitope..
  - Now your readily available and well characterized antibody will recognize the protein.

*

- Stoichiometry?
  - controls?
- Modifications?
  - controls?



When purifying a big complex one finds that at first 1) you are too rough – get few subunits, 2) as you learn to be more gentle get more subunits. How do you tell contaiminants from subunits?

Figure 10.7 page 256 Weaver 4th. Subunit structure yeast RNAP II. Shows stoichiometry, shows possibility of phosphorylation

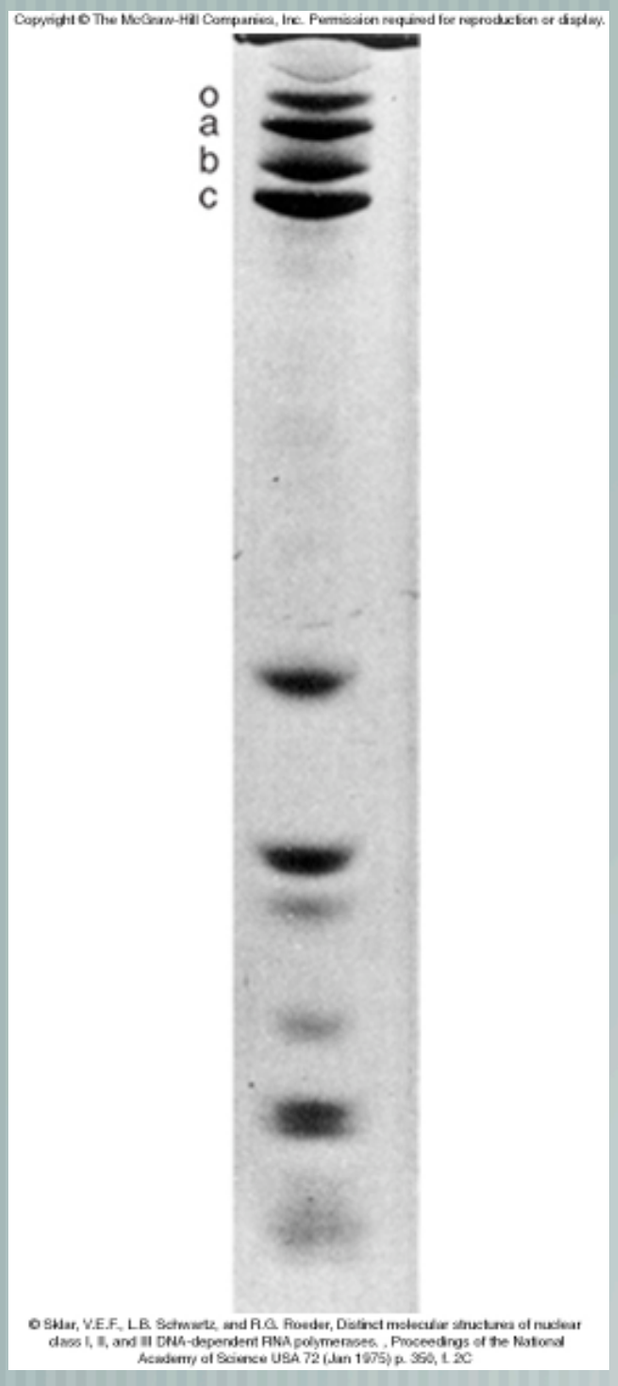
Table 10.2 Human and Yeast RNA Polymerase II Subunits

Subunit	Yeast Gene	Yeast Protein (kD)	Features
hRPB1	*RPB1*	192	Contains CTD; binds DNA; involved in start site selection; β′ ortholog
hRPB2	*RPB2*	139	Contains active site; involved in start site selection, elongation rate; β ortholog
hRPB3	*RPB3*	35	May function with Rpb11 as ortholog of the α dimer of prokaryotic RNA polymerase
hRPB4	*RPB4*	25	Subcomplex with Rpb7; involved in stress response
hRPB5	*RPB5*	25	Shared with Pol I, II, III; target for transcriptional activators
hRPB6	*RPB6*	18	Shared with Pol I, II, III; functions assembly and stability
hRPB7	*RPB7*	19	Forms subcomplex with Rpb4 that preferentially binds during stationary phase
hRPB8	*RPB8*	17	Shared with Pol I, II, III; has oligonucleotide/oligosaccharide-binding domain
hRPB9	*RPB9*	14	Contains zinc ribbon motif that may be involved in elongation: functions in start site selection
hRPB10	*RPB10*	8	Shared with Pol I, II, III
hRPB11	*RPB11*	14	May function with Rpb3 as ortholog of the α dimer of prokaryotic RNA polymerase
hRPB12	*RPB12*	8	Shared with Pol I, II, III

Source: Reprinted with permission from the *Annual Review of Genetics*, Volume 34, © 2000 by Annual Reviews. www.annualreviews.org

pink – essential for function
yellow – common to all three eukaryotic polymerases

o, a and b = 3 forms of Rpb1



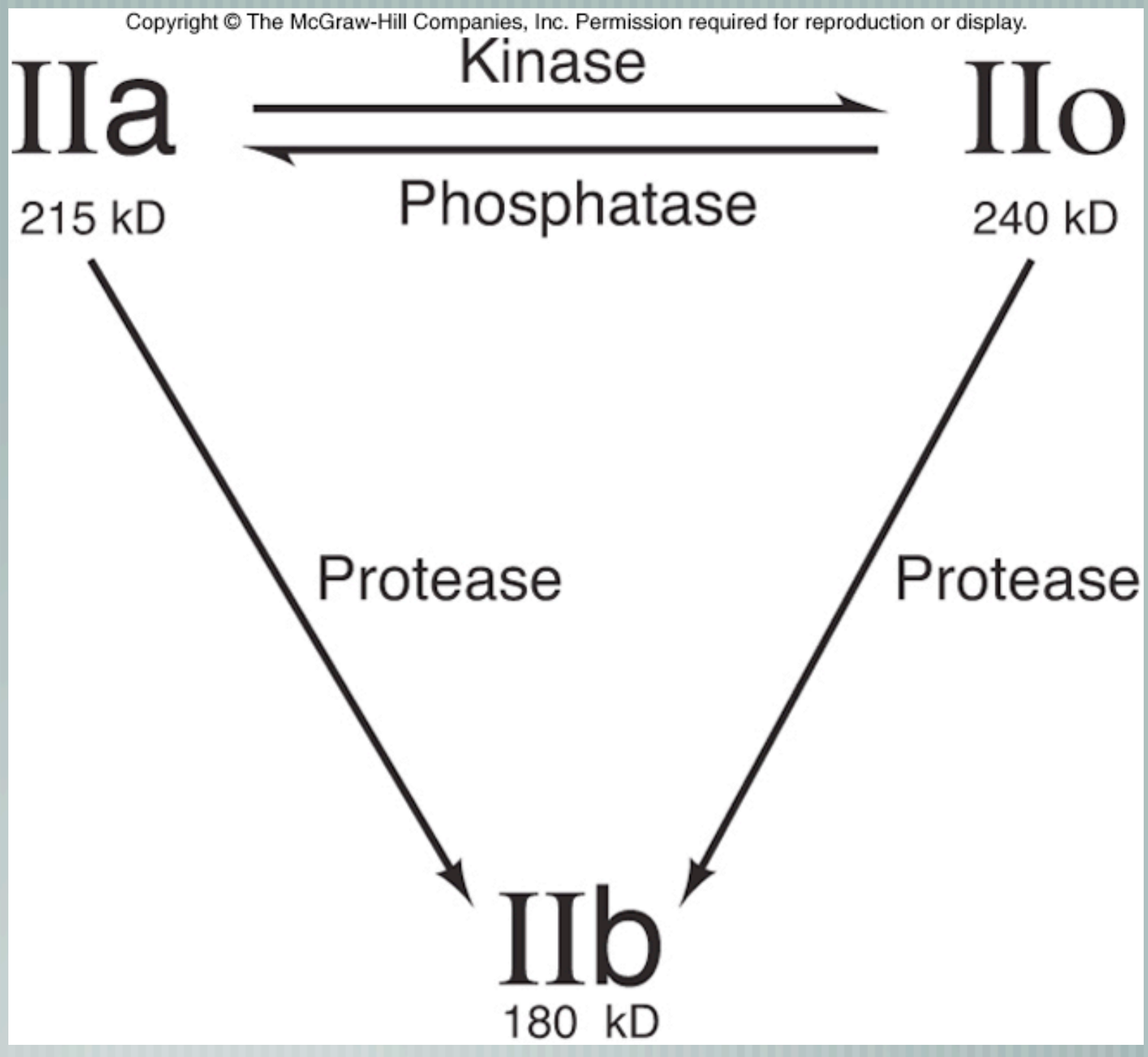
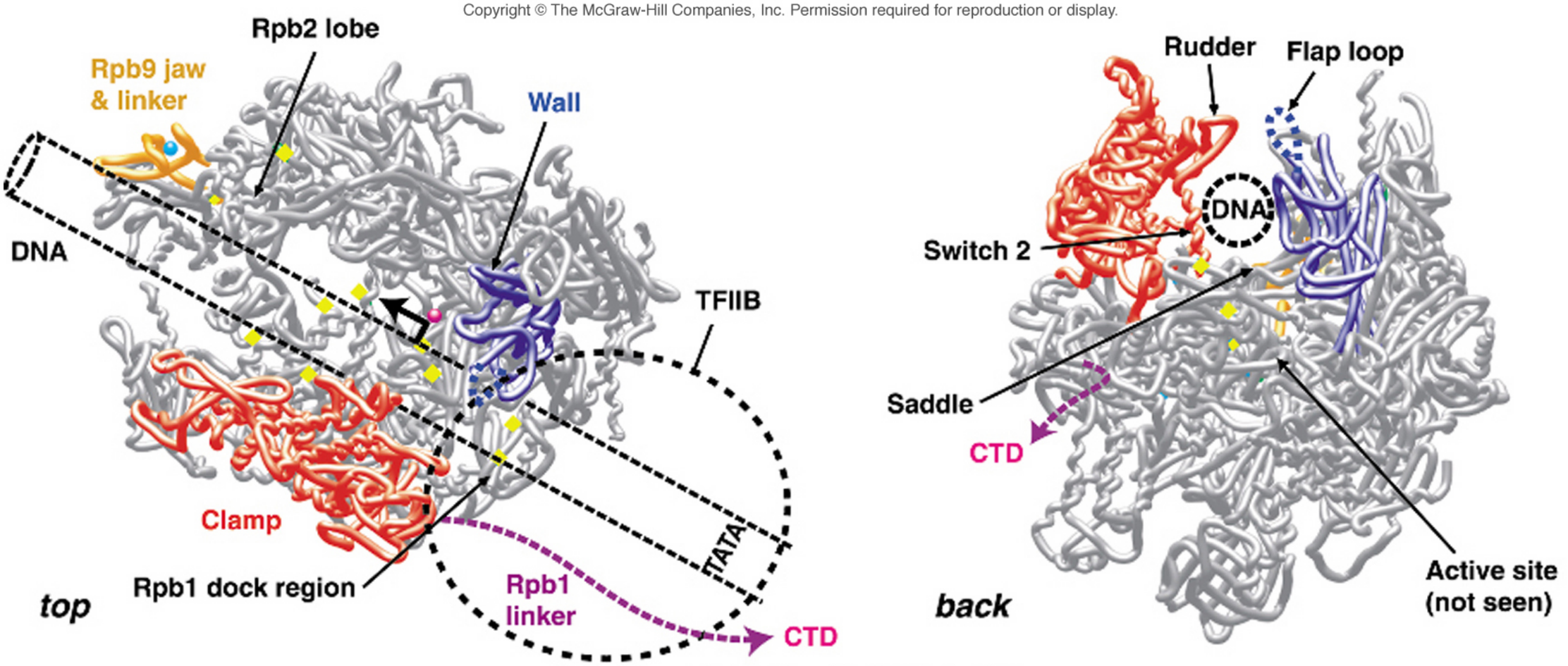


Figure 10.8 Weaver 4th ed

Fig 10.8 Weaver 4th ed.
3 forms of Rpb1 – how might one show that?



(Source: Cramer et al *Science* 292: p. 1873. © 2002 by the AAAS.)

Figure 10.14 Weaver 3rd edition

Promoters & regulatory sequences

- Core Promoters: fix & direct
  - exceptions
- Regulatory sequences: how much, when & where
  - exceptions

Biology is the science of exceptions.

Transcriptional Regulation

- Mammals regulate a minimum of 30,000 genes
- Many with multiple promoters
- DNA being regulated is wrapped in chromatin
- Combinatorial control

Can regulate nucleosome structure.

Core promoters are transcriptionally inactive without activation by regulatory proteins.

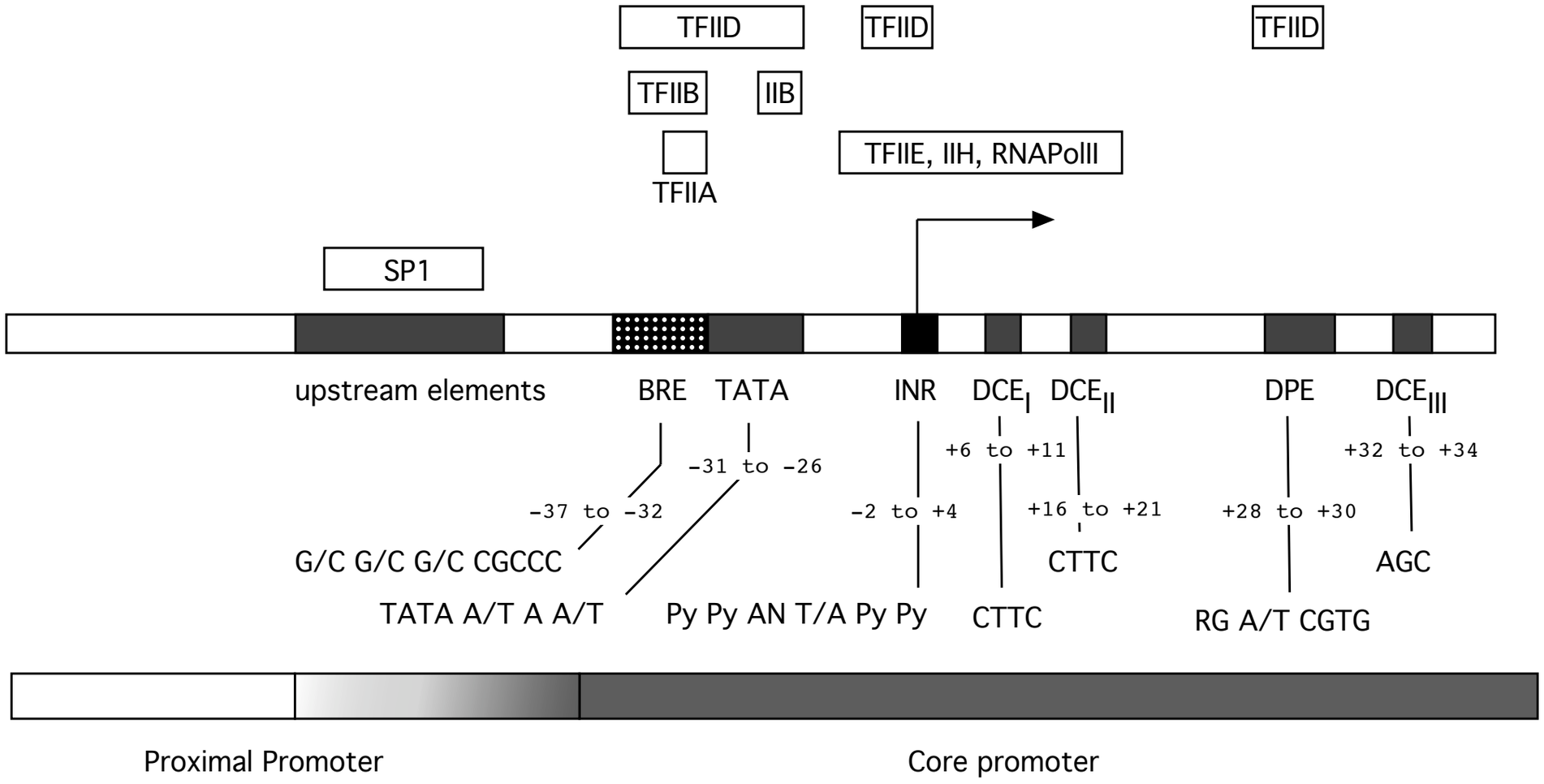
Core promoters used by RNAP II

Book refers to them as Class II promoters

- -40 to +50
- Preinitiation complex assembles here
- Determines start site and direction of transcription
- In vivo they are inactive or expressed very weakly. Need exogenous stimulation (exceptions exist). May have much more activity in vitro.

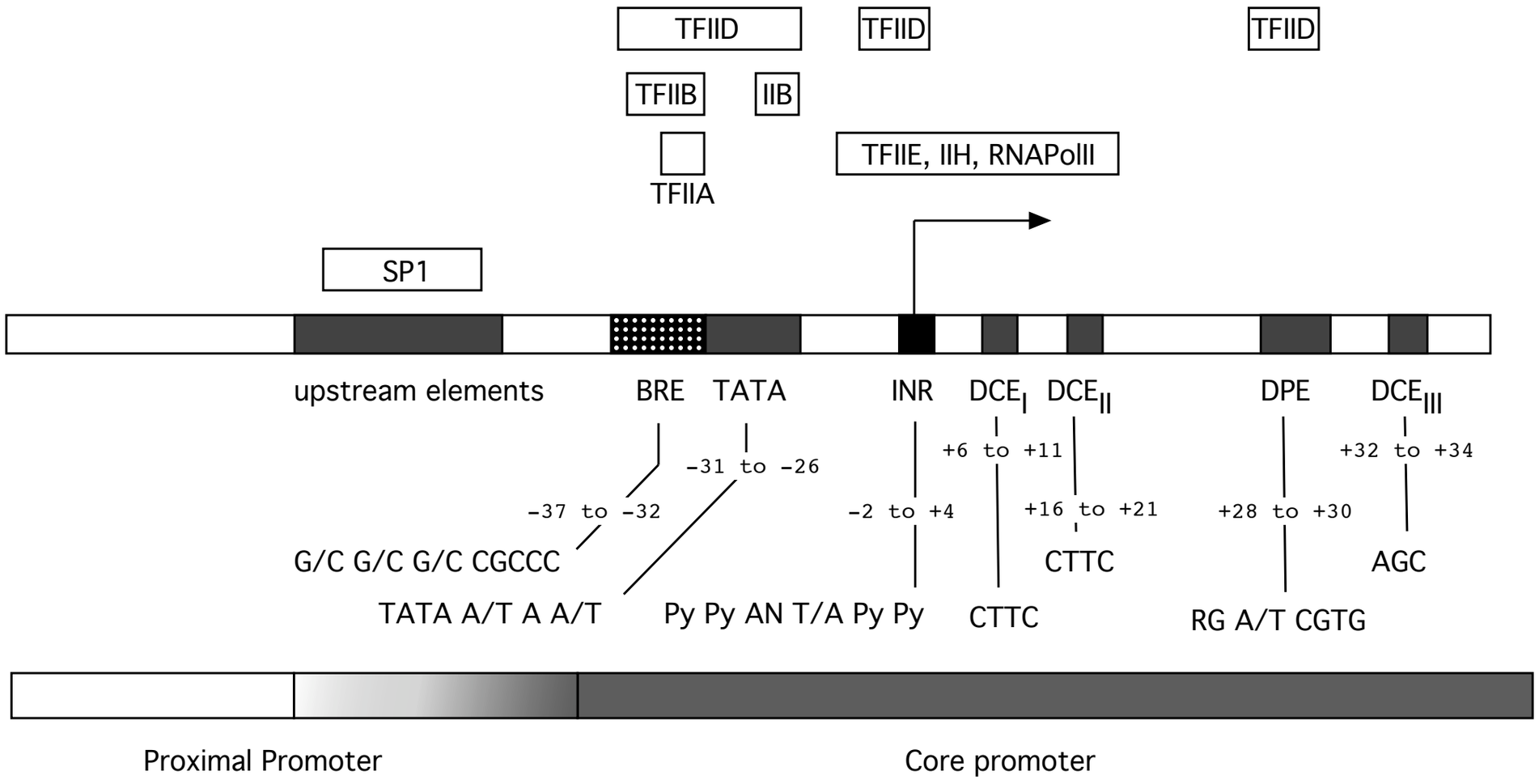
pg 287

RNAPII = RNA polymerase II



- TATA box
- Initiator element (Inr)
- Downstream core promoter element DPE is 7 nucleotides RGA/TCGTG about 30 bp downstream of +1. Often found in TATA-less promoters. Works with an Inr.
- BRE element at -32 to -38 interacts with TFIIB. (G/C)2G/ACGCC

pg 289. Drosophila initiator is TCA(G/T)T(TC).



A few common combinations
TATA or a DPE usually not both
TATA with DCE
TATA INR
INR DPE

MTE stands for "motif the ten element"
Found at +18 to +29
Found in Drosophila
Has not yet been shown to be important in mammals.
MTE requries INR
TATA MTE is common
MET DPR is common
MET can substitute for TATA and MTE

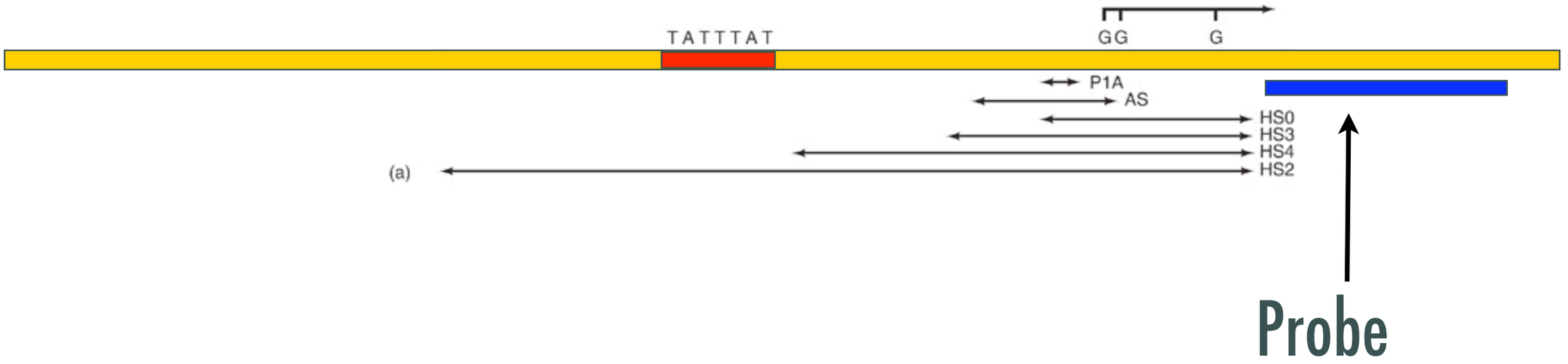
pg 289. Drosophila initiator is TCA(G/T)T(TC).

There exist many variants on the core promoter sequence. Why?

- May assemble slightly different pre-initiation complex with specific features required for proper regulation.
- The pre-initiation complex may bind to it less or more tightly. Why is this important?

Fig. 10.21

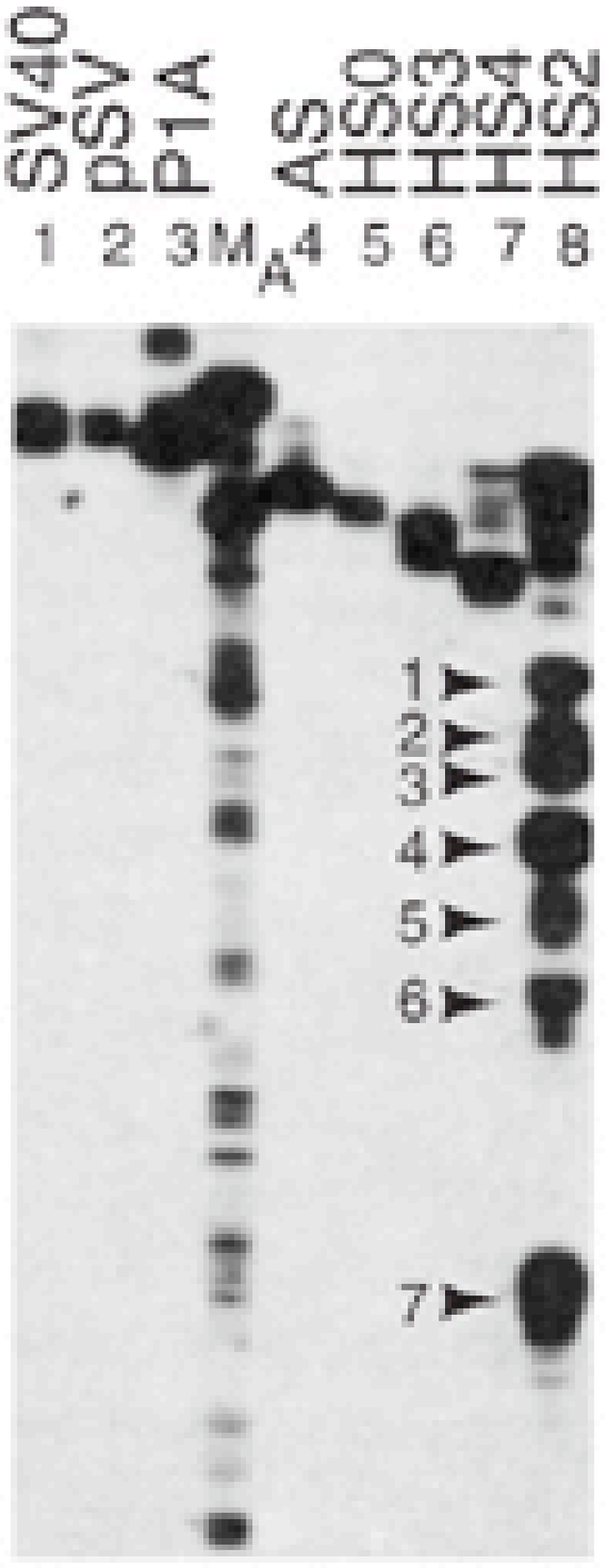
S1 protection

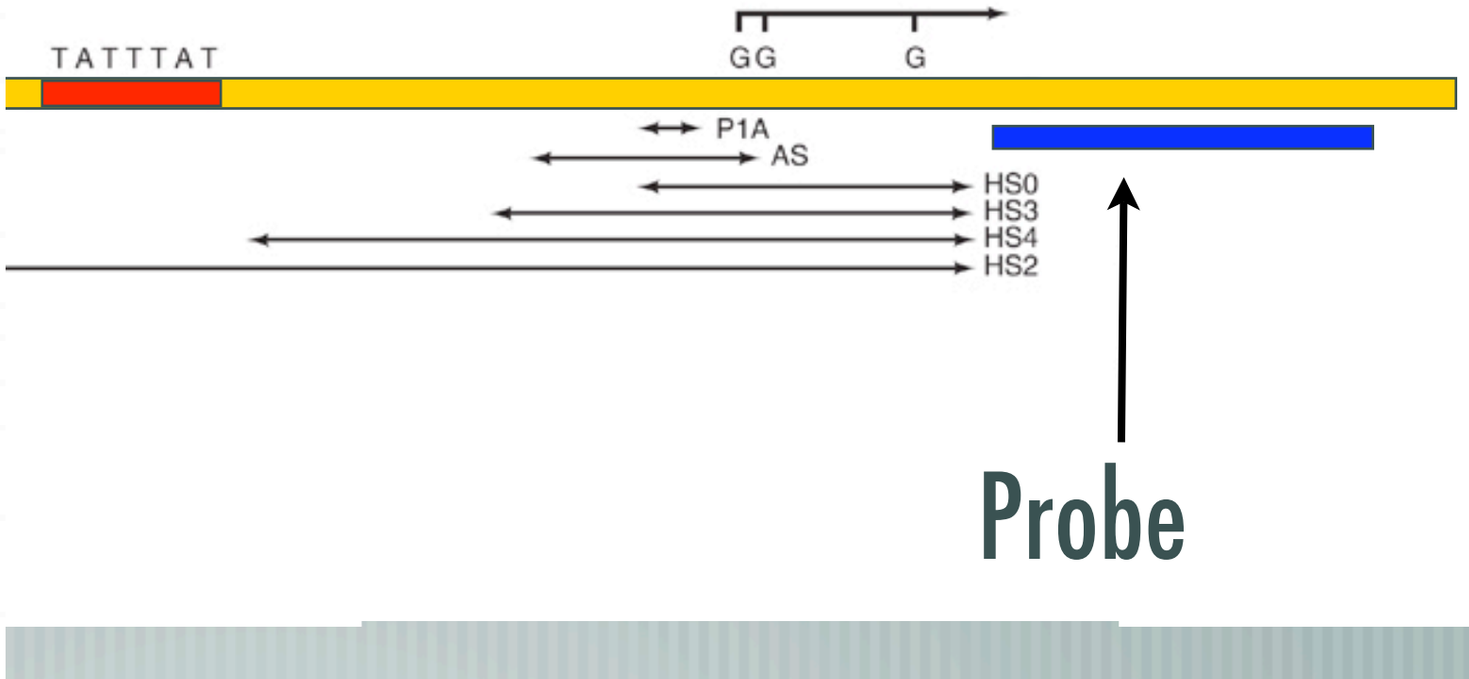


PSV=the entire thing (T-antigen segment from SV40)

Fig. 10.21

S1 protection

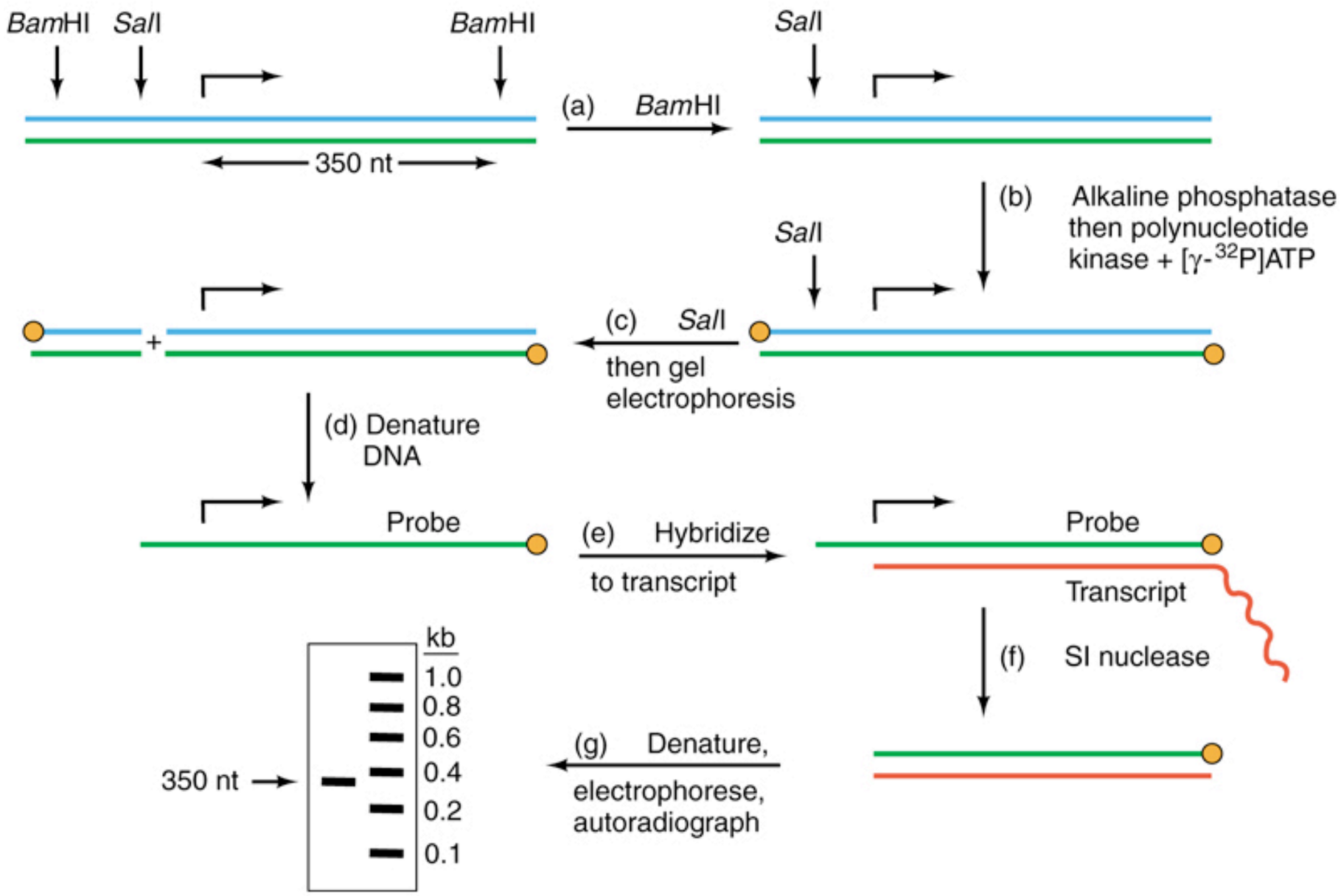




PSV=the entire thing (T-antigen segment from SV40)

Quantitative S1

Fig. 5.27



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Purpose: To determine the boundaries of exons and introns and perhaps the position of a trancription start site. One can also use it to determine the relative concentration of a nucleic acid.

Mapping - linker scanning

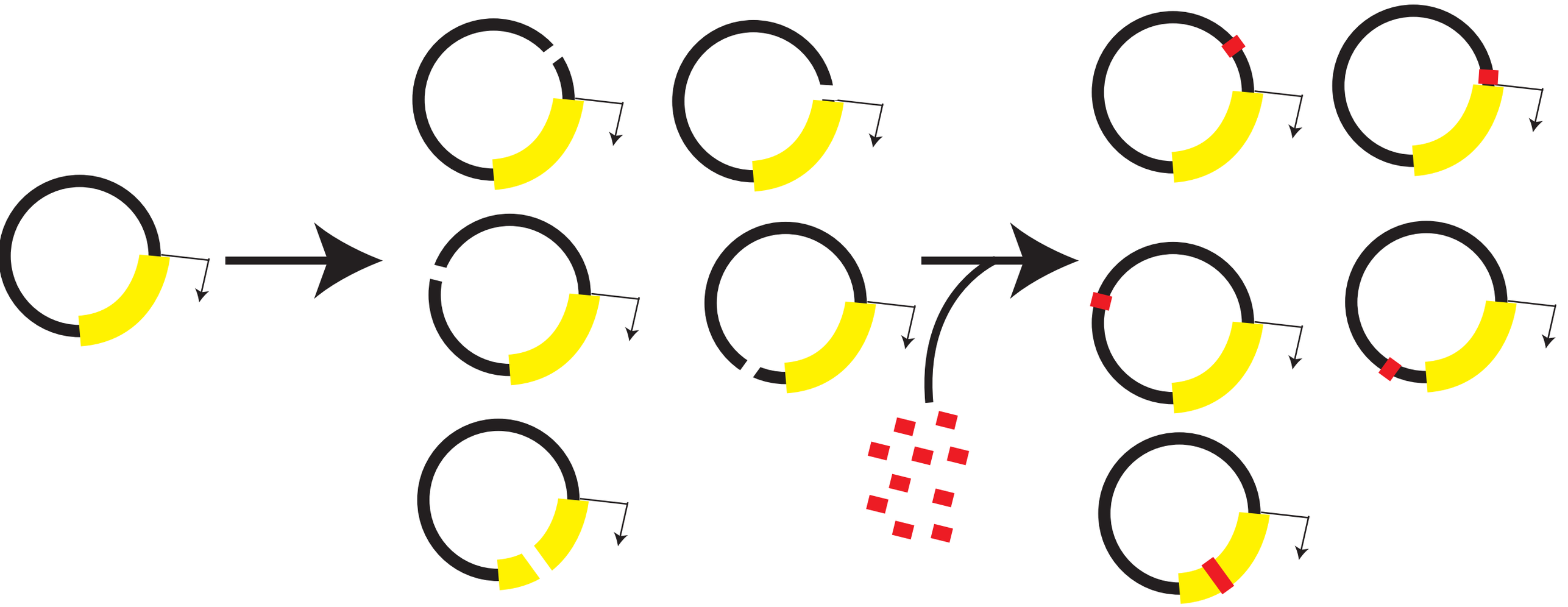
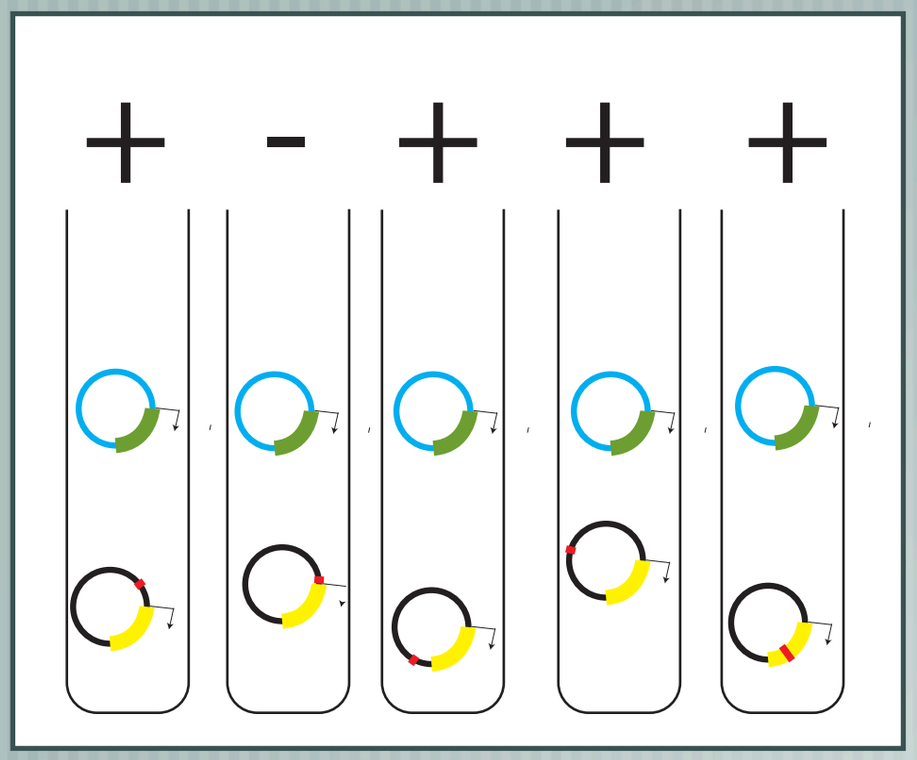


Figure 10.22

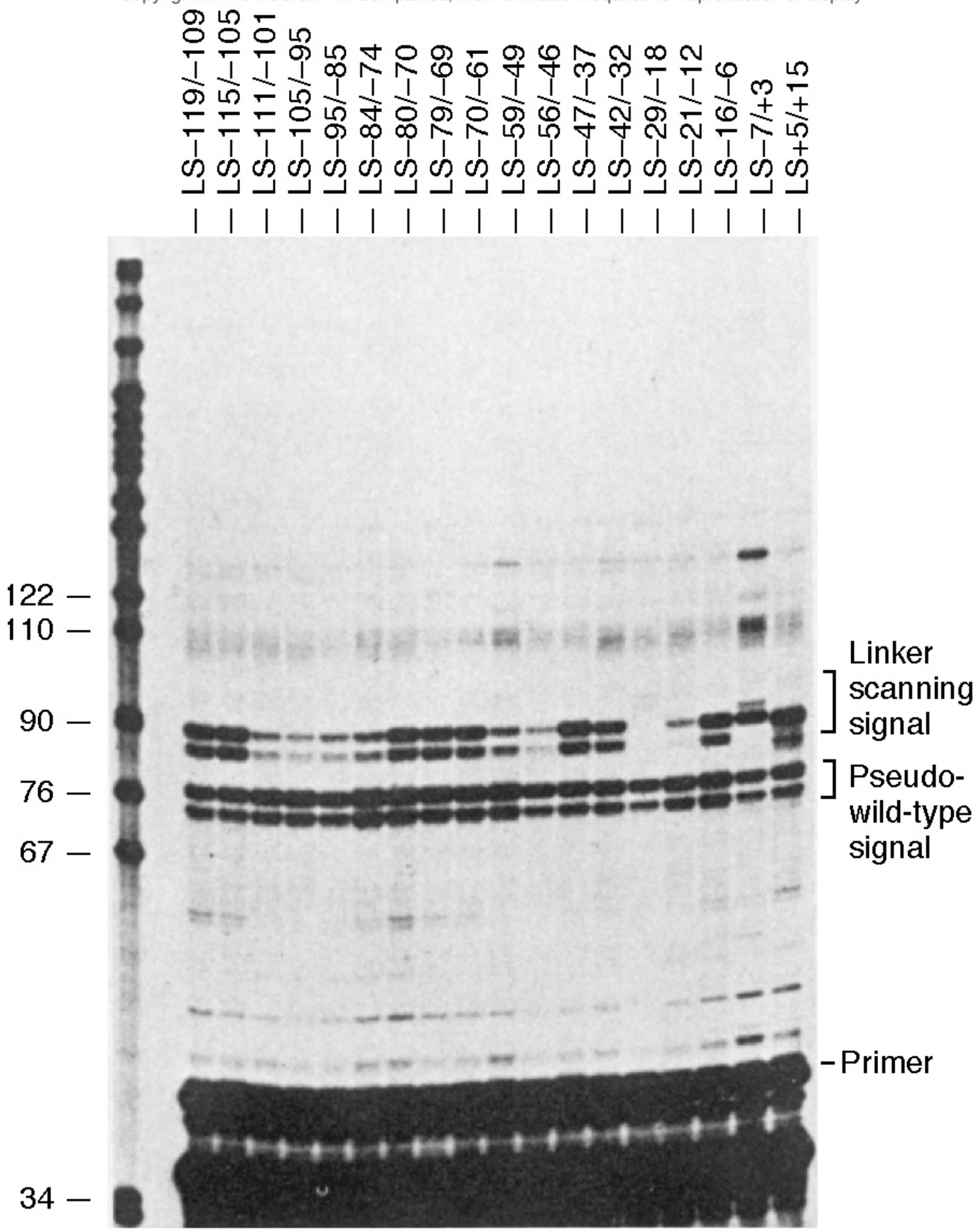
Endo + exonuclease to remove 10 bp.
Describe ligase

Controls?



Primer extension

Fig. 10.23 Weaver 4th



(Source: McKnight, S.L. and R. Kingsbury, Transcriptional control signals of a eukaryotic protein-coding gene. *Science* 217 (23 July 1982) p. 322, f. 5. © AAAS.)

Herpes Virus TK promoter. PRIMER EXTENSION. Plus minus refer to whether the 'example' expresses the yellow cassette. Green is identical but has different length - pseudo wildtype. TATA deleted by -29 to -18. GC Box at -85 to 105.

{McKnight and Kingsbury, 1982, #53952}

McKnight, S. L., and Kingsbury, R. (1982). Transcriptional control signals of a eukaryotic protein-coding gene. Science *217*, 316-324.

Transcription factors

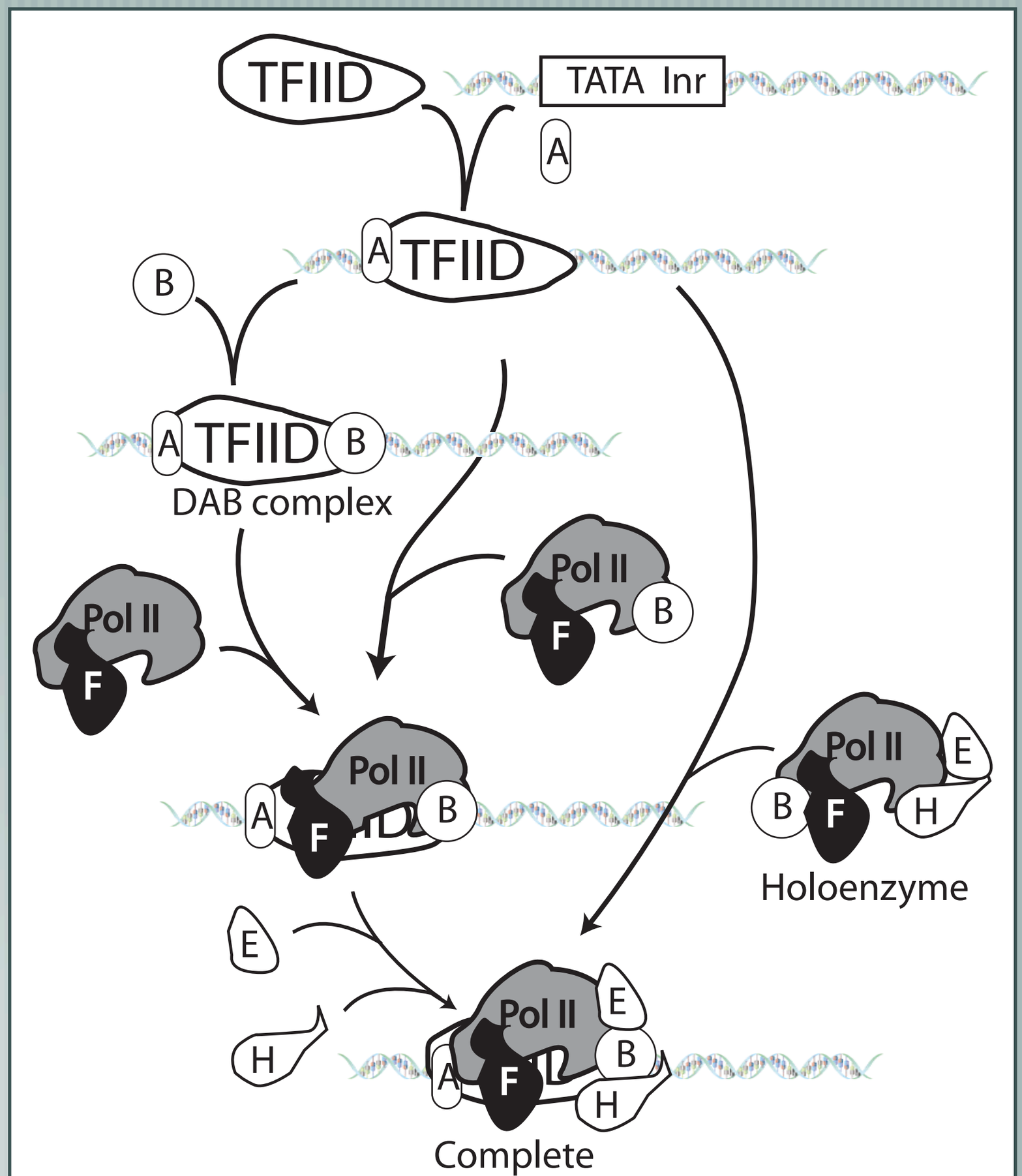
- Anything other than RNA polymerase that is required for transcription.
- In Eukaryotes, RNA polymerase holoenzyme cannot recognize promoters by itself.

General transcription factors

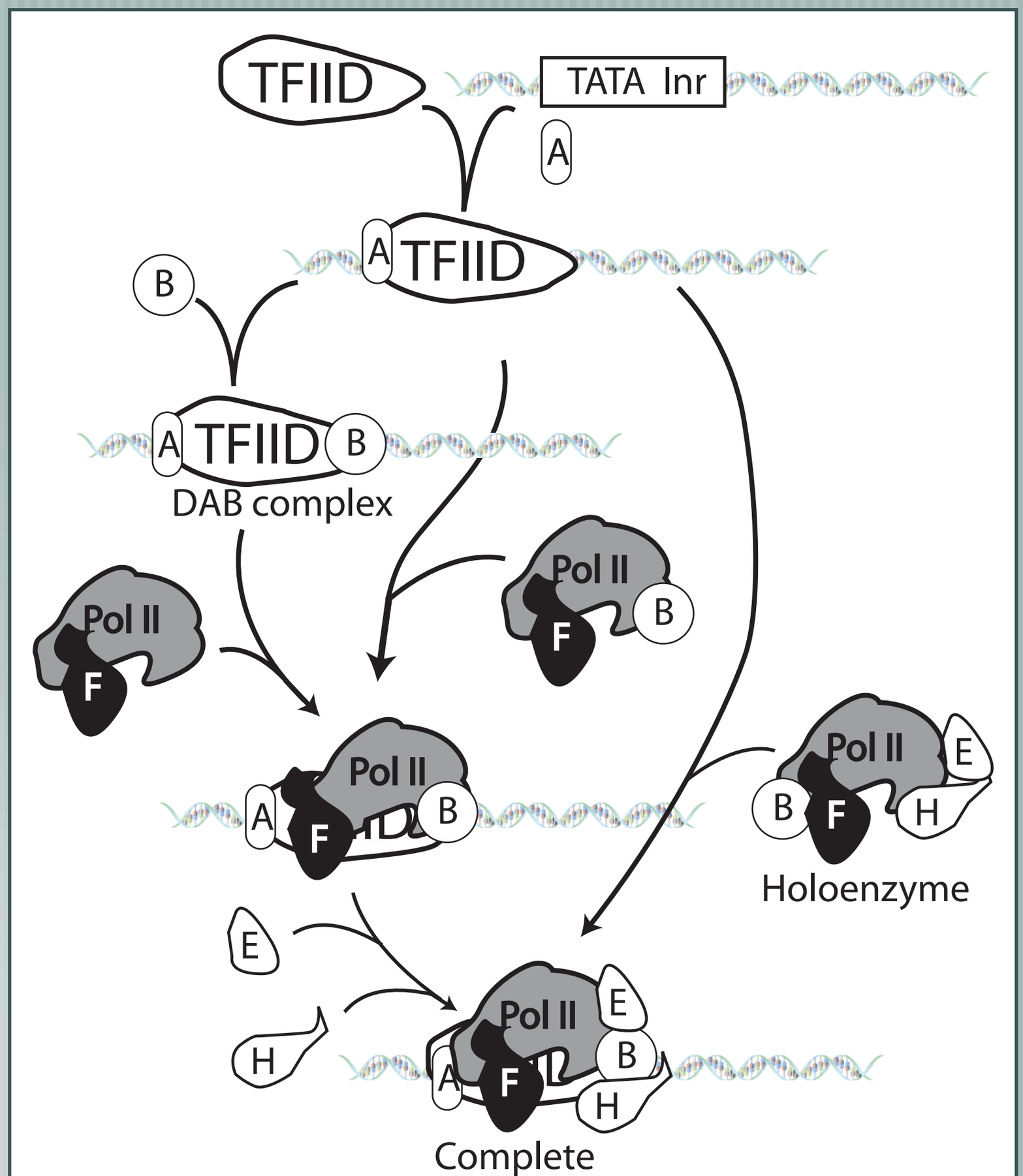
- Interact directly with core promoter. Determine site of intiation and direction of transcription. TFIIA, TFIIB, TFIID, TFIIF, TFIIH

Other factors have various names: inducible, regulatory, etc.

- TFIID=TBP + 8-10 TAFIIS
- TFIID binds minor groove of the TATA box.
- TFIIF - ATP dependent helicase activity
  2 proteins, one with homology to sigma
  Reduces affinity of polymerase for non-promoter DNA



- TFIIH - kinase that phosphorylates YSPTSPS (CTD domain)
- Unphosphorylated RNAP = RNAPIIA = initiation specific
- Phosphorylated RNAP = RNAPIIO = for chain elongation
- TFIIH also has helicase activity



52 repeads of the heptad.

TFIIF will bind prokaryotic RNA polymerase.

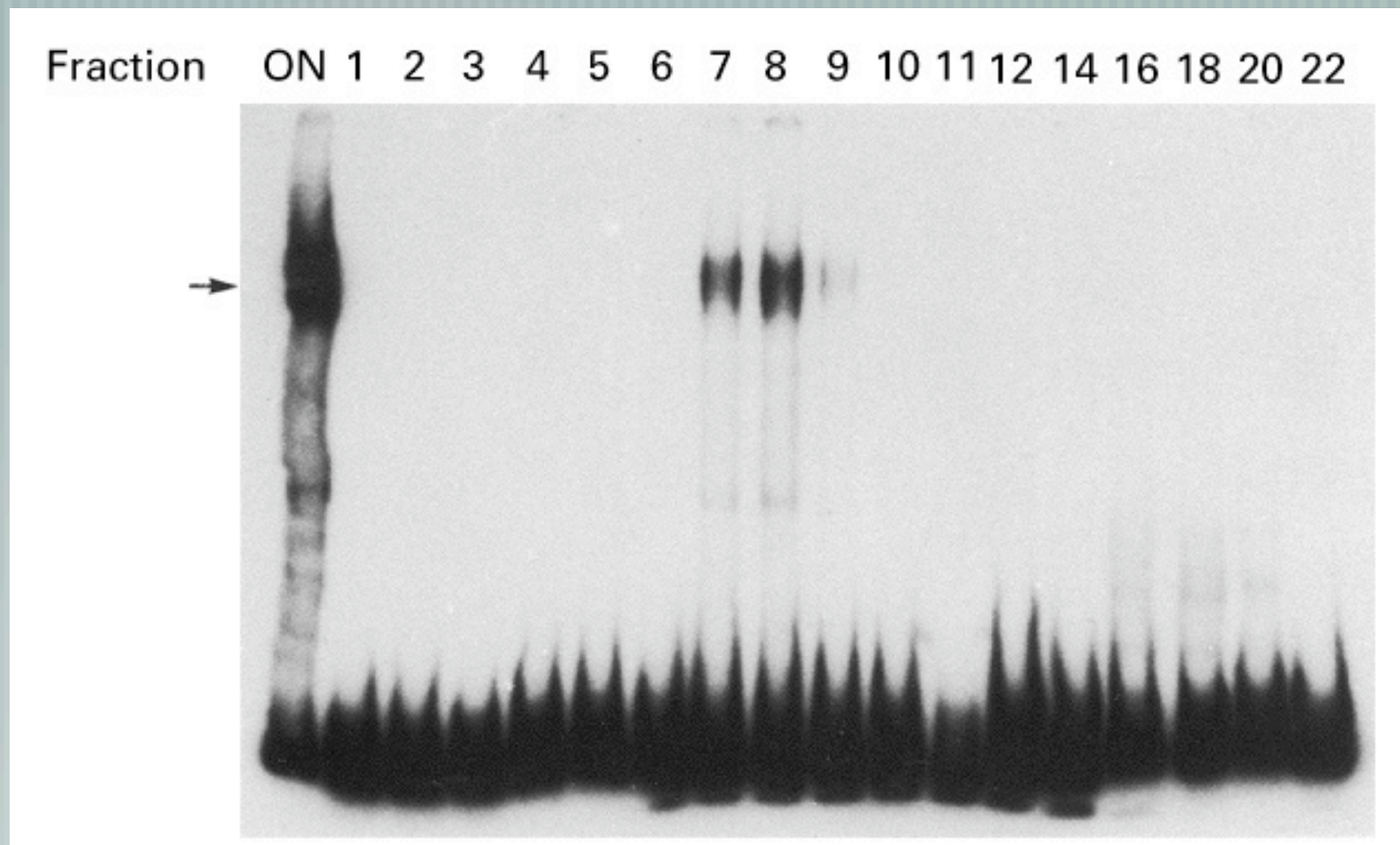
Note that this is similar to sigma=>Reduces affinity of polymerase for non promoter DNA.

Note: Polymerase II + F are a ‘unit’.

TFIIH is discussed on page 318 Weaver 3rd edition.

Gel Shift

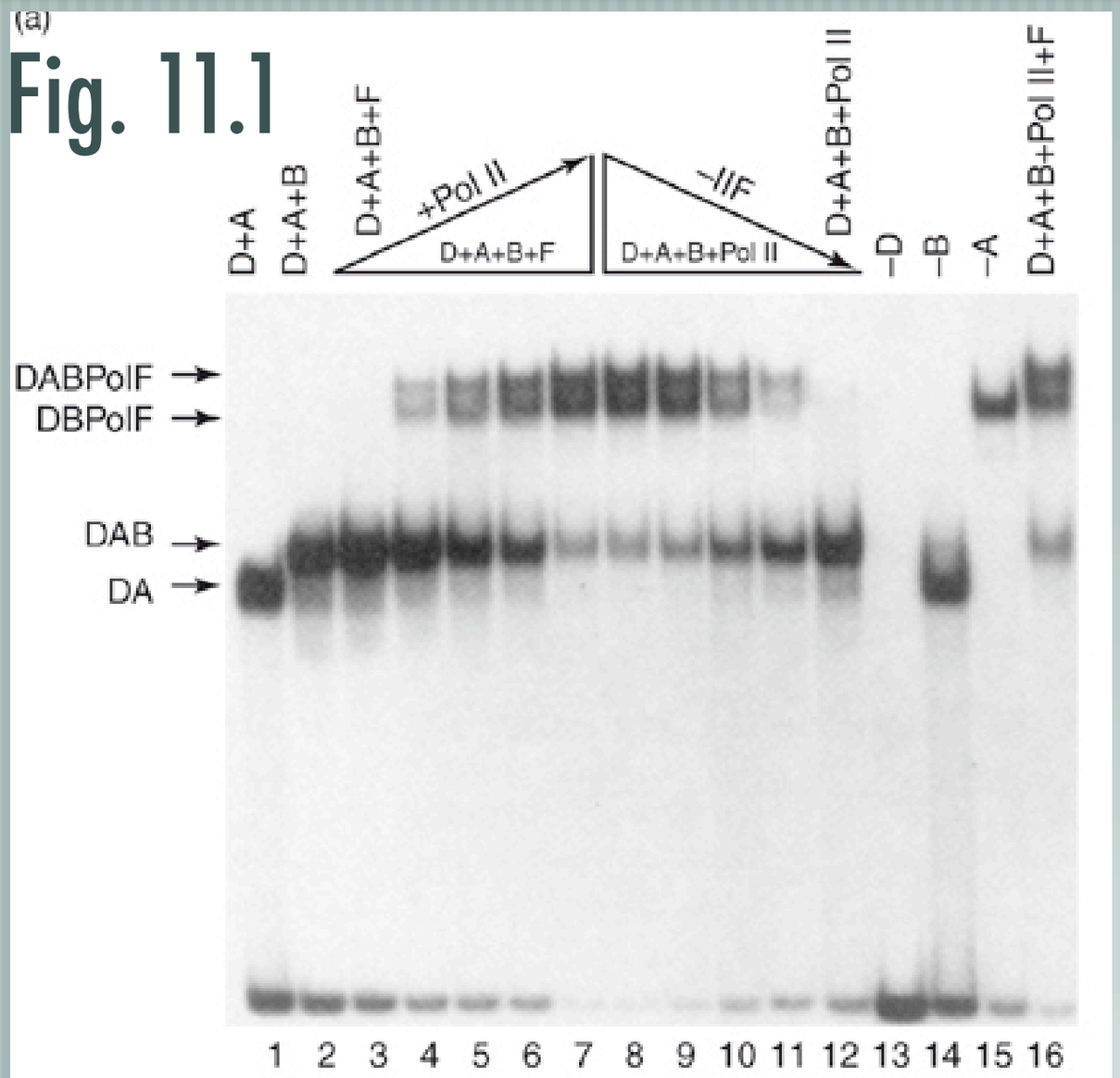
- End labeling
- Controls?



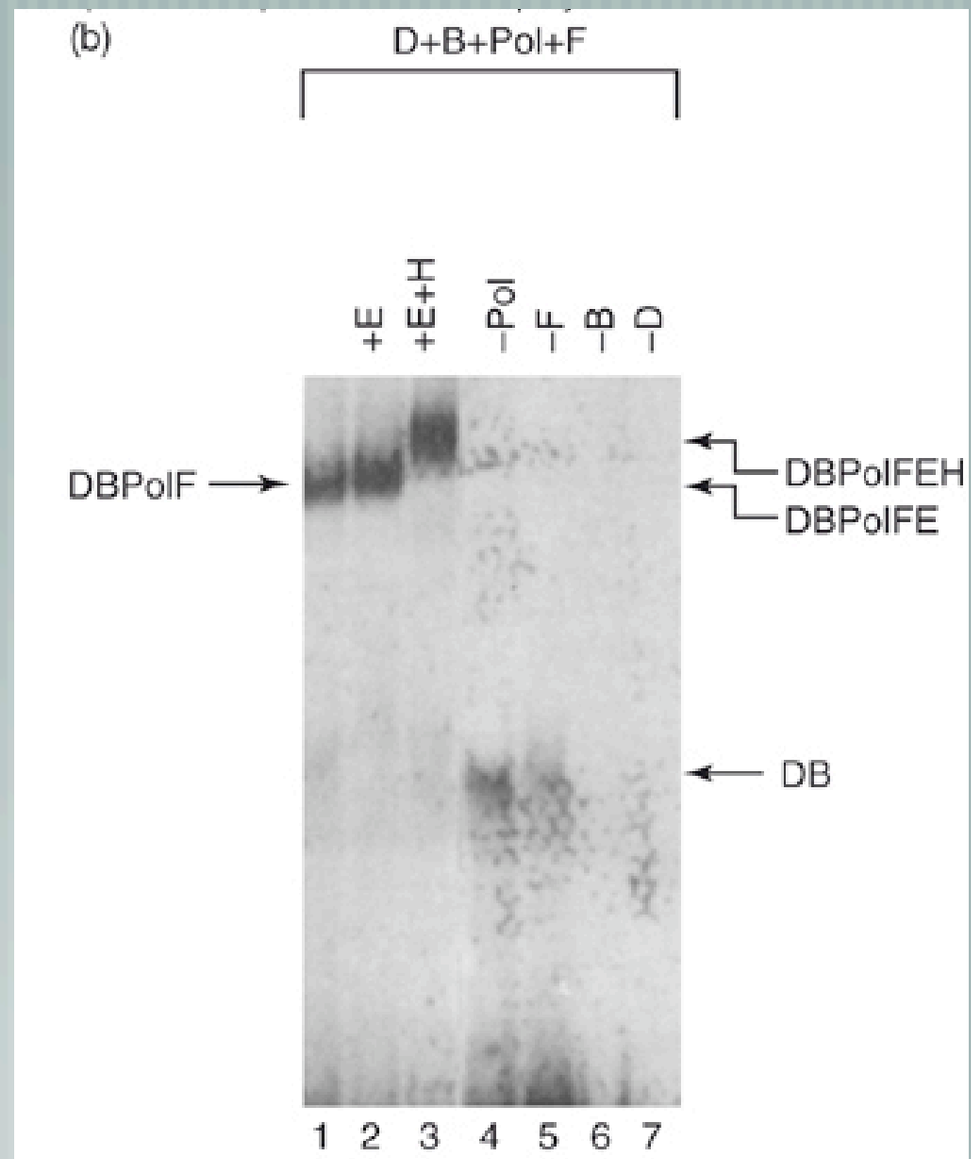
Gel Shift
Purification
End labeling: T4 polynucleotide kinase + gamma-32P-ATP.
Controls?

Watching it build

Fig. 11.1



© Flores, O., H. Lu, M. Killeen, J. Grenblatt, Z.F. Burton, and D. Reinberg, The Small Subunit of transcription factor IIF recruits RNA polymerase II into the preinitiation complex, Proceedings of the National Academy of Science USA, 88 (Nov 1991) p. 10001,



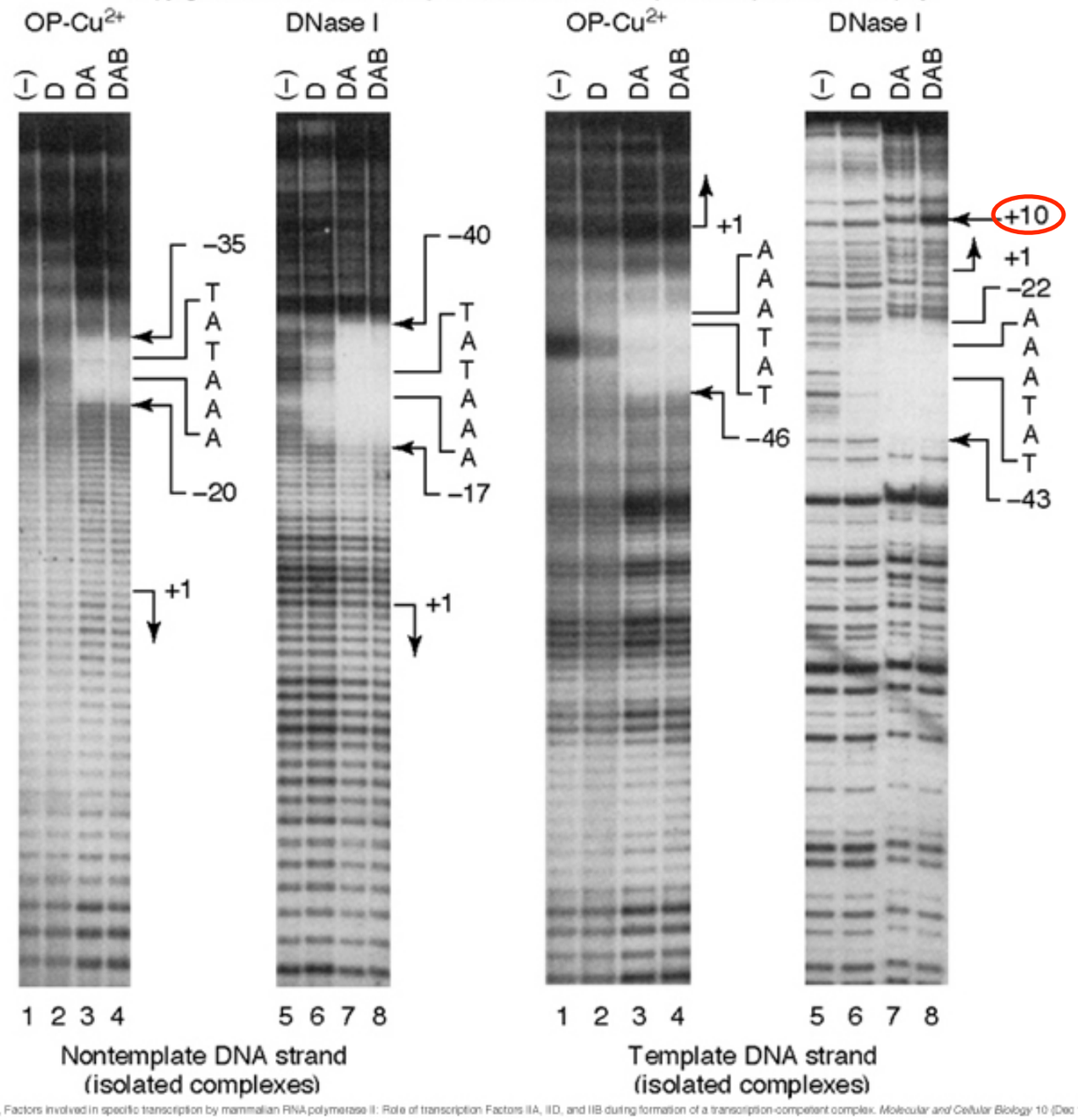
Cortes, P., O. Flores, and D. Reinberg, 1992. Factors involved in specific transcription by mammalian RNA polymerase II: Purification and analysis of transcription factor iiA and dentification of transcription factor IIJ. Molecular and Cellular Biology 1

Adenovirus major late promoter
in B they show that only DBPolF can accept E and H

Footprinting

D=TFIID
A=TFIIA
B=TFIIB

1,10 phenanthroline (OP)-copper complex



(Source: Maldonado E., I. Ha, P. Cortes, L. Weiss, and D. Reinberg, Factors involved in specific transcription by mammalian RNA polymerase II: Role of transcription Factors IIA, IID, and IIB during formation of a transcription-competent complex. *Molecular and Cellular Biology* 10 (Dec 1990) p. 6344, f. 9. American Society for Microbiology.)

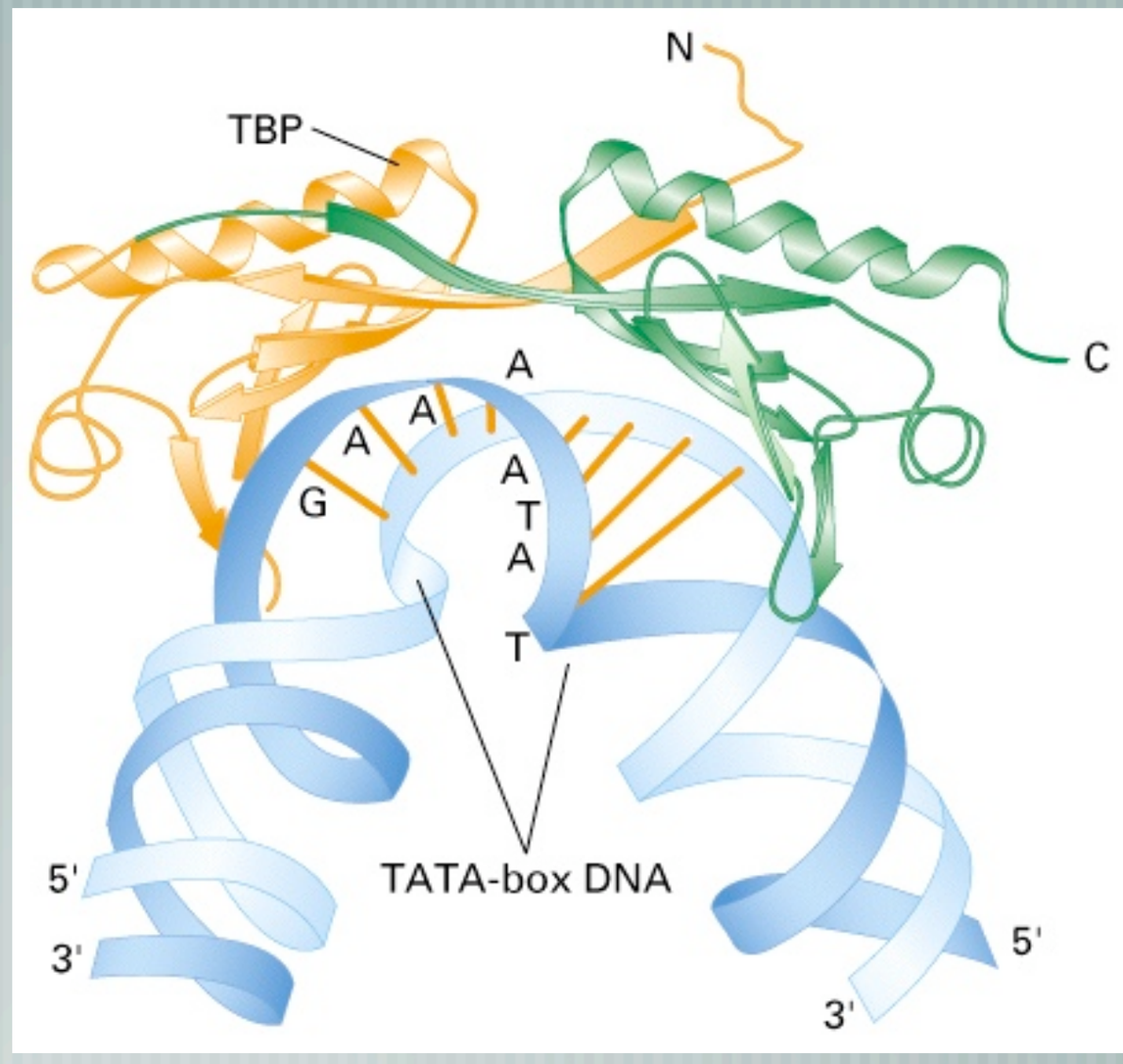
Four distinct complexes seen. This means that it can build in steps.
Calf intestinal phosphatase
T4 polynucleotide kinase + gamma-32P-ATP
Sequencing gel. Measurement with single bp resolution.
Figure 11.2

TFIID

- TFIID=TBP + 8-10 TAFIIS

TFIID: TBP can bend DNA

- Crystal structure of TBP suggested a saddle.
- TBP DNA co-crystal indicated that not like a saddle on a horse.



pg 306. TBP bends DNA

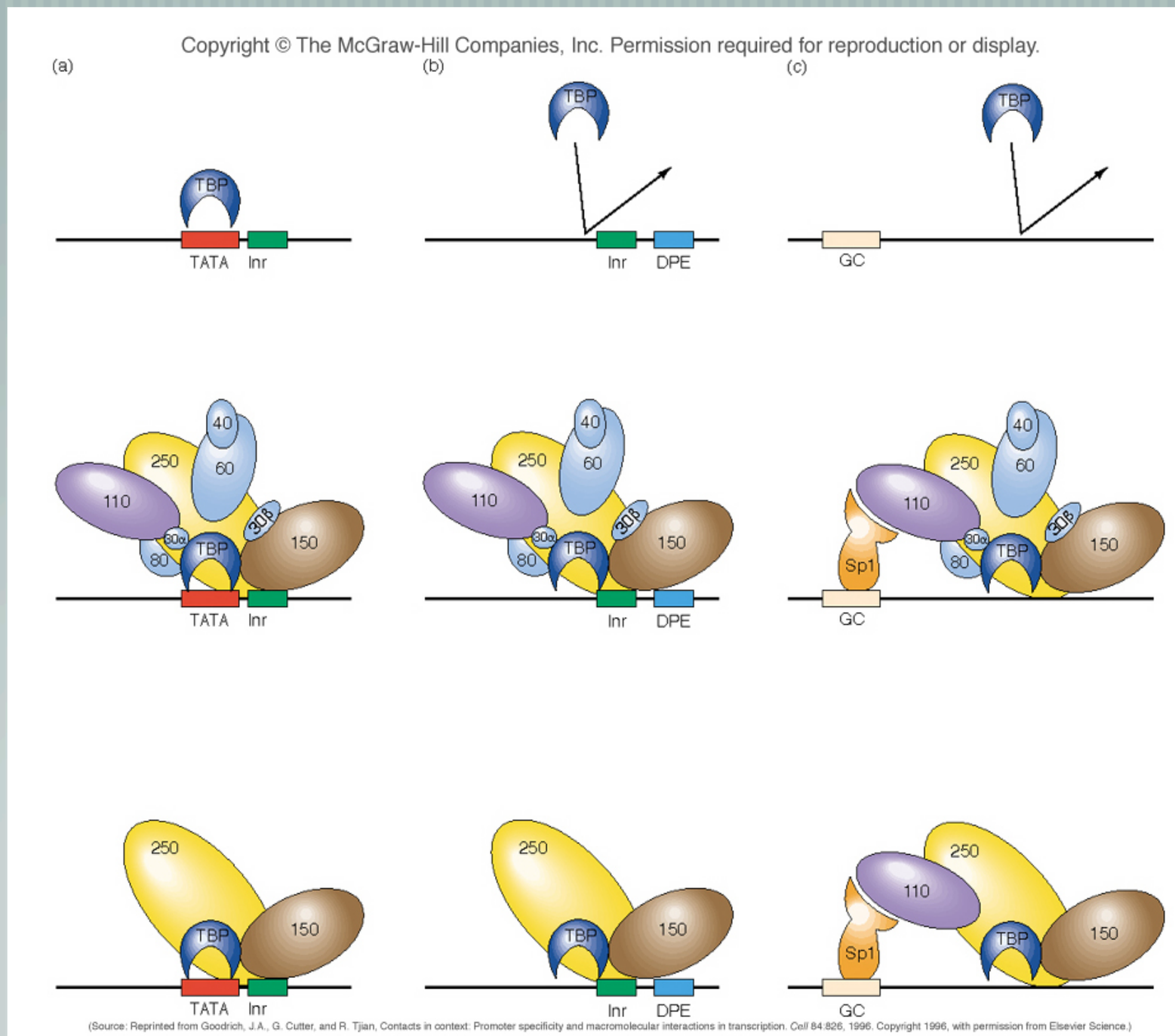
Saddle. But not sitting on the DNA like a horse's saddle sits on a horse. This one is sitting in a way that it bends the DNA.

TFIID: Who recognizes

TATA-less promoters are not recognized by TBP

TAF250 & TAF150 impart ability to recognize Inr & DPE

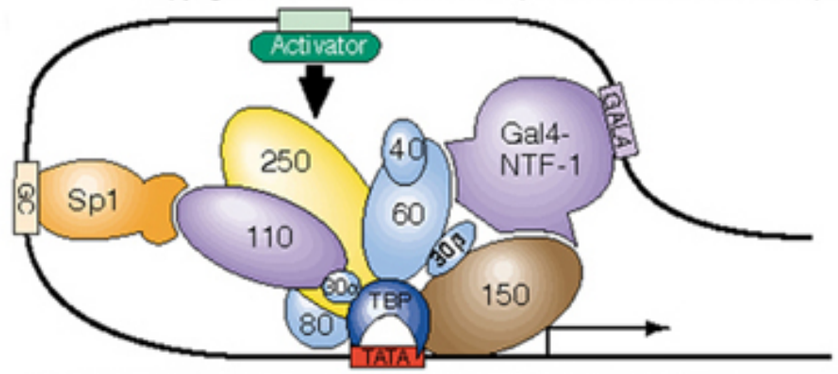
Other TF (Sp1) necessary for TATA, Inr & DPE-less promoters



(Source: Reprinted from Goodrich, J.A., G. Cutter, and R. Tjian, Contacts in context: Promoter specificity and macromolecular interactions in transcription. *Cell* 84:826, 1996. Copyright 1996, with permission from Elsevier Science.)

TBP has scaffold ability.

TFIID has many targets for interactions



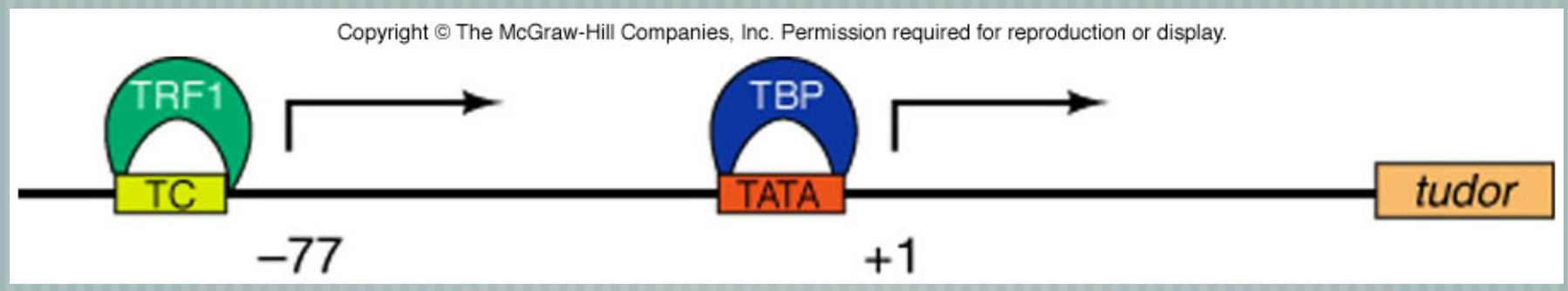
Holo-TFIID supports activation by an assortment of activators.

(Source: Reprinted from Chen, J.L., L.D. Attardi, C.P. Verrijzer, K. Yokomori, and R. Tjian, Assembly of recombinant TFIID reveals differential coactivator requirements for distinct transcriptional activators. *Cell* 79:101, 1994. Copyright © 1994, with permission from Elsevier Science.)

TAFs can have enzymatic activity

- TAFII250 - Histone acetyl transferase activity that acetylates lysine residues of histones. This can lead to remodeling of the chromatin.
- TAFII250 - Protein kinase activity that phosphorylates itself and TFIIF, TFIIA and TFIIE. Thought to modulate activity of initiation complex.

Other ways to recognize TATA-less promoters

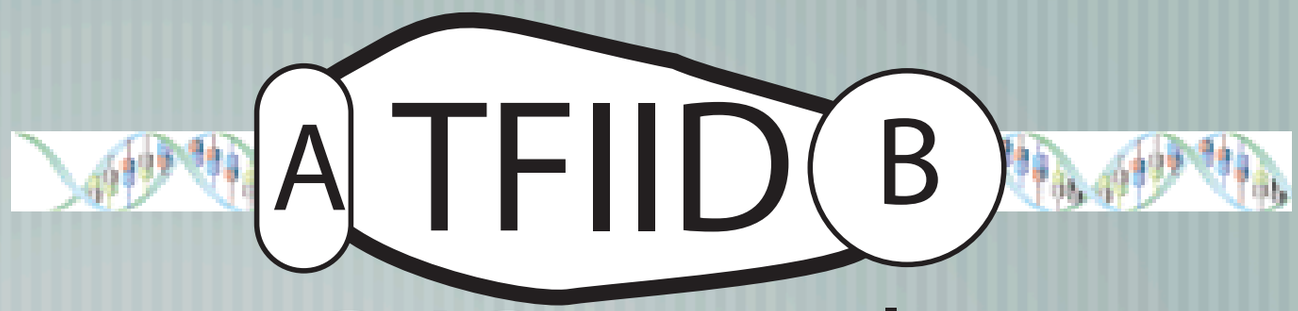


- Drosophila melanogaster
  TRF1 = TBP related factor. Interacts with TFIIA & B. Associates with nTAFs.
  TRF1 expressed in developing neural tissue.
- Other eukaryotes have
  TLF = TBP-like factor
  TFTC = TBP-free TAFII complexes

TC box = ATTGCTTTTCTT
Control of transcription by alternative TBPs!? Isn't this similar to the control of transcription in eukaryotes by alternative sigmas?

DAB complex

- TFIID adds first
- 2nd or 3rd is TFIIA
- 3rd or 2nd is TFIIB



DAB complex

- TFIIA stabilizes TFIID binding & interacts with activators
- TFIIB interacts with activators & helps position polymerase at +1

TFIIB

- The Bridge
- Required for binding of the Polymerase TFIIF complex to bind
- Binding of TFIIB can be regulated by other transcription factors
- One part of TFIIB binds TBP and the other which probably binds to Polymerase &/or TFIIF.

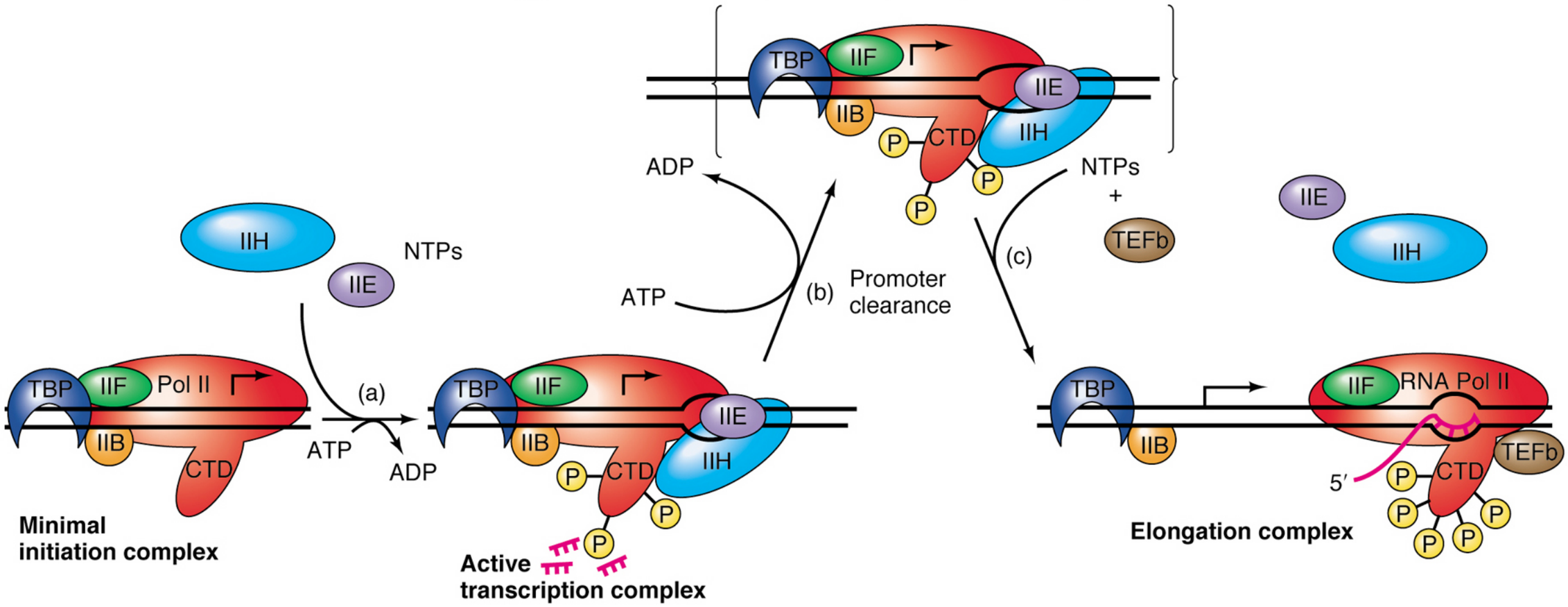
TFIIF

- Weak homology to part of bacterial sigma that binds core polymerase.
- Reduce non-specific binding to DNA.

TFIIH

- Phosphorylates CTD of RNAPII to convert it from IIA to IIO.
  - IIA binds TBP tightly. IIO does not.
- TFIIH contains 9 subunits.
  - Some are the kinase, some are ATP-dependent helicases.

Promoter Clearance



(Source: Modified from Goodrich, J.A. and T. Tjian. 1994. Transcription factors IIE and IIH and ATP hydrolysis direct promoter clearance by RNA polymerase II. *Cell* 77:145–56. Copyright 1994, with permission from Elsevier Science.)

ATP-dep DNA helicase activity of TFIIH is required for promoter clearance.

Note the abortive transcripts in the minimal initiation complex. Just like the ones that we say with the lac operon.

Note that TBP and IIB are left behind.

TFIIS

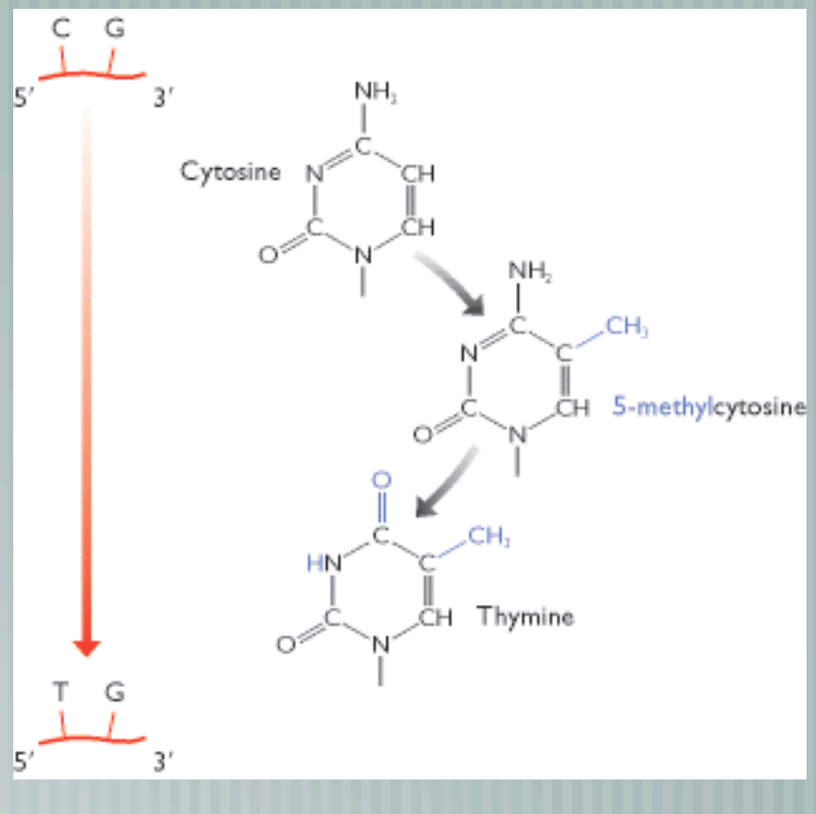
- RNA Polymerases pause at pause sites.
  - There is a chance that transcription will terminate at a pause site.
- TFIIS reduces pause time
- Stimulates proofreading - polymerase has RNase activity.
- Stimulates elongation

page 303 Weaver 4rd edition
This is an elongation factor

CpG Islands

CpG islands

CpG refers to the dinucleotide in which cytosine containing nucleotide is joined to a guanine containing nucleotide. The 'p' in the middle stands for the phosphate. The other strand of the DNA will have a GpC because of Watson-Crick base pairing. CpG dinucleotides are under-represented in the mammalian genome. In mammals most of them are methylated.



From Genomes 2 2nd edition TA Brown

Stats

300-3,000 bp - usually has to be at least 200 bp long to be counted and greater than 60% CpG

Near 40%-70% of mammalian promoters

CpG Islands

What is methylated?

CpG's are usually methylated when found in exons, transposable elements and satellite DNA. Methylated CpG's tend to persist because the hemimethylated version is recognized by an enzyme that then methylates the remainder.

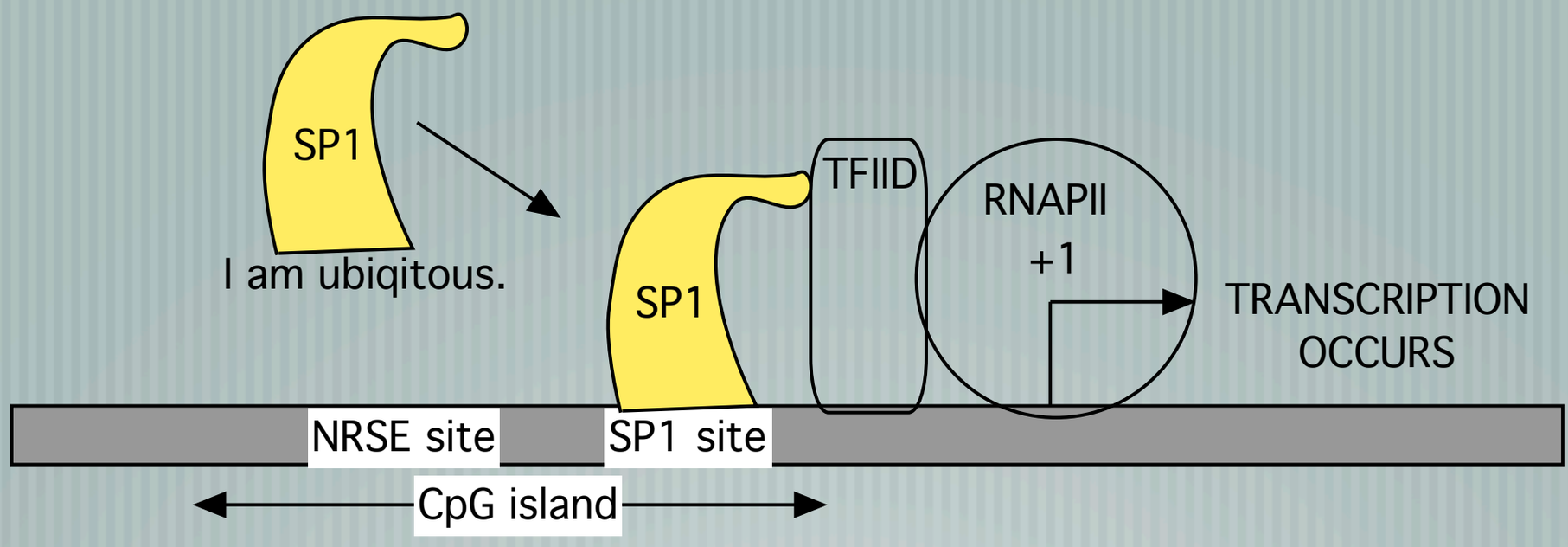
1) About 70% of CpG's are methylated in mammals

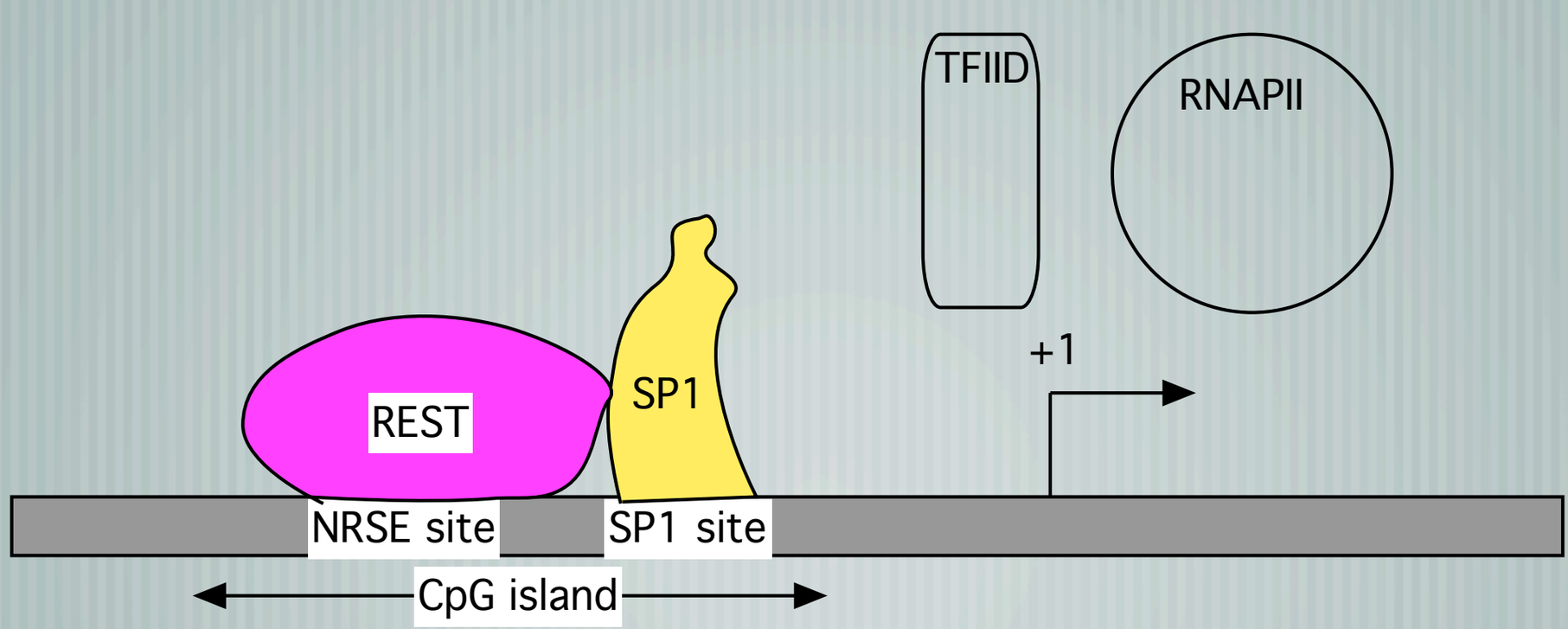
2) Satellite DNAs, transposons, other repetitive DNA (probably dead transposons) and intergenic DNA

Defense mech to silence non-coding DNA much of which is the product of invasion by transposons - defense against DNA of foreign origin.

3) EXONS!

CpG Islands


SP1
I am ubiqitous.
SP1
TFIID
RNAPII
+1
TRANSCRIPTION
OCCURS
NRSE site
SP1 site
CpG island


TFIID
RNAPII
REST
SP1
+1
NRSE site
SP1 site
CpG island

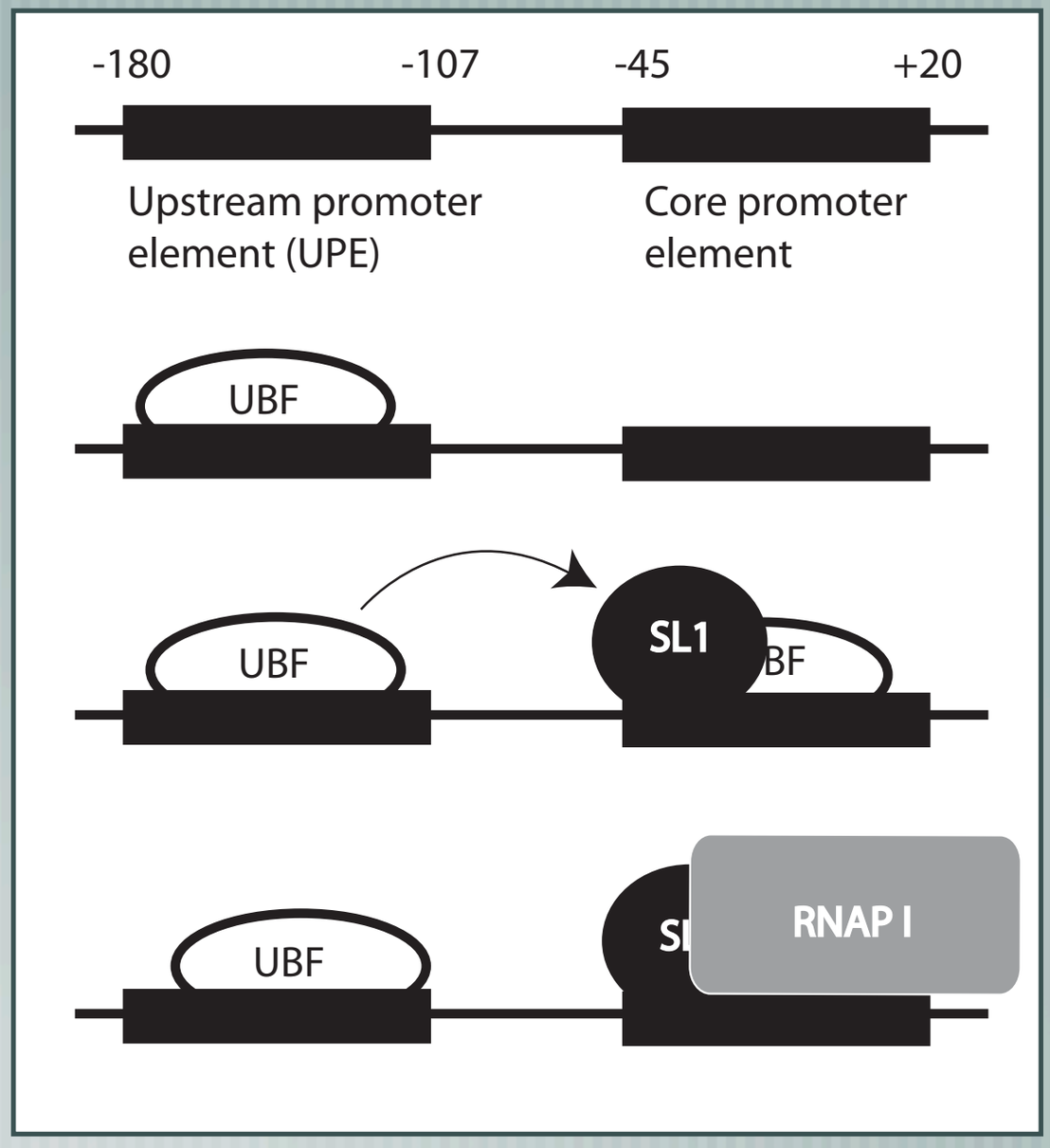
Poly I and III promoters next

- Class I factors refer to factors required used by RNA polymerase I for transcription
- Class III factors refer to factors required used by RNA polymerase III for transcription

RNA polymerase I

rRNA genes

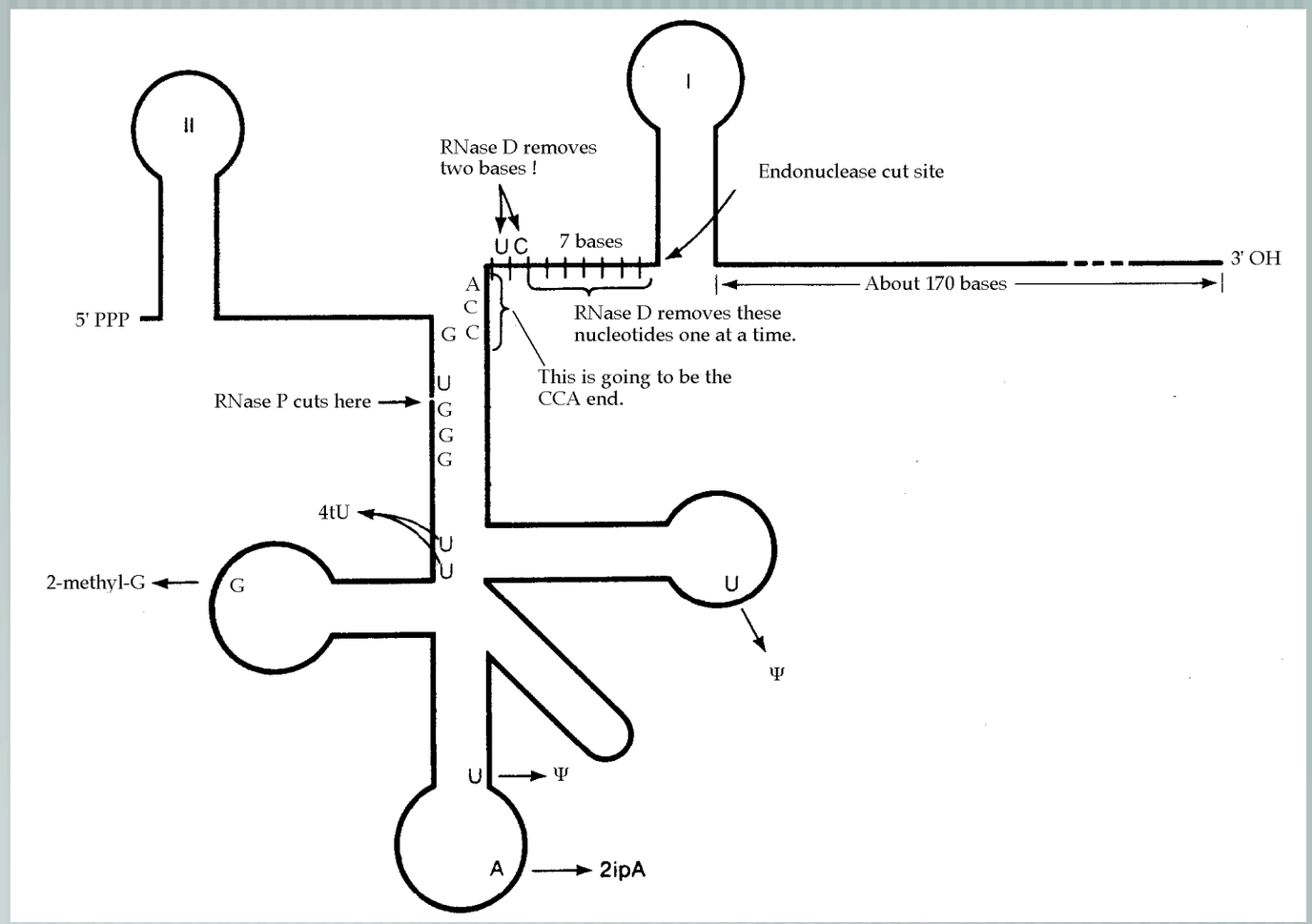
It was a surprise that ts mutations in TBP block transcription of poll genes. SL1 = TBP + 3 TAFS



pg 306 4th ed. ts mutations in TBP can block expression of RNApol I, II and III genes.

RNAP III

- tRNAs
- 5S rRNA
- U6 snRNA
- 7SL RNA
- 7SK RNA



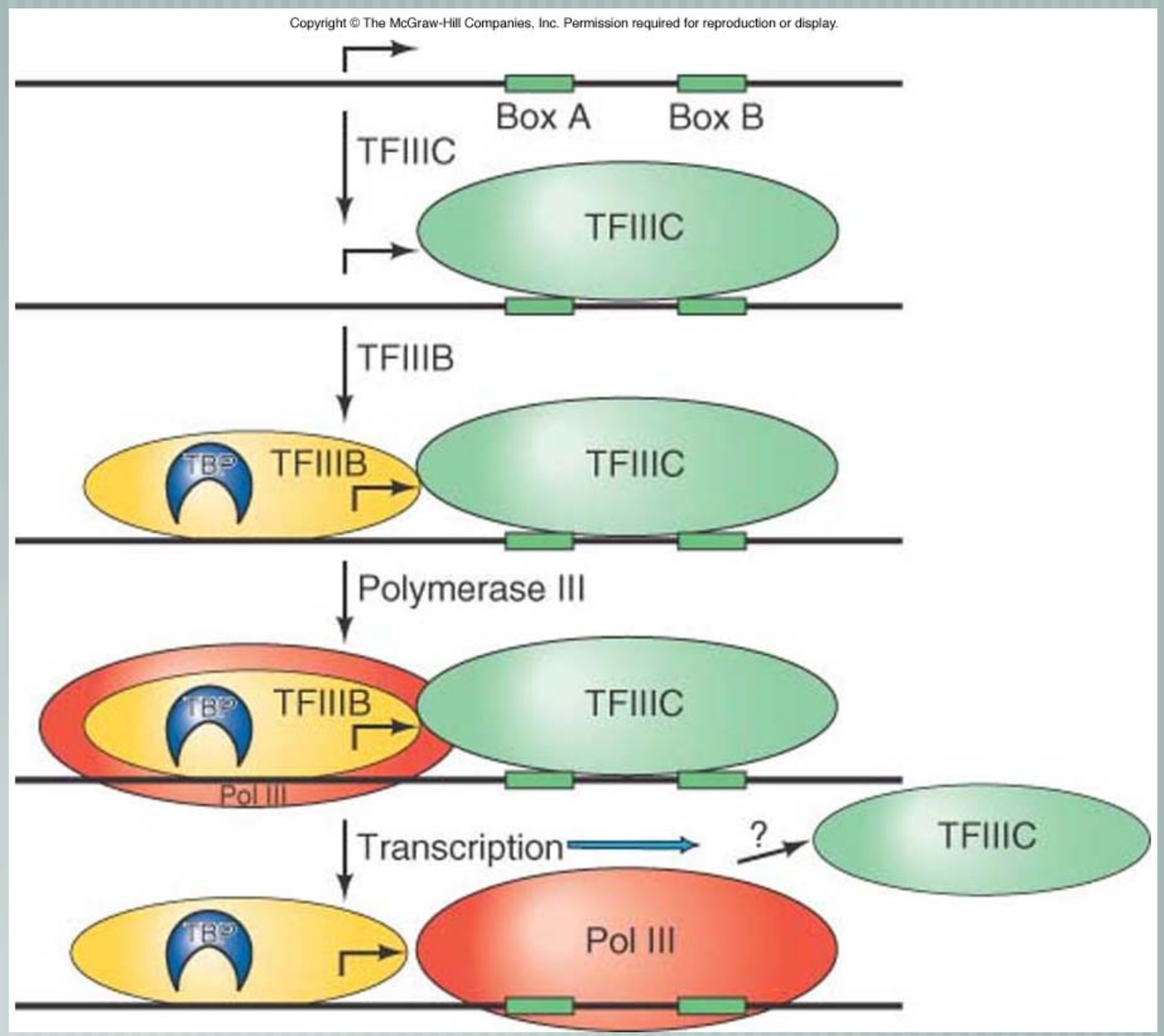
5S RNA component of large ribosomal subunit.

7SL RNA
Walter & Blobel. 1983. J Cell Biol 97:1693-1699. Signal recognition particle (SRP) is a ribonucleoprotein consisting of six distinct polypeptides and one molecule of small cytoplasmic 7SL- RNA. The particle was previously shown to function in protein translocation across and protein integration into the endoplasmic reticulum membrane.

7SK RNA - small cytoplasmic RNA. Function uncertain according to Human Molecular Genetics 2 1999.

RNAP III

- tRNAs
- 5S rRNA
- U6 snRNA
- 7SL RNA
- 7SK RNA



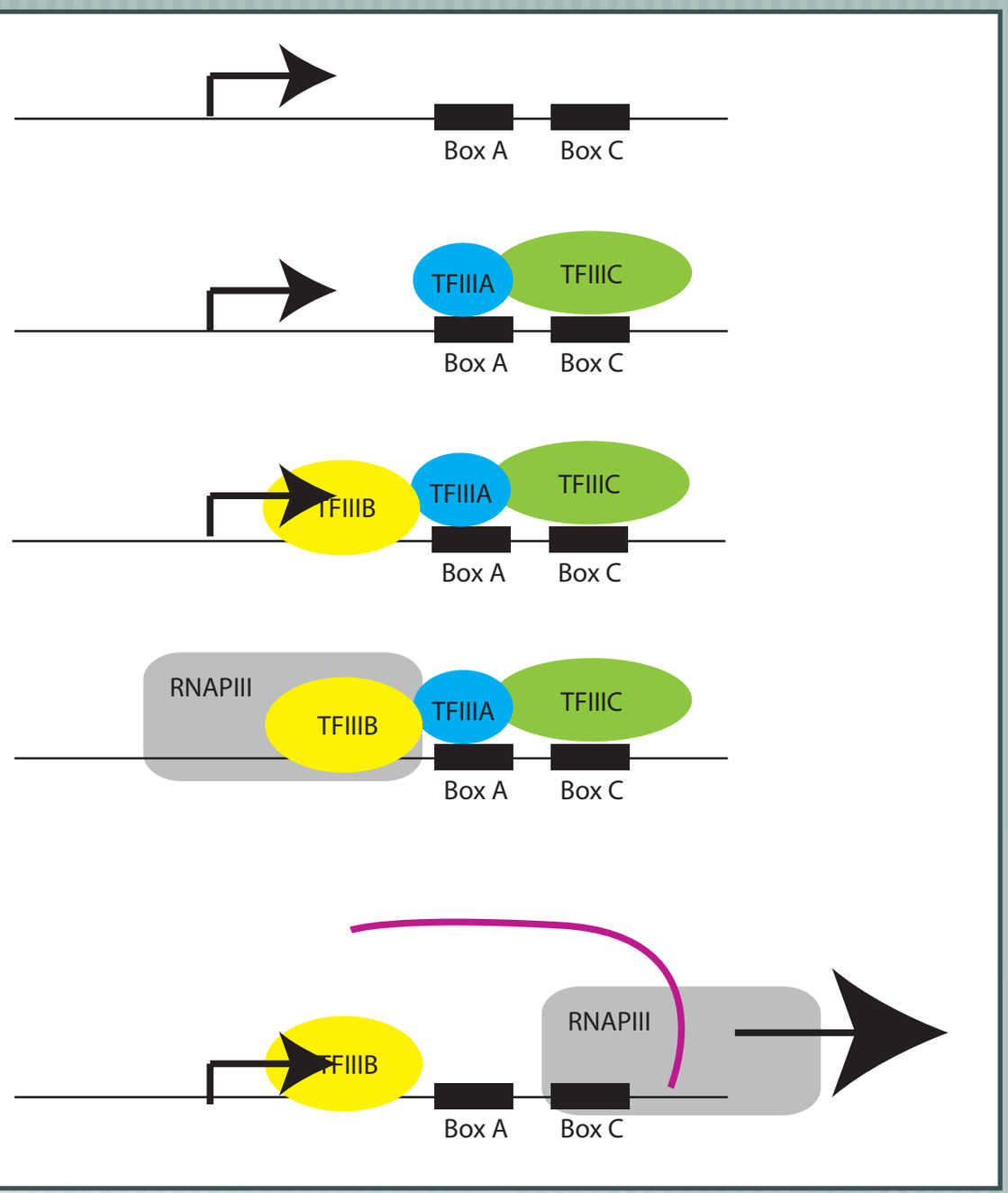
TFIIIA – first eukaryotic transcription factor identified – a Zn finger. pg 310 Weaver 4th edition.

end

RNAP III

- tRNAs
- 5S rRNA
- U6 snRNA
- 7SL RNA
- 7SK RNA

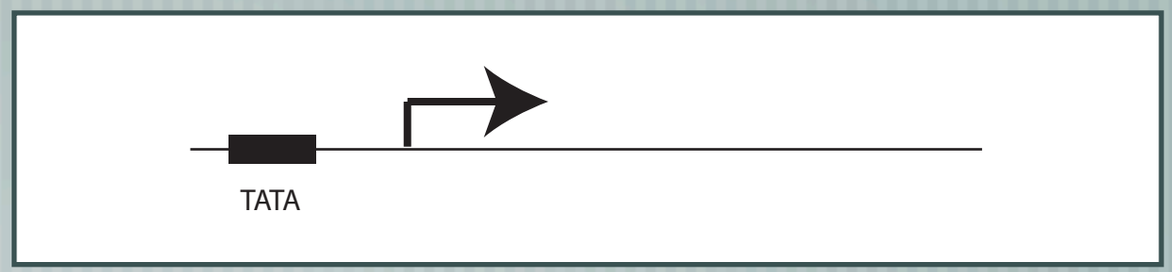
TFIIIB = TBP + TAFS



TFIIIC binds box C.
5S RNA genes no Box B. Have Box A & C.
TFIIIA binds Box A (Zn finger protein).

RNAP III

snRNAU6 gene

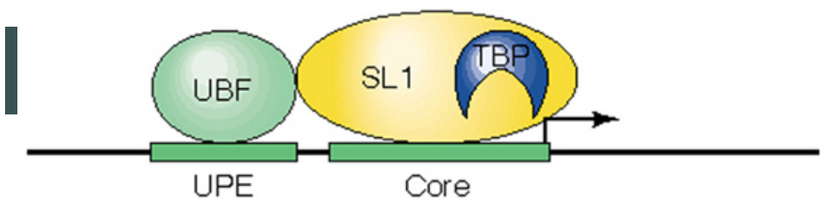


7SK RNA gene

TBP

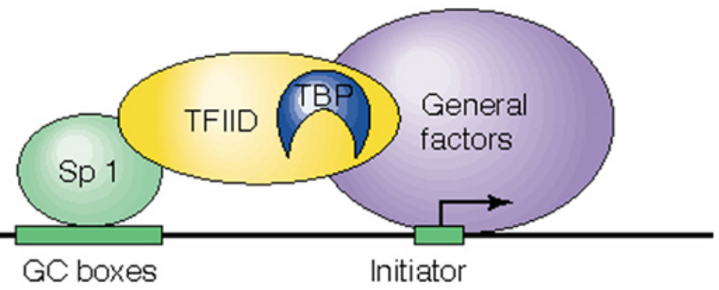
Class I
rRNA

RNAP I



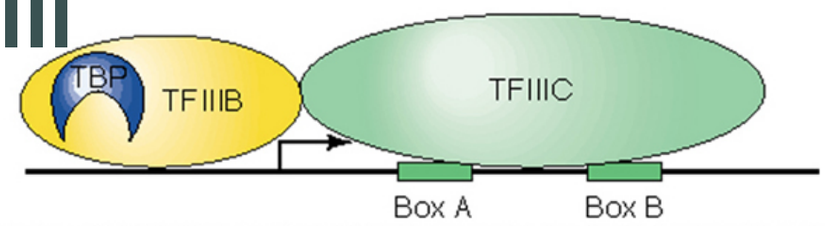
Class II
G_6I

RNAP II



Class III
VA_I

RNAP III



(Source: Reprinted from White, R.J. and S.P. Jackson, mechanism of TATA-binding protein recruitment to a TATA-less class III promoter. *Cell* 71:1051, 1992. Copyright © 1992, with permission from Elsevier Science.)

Organizing role, scaffold

From Human Molecular Genetics 2

Table 7.4. Functional diversity of RNA. From Human Molecular Genetics 2

A. RNA classes involved in assisting general gene expression

Ribosomal RNA (rRNA)
28S rRNA- Component of large cytoplasmic ribosomal subunit
5.8S rRNA-Component of large cytoplasmic ribosomal subunit
5S rRNA- Component of large cytoplasmic ribosomal subunit
18S rRNA- Component of small cytoplasmic ribosomal subunit
23S rRNA- Component of large mitochondrial ribosomal subunit
16S rRNA- Component of small mitochondrial ribosomal subunit

Transfer RNA (tRNA)
>40 different cytoplasmic
Binding to codons in mitochondrial or nuclear-encoded mRNA

tRNA; 22 types of mitochondrial tRNA

Small nuclear RNA (snRNA) including
U1 snRNA-Component of major spliceosome
U2 snRNA-Component of major spliceosome
U4 snRNA-Component of major spliceosome
U5 snRNA-Component of major and minor spliceosome
U6 snRNA-Component of major spliceosome
U4acat snRNA-Component of minor spliceosome
U6acat snRNA-Component of minor spliceosome
U11snRNA-Component of minor spliceosome
U12 snRNA-Component of minor spliceosome
U7 snRNA-Histone mRNA transcriptional termination

Small nucleolar RNA (snoRNA) - About 200 types, including
U3 snoRNA -rRNA processing
U8 snoRNA-rRNA processing
various box C/D snoRNAs-Site-specific methylation of the 2′ OH group of rRNA
various box H/ACA snoRNAs- Site-specific rRNA modification by formation of pseudouridine.

B. Other RNA classes

7SL RNA-Component of signal recognition particle for transporting proteins (see Section 1.5.4)
7SK RNA-Function uncertain
Telomerase RNA-Component of telomerase (Section 2.3.4)
XIST RNA-Regulatory gene imposing X-chromosome inactivation (Section 7.2.2)
H19 RNA-Imprinted gene, function unclear (Section 7.2.2)
SRA RNA-Encodes a steroid receptor coactivator

Don't try to memorize this or to learn it. Just a list that interested me.