RNA synthesis in Eukaryotes

Prokaryotic vs eukaryotic transcription

Prokaryotes:
- no membrane-bound nucleus
- transcription and translation are coupled

Eukaryotes:
- DNA is located in membrane-bound nucleus
- Transcription and translation are separated in space and time
There are 3 types of Eukaryotic RNA polymerases

**TABLE 28.2 Eukaryotic RNA polymerases**

<table>
<thead>
<tr>
<th>Type</th>
<th>Location</th>
<th>Cellular transcripts</th>
<th>Effects of α-amanitin</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Nucleolus</td>
<td>18S, 5.8S, and 28S rRNA</td>
<td>Insensitive</td>
</tr>
<tr>
<td>II</td>
<td>Nucleoplasm</td>
<td>mRNA precursors and snRNA</td>
<td>Strongly inhibited</td>
</tr>
<tr>
<td>III</td>
<td>Nucleoplasm</td>
<td>tRNA and 5S rRNA</td>
<td>Inhibited by high concentrations</td>
</tr>
</tbody>
</table>

- All have M.W > 500 kd
- Our discussion will focus on RNA Pol II

**Eukaryotic RNA Polymerase II**

RNA Pol II is responsible for transcription of DNA to heteronuclear RNA (hnRNA) (pre-mRNA)

- 8-12 subunits
- Two large subunits are responsible for RNA synthesis
- Some subunits are shared with the RNA Pol I and III complexes
Eukaryotic Promoters transcribed by RNA Pol II

Consensus sequence:

-90  -70  -25  +1

5'........ CAAT Box .......... GC Box ............. TATA box .......... (TATAAAA)

Promoter sequence

3'

Coding sequence

Start site

-70

5' CAAT Box (GGNCAATCT)

-25

TATA box (G66GC66)

• TATA box determines where transcription starts (required for most, but not all, type II promoters)
• GC box and CAAT box are present in some promoters - (regulate the frequency of transcription)
• GC box is characteristic for constitutively expressed genes

The TATA box is a highly conserved promoter element in eukaryotic DNA

Consensus sequence

<table>
<thead>
<tr>
<th>Base</th>
<th>17</th>
<th>22</th>
<th>13</th>
<th>7</th>
<th>97</th>
<th>7</th>
<th>85</th>
<th>63</th>
<th>88</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
<td>33</td>
<td>18</td>
<td>15</td>
<td>23</td>
<td>10</td>
<td>7</td>
<td>85</td>
<td>63</td>
</tr>
<tr>
<td>T</td>
<td>10</td>
<td>33</td>
<td>12</td>
<td>15</td>
<td>33</td>
<td>10</td>
<td>10</td>
<td>37</td>
<td>10</td>
</tr>
<tr>
<td>C</td>
<td>53</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>38</td>
</tr>
<tr>
<td>G</td>
<td>38</td>
<td>48</td>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

mRNA starts

A  \sim  50\%
G  =  25\%
C,U  \sim  25\%

15–26 Bases \text{ from Transcription}

5' T A T A A

-34 to

-26
Initiation of transcription in eukaryotes is more complicated than in bacteria

- RNA Pol II can not initiate transcription by itself: it requires generalized RNA Pol II transcription factors (TFII) (TFIIA, TFIIB, TFIID, TFIIF, TFIIE, TFIIH)
- The key initiation step is the recognition of TATA box by TBP (TATA box – binding protein; TFIID)

TATA box-binding protein (TBP) (part of TFIID)

- 32 kd protein tightly binds TATA box ($K_d = 1 \text{ nM}$)
- Forms specific H-bonds with the minor groove of the TATA box
- Bends and unwinds DNA duplex
- Provides a docking site for other TFs
TBP provides a docking site for other TFs

Formation of RNA Polymerase II pre-initiation complex by RNA Pol II generalized transcription factors (TFs)

IID contains TBP that binds TATA box

IIA stabilizes IID binding to promoter

IIB binds to IID

Pol II binds IIB

IIF begins unwinding DNA

IIE stimulates transcription

IIH has kinase and helicase activity

Basal transcription apparatus
Control of Gene expression

6 steps at which eukaryotic gene expression can be controlled

Figure 7-5. Molecular Biology of the Cell, 4th Edition.
Regulation of gene transcription

- The rate of RNA synthesis of a single gene can vary significantly

**Effects of Estrogen on Gene X mRNA levels**

**Effect of Age on Gene X mRNA levels**

Regulation of gene transcription

- The rate of RNA synthesis of different genes can vary significantly

mRNA levels vary from gene to gene
Interaction of DNA binding proteins with gene promoters is complex and can be regulated

1. Most genes are controlled on the level of transcription initiation (RNA polymerase binding to the promoter region).

2. Regulatory proteins (Transcription factors: activators and repressors) bind specific sequences within DNA, controlling the rate of transcription (usually transcriptional initiation).

General principles of gene regulation

(A) Regulator gene

Control sites

Structural genes

Genes to be expressed

Binding sites for activators and repressors
Interactions of regulatory proteins (transcription factors) with DNA

- Sequence-specific binding
- Non-covalent interactions including H-bonding and hydrophobic forces
- Most transcription factors have at least two structural domains: one is responsible for DNA binding and the other mediates transcriptional regulation
Transcription factor DNA binding is determined by protein folding

![Zinc finger (dimer)](image)

Figure 7-19. Molecular Biology of the Cell, 4th Edition.

A specific DNA sequence is recognized by the DNA binding motif of each individual transcription factor

5’T A A C A C C G T G C G T G T T G 3’
3’ A T T G T G G C A C G C A C A A C 5’

Figure 7-15. Molecular Biology of the Cell, 4th Edition.
Different zinc finger proteins bind different DNA sequences

How do transcription factors control gene transcription?
Eukaryotic gene expression

- Complex sets of regulatory elements in promoter regions that can act at a distance
- Eukaryotic DNA is organized in chromatin; therefore regulation of gene transcription involves chromatin remodeling
- Mechanisms used to control gene expression in eukaryotes are often more complex than those observed in prokaryotes

We will use RNA pol II transcribed genes for illustration

Transcriptional control in eukaryotes

- Regulatory proteins can alter the rate of transcription 100-fold
- These proteins activate and repress from short or long distances away

Figure 7–41. Molecular Biology of the Cell, 4th Edition.
Activation of transcription initiation by activator-dependent recruitment of RNA Pol II to the promoter: Histone remodeling and enzymatic modifications

145 bp duplex + Histone octamer (H2A, H2B, H3, H4)$_2$

(Nucleosomal core)

Inactive chromatin $\longleftrightarrow$ Active chromatin

Structural organization of the nucleosome

Figure 4–24 part 1 of 2. Molecular Biology of the Cell, 4th Edition.
Structural organization of the nucleosome: continued

Histones possess N-terminal tails

Tails extend out from the nucleosome

Tails may help pack nucleosomes together on the chromatin fiber

Figure 4–32. Molecular Biology of the Cell, 4th Edition.
Enzymatic modification of histones can control RNA Pol II access to promoters

Inactive (condensed) vs. Active (decondensed) chromatin

Figure 4–23. Molecular Biology of the Cell, 4th Edition.
**Histone acetylation and deacetylation**

- **Histone acetyltransferases**
  - Enzyme
  - Adds an acetyl group to a lysine in histone tail
  - Recruited to DNA by specific transcription factors (activators)
  - Coactivator

- **Histone deacetyltransferases**
  - Enzyme
  - Removes an acetyl group from a lysine in histone tail
  - Recruited to DNA by specific transcription factors (repressors)
  - Corepressor

**Covalent modification of core histone tails**
- Acetyl group
- Methyl group
- Phosphate group

![Histone acetyltransferase](image)

Figure 4–35 part 1 of 2. Molecular Biology of the Cell, 4th Edition.
Corepressors and coactivators can direct histone acetylation patterns at specific genes

(a) Repression-directed histone deacetylation

(b) Activator-directed histone hyperacetylation

Nuclear hormones

cortisol

estradiol

testosterone

thyroxine

Figure 15–12 part 1 of 2. Molecular Biology of the Cell, 4th Edition.
Nuclear hormones

- Small
- Hydrophobic
- Site of action is the nucleus
- Bind to nuclear receptors
- Regulate transcription
  Facilitate chromatin remodeling


Molecular Basis of Thyroid (Nuclear) Hormone Action
### Human Nuclear Hormones and Cognate Receptors
*(Thyroid, Retinoid, and Steroid Hormones)*

<table>
<thead>
<tr>
<th>Nuclear Hormone Receptor</th>
<th>Hormone</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR</td>
<td>Thyroid hormone</td>
</tr>
<tr>
<td>AR</td>
<td>Dihydrotestosterone</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen</td>
</tr>
<tr>
<td>GR</td>
<td>Cortisol</td>
</tr>
<tr>
<td>MR</td>
<td>Aldosterone</td>
</tr>
<tr>
<td>PR</td>
<td>Progesterone</td>
</tr>
<tr>
<td>RAR</td>
<td>trans-Retinoic Acid</td>
</tr>
<tr>
<td>VDR</td>
<td>1,25-Dihydroxyvitamin D₃</td>
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</tbody>
</table>

### Human Orphan Nuclear Receptors

<table>
<thead>
<tr>
<th>Orphan Nuclear Receptor</th>
<th>Naturally Occurring Ligand</th>
<th>(Synthetic Ligand)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR</td>
<td>Fatty Acids</td>
<td>(thiazolidinediones, fibrates)</td>
</tr>
<tr>
<td>RXR</td>
<td>9-cis-retinoic acid</td>
<td></td>
</tr>
<tr>
<td>ROR</td>
<td>Stearic acid, cholesterol</td>
<td></td>
</tr>
<tr>
<td>PXR</td>
<td>Pregnanes, bile acids, (rifampicin)</td>
<td></td>
</tr>
<tr>
<td>LXR</td>
<td>Cholesterol metabolites</td>
<td></td>
</tr>
<tr>
<td>FXR</td>
<td>Bile acids (GW4064)</td>
<td></td>
</tr>
<tr>
<td>CAR</td>
<td>3α,5α-Androstanol, (TCPBOP)</td>
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</tr>
<tr>
<td>HNF4</td>
<td>Fatty acids</td>
<td>(Diethylstilbestrol), (4-Hydroxytamoxifen)</td>
</tr>
<tr>
<td>ERR</td>
<td>-</td>
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</tr>
<tr>
<td>DAX</td>
<td>-</td>
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<td>GCNF</td>
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<td>NGF-1B</td>
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<td>TLX</td>
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<td></td>
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<tr>
<td>COUP-TF</td>
<td>-</td>
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</tr>
</tbody>
</table>
Nuclear receptor: DNA-binding domain and ligand binding domain

AGGTCA

N-terminal
DNA-binding domain
Ligand-binding domain
C-terminus

Ligand-binding pocket

1. Unliganded TR recruits histone deacetyltransferases and keeps chromatin compacted

2. Liganded TR releases HDAC complex

3. Liganded TR recruits histone acetyltransferases leading to histone acetylation and chromatin relaxation

6. Relaxed chromatin allows recruitment of basal transcriptional machinery and RNA Pol II

3. Liganded TR recruits histone acetyltransferases leading to histone acetylation and chromatin relaxation

4. Acetylated histones bind chromatin remodeling complexes (engines). The complexes bind to acetylated lysines through a bromodomain. The remodeling complex physically moves the nucleosome core in a reaction requiring ATP.
5. TBP is recruited to the TATA box by specific TAFs. These TAFs bind to acetylated lysines through a bromodomain.

6. Relaxed chromatin and TBP allows recruitment of the remaining basal transcriptional machinery and RNA Pol II.