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
Many Factors Affect the Regulation of Transcription by RNA Polymerase II
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
Sequence-specific Factors Typically Bind in Clusters
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

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

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

Sequence-specific Factor

Labeled double-stranded DNA fragment

Electrophoresis

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

"Supershift"

Autoradiography of Labeled DNA Fragments
Sequence-specific DNA Affinity Chromatography

Protein Fraction + Nonspecific Competitor DNA

Sequence-specific Proteins

Non-specific Proteins

Sequence-specific DNA Affinity Resin

Specific DNA Recognition Sites

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
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How Do Enhancers Work?

Transcriptional Enhancers

Promoters

Boundary or Insulator Elements
Enhancer-Promoter Specificity vs. Insulator/Boundary Function

Specific Enhancer-Promoter Interactions

Insulation of Enhancer Function by Boundary Elements
What Happens When You See an Increase in Transcription in a Population of Cells?

**Probability (On or Off) Model**
Enhancers Increase the Probability of Transcription but not the Amount of Transcription in a Cell

**Progressive Response Model**
Enhancers Uniformly Increase the Amount of Transcription in a Cell in a Continuous Manner
A Facilitated Tracking Model for Enhancer Function

Sequence-specific Activators Bind to the Enhancer

A Small Loop Is Formed as Activators and Coactivators Begin Tracking

Enhancer-Promoter Connection Is Established