DNA the Molecule of Life

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)

Transcriptional Enhancers
Proximal Promoter
Core Promoter
Protein-coding Region

DNA

Transcribed Region

Transcription
Pre-mRNA Splicing
Translation

pre-mRNA
mRNA
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

Ubiquitinylating Enzymes
ATP-utilizing Chromatin Remodeling Factors
CpG Methylation
HDAC Complexes
p300/CBP
ARC/TRAP/DRIP/SMCC/NAT/Mediator/SRB/CRSP complex
Boundary Elements
Elongation Factors
Heterochromatin
HMG Proteins
Protein Methyltransferases
RNAi Machinery
Chromatin Assembly Factors
Polycomb Group Proteins
Protein Acetyltransferases
Promoter- and Enhancer-binding Factors
Basal/General Transcriptional Machinery
Dr1-Drap1/NC2 Mot1 NOT proteins Srb10-Srb11
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
Three Different Perspectives of Transcriptional Regulation

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Basal Transcription Machinery – Synthesis of mRNA
Sequence-specific DNA-binding Regulatory Factors

Promoter- and Enhancer-binding Factors
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
Genetic Component of Transcriptional Regulation
Epigenetic Components of Transcriptional Regulation

- Ubiquitylating Enzymes
- ATP-utilizing Chromatin Remodeling Factors
- CpG Methylation
- HDAC Complexes
- KInases
- Polycomb Group Proteins
- Protein Acetyltransferases
- Protein Methyltransferases
- Chromatin Assembly Factors

Heterochromatin

HMG Proteins

RNAi Machinery
Many Factors Affect the Regulation of Transcription by RNA Polymerase II
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

- -37 to -32  ~-31 to -26

-2 to +4

+18 to +27  +28 to +33

BRE  TATA  Inr  MTE  DPE

TFIIB Recognition Element  TATA Box  Initiator  Motif Ten Element  Downstream Promoter Element

Dm: TCA^G^T^T
Hs: PyPy^A^N^T^PyPy

GGG CCA CGCC TATA A TAA A G
CGA C G C GC A AC
AG A C G T T C
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.


- **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 TFIIF into the transcription preinitiation complex (PIC).

- **TFIIF** – nine polypeptides. Core TFIIF 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

~37 to -32  ~31 to -26  -2 to +4  +18 to +27  +28 to +33

BRE  TATA  Inr  MTE  DPE

TFIIB Recognition Element  TATA Box  Initiator  Motif Ten Element  Downstream Promoter Element

GGGCGGCC  TATA  A  TAA  A

Dm: TCA GTT

Hs: PyPy ANATPyPy

CGAAGCGCAAC  AGACG

GGGCGGCC  A  G  T  T  C
TATA- versus DPE-dependent Core Promoters
A Role for Core Promoters in Enhancer Specificity
"Runoff" Transcription Assay (In Vitro)

Double-stranded DNA Template (Linear DNA) → In Vitro Transcription (with labelled rNTPs) → Radiolabelled RNA

Analyze Radiolabelled RNA by Agarose Gel Electrophoresis and Autoradiography
"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' Analyze Radiolabelled RNA by Agarose Gel Electrophoresis and Autoradiography 3'
Primer Extension Analysis of RNA

Double-stranded DNA Template (linear or circular)

1. **In Vitro Transcription (with unlabelled rNTPs)**
   
   5' RNA 3'

2. **Anneal Labelled Oligonucleotide Primer**
   
   5' RNA 3' 5'

3. **Extend with Reverse Transcriptase (+ dNTPs)**
   
   5' RNA 3' 5'

4. **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

-37 to -32  ~-31 to -26  -2 to +4  +18 to +27  +28 to +33

BRE  TATA  Inr  MTE  DPE

TFIIB Recognition Element  TATA Box  Initiator  Motif Ten Element  Downstream Promoter Element

GGGCGCC  TATA  A  A  G

Dm: TCAAGTT
Hs: PyPyANPyPy

CGAAGCACGACC  AGACGTTAC
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
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

Pol II
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

Partial DNase I digestion gives single-stranded nicks

Autoradiography of Labelled DNA Fragments

Electrophoresis

No Factor (Control/Reference) + Sequence-specific Factor

FOOTPRINT
Mutation of the DPE Reduces Binding of TFIID
Gel Mobility Shift Analysis of Sequence-specific DNA-binding Proteins

Sequence-specific Factor

Labelled double-stranded DNA fragment

No Factor (Control/Reference) + Sequence-specific Factor + Sequence-specific Factor + Antibody

"Supershift"

Autoradiography of Labelled DNA Fragments

Electrophoresis
Sequence-specific DNA Affinity Chromatography

Protein Fraction + Nonspecific Competitor DNA

Sequence-specific Proteins + Nonspecific Proteins

Specific DNA Recognition Sites

Sequence-specific DNA Affinity Resin

Sequence-specific DNA Affinity Resin

Wash Resin and Elute Purified Sequence-specific DNA-binding Protein
Chromatin Immunoprecipitation (ChIP) Analysis

1. Formaldehyde fixation of chromatin in living cells
2. Sonication
3. Chromatin purification and immunoprecipitation
4. Reversal (hydrolysis) of crosslinks
5. Analysis of immunopurified DNA sequences (typically, by PCR)

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