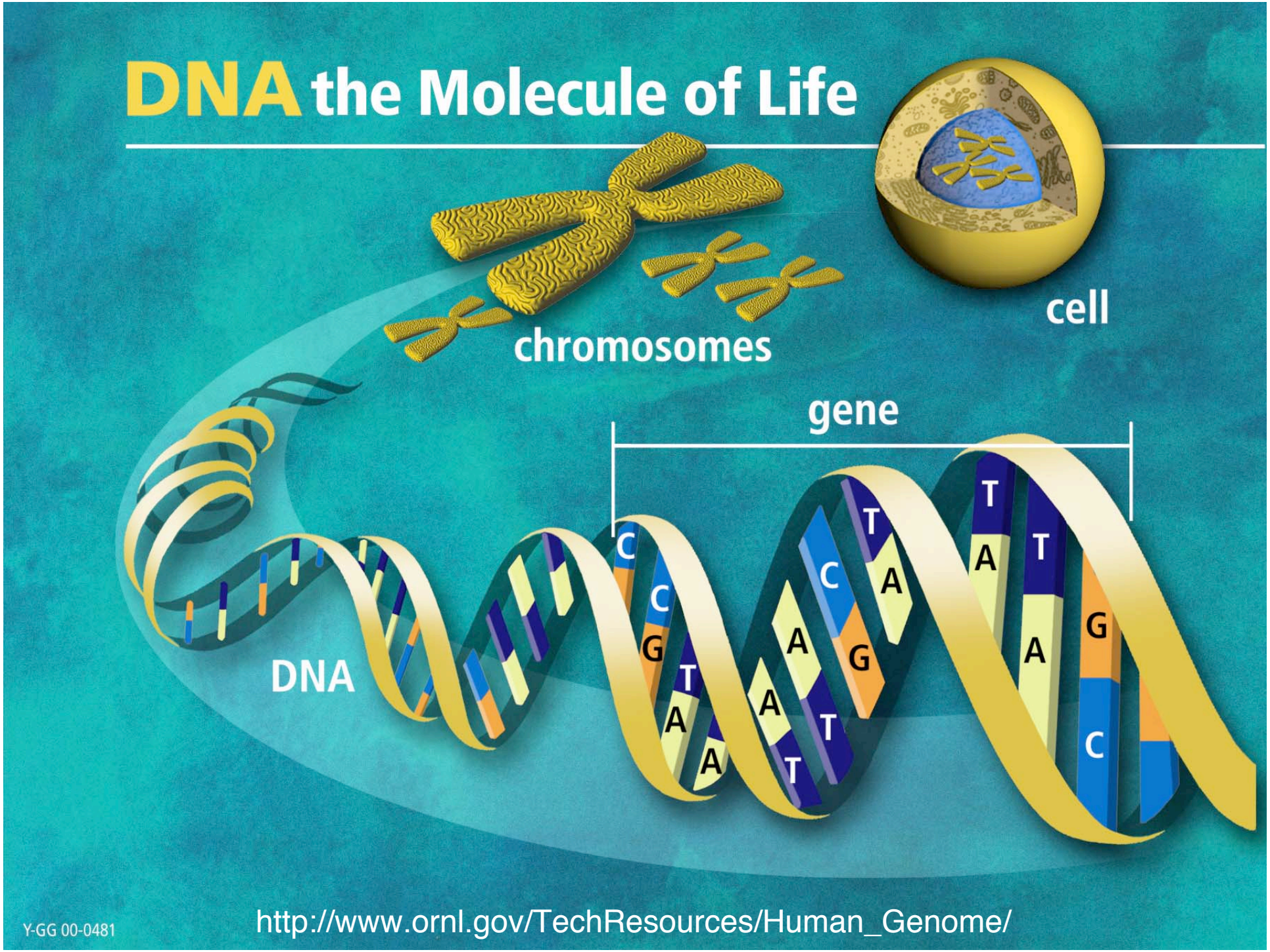
DNA the Molecule of Life
chromosomes
cell
gene
DNA
C
C
G
T
A
A
A
T
A
G
C
T
A
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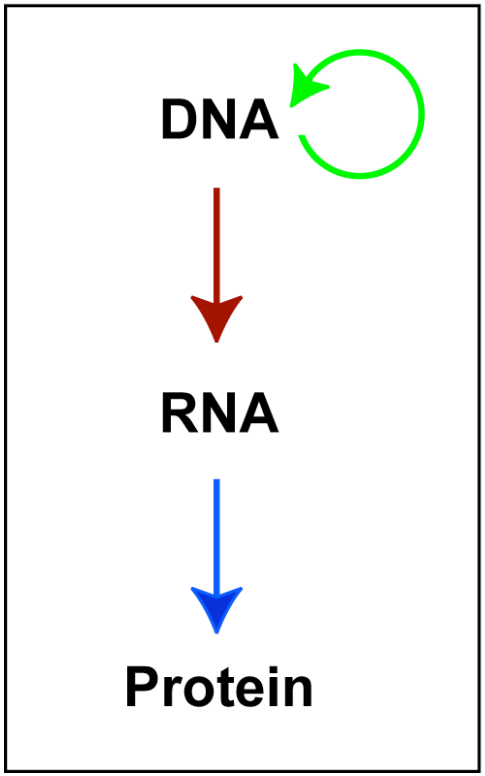
C
Y-GG 00-0481
http://www.ornl.gov/TechResources/Human_Genome/

Central Dogma of Molecular Biology



DNA = Master Copy of Genetic Information

RNA = Temporary (Disposable) Copy of DNA

Protein = Ultimate Product of Gene Expression

Replication = Duplication of DNA prior to Cell Division

Transcription = Synthesis of RNA from DNA Template

Translation = Synthesis of Protein from RNA Template

There Are Three RNA Polymerases in Eukaryotes

RNA Polymerase I: Synthesis of Ribosomal RNA (rRNA)

RNA Polymerase II: Synthesis of Messenger RNA (mRNA)

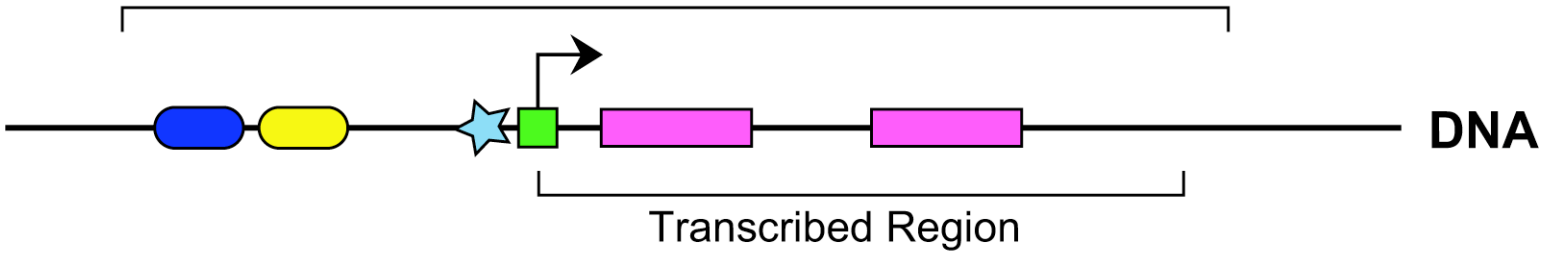
RNA Polymerase III: Synthesis of Transfer RNA (tRNA) and 5S RNA

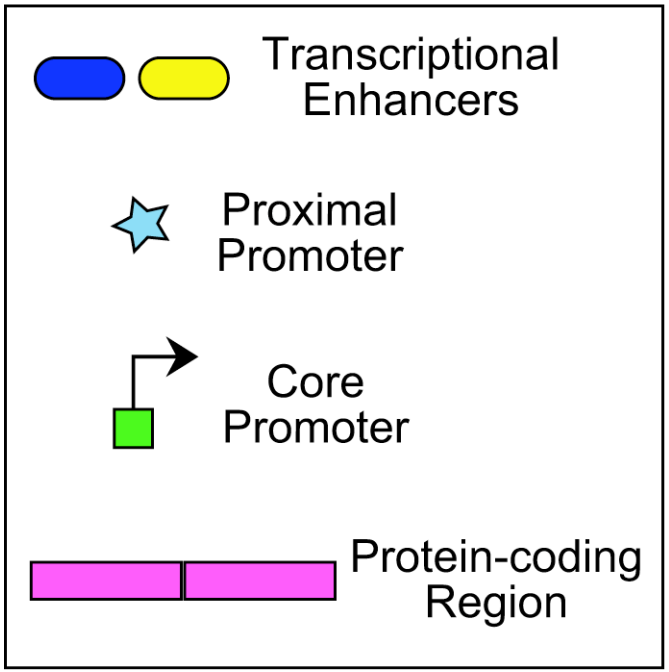
Eukaryotes = organisms in which cells have nuclei (*e.g.*, fungi, insects, plants, mammals)

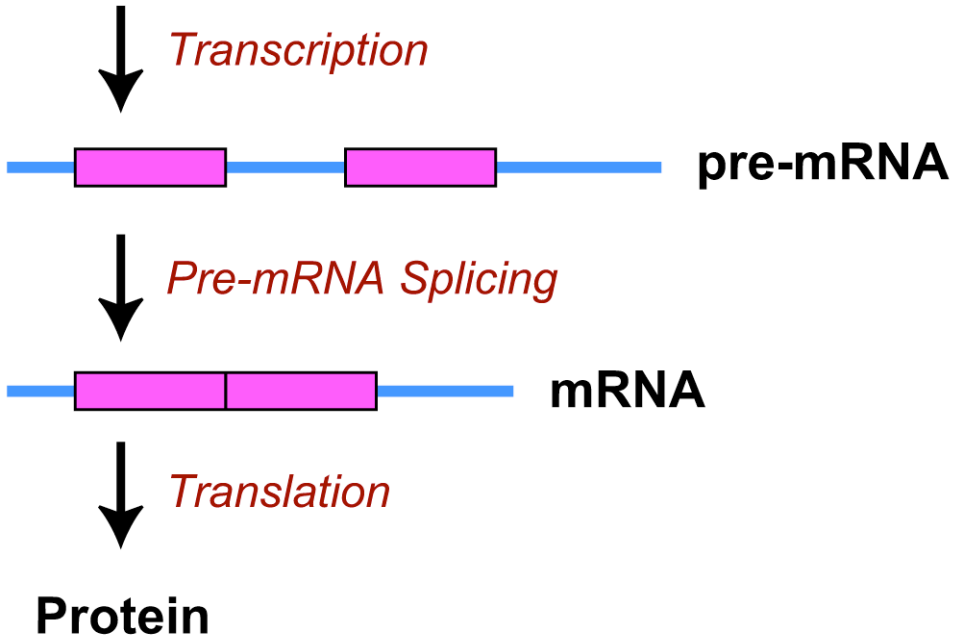
Prokaryotes = organisms in which cells do NOT have nuclei (*e.g.*, bacteria)

What Is a Gene?

Gene = Segment of DNA that encodes a specific function (typically, a protein)





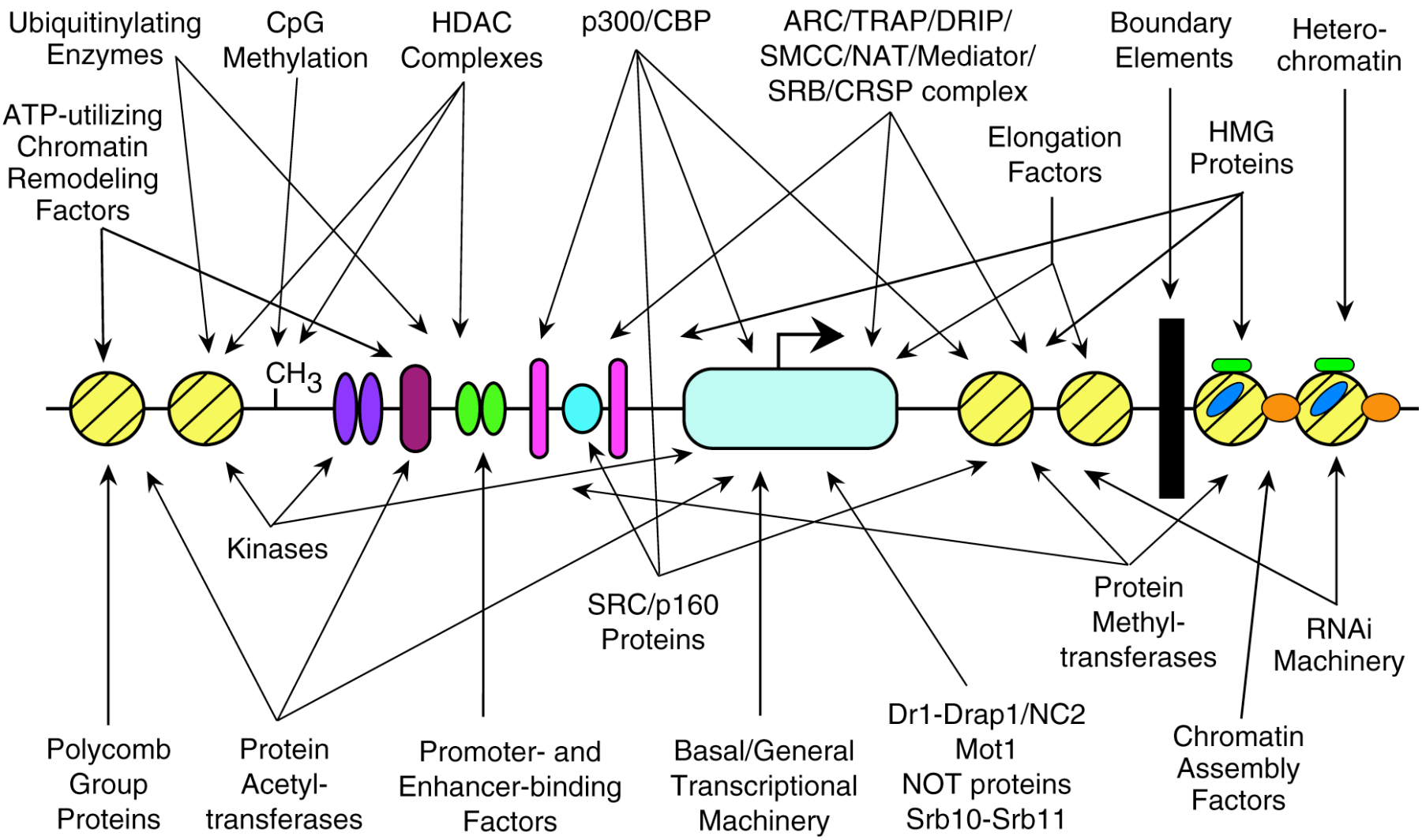


Gene Expression = gene activation

How is the Activity of Each of the Tens of Thousands of Genes Regulated?

- Events prior to transcription (*e.g.*, signalling pathways, changes in chromatin structure)
- Transcriptional regulation
- Post-transcriptional regulation (*e.g.*, splicing, translation)

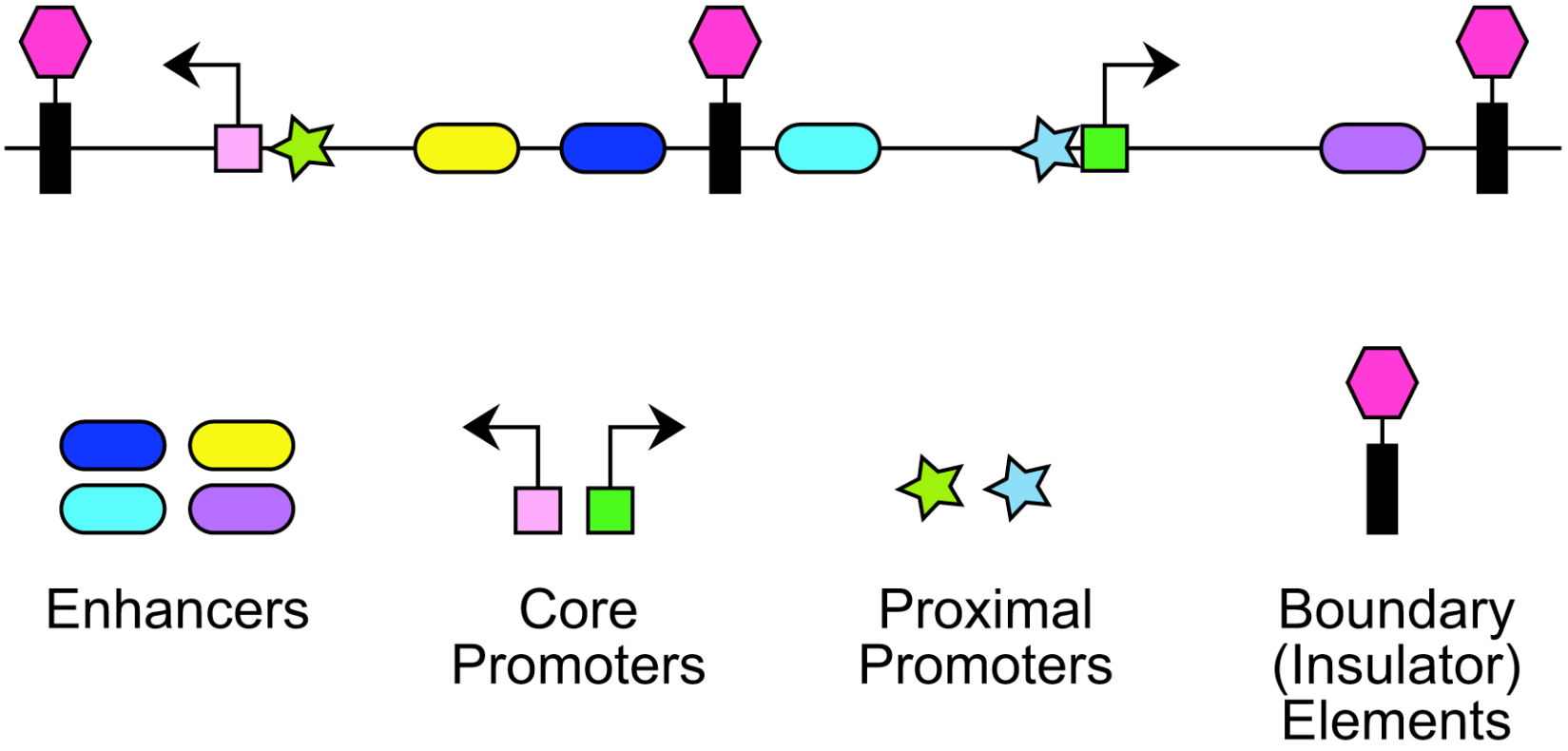
Many Factors Affect the Regulation of Transcription by RNA Polymerase II



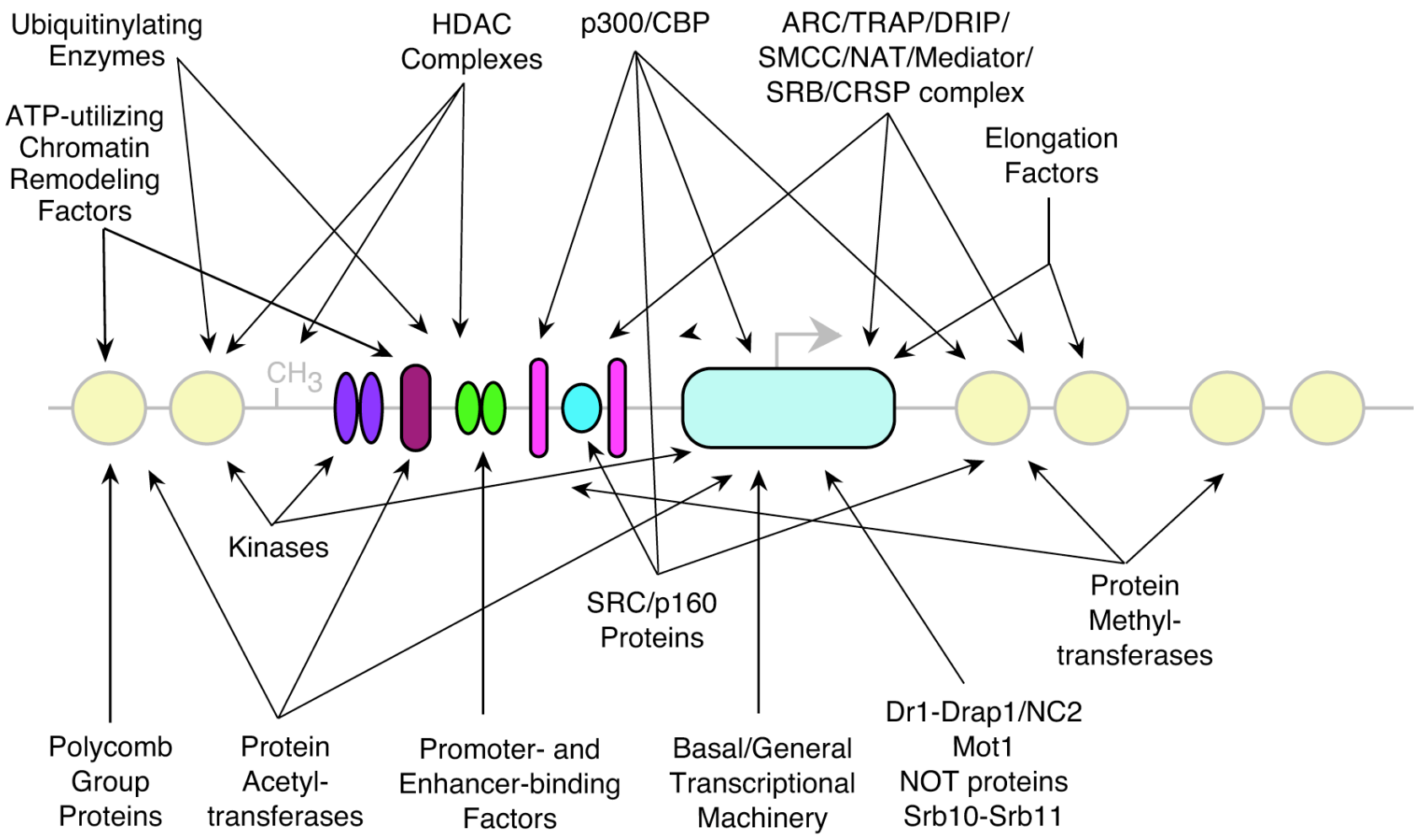
Three Different Perspectives of Transcriptional Regulation

- Cis-acting DNA Elements vs. Trans-acting Protein Factors
- Basic (Basal) Transcription vs. Regulatory Processes
- Genetic (involving DNA sequence) vs. Epigenetic (not involving primary DNA sequence) Phenomena

DNA Regulatory Elements (cis elements) for Transcription of Protein-coding Genes by RNA Polymerase II



Trans-acting Protein Factors Involved in Transcriptional Regulation



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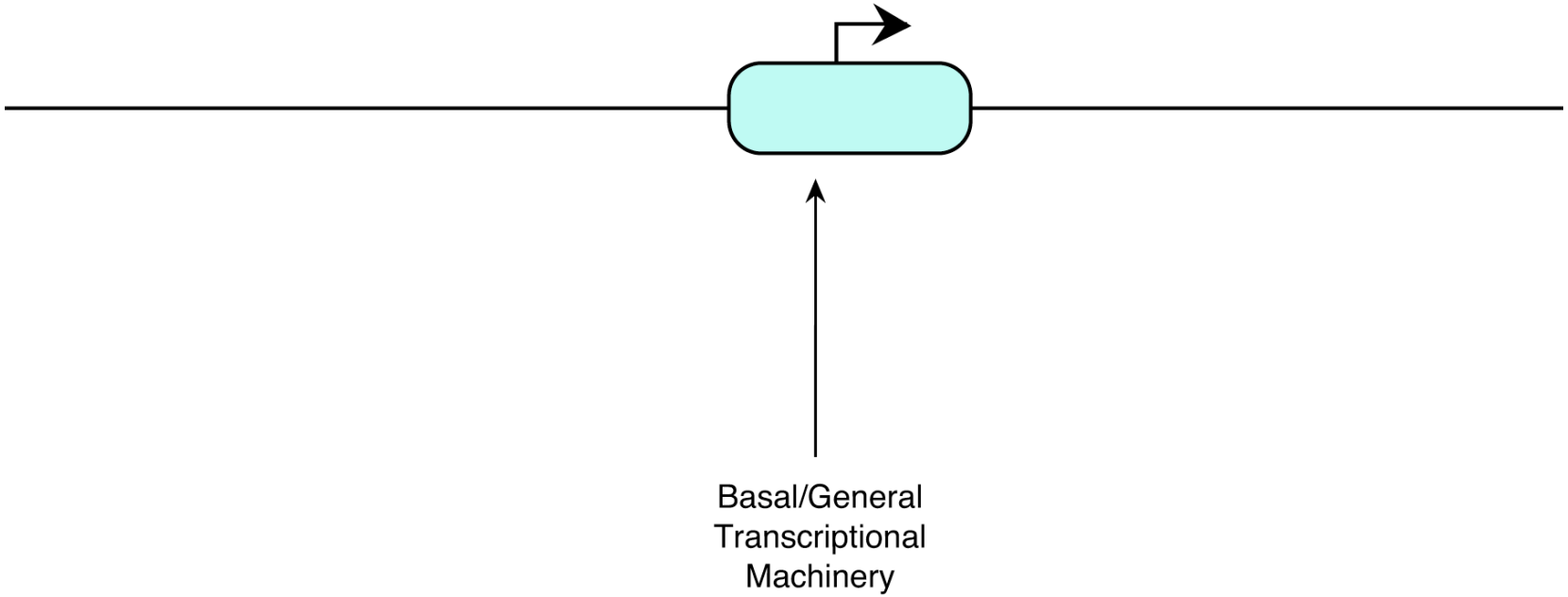
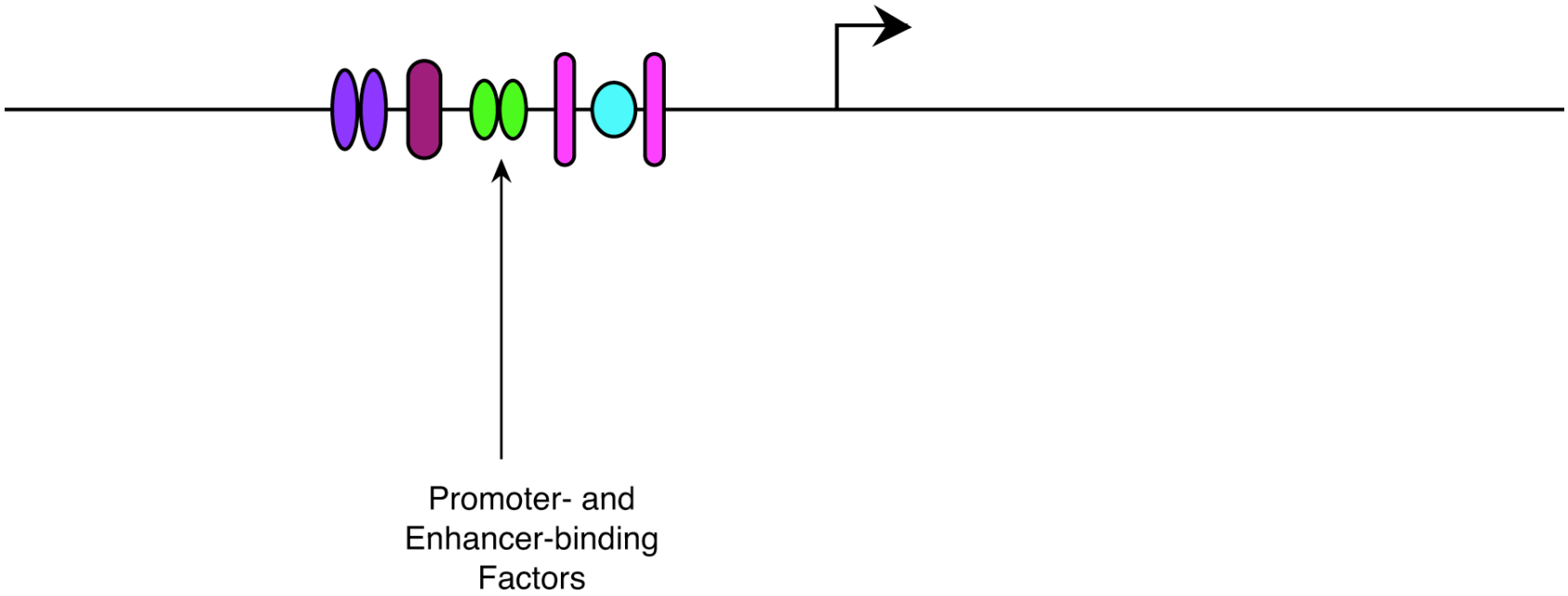
Basal Transcription Machinery – Synthesis of mRNA



Sequence-specific DNA-binding Regulatory Factors



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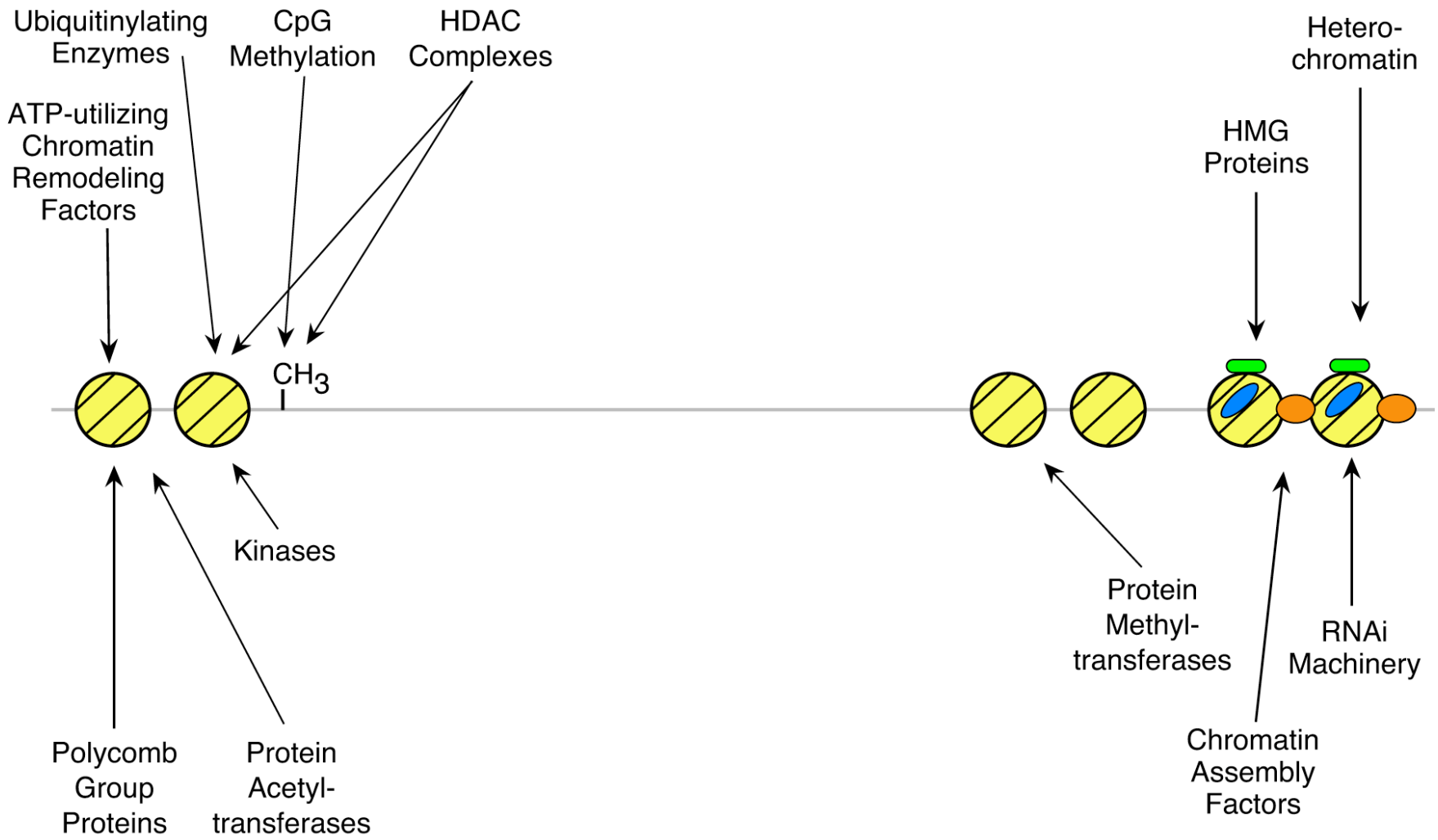
Genetic Component of Transcriptional Regulation

Epigenetic Components of Transcriptional Regulation



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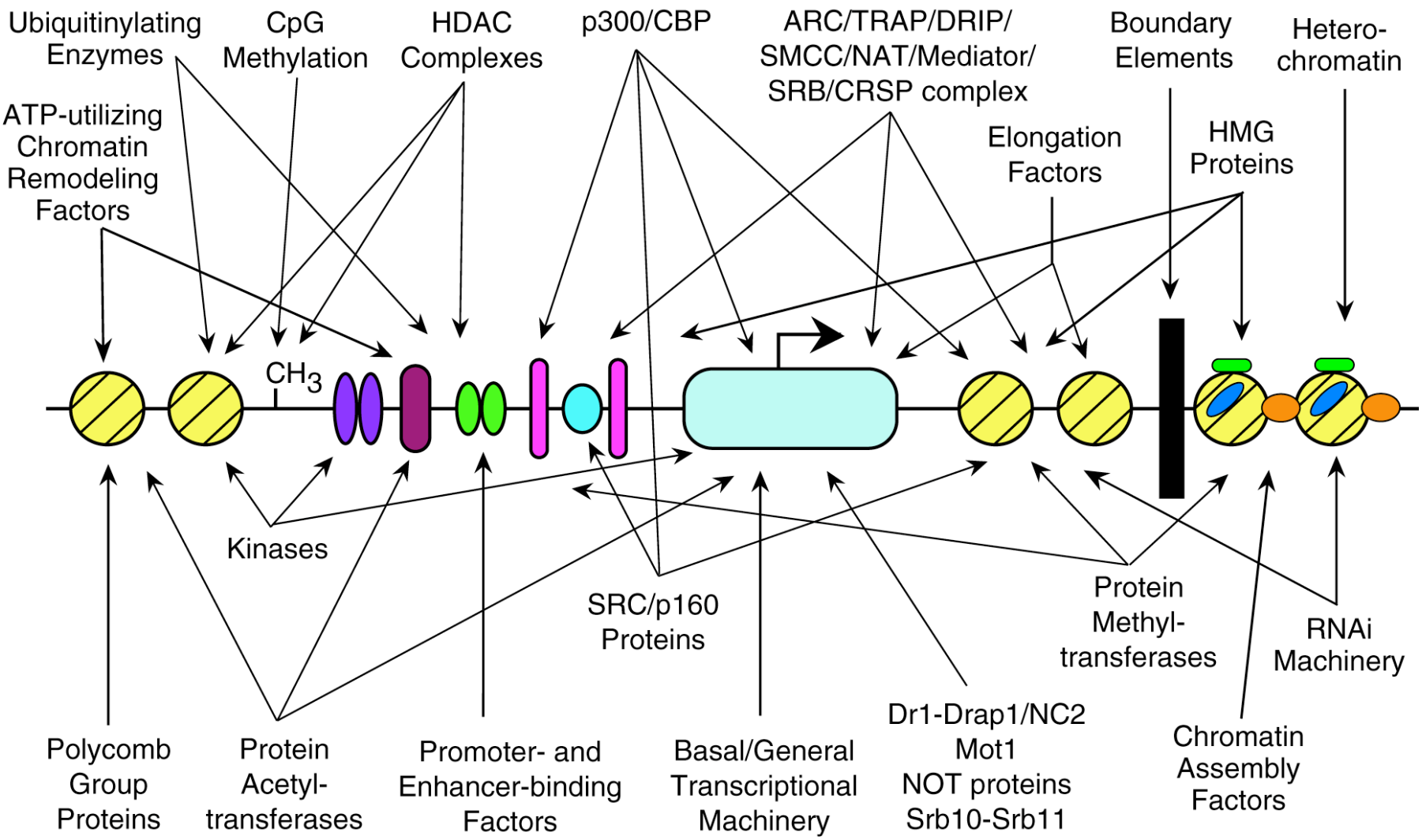
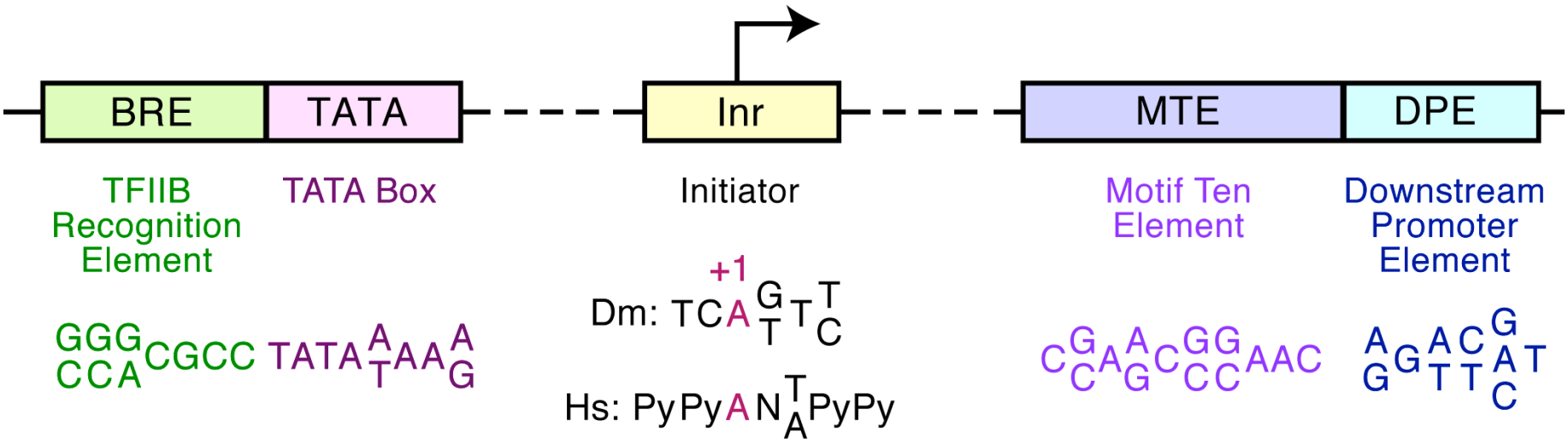


Specific Topics

- Basal transcription by RNA polymerase II
- Sequence-specific DNA-binding factors
- How might enhancers work?
- Chromatin structure – Introduction
- Covalent modification of histones
- Chromatin remodeling factors
- Chromatin assembly

Core Promoter Elements



Basal ('General') Transcription Factors for RNA Polymerase II

- TFIID – consists of TBP (TATA-box binding protein) + TAFs (TBP-associated factors). Binds to core promoter motifs. TAFs interact with activator proteins. The first step in basal transcription is probably binding of TFIID to the core promoter.

- TFIIA – three (or two) small subunits. Increases affinity of TBP for DNA in vitro. Not needed for transcription in vitro. Could be an anti-inhibitor.

- TFIIB – one subunit of 35 kDa. Binds to TBP and the BRE.

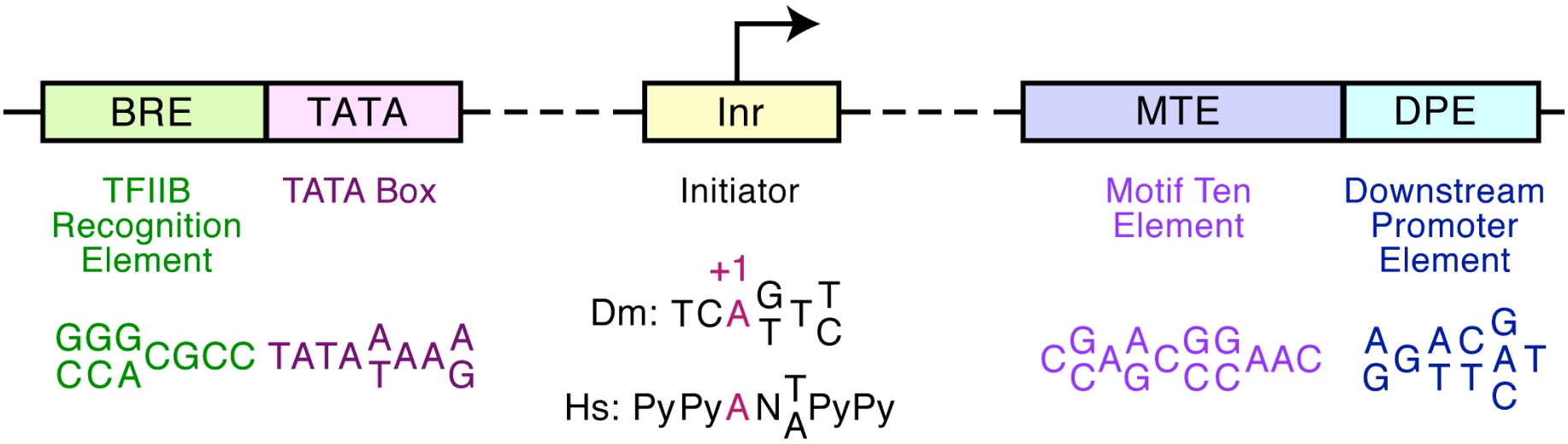
- RNA Polymerase II – consists of two large subunits (IIa and IIb) as well as about eight smaller subunits. Unique feature of largest (IIa) subunit is the C-terminal domain (CTD), which is an imperfectly-repeated heptapeptide motif, YSPTSPS.

- TFIIF – also known as RAP30/74. Binds to RNA polymerase II. Two subunits of 30 and 74 kDa. Functions in transcription initiation and elongation.

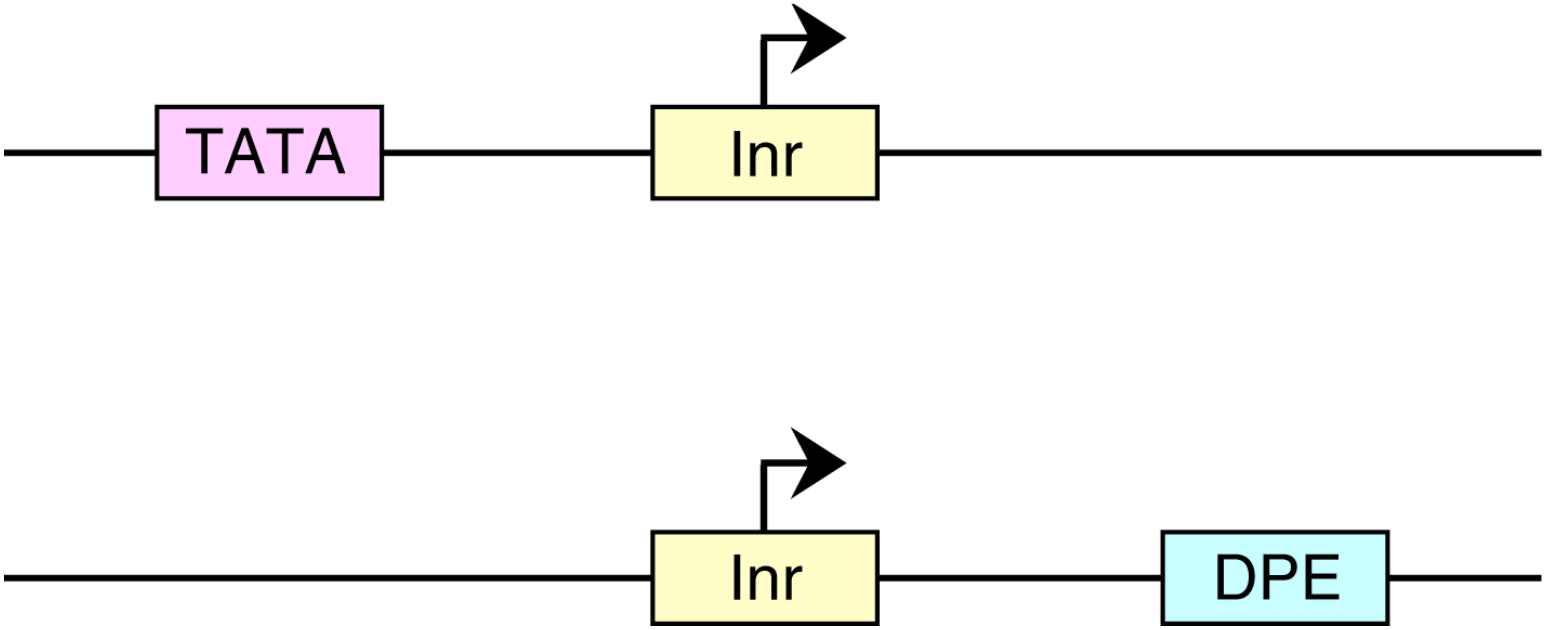
- TFIIE – two polypeptides of 34 and 56 kDa. Required for assembly of TFIIH into the transcription preinitiation complex (PIC).

- TFIIH – nine polypeptides. Core TFIIH has six subunits, which include 5'–>3' and 3'–>5' DNA helicases, and is also involved in nucleotide excision repair. Also has a three subunit Cdk7/MO15 + Cyclin H + MAT1 kinase complex that phosphorylates Ser5 of the CTD during transcription initiation.

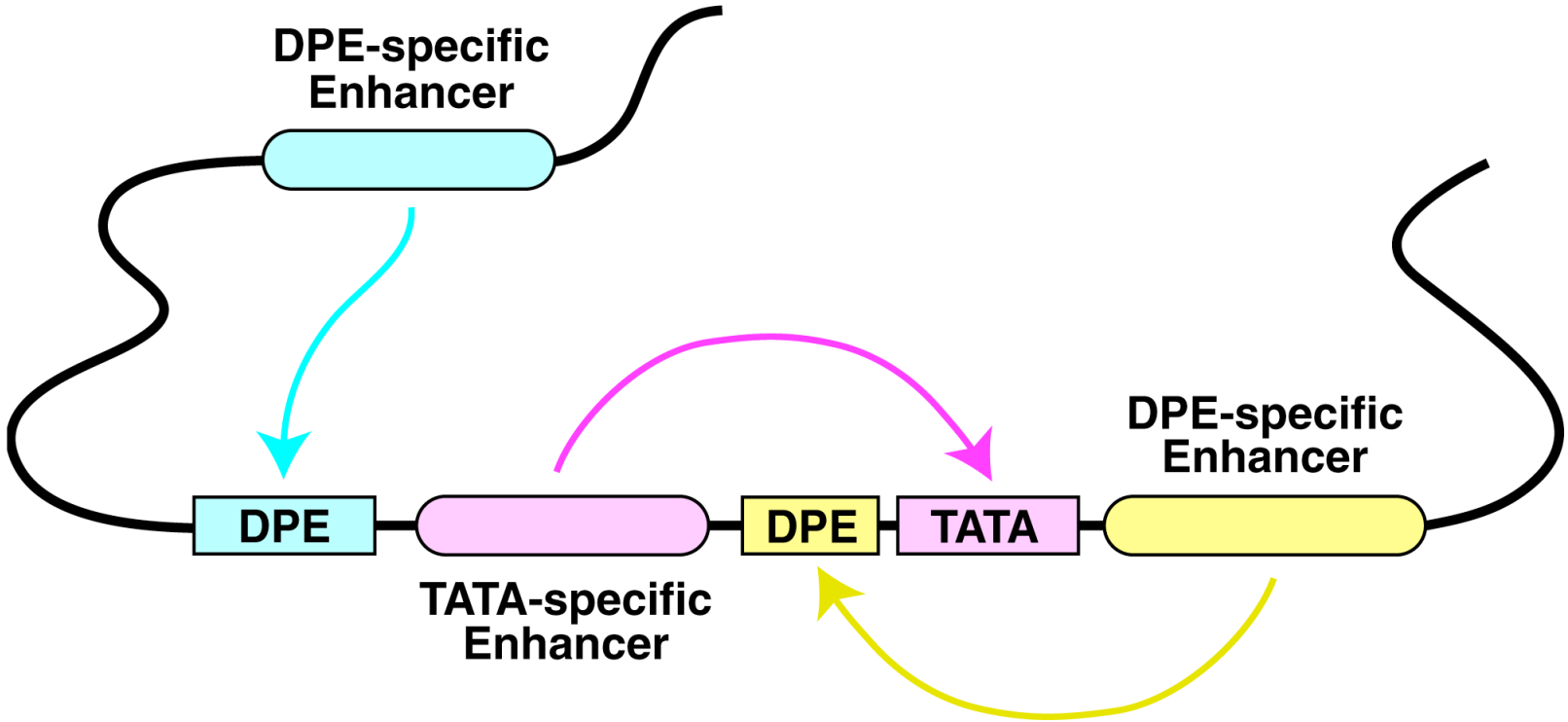
Core Promoter Elements



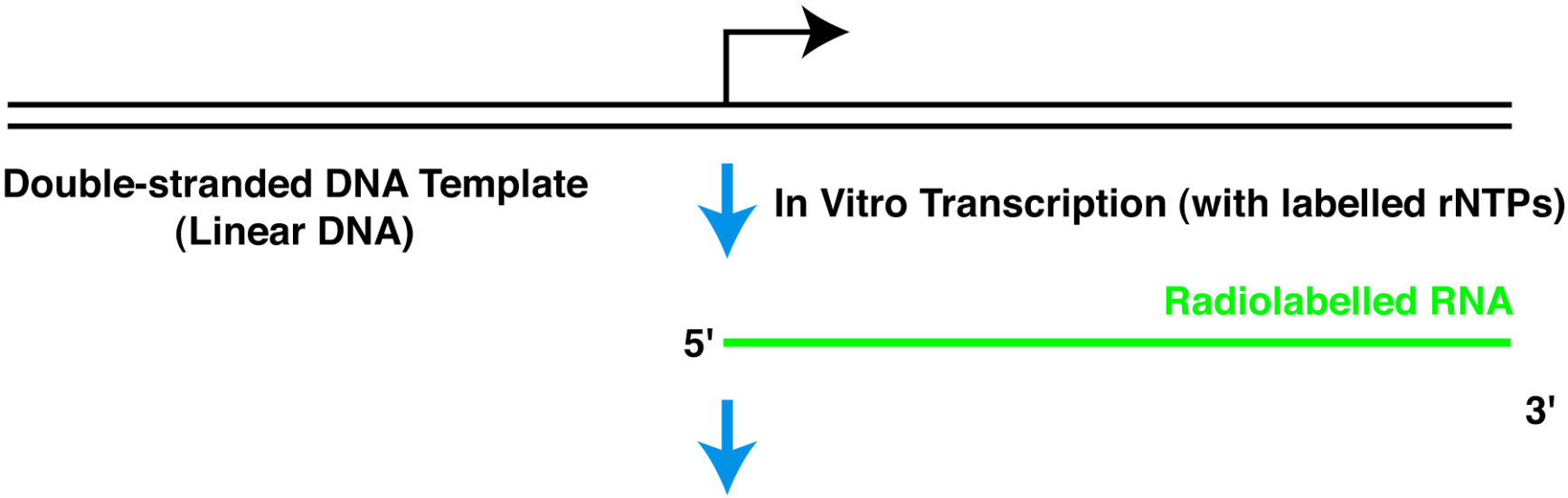
TATA- versus DPE-dependent Core Promoters



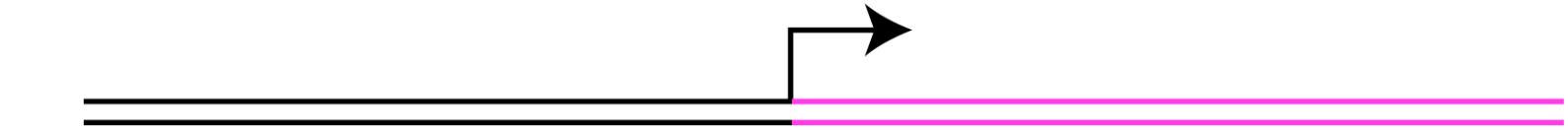
A Role for Core Promoters in Enhancer Specificity



"Runoff" Transcription Assay (In Vitro)



"G-less Cassette" Variation of Runoff Transcription Assay



Special Double-stranded DNA Template
That Is Lacking G Residues
Downstream of the +1 Start Site

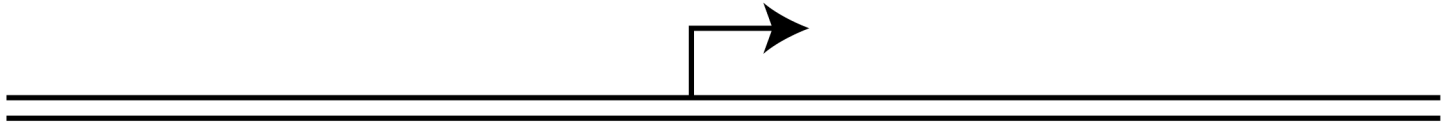
In Vitro Transcription with ONLY ATP, CTP, UTP

Radiolabelled RNA Lacking G Nucleotides

5' ——— 3'

Analyze Radiolabelled RNA by Agarose Gel Electrophoresis and Autoradiography

Primer Extension Analysis of RNA



Double-stranded DNA Template
(linear or circular)

In Vitro Transcription (with unlabelled rNTPs)

RNA
5' 3'

Anneal Labelled Oligonucleotide Primer

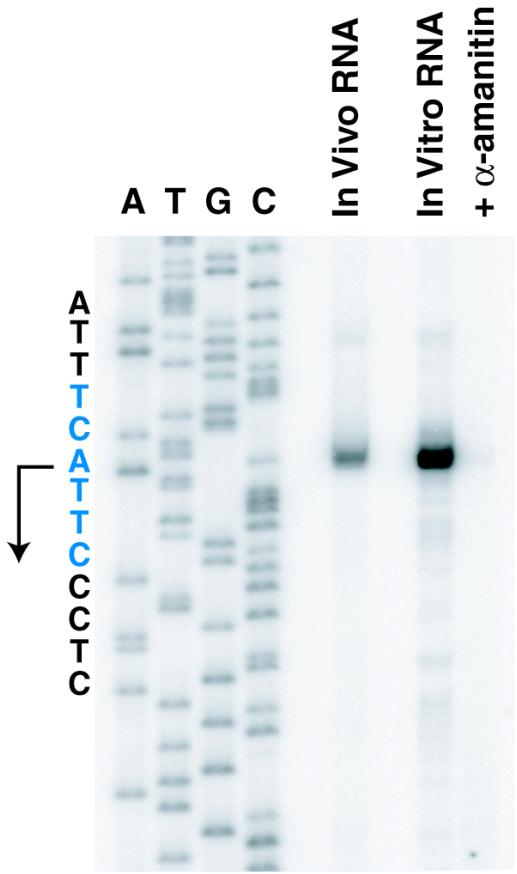
RNA
5' 3'
5'

Extend with Reverse Transcriptase (+ dNTPs)

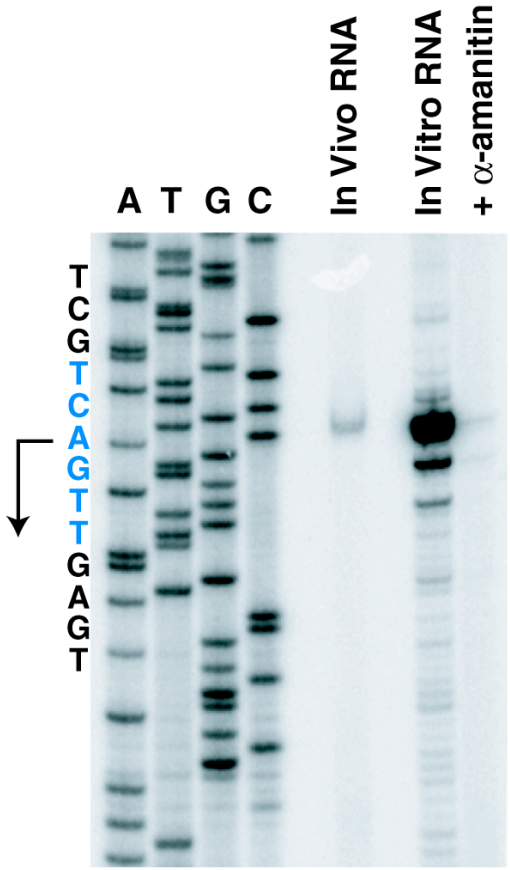
RNA
5' 3'
5'

Analyze on Polyacrylamide-Urea Gel (= same as DNA Sequencing Gel)

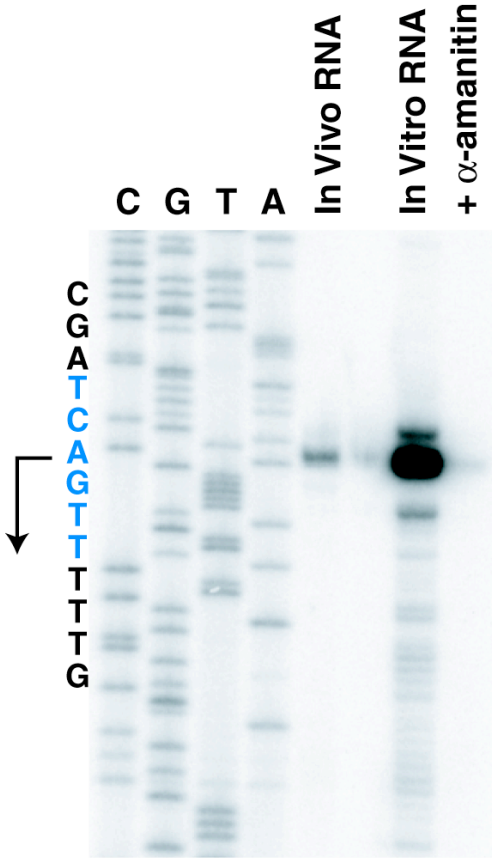
Mapping of In Vivo and In Vitro Start Sites of MTE-containing Promoters



CG10479

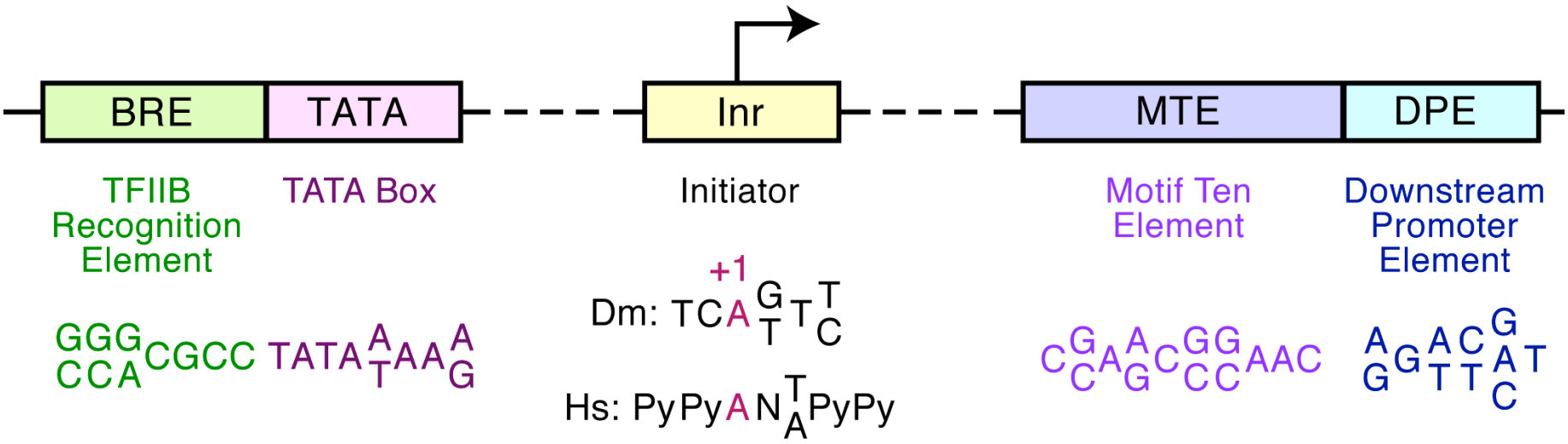


CG15695



Tollo

Core Promoter Elements



Specific Topics

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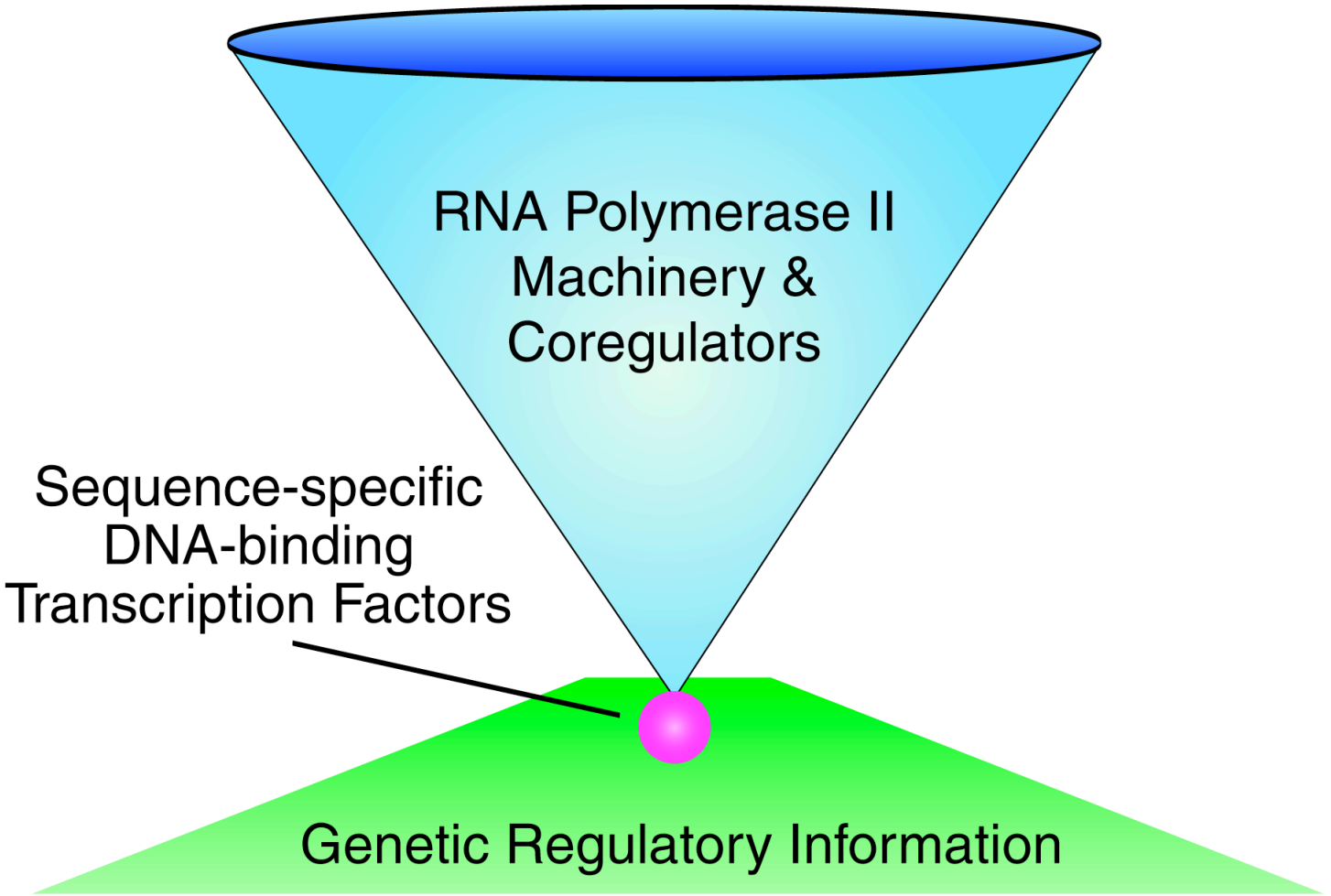
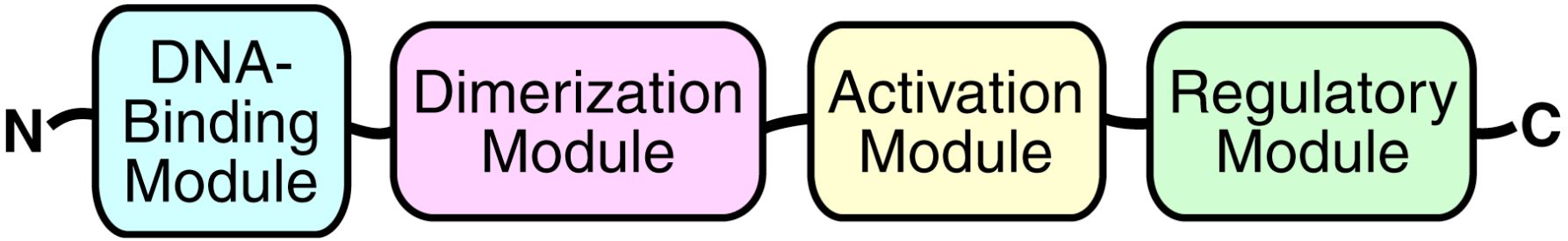
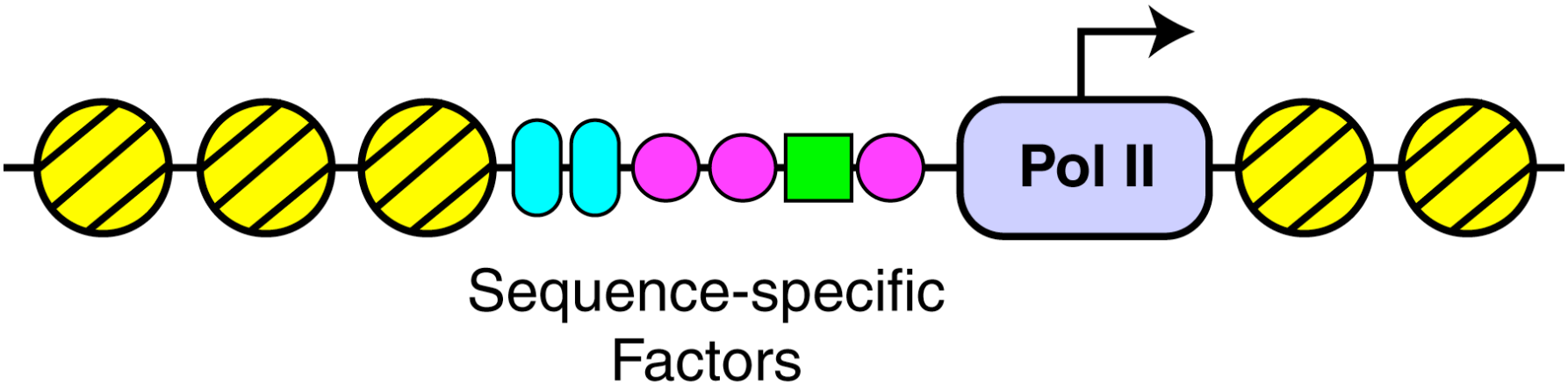
Sequence-specific DNA-binding Transcription Factors Are the Apex at the Interface of Genetic Regulatory Information and the Inverted Cone of Other Transcription Factors



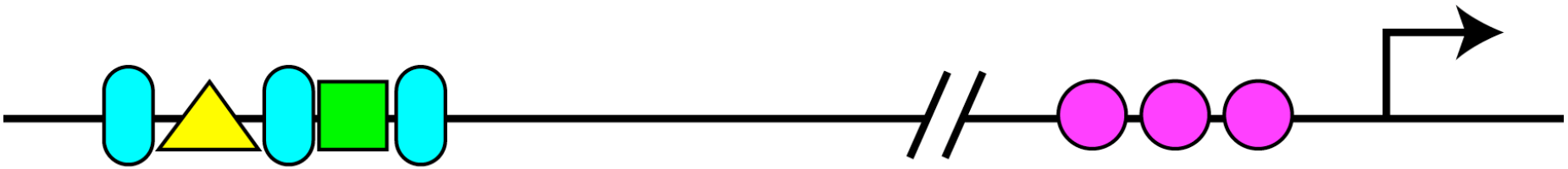
Sequence-specific Transcription Factors Are Modular



Chromatin Is an Integral Component of Transcription



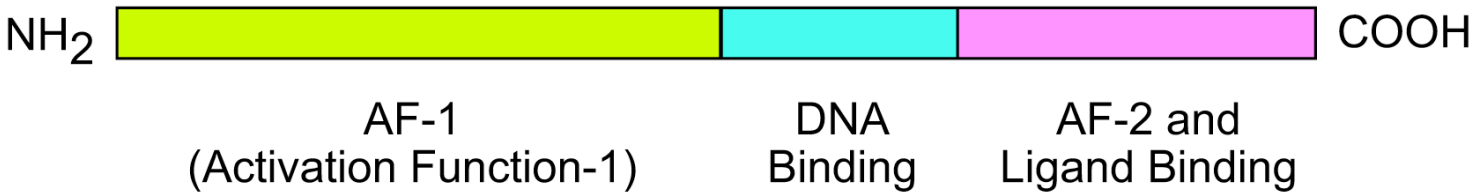
Sequence-specific Factors Typically Bind in Clusters



Enhancer

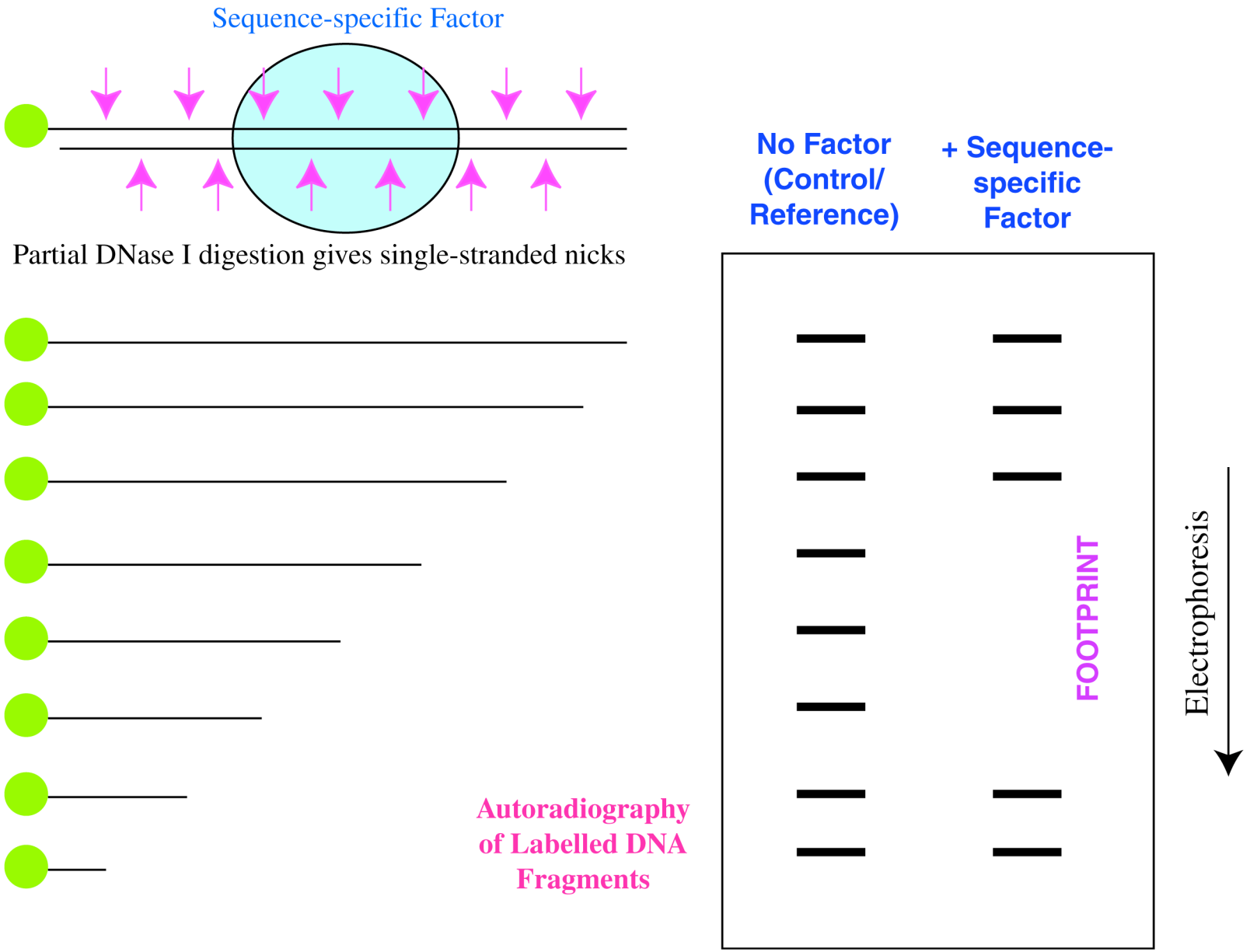
Proximal Promoter

Nuclear Receptors Are an Interesting Family of Sequence-specific DNA-binding Transcription Factors

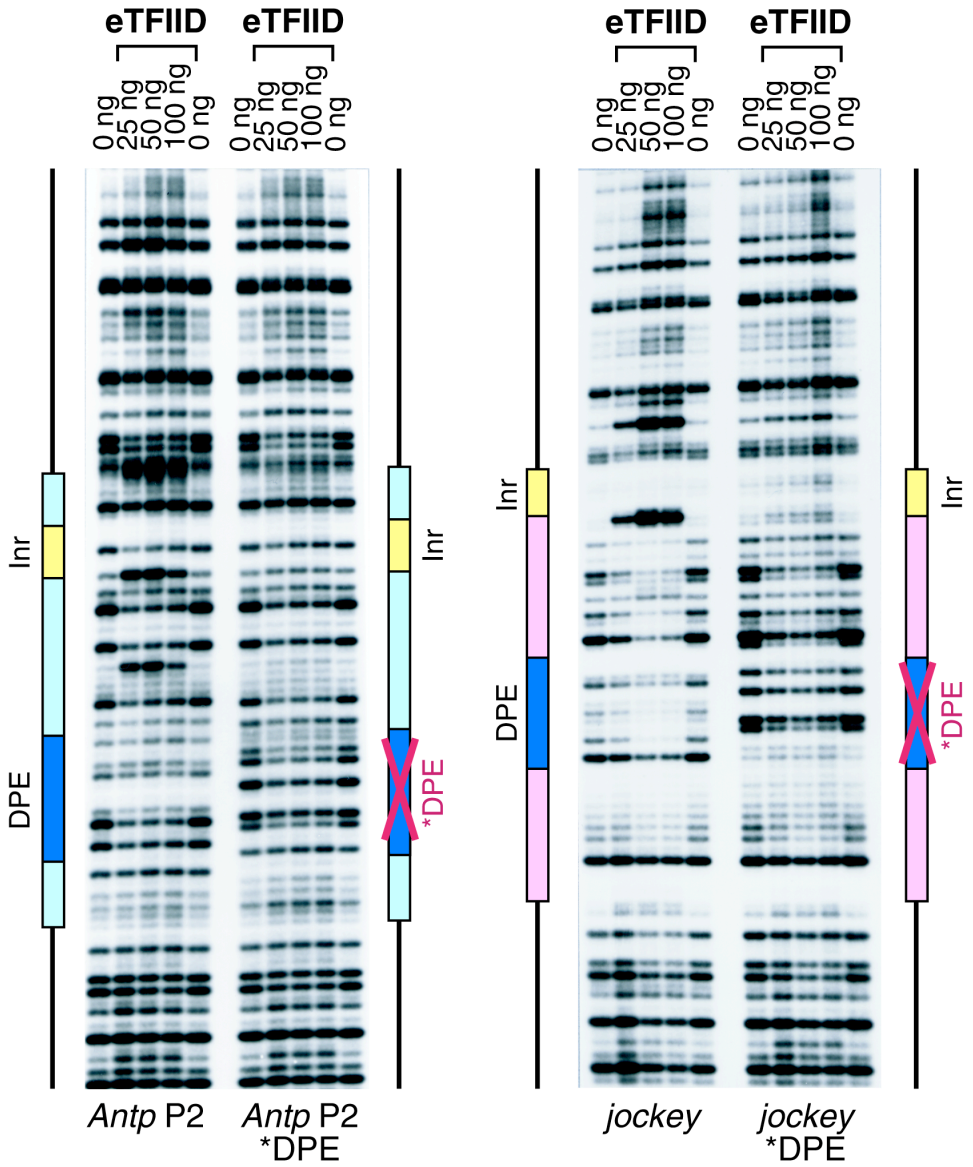


- Sequence-specific DNA-binding proteins
- Upon binding of their cognate ligands (agonists), they activate transcription.
- Thus, nuclear receptors function as both the receptor for the signals (agonists) as well as sequence-specific DNA-binding transcriptional activators.
- Inactivated by antagonists, which are ligands that resemble the agonists, but block activation functions.
- Examples include estrogen receptor, androgen receptor, glucocorticoid receptor, vitamin D receptor, thyroid hormone receptor.

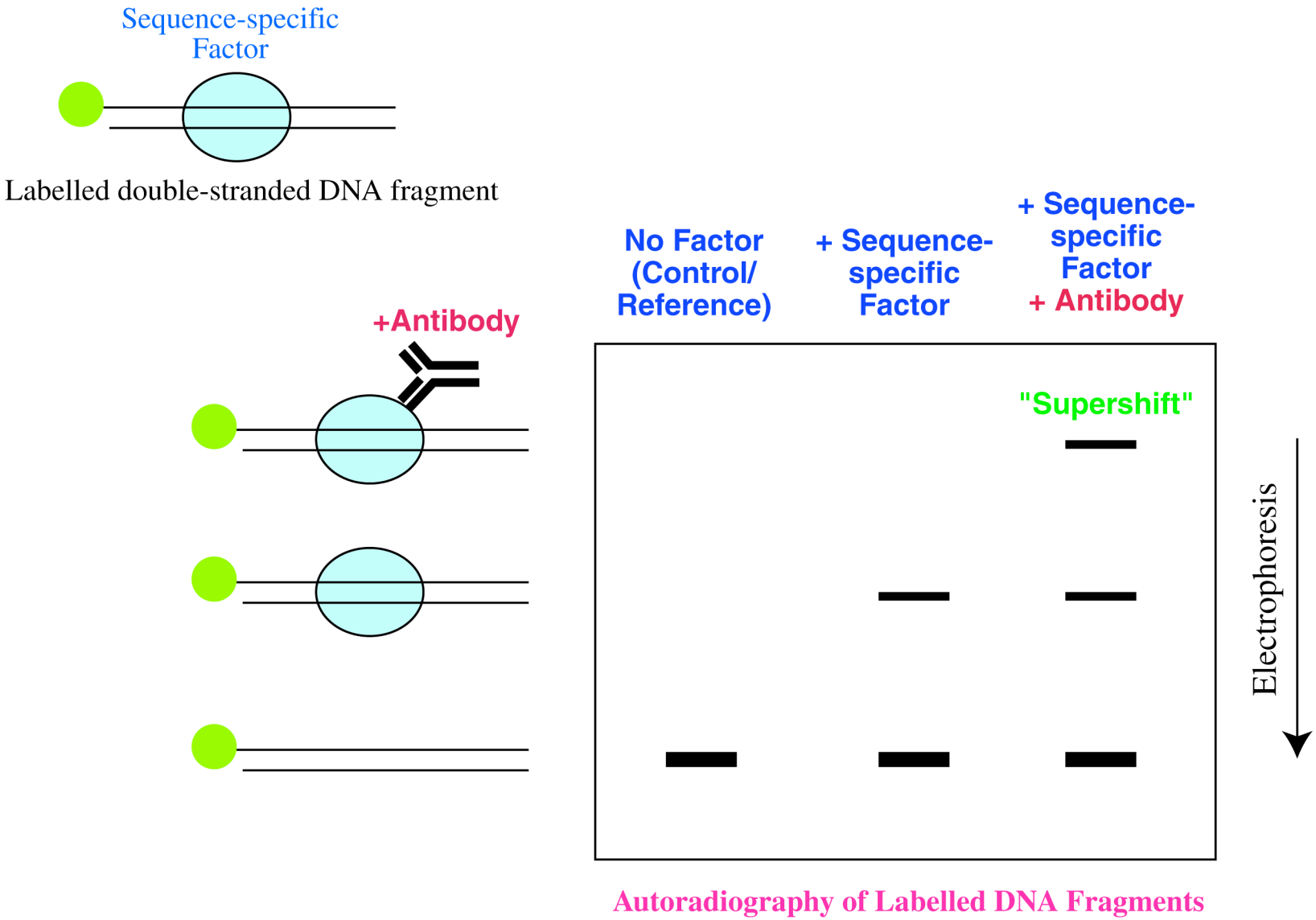
DNase I Footprinting Analysis of Sequence-specific DNA-binding Proteins



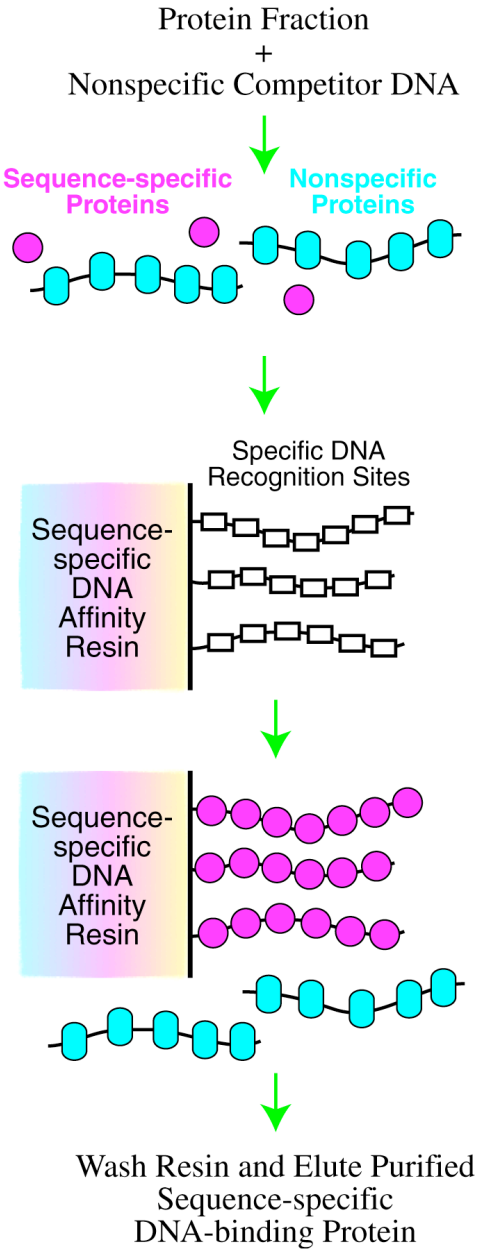
Mutation of the DPE Reduces Binding of TFIID



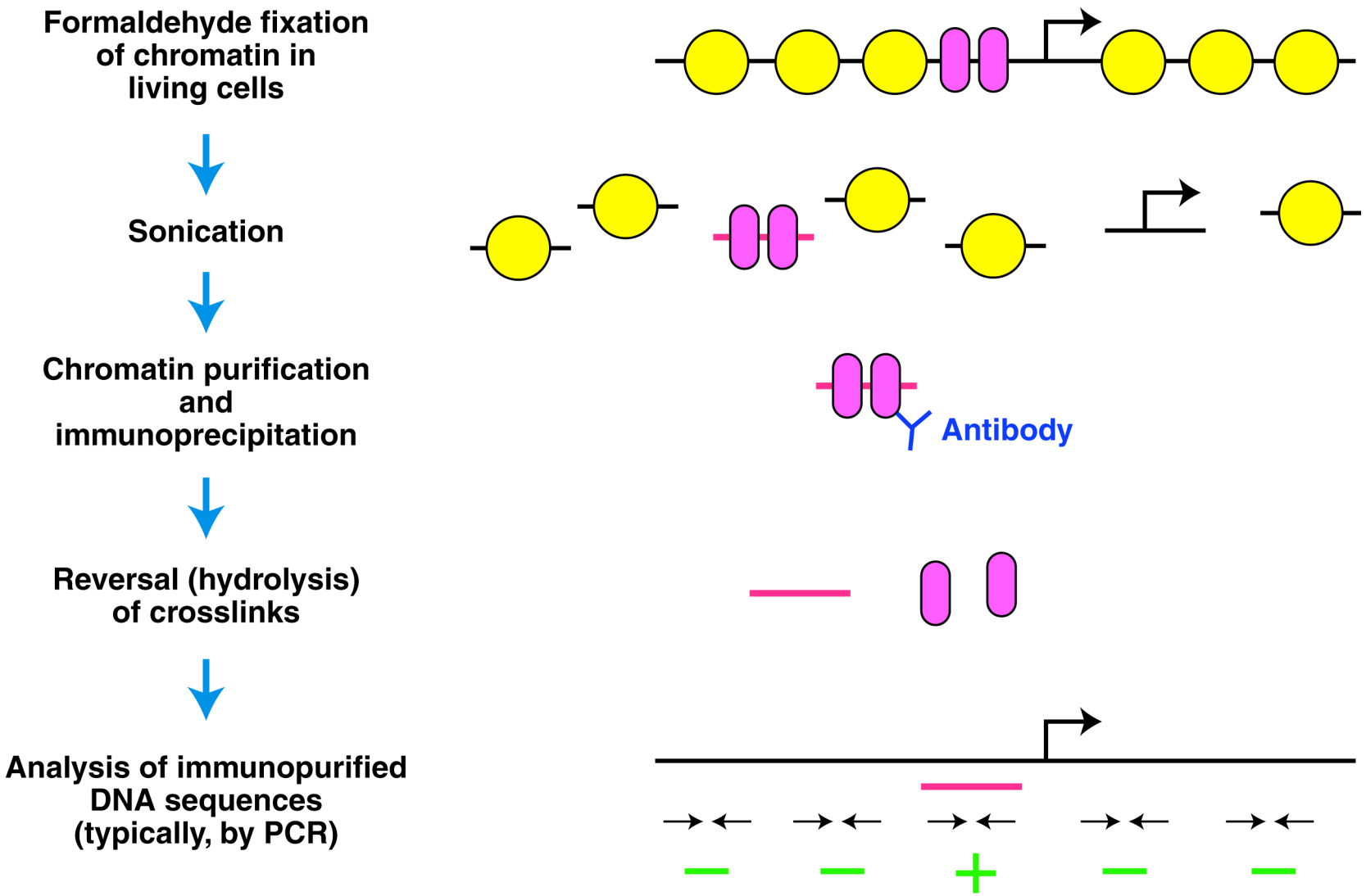
Gel Mobility Shift Analysis of Sequence-specific DNA-binding Proteins



Sequence-specific DNA Affinity Chromatography



Chromatin Immunoprecipitation (ChIP) Analysis



Adapted from: Orlando, V. (2000) *Trends Biochem. Sci.* **25**, 99-104.

Sequence-specific DNA-binding Transcription Factors (RNA Pol II)

- Modular Structure
  - Sequence-specific DNA-binding Modules
  - Transcriptional Activation/Repression Modules
  - Regulatory Modules (inter- or intramolecular)
  - Multimerization Modules (homo- and heterotypic interactions)
- Regulate Transcription via Recruitment of Coactivators and Corepressors
- Chromatin Is an Integral Component in the Function of Sequence-specific Factors
- Sequence-specific Factors Can Be Regulated by Post-translational Modifications
- Sequence-specific Factors Are Often Members of Multiprotein Families
- Recognition Sites for Sequence-specific Factors Tend to Be Located in Clusters
- Sequence-specific Factors Typically Bind to DNA with Relatively Low Specificity
- Sequence-specific Factors Can Affect Transcription Initiation and/or Elongation
- Some Factors Are Commonly Found in Proximal Promoter Regions
- Sequence-specific Factors Bind to Boundary/Insulator Elements
- Some Sequence-specific Factors Can Bend DNA