Principles of

MOLECULAR BIOLOGY

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Chapter 13

Eukaryotic Transcription

Parameter	prokaryotic	eukaryotic
RNA Polymerase	1 for all types	3; each for every type:I, II, III
Binding to promoter	Direct	indirect
Transcription factors	few	numerus
amanitin	not sensitive	Sensitive(II, I)
Transcript processing	no	yes
promoter	simple	complicated
nucleosomes	absent	present

RNA Polymerase II Structure

Nuclear RNA polymerases have limited synthetic capacities

- None of the RNA polymerases can initiate transcription from specific start sites within double-stranded DNA
- All require assistance of other proteins
- Each polymerase requires its own specific set of transcription factors

Introduction to Eukaryotic Nuclear RNA Polymerases

The eukaryotic cell nucleus has three different kinds of RNA polymerase

TABLE 13.1 Comparing the Three Eukaryotic RNA Polymerases

Enzyme	Location	RNA Products	Sensitivity to α-amanitin	Sensitivity to actinomycin D
RNA polymerase I	Nucleolus	Pre-rRNA (leading to 5.8S, 18S, and 28S rRNA)	Resistant	Very sensitive
RNA polymerase II	Nucleoplasm	Pre-mRNA and some small nuclear RNAs (snRNAs)	50% inhibition at 0.02 μg/mL	Slightly sensitive
RNA polymerase III	Nucleoplasm	tRNA, 5S rRNA, U6 snRNA (spliceosomal RNA), and 7SL RNA (signal recognition particle RNA)	50% inhibition at 20 μg/mL	Slightly sensitive

Figure 13.F02A: α-Amanitin. (a) Amanita phalloides (also known as the Death Cap). (b) Structural formula of α-amanitin.

(Part a © Niels-DK/Alamy Images. Part b adapted from Defendenti, C., et al. 1998. Forensic Sci Int 92:59–68.)

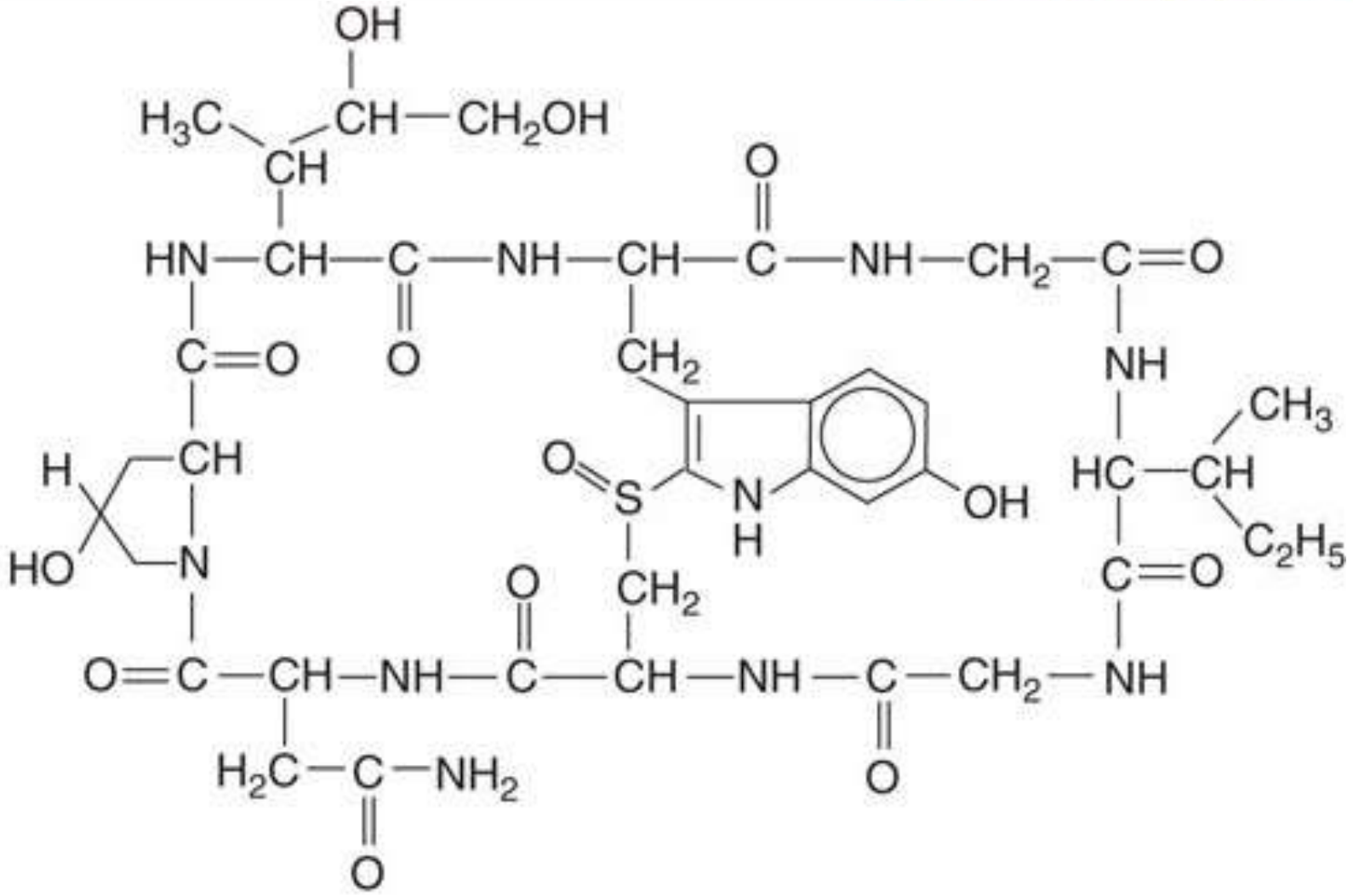


Figure 13.F02B: α-Amanitin. (a) Amanita phalloides (also known as the Death Cap). (b) Structural formula of α-amanitin.

(Part a © Niels-DK/Alamy Images. Part b adapted from Defendenti, C., et al. 1998. Forensic Sci Int 92:59–68.)

Core Promoter for Protein-Coding Genes

The core promoter for protein-coding genes extends from 40bp upstream of the transcription start site to 40bp downstream from this site

- A consensus TATAXAX (where X is an A or T) is present 25 to 30 bases upstream of transcription start sites for RNA Pol II genes
- Known as the **TATA box**
- Found primarily in highly expressed gene promoters
  - TATA-less promoters have other short sequences that replace the TATA box
- The DNA region that includes these elements is the **core promoter**

Core Promoter for Protein-Coding Genes

Other components of the core promoter

- **Initiator (Inr) element** flanks the start site
- **Downstream promoter element** (DPE)
- **TFIIB recognition elements** (BRE)
  - BRE^u and BRE^d flank the TATA box

In ≈10,000 known human core promoters

- Inr is present in about half
- DPE and BRE present in about a quarter
- TATA boxes present in about one-eighth

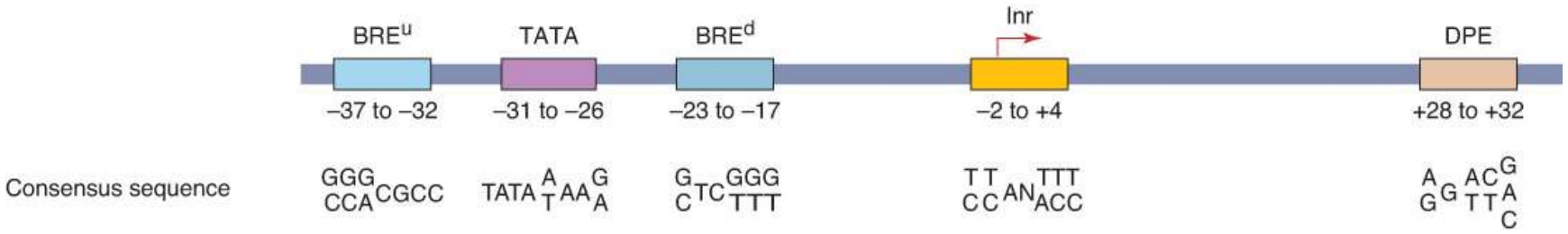


Figure 13.F07: Core promoter elements that contribute to basal transcription in multicellular animals.

(Adapted from Maston, G. A., et al. 2006. Annu Rev Genom Hum Genet 7:29–59.)

Eukaryotic Promoter

Site where other regulatory proteins bind to enhance transcription

A site where regulatory proteins can bind to enhance transcription

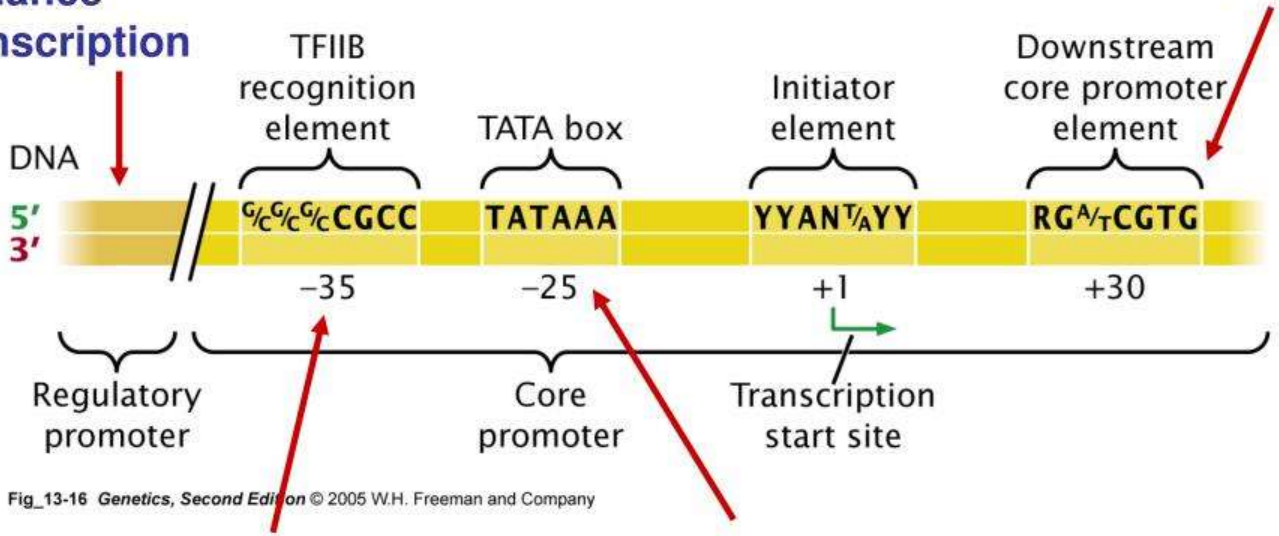


Fig_13-16 *Genetics, Second Edition* © 2005 W.H. Freeman and Company

Sequence recognized by a transcription factor

Sequence where DNA is denatured determining where transcription starts

General Transcription Factors: Basal Transcription

RNA polymerase II requires the assistance of general transcription factors to transcribe naked DNA from specific transcription start sites

- The general transcription factors
    - TFIIA, TFIIB, TFIID, TFIIF and TFIIH
  - The first two letters TF indicate general transcription factor
  - Roman numeral II signifies RNA polymerase II
  - Final letter based on protein fractionation scheme

General Transcription Factors: Basal Transcription

TABLE 13.3 General Transcription Factors

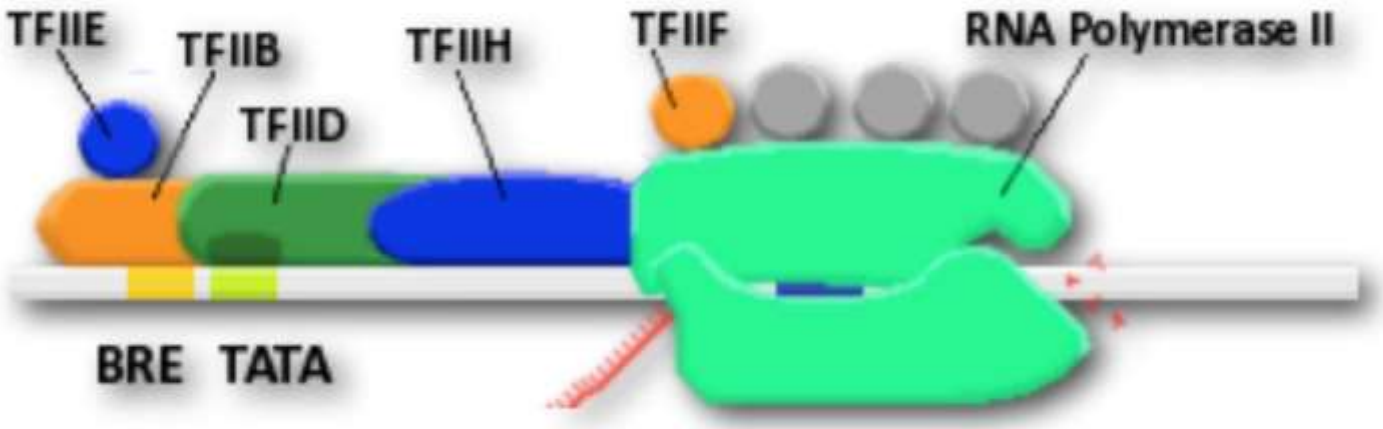
Factor	No. of Subunits	Functions
TFIIA	2	Stabilizes TBP and TFIID binding. Blocks the inhibitory effects of TAF1 and other proteins.
TFIIB	1	Stabilizes TFIID-promoter binding. Contributes to transcription start site selection. Helps recruit RNA polymerase II • TFIIF complex to the core promoter.
TFIID (TBP and TAFs)	14	Binds to the TATA box, Inr, and DPE. It can deform promoter DNA and serve as a platform for the assembly of TFIIB.
TFIIE	2	Helps to recruit TFIIH to the core promoter and is required for promoter melting.
TFIIF	3	Binds RNA polymerase II and is involved in recruiting the polymerase to the pre-initiation complex. Required to recruit EFIIE and EFIIH to the pre-initiation complex.
TFIIH	10	Functions in transcription and DNA repair. It has kinase and helicase activities and is essential for open complex formation.

General Transcription Factors: Basal Transcription

TFIID or its TBP subunit must bind to a TATA core promoter before other general transcription factors can do so

- **TATA binding protein** (TBP) is the binding subunit in TFIID
- **TBP associated factors** (TAFs) bind to elements in TATA-less promoters

Eukaryotic Transcription Complex

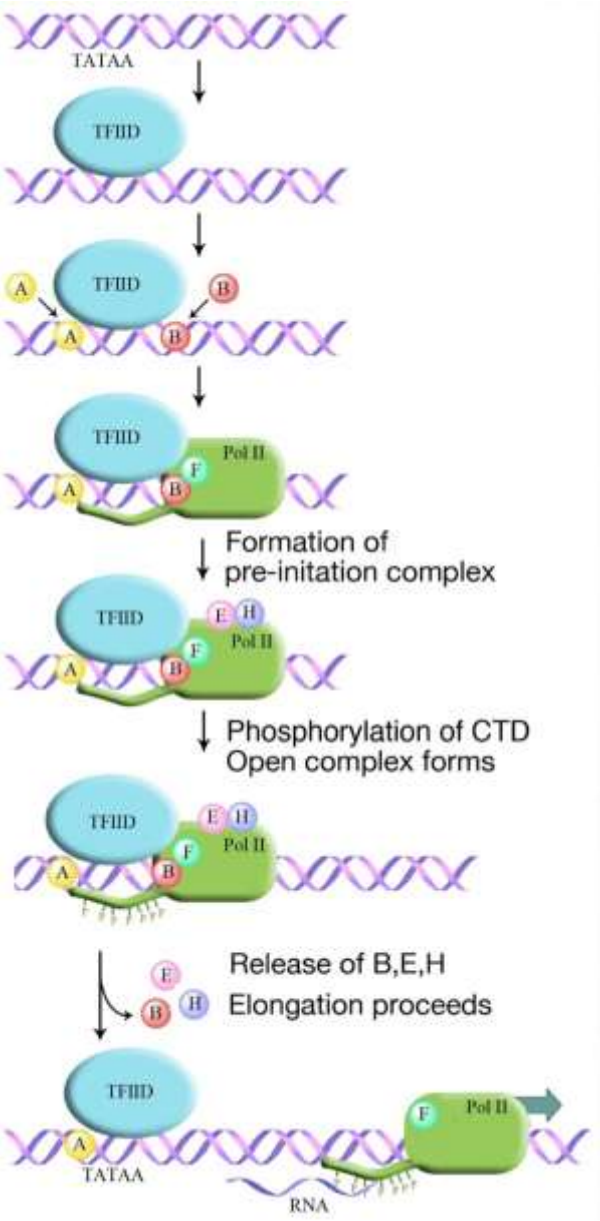


Transcription Elongation

The C-terminal domain of the largest RNA polymerase subunit must be phosphorylated for chain elongation to proceed

- The carboxy terminal domain (CTD) contains tandem repeats of an unusual heptapeptide
  - Tyr-Ser-Pro-Thr-Ser-Pro-Ser
- Five of the seven residues can be phosphorylated
  - Must be dephosphorylated for Pol II to assemble into the prinitiaition complex
  - Phosphorylation is necessary for elongation
- TFIIH phosphorylates Ser-5 permitting promoter clearance

Transcription Complex Assembly



Transcription Elongation

A variety of transcription elongation factors helps to suppress transient pausing during elongation

- Reverse movement of Pol II occurs called **backtracking**
- In the paused state (2-4 nucleotides) it can return to the elongation stage without assistance
- In the arrested state (7-14 nucleotides) TFIIS is required to reactivate transcription
  - When an error occurs the polymerase backtracks
  - The mismatch is removed and transcription can resume

Regulatory Promoters, Enhancers and Silencers

Enhancers stimulate transcription and silencers block transcription

- **Enhancers** are distance and orientation independent
  - First identified in SV40 early gene
  - 72 bp repeat stimulates transcription by 100-fold in in-vitro studies
  - Can work even when located 1,000s of bp upstream or downstream
  - Can work inserted forwards or backwards
- Eukaryotes also have **silencers** that repress transcription

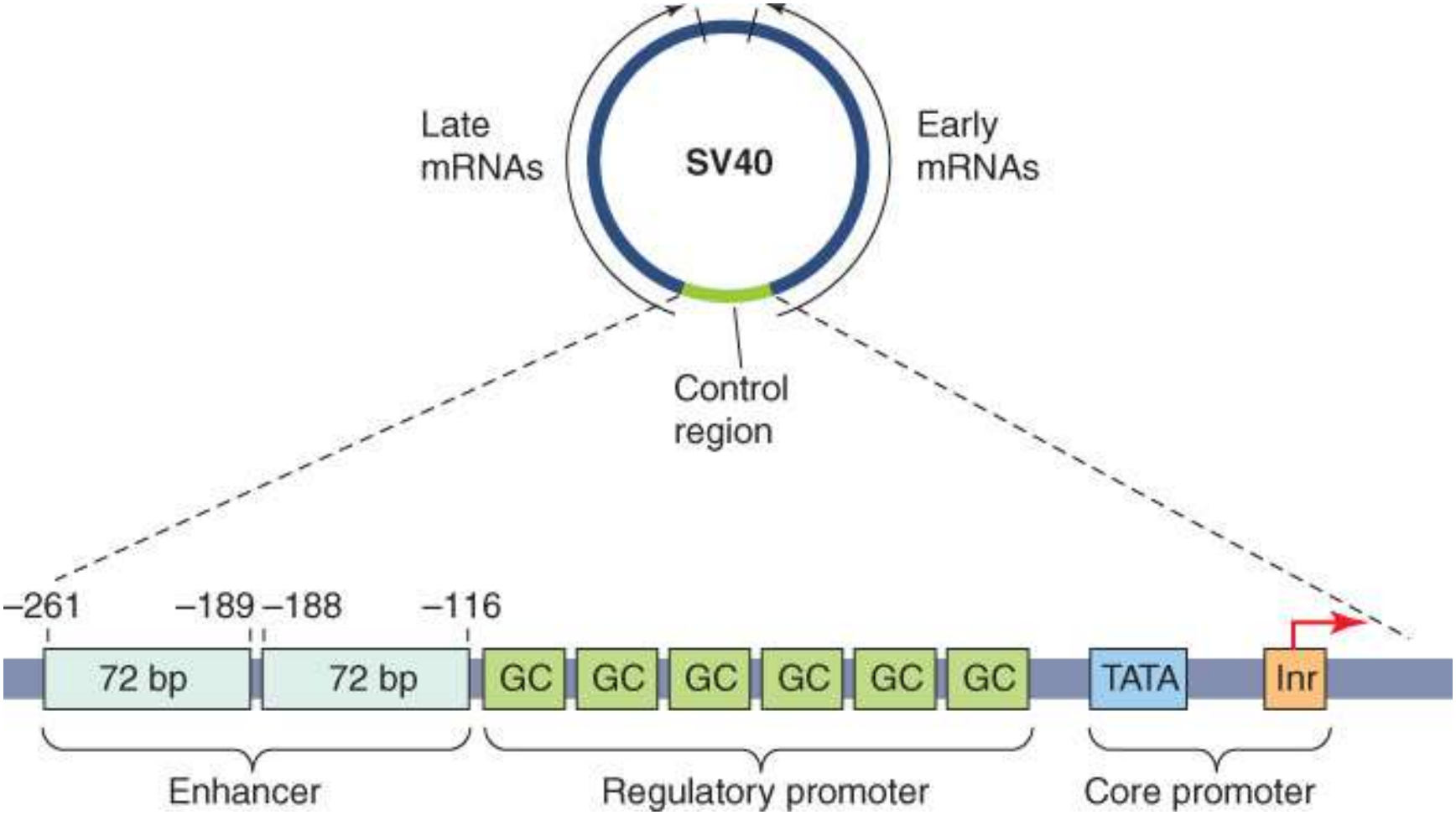


Figure 13.F19: SV40 early transcription unit enhancer and promoter regions.

Epigenetic Modifications

Cells remodel or modify chromatin to make the DNA in chromatin accessible to the transcription machinery

- Eukaryotic cells use ATP-dependent chromatin remodeling complexes
  - Reposition nucleosomes
  - Eject nucleosomes
  - Unwrap nucleosomes
  - Exchange or eject histone dimers

Epigenetic Modifications

Histone modification influences transcription of protein-coding genes

- Active genes have acetylated histones
  - **Histone acetyltransferase** (HAT)
  - Acetylation takes place on specific lysine.
- Chromatin remodeling complexes respond to advancing transcription by:

1) displacing histones from DNA onto chaperones
   - Reassemble after the DNA region has been transcribed

2) Only one H2A·H2B heterodimer needs to be removed

Epigenetic Modifications

DNA methylation plays an important role in determining whether chromatin will be silenced or actively expressed in vertebrates

- Many vertebrate core promoters are in **CpG islands**
- Methyl transferase add methyl groups to specific lysine and arginine.
- Methylation of C in these regions can lead to gene silencing
  - Methyl-CpG prevents transcription factor binding
  - Methyl-CpG acts as a signal for histone modification
    - Many genes are silenced by this mechanism

Epigenetic Modifications

Epigenetics is the study of inherited changes in phenotype caused by changes in chromatin other than changes in DNA sequence

- Patterns of histone modification and DNA methylation are inheritable
- Since these patterns determine which DNA segments will be transcriptionally active and silent, this is a form of inheritance that is not directly determined by DNA sequence
  - **Epigenetics** is term used to describe these patterns of inheritance