11.1 Overview of Eukaryotic Gene Control and RNA Polymerases (p.448-454)

1. transcriptional control
2. control elements
3. Core RNA Pol: α₂ββ’ω
4. Pol II - mRNAs, some
5. CTD

Analysis of transcription of multiple genes
Run-on transcription analysis:
1. isolate nuclei
2. incubate with[^32P]-labeled NTP
   ____-actively transcribing Pol will incorporate label
3. isolate RNA
4. use labeled RNA to probe membrane containing specific sequences (i.e. DNA corresponding to genes of interest)
Results of run-on transcription analysis
pattern of specific sequences for genes of interest

What can you conclude about gene expression for:
• genes 1-12?
• actin(A), tubulins (α, β)?
• tRNA?

How are transcriptional control elements identified?
Test the effect of specific DNA sequences on transcription
Reporter genes - easily assayed gene products
1. X-gal + galactose + BLUE β-galactosidase (LacZ)
2. luciferin → light emission luciferase
3. GFP - fluoresces green under UV light

Eukaryotic Regulatory Elements

PROMOTER - DNA sequence that regulates transcription of a gene
TRANSCRIPTION FACTORS - proteins that regulate transcription

UPSTREAM(–) →1 DOWNSTREAM(+) RNA polymerase Start site on template strand Stop site on template strand

5' Deletion analysis of transcription control elements
1. create reporter genes
2. test expression of reporter (in vivo or in vitro)
3. assay production of reporter gene product
Test sequences:

- **Upstream region of TTR DNA**

  5' → Start → 3'

  1. **Recombinant DNA techniques**

  2. **5'-deletion series**

Put reporter into expression system:

- **5'-deletion mutants**

  1. 2. 3. 4. 5.

  4. **Transfect each type of plasmid (1-5) separately into cultured cells**

Fuse test sequences to reporter gene:

- **Plasmid vector**

  - 1. 2. 3. 4. 5.

  - 2. **Ligate into vector carrying reporter gene**

  - 3. **Transform E. coli and isolate plasmid DNAs**

  - 5'-deletion mutants

Assay reporter expression:

- **Reporter plasmid**

- **Reporter mRNA**

- **Preparation of cell extract and assay activity of reporter enzyme**

Plasmid no. | Reporter-gene expression
--- | ---
1 | +++
2 | +++
3 | +
4 | +
5 | +

**What can you conclude about regions important for expression from this transcriptional control element?**
3 Eukaryotic RNA Pol

<table>
<thead>
<tr>
<th>Type</th>
<th>RNA genes</th>
<th>α-amanitin</th>
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<tbody>
<tr>
<td>Pol I</td>
<td>pre-rRNA (28S, 5.8S, 18S)</td>
<td>-</td>
</tr>
<tr>
<td>Pol II</td>
<td>mRNAs (most)</td>
<td>+++</td>
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<tr>
<td></td>
<td>snRNAs (most)</td>
<td></td>
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<tr>
<td></td>
<td>(miRNAs)</td>
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<tr>
<td>Pol III</td>
<td>tRNAs 5S rRNA</td>
<td>+</td>
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RNA Pol II Carboxyl-Terminal Domain (CTD) - 7 a.a. repeats

1. initiation - unphosphorylated CTD
2. elongation - phosphorylated CTD

11.2 Regulatory Sequences in Protein-Coding Genes (p. 454-458)

1. multiple control regions
2. TATA boxes, Initiator sequences, CpG islands
3. promoter proximal elements
4. enhancers
5. cell specific function
3 General Promoter Elements

1. TATA box - positions Pol II for txn

2. Initiator - at start site

\[ 5' \text{Y-Y} +1 \text{A} - N/T/A-Y-Y-Y \ 3' \]

3. CpG Island - usually 20-50nt upstream of "start" site

Elements that regulate promoters

1. Promoter-proximal: up to 200bp upstream of +1

Identification of transcription control elements by linker-scanning mutations

Multiple Transcription Control Elements

Recall:
11.3 Activators and Repressors of Transcription (p. 458-468)

1. activator and repressor proteins bind regulatory sequences
2. DNA binding + activator or repressor domain(s)
3. motifs in DNA
4. dimerization
5. cooperative binding
6. co-activators and co-repressors
7. enhancesome

Experimental detection of txn factors and DNA binding sites

DNase I footprinting
1. $^{32}$P-label DNA at one end
2. incubate DNA with protein sample (fractionated extract)
3. add DNase I
4. isolate DNA

Electrophoretic Mobility Shift Assay (EMSA)
1. incubate $^{32}$P-labeled DNA probe with protein
2. electrophorese under non-denaturing conditions
3. detect

5. denature and separate by electrophoresis
6. detect labeled DNA fragments
How do you identify the protein that binds the DNA control element?

Sequence-specific DNA affinity chromatography:
1. fractionate nuclear extract by column chromatography
2. use DNase I footprinting or EMSA to find fractions with binding activity
3. add fractions to new column containing DNA sequences for the control element
4. identify protein by a. a. sequencing

Functional tests

In vitro - Does protein affect transcription from template containing the promoter element?

In vivo - Does protein X affect expression of a reporter gene within a cell?

Activators and Repressors are Modular Proteins

Examples

1. DNA-binding domain
2. Activation domain
3. Flexible protein domain
Experimental Identification of Separate Functional Domains

(a) Reporter-gene construct

UAS_{GAL} TATA box

\[ \text{lacZ gene} \]

Classes of DNA binding protein motifs

1. Homeodomain - often regulate developmental genes
   - helix-loop-helix

2. Zinc-finger - C\textsubscript{2}H\textsubscript{2} or C\textsubscript{4}
   - nuclear receptors

3. Leucine-zipper - dimers
   - basic zipper (bZip)

4. Basic Helix-Loop-Helix (bHLH)
   - dimers
Combinations of txn factors = complex regulation

Homodimers & Heterodimers

Cooperative DNA binding

1. Activation domains - often acidic
   - co-activators - proteins or ligands
2. Repression domains
   - co-repressors - proteins or ligands

Co-factors can change the activity of a protein

RAR + retinoic acid → ACTIVATE
RAR → REPRESS

Enhancesome:
DNA sequences
activators (dimers, co-factors)
DNA bending