Section 6

Information Flow
Antibiotic targets

Inhibition of cell wall synthesis: Penicillins, cephalosporins, bacitracin, vancomycin

Inhibition of protein synthesis: Chloramphenicol, erythromycin, tetracyclines, streptomycin

Inhibition of nucleic acid replication and transcription: Quinolones, rifampin

Enzymatic activity, synthesis of essential metabolites

Injury to plasma membrane: Polymyxin B

Inhibition of synthesis of essential metabolites: Sulfanilamide, trimethoprim
Antibiotic targets

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Learning Goal throughout upcoming sections:

• Explain the mechanism of action of the transcriptional, translational and DNA synthesis inhibitors
Explain the mechanism of action of the transcriptional and translational inhibitors

Requires knowledge of:

• How information is converted from gene to gene product (process of transcription and translation).
• Structure and function of key molecules involved in transcription.
• Structure and function of key molecules involved in translation.
• Regulation of transcriptional initiation.
• Differences in gene expression between prokaryotes and eukaryotes.
• Relationship of DNA mutation to protein structure and function.
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  – Compare and contrast transcription and translation.
Why is the strain on the left purple while the one on the right is not?


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http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab2/sainsol.html
Two interpretations of the observation:

1. One strain has genes for pigment biosynthesis, the other doesn’t.

2. Both strains possess the genes for pigment biosynthesis but expression patterns differ.
Two interpretations of the observation:

✓ 1. One strain has genes for pigment biosynthesis, the other doesn’t.

2. Both strains possess the genes for pigment biosynthesis but expression patterns differ.

In this case, interpretation #1 happens to be the correct explanation. That knowledge is gained from experimental results not described.
We can apply the same logic to explain why some strains produce antibiotics and others don’t.

- Insert photo of student antibiotic screen plate if available
- Alternatively, insert photo showing student patches of different morphology
Gene Expression

Process by which information in a gene is converted into functional gene product (usually a protein, but can be RNA)

DNA (gene) → transcribed into mRNA

Inside of cell

mRNA → translated into polypeptide(s)

DNA → tRNA, rRNA, mRNA

Transcription

Translation

RNA → tRNAs

Ribosome

Polypeptide(s)
Enzyme gene $\rightarrow$ Enzyme mRNA $\rightarrow$ Enzyme

Enzyme #1

A $\rightarrow$ B

Pigment precursor $\rightarrow$ Purple Pigment
Gene for enzyme missing:
• No transcription of enzyme
• No translation of enzyme
• No enzyme protein
• No purple pigment accumulates
Genes encode the instructions for building *gene products* (usually proteins but also some RNAs) that carry out regulatory, enzymatic or structural roles in the cell.

The purple colony is genetically identical to the white colony except the white colony is missing one gene.
Biosynthetic pathways involve multiple enzymes. Disruption of one can disrupt the entire pathway.

Dihydropteroate diphosphate (DHPP) + \( p \)-aminobenzoic acid (PABA)

\[ \text{Dihydropteroate synthase (DHPS)} \]

Dihydropteroic acid

\[ \text{Other enzymes} \]

Dihydrofolate reductase (DHFR)

Dihydrofolic acid (DHF)

\[ \text{Thymidine synthesis} \]

\[ \text{Amino acid synthesis} \]

tetrahydrofolic acid (THF)
Likewise, all enzymes required for antibiotic synthesis must be present (and expressed) for antibiotic production.
Bacteria organize genes into operons

• Groups of genes whose products are used under the same condition are grouped together in bacteria so that they can be regulated (turned on and off) using the same regulatory switches.

• Examples
  – Groups of enzymes used in the same biosynthetic pathway
  – Proteins that, together, compose a structure (e.g. flagellar proteins)
  – Regulatory proteins used under the same conditions (e.g. proteins that regulate sporulation).
There are many human conditions resulting from a single gene defect

- Cystic fibrosis—defect in gene encoding protein that transports ions across epithelial cell membrane.
- Sickle cell anemia—defect in gene encoding hemoglobin; hemoglobin is produced, but with altered conformation due to single amino acid change.
- Color blindness—defect in single gene encoding a photopigment in the retina.
- Phenylketonuria—a metabolic disorder resulting in intellectual disability; loss of gene encoding enzyme that catalyzes conversion of phenylalanine to tyrosine.
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What do you know about transcription? What key molecules are involved and what is their role?
RNA synthesis

• Requires DNA as a template.
• RNA polymerase binds double-stranded DNA, causes hydrogen bonds to separate, catalyzes synthesis of RNA nucleotide polymer complementary to template DNA.
• Occurs 5’ to 3’ (monomer nucleotides are added to the 3’ –OH).
Transcription: RNA synthesis from a DNA template

- Generic nucleotide monomer structure
- Nucleic acid polymer structure
- Structural differences between RNA and DNA
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What do you know about the molecular mechanics of translation?

What molecules are involved?
In the schematic diagram, label each of the following molecules:

- mRNA
- ribosomal small subunit
- ribosomal large subunit
- tRNA
- amino acid
- codon
- anticodon
Active learning option #1—ANSWER
Arrange the following in order:

A.

B.

C.

D.

E.

Active learning option #2
Arrange the following in order:

A.

B.

C.

D.

E.

1. C
2. B
3. E
4. A
5. D
Translation strip sequence

- Ribosome reaches a stop codon, release factor binds to A-site.
- Peptide bond between tRNA and polypeptide is hydrolyzed, releasing polypeptide.
- Ribosome small subunit binds mRNA at a ribosome binding site.
- Initiator charged (aminoacyl)-tRNA and large subunit associate with the ribosome/mRNA complex.
- Ribosome small subunit “slides” along mRNA until translational start (AUG) sequence is recognized.
- Peptide bond is formed, uncharged initiator tRNA exits ribosome.
- Coding amino acids enter A-site, peptide bonds are formed, then uncharged tRNAs exit ribosome.
- New charged tRNAs enter the A-site of the complete ribosome; initiator tRNA occupies P-site.
Translation strip sequence

1. Ribosome small subunit binds mRNA at a ribosome binding site
2. Ribosome small subunit “slides” along mRNA until translational start (AUG) sequence is recognized.
3. Initiator charged (aminoacyl)-tRNA and large subunit associate with the ribosome/mRNA complex.
4. New charged tRNAs enter the A-site of the complete ribosome; initiator tRNA occupies P-site.
5. Peptide bond is formed, uncharged initiator tRNA exits ribosome.
6. Coding amino acids enter A-site, peptide bonds are formed, then uncharged tRNAs exit ribosome.
7. Ribosome reaches a stop codon, release factor binds to A-site.
8. Peptide bond between tRNA and polypeptide is hydrolyzed, releasing polypeptide.
Translation movie

http://www.youtube.com/watch?v=5bLEDd-PSTQ
Transcribe the following DNA:

3’ TAC CGA ACG 5’ DNA
Transcribe the following DNA:

3’ TAC CGA ACG 5’

Remember that in RNA, thymine (T) is replaced by uracil (U)
ANSWER

3’ TAC CGA ACG 5’

transcription

5’ AUGGCUUUGC 3’

DNA

mRNA
Translate the following mRNA:

**5’ AUGGCUUUUGC 3’**

<table>
<thead>
<tr>
<th>First letter</th>
<th>Second letter</th>
<th>Third letter</th>
</tr>
</thead>
<tbody>
<tr>
<td>U</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td></td>
<td>UUC</td>
<td>U</td>
</tr>
<tr>
<td></td>
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<td>G</td>
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<tr>
<td></td>
<td>GUG</td>
<td></td>
</tr>
</tbody>
</table>

The translation of **5’ AUGGCUUUUGC 3’** is **Met-Ile-Phe**.
ANSWER

5’ AUGGCUUUGC 3’

mRNA

N – Met–Ala–Cys–C

polypeptide
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Section 7

• Regulation of transcriptional initiation.
• Differences in gene expression between prokaryotes and eukaryotes.

Section 8

• Relationship of DNA mutation to protein structure and function.