Prokaryotic DNA organization

- Circular DNA
- Condensed by packaging proteins (e.g. H-NS, IHF)
- Supercoiled
Bidirectional replication

- Replication starts at ori (oriC in *E. coli*)
- Continues bidirectionally
- Chromosome attached to plasma membrane

Fig. 11.11
Rolling circle replication

Used by plasmids and some bacteriophage

Fig. 11.12
DNA polymerases

- 3 DNA polymerases
- Synthesize 5´-3´
- DNA pol III = DNA replication
- DNA pol I, II = DNA damage repair
Quinolones

Nalidixic acid

Norfloxacin

Ciprofloxacin

Fig. 35.5
Fig. 35.6

Quinolones → ATP → DNA gyrase cuts both strands of one DNA

One DNA strand passed through the other strand

Break in the DNA sealed
DNA replication

Fig. 11.16
Fig. 11.16

- Leading strand template
- Lagging strand template
- DNA Polymerase III
- Fork movement
- Completed Okazaki fragment
- DNA polymerase I replacing RNA primer
- DNA ligase joining fragments
DNA ligase

- Can ligate 5′-PO₄ to 3′ OH without insertion of nucleotide

Fig. 11.17
Creating a recombinant plasmid

Fig. 14.4
Steps in cloning a gene

• Isolate plasmid vector
  – Plasmid DNA isolation

• Isolate gene of interest
  – Chromosomal DNA isolation
  – Polymerase chain reaction

• Cut plasmid and gene of interest with same restriction endonuclease
Polymerase chain reaction (PCR)

- Requires DNA polymerase that is not inactivated by high temperatures

- Taq, Vent polymerases isolated from thermophiles
Restriction endonucleases

Fig. 14.2
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Microbial Source</th>
<th>Recognition Sequence(^a)</th>
<th>End Produced(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alu</em>I</td>
<td><em>Arthrobacter luteus</em></td>
<td>5’—A—G↓C—T—3’</td>
<td>C—T—3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’—T—C—G—A—5’</td>
<td>G—A—5’</td>
</tr>
<tr>
<td><em>BamHI</em></td>
<td><em>Bacillus amyloliquefaciens H</em></td>
<td>5’—G↓G—A—T—C—C—3’</td>
<td>G—A—T—C—C—3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’—C—T—A—G—G—5’</td>
<td>G—5’</td>
</tr>
<tr>
<td><em>EcoRI</em></td>
<td><em>Escherichia coli</em></td>
<td>5’—G↓A—A—T—T—C—3’</td>
<td>A—A—T—T—C—3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’—C—T—T—A—A—G—5’</td>
<td>G—5’</td>
</tr>
<tr>
<td><em>HaeIII</em></td>
<td><em>Haemophilus aegyptius</em></td>
<td>5’—G—G↓C—C—3’</td>
<td>C—C—3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’—C—C—G—G—5’</td>
<td>G—G—5’</td>
</tr>
<tr>
<td><em>HindIII</em></td>
<td><em>Haemophilus influenzae b</em></td>
<td>5’—A↓A—G—C—T—T—3’</td>
<td>A—G—C—T—T—3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’—T—T—C—G—A—A—5’</td>
<td>A—5’</td>
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<tr>
<td><em>NcoI</em></td>
<td><em>Nocardia otitidis-caviarum</em></td>
<td>5’—G—C↓G—G—C—C—C—3’</td>
<td>G—G—C—C—C—3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’—C—G—C—C—G—G—5’</td>
<td>C—G—5’</td>
</tr>
<tr>
<td><em>PstI</em></td>
<td><em>Providencia stuartii</em></td>
<td>5’—C—T—G—C—A↓G—3’</td>
<td>G—3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’—G—A—C—G—T—C—5’</td>
<td>A—C—G—T—C—5’</td>
</tr>
<tr>
<td><em>SalI</em></td>
<td><em>Streptomyces albus</em></td>
<td>5’—G—T—C—G—A—C—3’</td>
<td>T—C—G—A—C—3’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’—C—A—G—C—T—G—5’</td>
<td>G—5’</td>
</tr>
</tbody>
</table>

\(^a\)The arrows indicate the sites of cleavage on each strand.

\(^b\)Only the end of the right-hand fragment is shown.
Fig. 14.11
Fig. 14.11

Complementary base pairing followed by ligation

Transform Ca^{2+}-treated Tet'^+ E. coli

Culture bacteria in medium with tetracycline

Petri dish

Tetracycline-resistant colony from transformed cell
<table>
<thead>
<tr>
<th>Peptide or Protein</th>
<th>Potential Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_1$-antitrypsin</td>
<td>Treatment of emphysema</td>
</tr>
<tr>
<td>$\alpha$-, $\beta$-, and $\gamma$-interferons</td>
<td>As antiviral, antitumor, and anti-inflammatory agents</td>
</tr>
<tr>
<td>Blood-clotting factor VIII</td>
<td>Treatment of hemophilia</td>
</tr>
<tr>
<td>Calcitonin</td>
<td>Treatment of osteomalacia</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>Treatment of wounds</td>
</tr>
<tr>
<td>Erythropoetin</td>
<td>Treatment of anemia</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Growth promotion</td>
</tr>
<tr>
<td>Insulin</td>
<td>Treatment of diabetes</td>
</tr>
<tr>
<td>Interleukins-1, 2, and 3</td>
<td>Treatment of immune disorders and tumors</td>
</tr>
<tr>
<td>Macrophage colony stimulating factor</td>
<td>Cancer treatment</td>
</tr>
<tr>
<td>Relaxin</td>
<td>Aid to childbirth</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>Plasma supplement</td>
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<tr>
<td>Somatostatin</td>
<td>Treatment of acromegaly</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>Anticoagulant</td>
</tr>
<tr>
<td>Tissue plasminogen activator</td>
<td>Anticoagulant</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>Cancer treatment</td>
</tr>
</tbody>
</table>
Transcription of RNA

- mRNA
- rRNA
- tRNA
- sRNA

Fig. 11.21
Prokaryotic Promoter

- 35 region
  TTGACA
  RNA polymerase recognition site

- 10 region
  TATAAT
  RNA polymerase binding site

Consensus sequences

Template strand

5’- CCCCCAGGGCTTTTACACTTTATGCTTTCCGGCTCGTAT
3’- GGGGTCCGAAATGTGAAATACGAAGGGCCGAGCATA

GTTGTGTGGAAAT

TGTGAGC- 3’
ACACTCG- 5’

CAACACACCTTA
+1

Region unwound by RNA polymerase in open complex

Beginning of RNA chain

Fig. 11.22
Fig. 12.2
Prokaryotic Terminator

- **Rho-independent**
  - 6 uridines follow hairpin
  - Requires no accessory proteins

- **Rho-dependent**
  - No uridines after hairpin
  - Requires Rho to displace RNA polymerase
Rifampin

- Binds to RNA polymerase
- Inhibits transcription, killing cell
Polycistronic mRNA

- Multiple genes on single transcript
- Transcription from a single promoter
- Usually genes on a polycistronic mRNA are related to a specific function or structure
- No introns in within genes

Fig. 12.1
Coupled transcription-translation

Fig. 12.6
Prokaryotic Ribosome

30S
(0.9 × 10^6 daltons)

16S rRNA
+ 21 polypeptide chains

70S
(2.8 × 10^6 daltons)

50S
(1.8 × 10^6 daltons)

5S rRNA
+ 23S rRNA
+ 34 polypeptide chains

Fig. 12.12
Translational Domain

- Formed by association of 30S and 50S subunits
- 16S rRNA binds to and aligns mRNA

16S rRNA: 3'-UUCCUU-5'
mRNA: 5'-AAGGAA-3'

Fig. 12.12
tRNA structure

Fig. 12.7
Amino-acyl tRNA = “Charged” tRNA
-cognate amino acid attached to 3’ end

Fig. 12.10
Table 11.1 The Genetic Code

<table>
<thead>
<tr>
<th>First Position (5' End)</th>
<th>Second Position</th>
<th>Third Position (3' End)</th>
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</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td><strong>U</strong></td>
<td><strong>G</strong></td>
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<tr>
<td>AUU</td>
<td>UCU</td>
<td>UAU</td>
</tr>
<tr>
<td>AUC</td>
<td>UCC</td>
<td>UAC</td>
</tr>
<tr>
<td>AUA</td>
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<tr>
<td>AUG</td>
<td>UCG</td>
<td>UAG</td>
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<tr>
<td><strong>C</strong></td>
<td><strong>C</strong></td>
<td><strong>C</strong></td>
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<tr>
<td>CUU</td>
<td>CCU</td>
<td>CAU</td>
</tr>
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<td>CAC</td>
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<tr>
<td>GUU</td>
<td>GCU</td>
<td>GAU</td>
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<td>GUC</td>
<td>GCC</td>
<td>GAC</td>
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<td>GUA</td>
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<td>GAA</td>
</tr>
<tr>
<td>GUG</td>
<td>GCG</td>
<td>GAG</td>
</tr>
</tbody>
</table>

*The code is presented in the RNA form. Codons run in the 5’ to 3’ direction. See text for details.*
Initiation of Protein Synthesis

Fig. 12.14
Alignment to allow in-frame translation

Fig. 11.19
Initiation of Protein Synthesis

```
tRNA  3'-'UAC-5'
mRNA  5'-'AUG-3'
```

Fig. 12.14
Prokaryotic Initiator tRNA

\[
\text{CH}_3 - \text{S} - \text{CH}_2 - \text{CH}_2 - \text{CH} - \text{C} - \text{tRNA}^{f\text{Met}}
\]

Fig. 12.13
Elongation

Fig. 12.15

Binding AA-tRNA to A site

AA-tRNA-GTP-EF-Tu complex

Peptide bond formation
Peptide Bond Formation

Fig. 12.16
Termination

Fig. 12.17
Tetracyclines

- Includes doxycycline, chlortetracycline
- Binds to 30S subunit
- Interferes with aminoacyl-tRNA binding to A site

Fig. 35.9
Macrolide antibiotics

- Includes clindamycin, azithromycin
- Binds 23S rRNA of 50S subunit
- Inhibits peptide chain elongation

Fig. 35.11
Aminoglycoside antibiotics

- Includes streptomycin, gentamicin, neomycin, tobramycin, kanamycin
- Binds to 30S subunit
- Inhibits protein synthesis
- Causes misreading of mRNA

Fig. 35.10