Come to lectures! All exam questions will be from lectures.
Supplemental and updated course materials will be posted on the course web site regularly; http://mcb.berkeley.edu/courses/mcb110
Interrupt me in lecture to ask questions
We discuss many important concepts and principles in the context of experiments
Come to my office hours – Fridays from 3 to 5 PM
Make an appointment outside office hours by email qzhou@berkeley.edu

Midterm: Monday, Nov. 5 in 100 GPB from 6:30 to 8:30 PM

Why regulation of gene expression is important?
• Cellular function is largely dictated by the set of macromolecules inside the cell.
• Different macromolecules accumulate to different levels under different growth conditions and in different cell types.
• Diseases can be caused by aberrant control of gene expression: too much or too little of a protein; wrong time and wrong place for a protein.
Transcription of DNA into RNA by RNA polymerase---an overview

1. Requires DNA template, four ribonucleotide 5’ triphosphates, Mg\(^{2+}\).

2. De novo synthesis: does not require a primer. Low fidelity compared to DNA polymerase: errors 1/10^4-10^5 (10^5 higher than DNA polymerase).

3. Activity highly regulated in vivo: at initiation, elongation and termination.

4. The nucleotide at the 5’ end of an RNA strand retains all three of its phosphate groups; all subsequent nucleotides release pyrophosphate (PPi) when added to the chain and retain only their α phosphate (red).

5. The released PPI is subsequently hydrolyzed by pyrophosphatase to Pi, driving the equilibrium of the overall reaction toward chain elongation.

6. Growth of the transcript always occurs in the 5’-to-3’ direction.
**E. coli RNA polymerase holoenzyme bound to DNA**

A single RNA polymerase makes multiple types of RNAs (rRNA, tRNA and mRNA) in prokaryotes.

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### Subunit Stoichiometry in holoenzyme Role
- α: 2 Binds regulatory sequences/proteins
- β: 1 Forms phosphodiester bonds
- β': 1 Binds DNA template
- σ: 1 Promoter recognition
- ω: 1 RNAP assembly

**Typical E.coli promoters recognized by an RNA polymerase holoenzyme containing σ70**

<table>
<thead>
<tr>
<th>Consensus sequence</th>
<th>UP element</th>
<th>-35 Region</th>
<th>Spacer</th>
<th>-10 Region</th>
<th>Spacer</th>
<th>RNA start</th>
</tr>
</thead>
<tbody>
<tr>
<td>rmb P1</td>
<td>AGAAAATATTTAATTTTTCCT NN GTOTCA N₁₆ TATAAT N₄ A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ptp</td>
<td>TTGACA N₁₇ TTAACT N₇ A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lac</td>
<td>TTTACA N₁₇ TAGTT N₄ A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>recA</td>
<td>TTGATA N₁₆ TATAAT N₇ A</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>araBAD</td>
<td>CTGACG N₁₈ TACTGT N₄ A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Strong promoters: close to consensus sequences and spacing
Weak promoters: contain multiple substitutions at the -35 and -10 regions
Biochemical studies of bacterial RNA polymerase

1. DNA binding assay- DNase I footprinting to look for polymerase-bound promoters

2. Role of individual subunits- dissociation of holoenzyme by column chromatography

DNase I footprinting: a common technique for identifying protein-binding sites in DNA.
1. A DNA fragment is labeled at one end with $^{32}$P (red dot).
2. Portions of the sample then are digested with DNase I in the presence and absence of a protein that binds to a specific sequence in the fragment.
3. A low concentration of DNase I is used so that on average each DNA molecule is cleaved just once (vertical arrows).
4. The two samples of DNA then are separated from protein, denatured to separate the strands, and electrophoresed. The resulting gel is analyzed by autoradiography, which detects only labeled strands and reveals fragments extending from the labeled end to the site of cleavage by DNase I.
Ion-exchange chromatography

Dissociation of RNAP and purification of σ by ion-exchange chromatography

Carboxymethyl-(-CO$_2^-$) or phospho-(-PO$_3^-$) cellulose

Fraction number

[NaCl] [protein]
The dissociable sigma subunit gives promoter specificity to prokaryotic RNA polymerase (RNAP)

Core enzyme

No specific promoter binding; tight non-specific DNA binding ($K_d \sim 5 \times 10^{-12}$ M)

Holoenzyme

Specific promoter binding; weak non-specific DNA binding ($K_d \sim 10^{-7}$ M); finds promoter 10,000 times faster.

Transcription initiation by prokaryotic RNA polymerase

Holoenzyme “sliding and scanning”

Promoter -35 -10

Closed complex

rNTPs PPI

Open complex; initiation

Sigma separates from the core once a few phosphodiester bonds are formed

5’pppA mRNA
Interactions of various sigma factors of *E. coli* with the same core polymerase to form holoenzymes with different promoter-binding specificity

<table>
<thead>
<tr>
<th>Sigma Factor</th>
<th>Promoters Recognized</th>
<th>Promoter Consensus</th>
</tr>
</thead>
<tbody>
<tr>
<td>σ70</td>
<td>Most genes</td>
<td>-35 Region TTGACAT</td>
</tr>
<tr>
<td>σ32</td>
<td>Genes induced by heat shock</td>
<td>-10 Region TATAAT</td>
</tr>
<tr>
<td>σ28</td>
<td>Genes for motility and chemotaxis</td>
<td>-10 Region TATAAT</td>
</tr>
<tr>
<td>σ38</td>
<td>Genes for stationary phase and stress response</td>
<td>-10 Region TATAAT</td>
</tr>
<tr>
<td>σ54</td>
<td>Genes for nitrogen metabolism &amp; other functions</td>
<td>-24 Region CTGGNA</td>
</tr>
</tbody>
</table>

Heat-shock response:
High temperature induces the production of σ32, which binds to the core polymerase to form a unique holoenzyme for recognition of the promoters of heat-shock induced genes.

Transcriptional elongation: Movement of transcription bubble (17-bp, 1.6 turns of B-DNA duplex)

Speed of movement: 50-90-nt/sec

Supercoiling of DNA during transcription causes a requirement for topoisomerases
Rho-independent prokaryotic transcription termination

The core polymerase pauses after synthesizing a hairpin. If the hairpin is really a terminator, RNA will dissociate from the DNA strand as the A-U pairing is unstable. Once the RNA is gone, DNA duplex reforms and the core is driven off.

Rho-dependent transcription termination

Rho: forms RNA-dependent hexameric helicase/ATPase, translocates along RNA 5'-to-3', pulling RNA away when it reaches the transcription bubble.