The Molecular Biology of Gene Regulation

The Molecular Biology of Gene Regulation

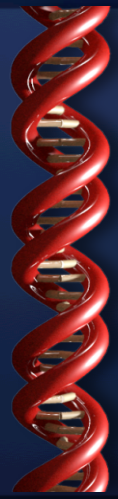
The discovery of transcription factors—key molecules that regulate the use of genetic information in the genome.

Transcription factors are involved in many fundamental aspects of biology including embryonic development, cellular differentiation and cell fate.

Another reason Transcription Regulation is Important

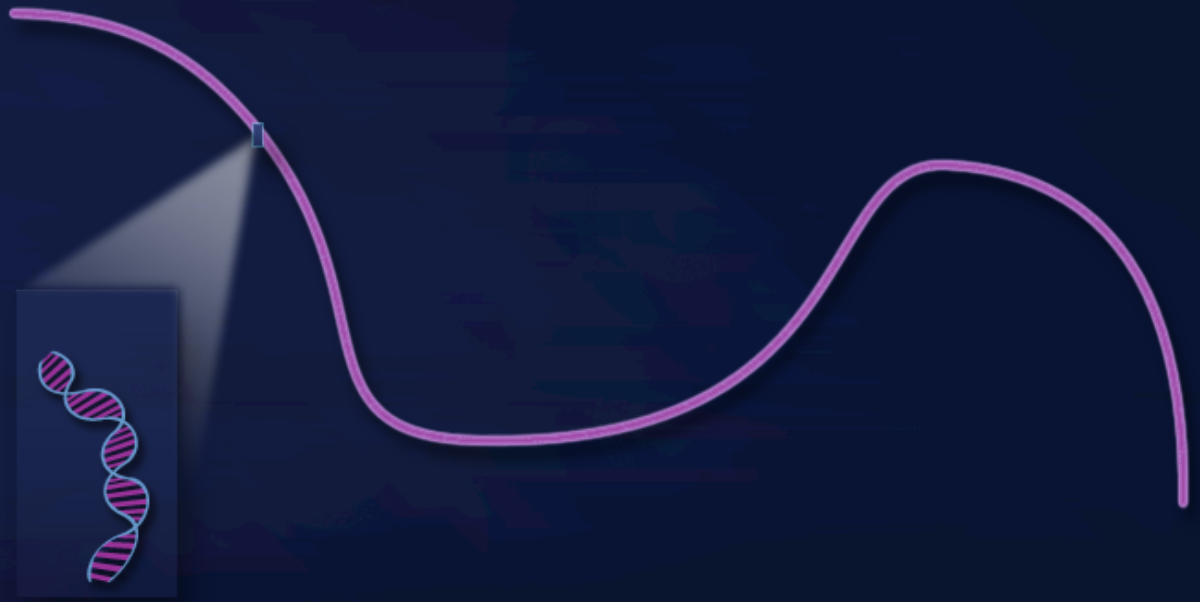
Transcription factors play key role in many diseases

Cancer
Diabetes
Infectious Agents
Inflammation
Huntington's
Parkinson's



Highly specific drugs
Rapid diagnosis

Organization of Genes in the Genome



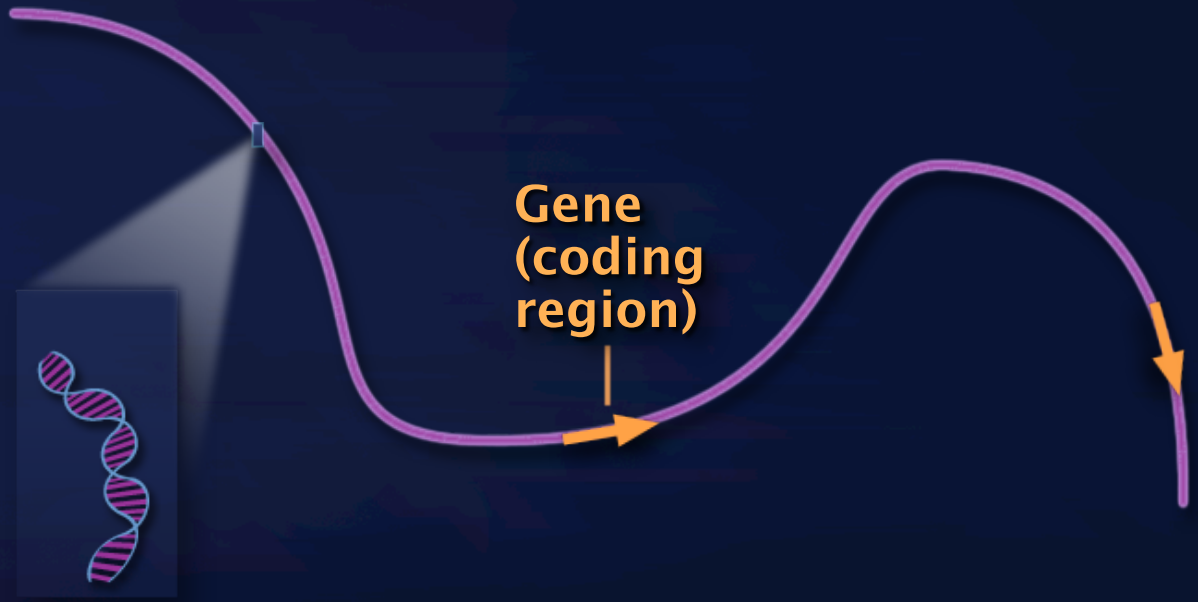
Human genome has 3 billion base pairs and ~22,000 genes.

Flow of Biological Information – The Process of Gene Expression

DNA → RNA → Protein

Transcription Translation

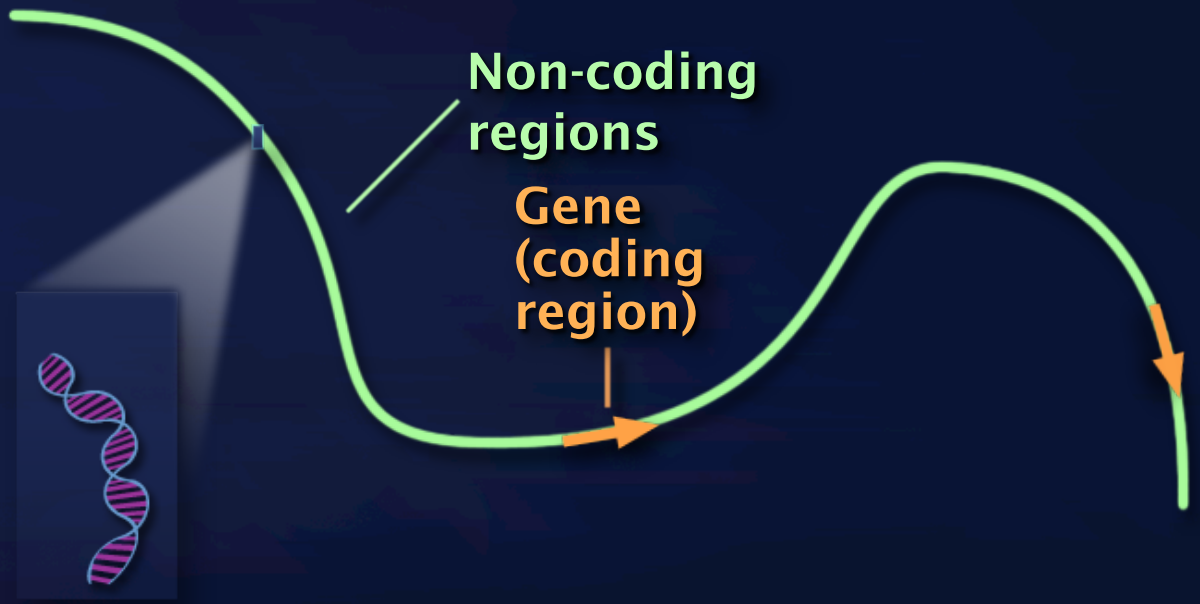
Organization of Genes in the Genome



Human genome has 3 billion base pairs and ~22,000 genes.

Only ~3% of DNA is protein coding sequence.

Organization of Genes in the Genome

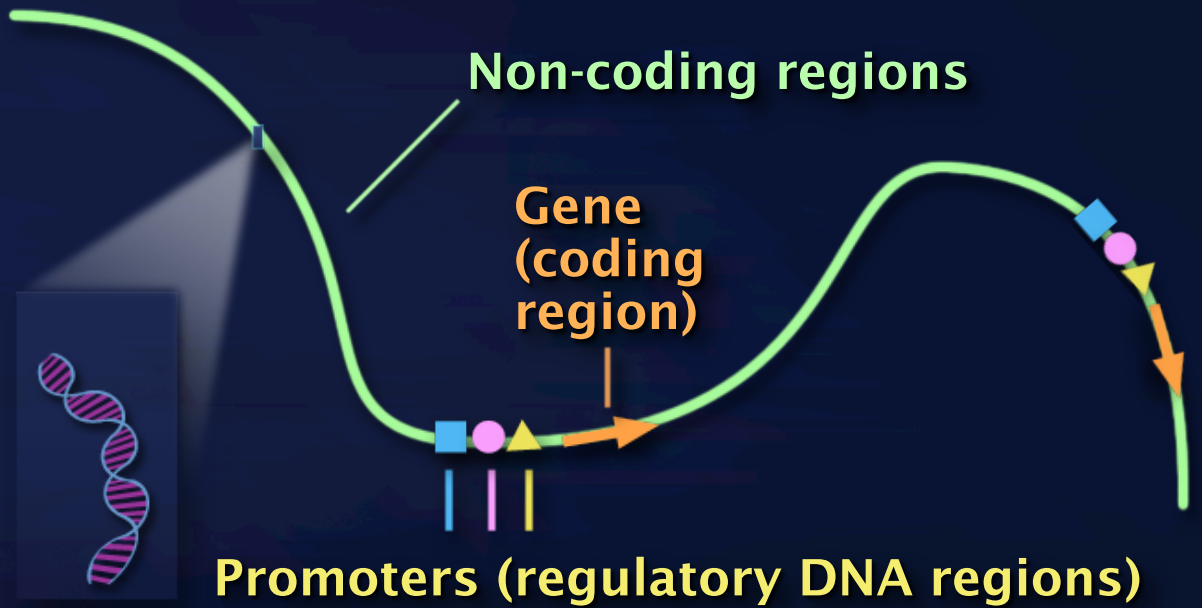


Human genome has 3 billion base pairs and ~22,000 genes.

Only ~3% of DNA is protein coding sequence.

The majority of the DNA in chromosomes is used for other purposes

Organization of Genes in the Genome



Human genome has 3 billion base pairs and ~22,000 genes.

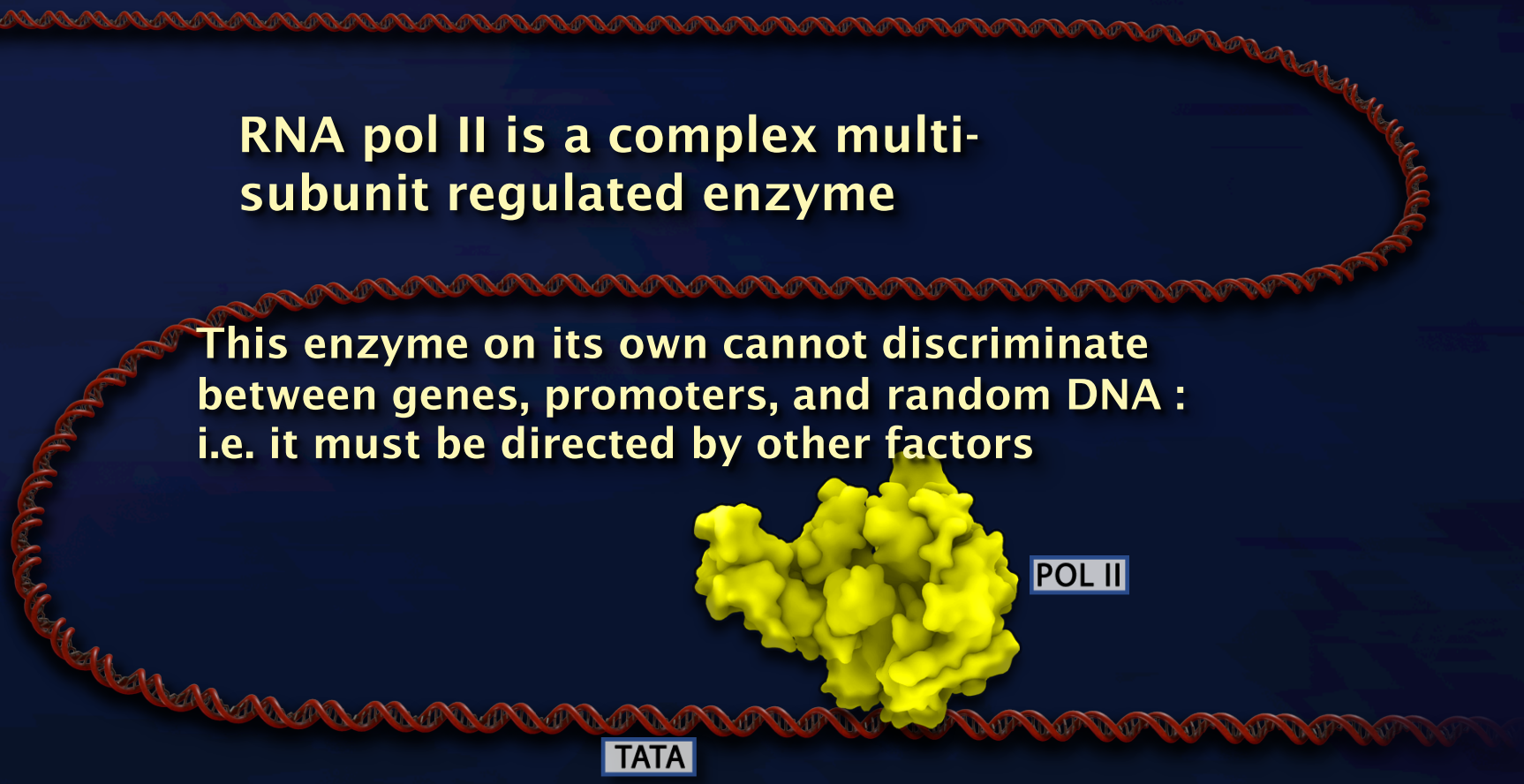
Only ~3% of DNA is protein coding sequence.

An important component of the remaining DNA are regulatory sequences.

RNA Polymerase II is an enzyme that transcribes DNA to RNA

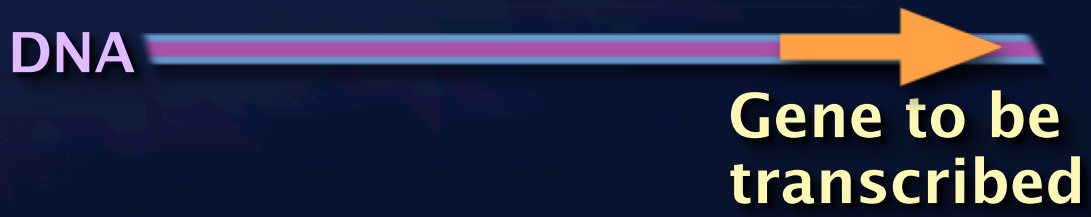
RNA pol II is a complex multi-subunit regulated enzyme

This enzyme on its own cannot discriminate between genes, promoters, and random DNA : i.e. it must be directed by other factors



Hunting for Elusive and Specialized Proteins that Recognize Regulatory DNA and Control Gene Expression

How do the right genes get expressed in the right cells and at the right times ?

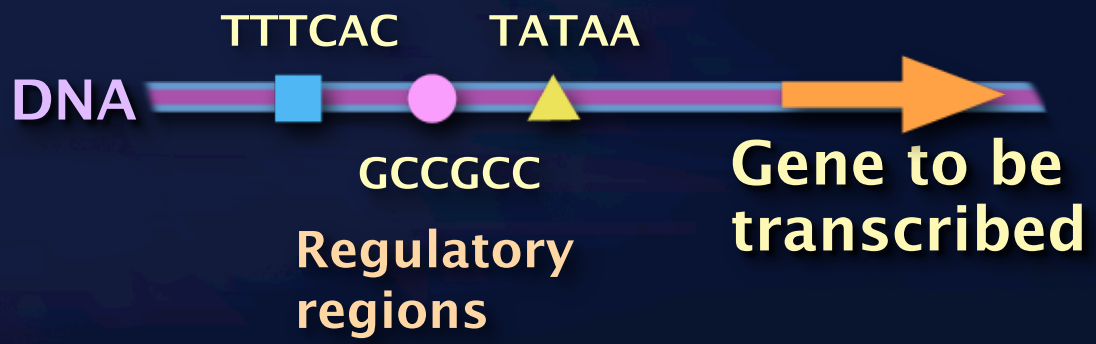


Two major approaches:

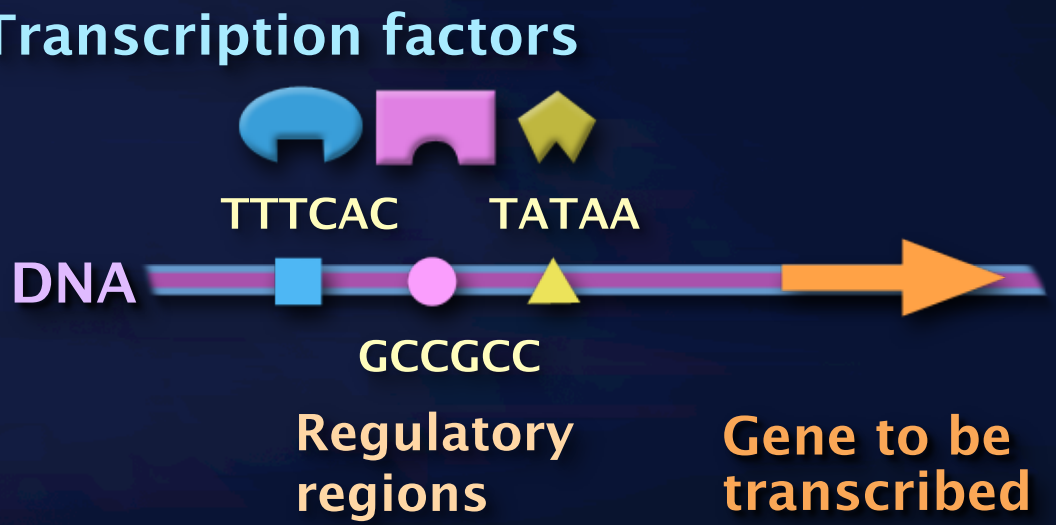
In vitro Biochemistry

In vivo Genetics (model organisms)

Transcription Factors are Specialized Proteins that Control Gene Expression

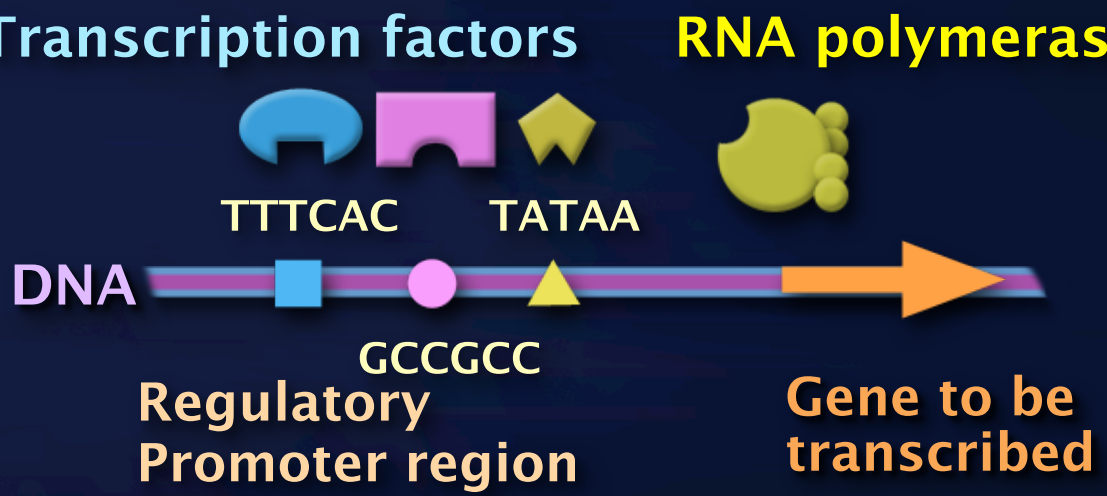


Proteins that Control Gene Expression



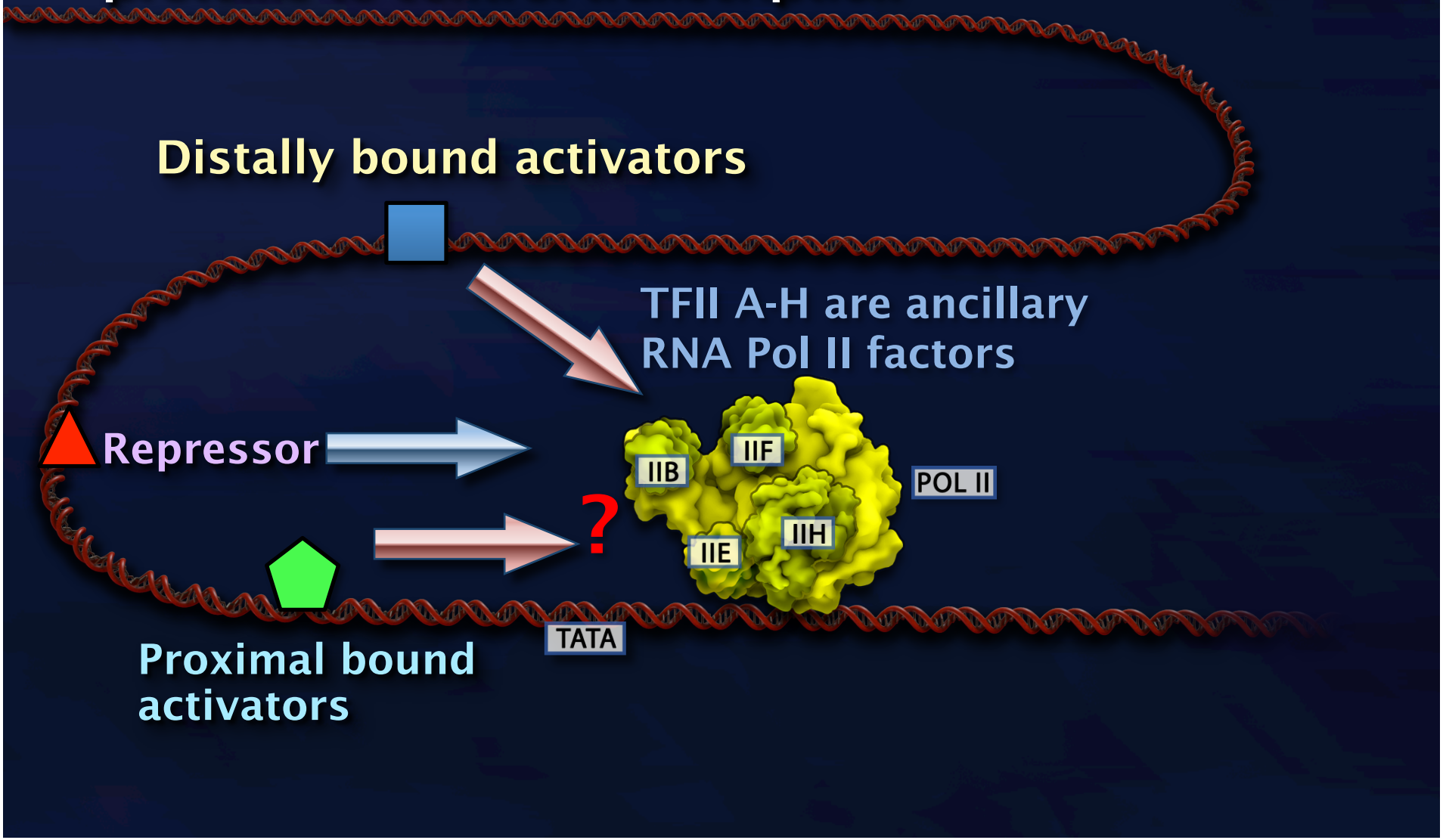
A major family of transcription factors are sequence-specific DNA binding proteins.

Proteins that Control Gene Expression



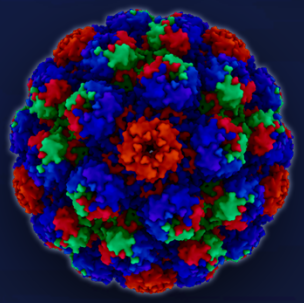
Transcription factors recruit and instructs RNA pol II to initiate RNA synthesis at specific genes by recognizing and binding to DNA elements called promoters.

RNA Pol II requires a group of >85 associated factors and regulatory proteins to control transcription

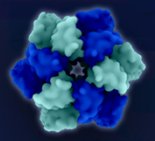


Discovering the First Eukaryotic Gene Specific Transcription Factor

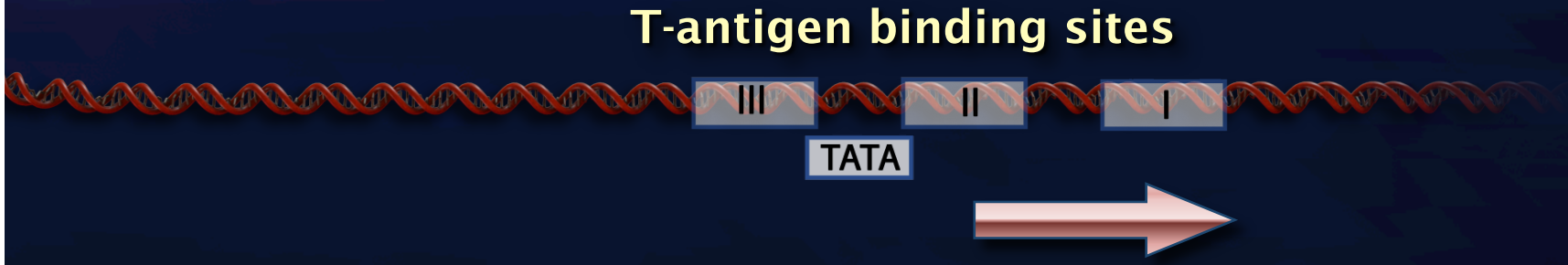
SV40 was first viral genome sequenced.



SV40

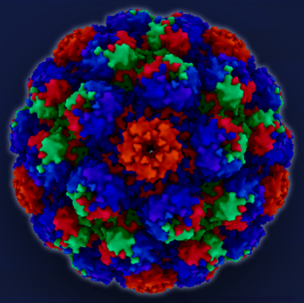


T-antigen:
Repressor

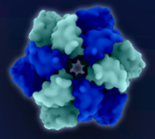


Discovering the First Eukaryotic Gene Specific Transcription Factor

SV40 was first viral genome sequenced.



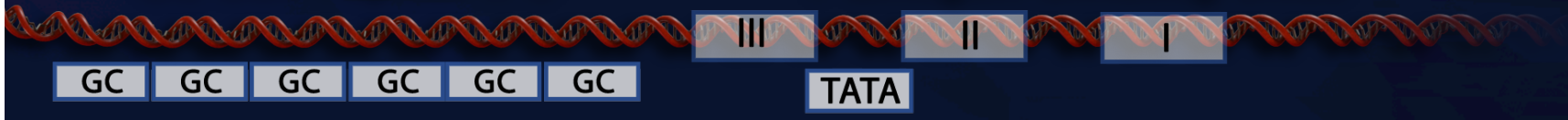
SV40



T-antigen:
a sequence
specific DNA
Binding factor

GC box repeat x 6

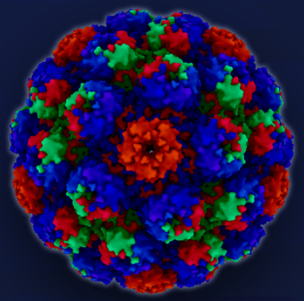
T-antigen binding sites



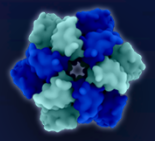
Promoter Proximal
Activator Binding Sites

Transcription start site
for early gene expression

Discovering the First Eukaryotic Gene Specific Transcription Factor

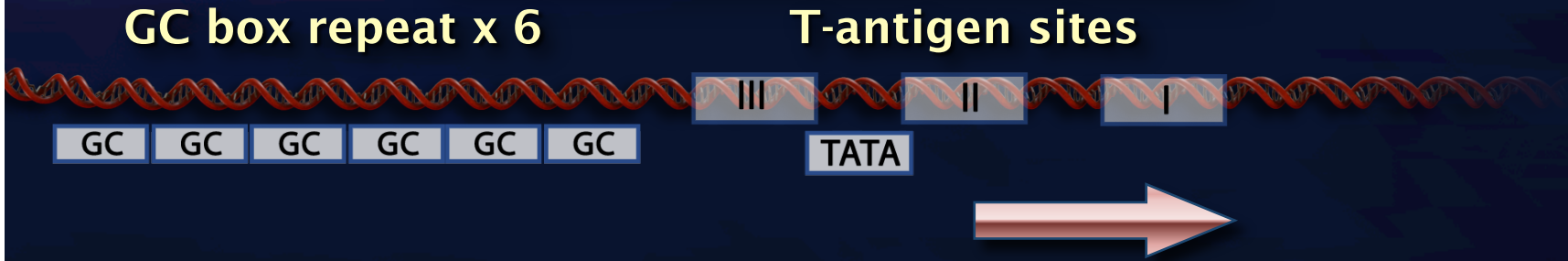


SV40



T-antigen:
Repressor

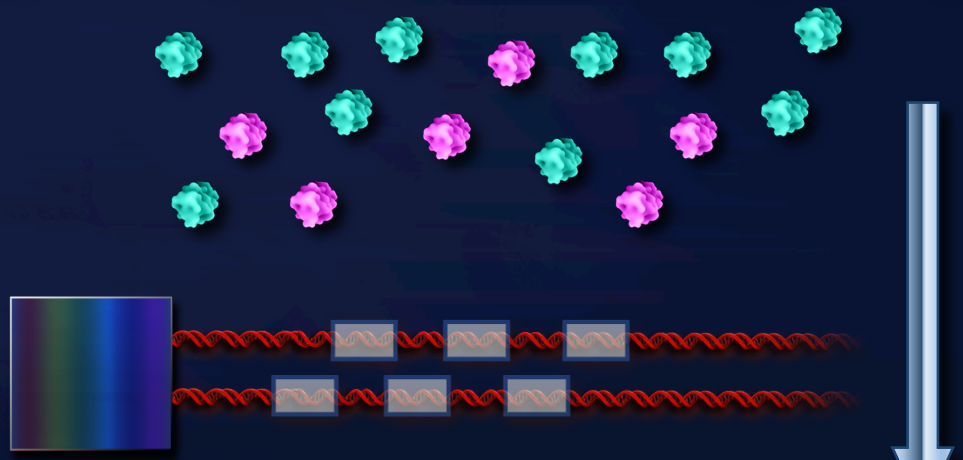
What host molecule binds to the GC boxes?



Isolating Sequence-Specific DNA-Binding Proteins

Concept

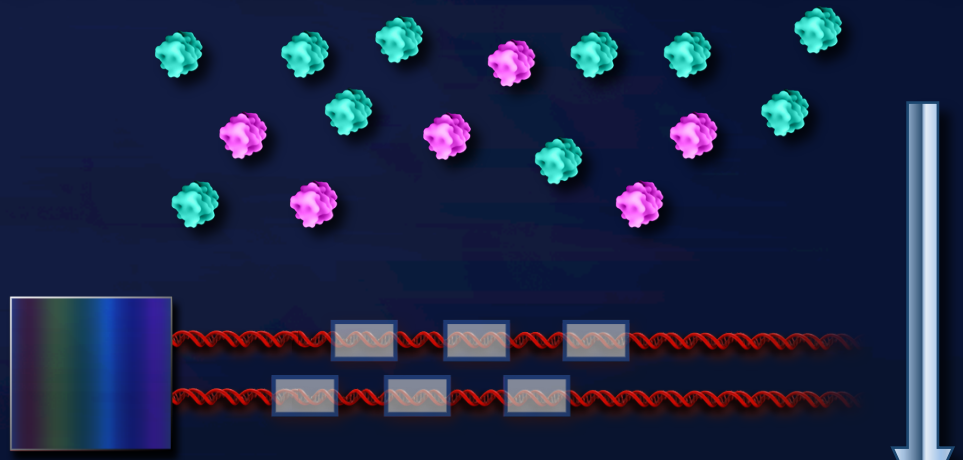
Sequence-specific proteins Nonspecific proteins



DNA with specific recognition sequences attached to resin

Isolating Sequence-Specific DNA-Binding Proteins

Sequence-specific proteins Nonspecific proteins



DNA with specific recognition sequences attached to resin

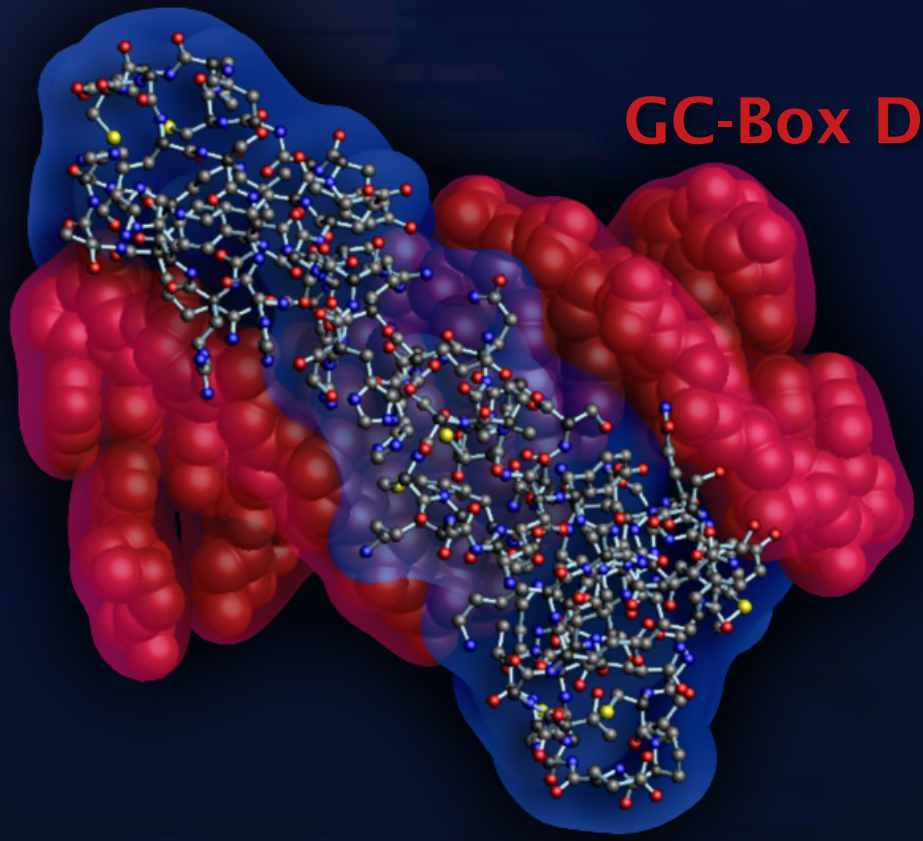
Add Nonspecific DNA

Isolating Sequence-Specific DNA-Binding Proteins

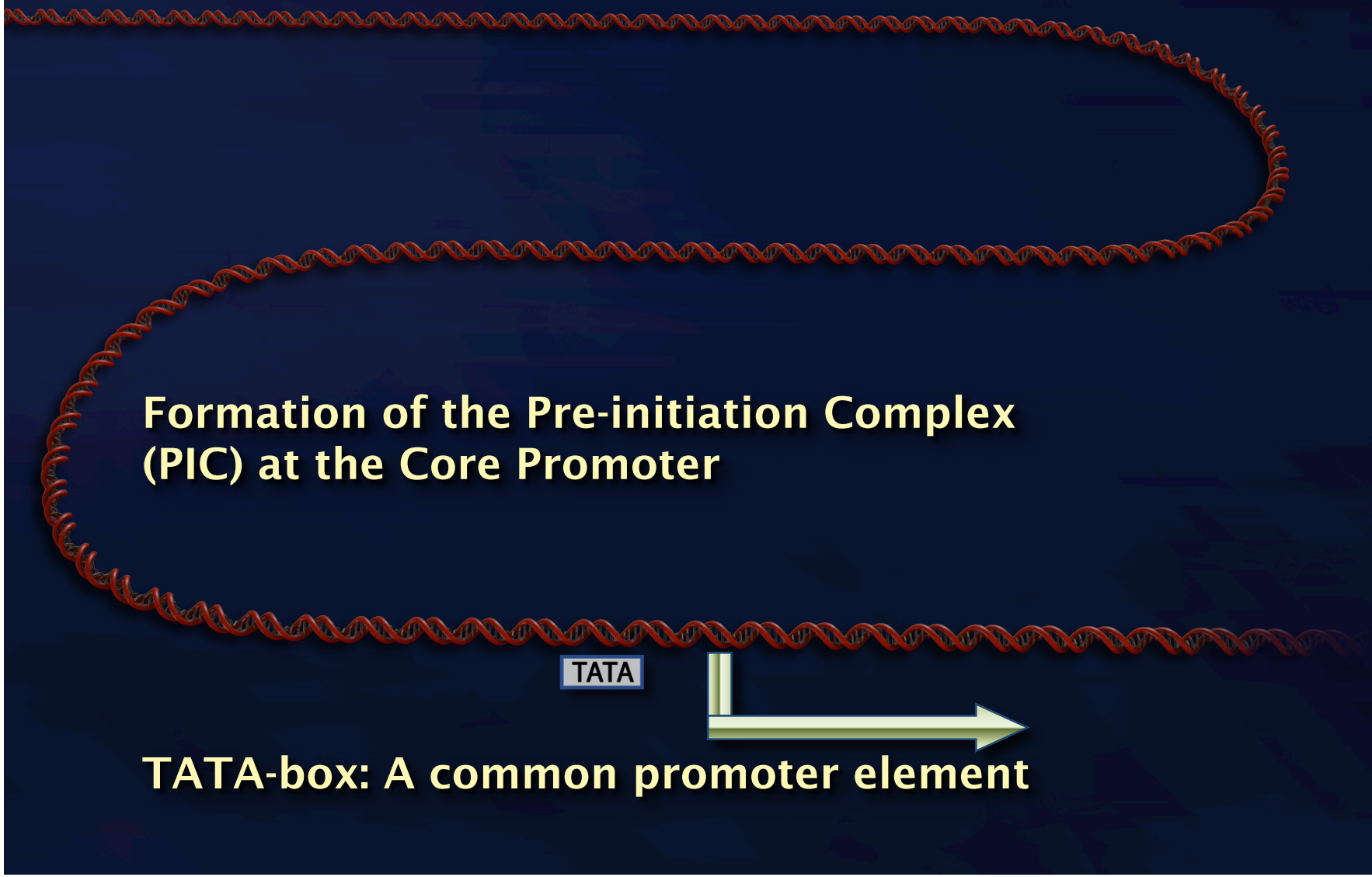
Use solid phase specific DNA sequences coupled with non-specific competitor DNA in solution to achieve high selectivity

Biochemical purification and molecular cloning of Human Transcription Factor Sp1, a Potent Activator

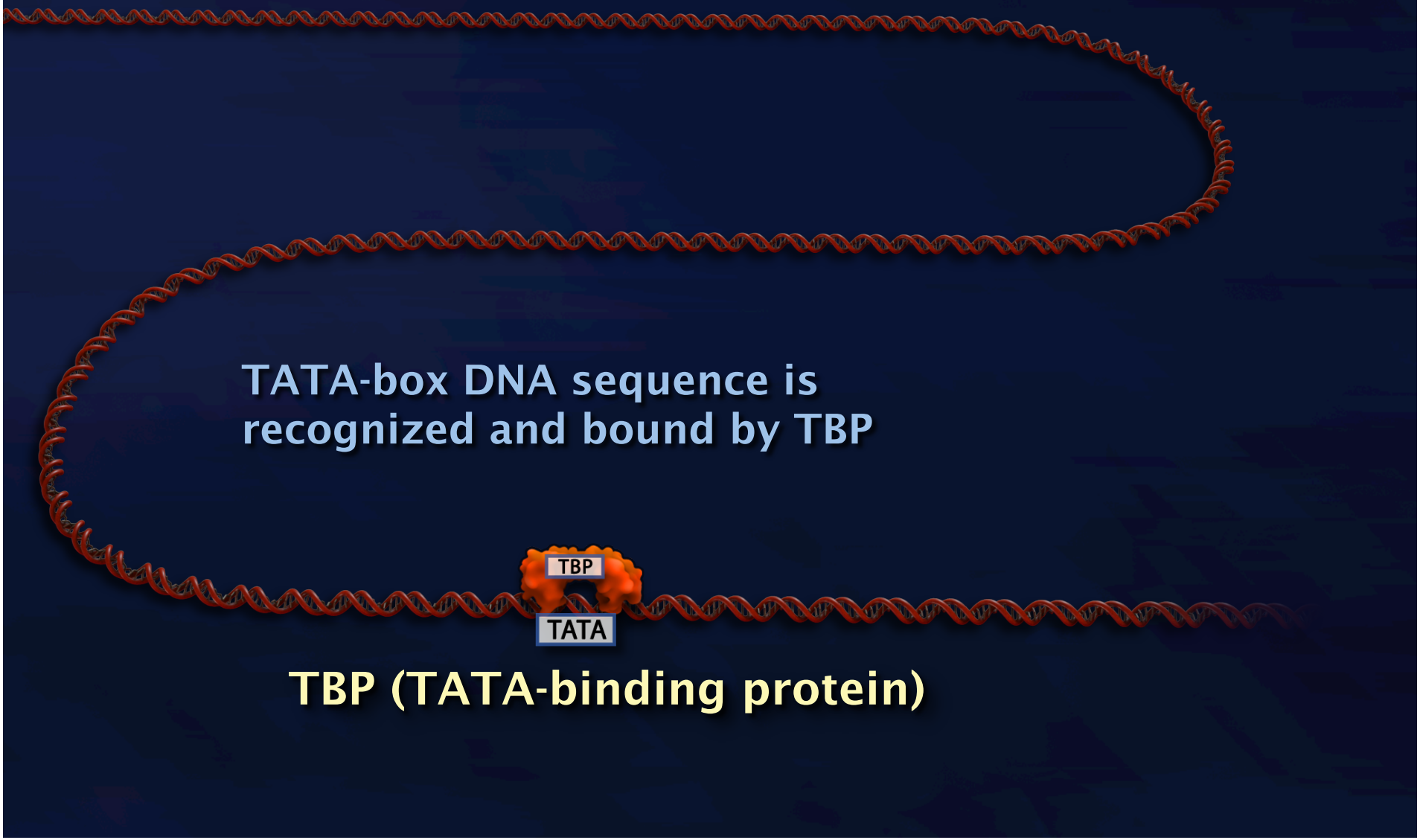
SP1 Binds to DNA via Three Zinc-Finger Domains



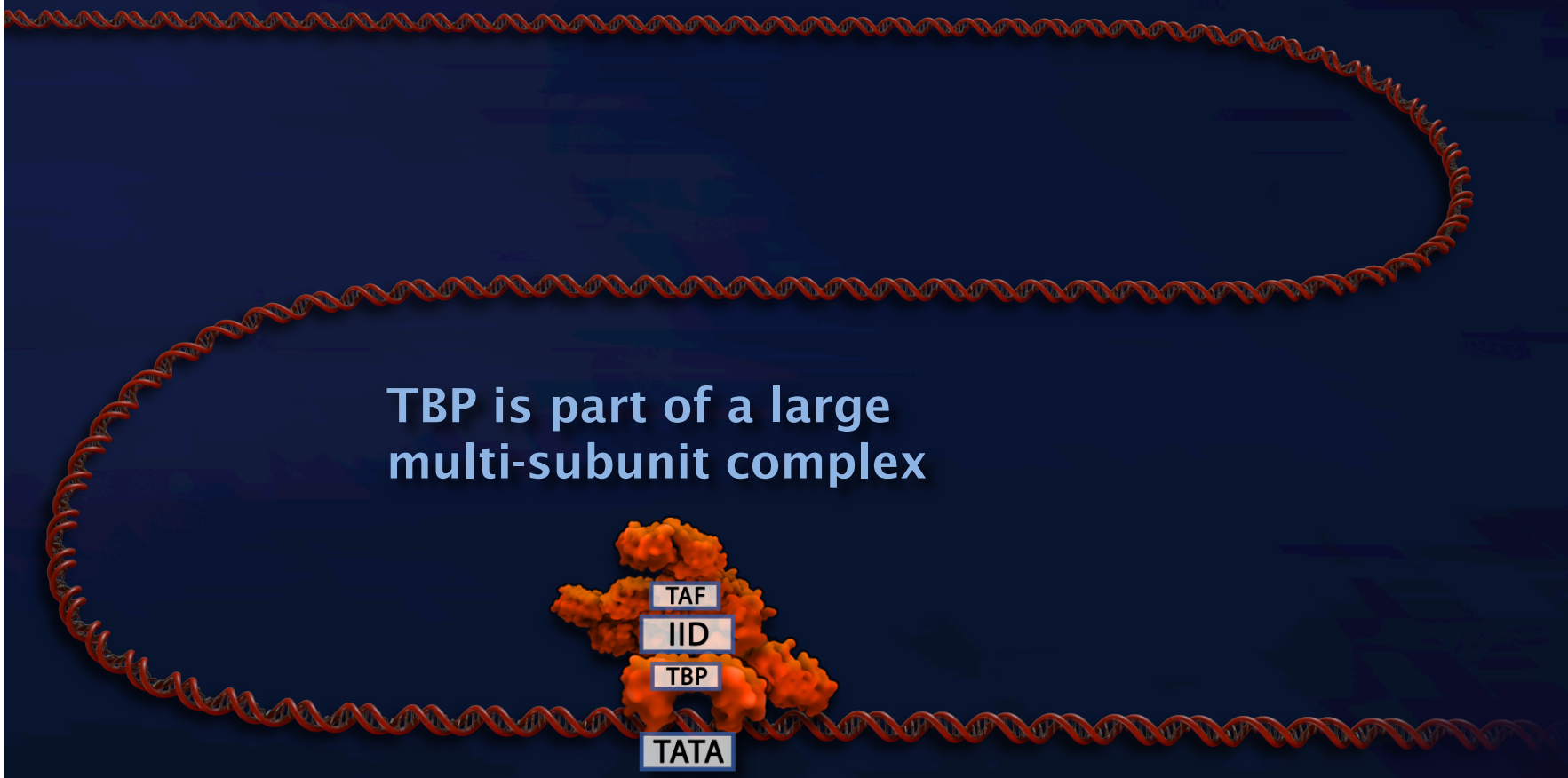
How Initiation of Transcription Works



How Initiation of Transcription Works

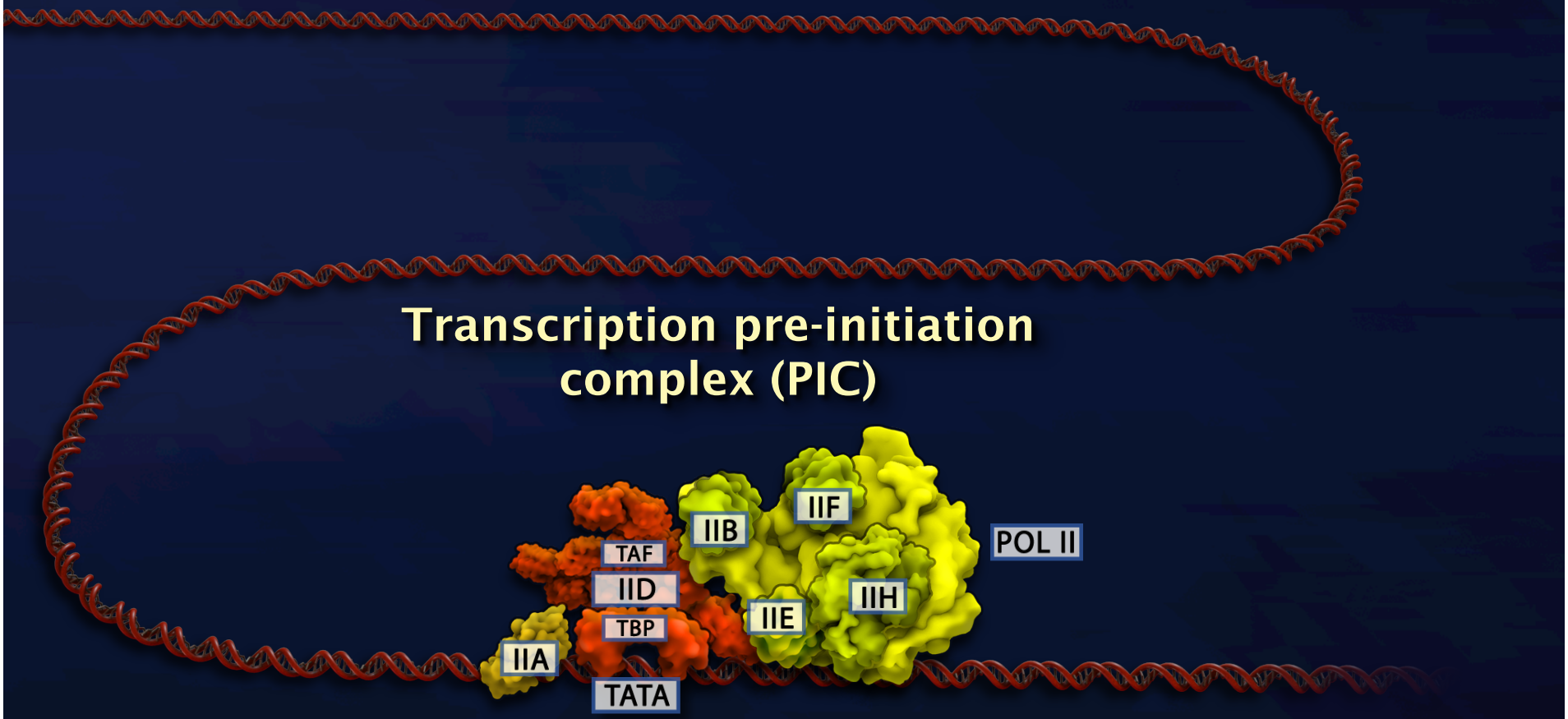


How Initiation of Transcription Works



TBP + 12-15 TAF Subunits = TFIID
TAF = TBP associated factors

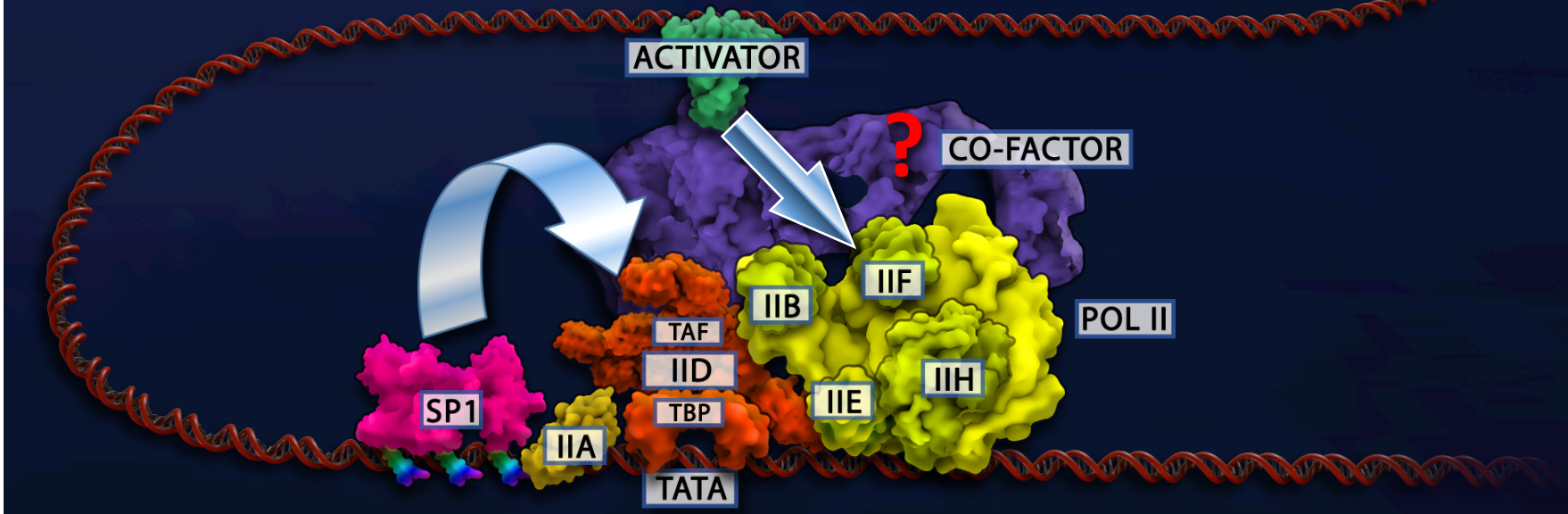
How Initiation of Transcription Works



TFIIA + TFIIB + TFIID TFIIE + TFIIF +
TFIIH + RNA Pol II forms the **PIC**

How Initiation of Transcription Works

PIC : An ensemble of Multi-subunit transcription factors and co-factors



Transcription can be activated or repressed by proximal and distal promoter factors

Transcription Animation