Basic Molecular Biology (2)

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http://www.soest.hawaii.edu/marinefungi/OCN403webpage.htm
Overview of Last Lecture

- Major difference of prokaryote and eukaryotes
- Central dogma and genetic info flow (Prok- & euk)
- Major difference of DNA & RNA
- DNA replication and PCR
- Transcription and the production of mRNA (ProK&Euk)
- Translation, protein function and structure
- Ribosome and its major components
Gene Expression Regulation

1. Two major modes

1. The activity of preexisting enzyme.
2. The amount of an enzyme.
3. These two modes work together in cell.

An overview of the regulation mechanisms. The product of gene A is enzyme A and is produced constitutively. Enzyme B is also synthesized constitutively but its activity can be inhibited. The synthesis of the product of gene C can be controlled at the level of translation. The synthesis of the product of gene D can be controlled at the level of transcription.
2. Antisense regulation

If two RNAs base-pair form complementary structure, translation will be blocked! The underlying mechanism for RNAi.
Feedback inhibition of enzyme activity.

Feedback inhibition in a branched biosynthetic pathway. N-acetyl glutamate synthase (AGS) & g-glutamyl kinase (GK).
Gene Expression Regulation

Mechanism of enzyme inhibition by an allosteric effector

- Allosteric site
- Allosteric effector
- Substrate binding site
- Substrate

Conformational change in substrate binding site; enzyme reaction inhibited

Substrate cannot bind
No enzyme reaction

Enzyme reaction proceeds
4. Modification of enzymes

a) Regulation of glutamine synthetase by covalent modification.

b) Relation of the # of AMP added and the enzyme activity.
Enzyme can also be controlled at the level of protein processing.
6. Regulation of transcription

Specific DNA binding proteins.
1) dimers that combine specifically with two sites on the DNA.
2) The specific DNA sequences that interact with the protein are inverted repeats.
3) The operator sequence of the lactose operon is shown and the inverted repeats, which are sites at which the lac repressor makes contact with the DNA (in shaded boxes).
Gene Expression Regulation

7. The helix-turn-helix structure of common DNA binding proteins in prokaryotes

Examples:

a) Lambda repressor
b) The lac and trp repressor of E. coli
8. Simple models of eukaryotic DNA binding proteins

a) Helices are represented by cylinders. Recognition helices are the DNA binding domains.

b) The zinc finger structure. The amino acids holding the Zn\(^{2+}\) ion always include at least two cysteine residues (C) with the other residues being histidine (H).

c) The leucine zipper structure. The leucine residues (shown in yellow) are always spaced exactly every seven amino acids. The interaction of the leucine side chains helps hold the two helices together.
9. DNA Structure & protein binding sites

DNA binding proteins usually bind the major grooves.
Regulation of transcription

1. Negative control - Repression and induction in bacteria

addition of arginine repress enzymes involved in arginine synthesis

The process of enzyme repression. In the case of the argCBH operon the repressor would be the arginine repressor and the corepressor would be the amino acid arginine.
Addition of lactose induce β-galactosidase production. The process of enzyme induction. In the case of the lac operon the repressor would be the lac repressor and the inducer would be allolactose.
Regulation of transcription

2. Positive control in bacteria

Positive control of enzyme induction.

(a) In the absence of an inducer, neither the activator protein nor the RNA polymerase can bind to the DNA.

(b) An inducer molecule binds to the activator protein, which in turn binds to the activator binding site. This allows RNA polymerase to bind to the promoter and begin transcription.

(c) In the case of the malEFG operon, the activator protein would be the maltose activator protein and the inducer would be the sugar maltose.
Regulation of transcription

Some activator proteins interact with RNA polymerase:
(a) The activator binding site is near the promoter.
(b) The activator binding site is several hundred base pairs from the promoter.
(c) In this case, the DNA must be looped to allow the activator and the RNA polymerase to contact.
Regulation of transcription

3. Control by two-component systems in bacteria

a) A sensor kinase in the cell membrane can phosphorylate itself in response to an environmental signal.

b) The phosphoryl group is then transferred to the other main component, a response regulator.

c) The phosphorylated response regulator serves as a repressor.

d) There must also be a phosphatase in the system to cycle the response regulator.
Regulation of transcription

4. Regulation in eukaryotes

1) Transcription and translation take place in separate compartments in the cell. Some of previous regulations may not occur in eukaryotes.

2) Most active eukaryotic cells transcribe a common (basal) set of structural genes that maintain (household) cellular functions.

3) Control can occur at the levels of transcription, mRNA splicing, and translation. Therefore, regulation in eukaryote is generally more complicated than that in bacteria.
Regulation of transcription

4) In general, the control of transcription in eukaryotes is mediated by proteins that are collectively classified as transcription factors.

- Initiator sequence surrounding the start point
- TATA box at about –25 bp
- TATA+Initiator = core promoter
- A transcription factor binds to the TATA box before RNAPoly II
Regulation of transcription

Transcription factors

- A basal transcription factor is always required to allow RNApoly to bind to DNA.
- For RNApoly II, TFIID binds to the TATA box. This is the basal transcription factor.
- More TFs bind to TFIID through protein-protein interactions to form the pre-initiation complex.
- Then RNApoly binds
- *Many* TFs may be involved.
DNA Manipulation Enzymes

1. Restriction endonuclease
# DNA Manipulation Enzymes

**Table 14.2**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Microbial Source</th>
<th>Recognition Sequence</th>
<th>End Produced</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>AluI</em></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-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>HinIII</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>
</tr>
<tr>
<td><em>NolI</em></td>
<td><em>Nocardia otitidis-caviarum</em></td>
<td>5′-G-C-G-C-G-C-C-3′</td>
<td>G-G-C-G-C-C-3′</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3′-C-G-C-G-G-C-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>SauI</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>

*The arrows indicate the sites of cleavage on each strand.*
*Only the end of the right-hand fragment is shown.*
2. DNA ligase
Functions: Link DNA strands together by forming phosphodiester bonds between the 5’-phosphate and the 3’-OH termini of the discontinuous stands.
## DNA Cloning Vector

### 1. General introduction

<table>
<thead>
<tr>
<th>Type</th>
<th>Vector</th>
<th>Restriction Sequences Present</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasmid (E. coli)</td>
<td>pBR322</td>
<td><em>BamHI</em>, <em>EcoRI</em>, <em>HaeIII</em>, <em>HindIII</em>, <em>PstI</em>, <em>SacI</em>, <em>XorI</em></td>
<td>Carries genes for tetracycline and ampicillin resistance</td>
</tr>
<tr>
<td>Plasmid (yeast/E. coli hybrid)</td>
<td>pYe(CEN3)41</td>
<td><em>BamHI</em>, <em>BglII</em>, <em>EcoRI</em>, <em>HindIII</em>, <em>PstI</em>, <em>SacI</em></td>
<td>Multiplies in <em>E. coli</em> or yeast cells</td>
</tr>
<tr>
<td>Cosmid (artificially constructed E. coli plasmid carrying lambda cos site)</td>
<td>pJC720</td>
<td><em>HindIII</em></td>
<td>Can be packaged in lambda phage particles for efficient introduction into bacteria; replicates as a plasmid; useful for cloning large DNA inserts</td>
</tr>
<tr>
<td>YAC (yeast artificial chromosome)</td>
<td>pYAC</td>
<td><em>SmaI</em>, <em>BamHI</em></td>
<td>Carries gene for ampicillin resistance; multiplies in <em>Saccharomyces cerevisiae</em></td>
</tr>
<tr>
<td>BAC (bacterial artificial chromosome)</td>
<td>pBAC108L</td>
<td><em>HindIII</em>, <em>BamHI</em>, <em>NorI</em>, <em>SmaI</em>, and others</td>
<td>Modified F plasmid that can carry 100–300 kb fragments; has a <em>cosN</em> site and a chloramphenicol resistance marker</td>
</tr>
<tr>
<td>Virus</td>
<td>Charon phage</td>
<td><em>EcoRI</em>, <em>HindIII</em>, <em>BamHI</em>, <em>SstI</em></td>
<td>Constructed using restriction enzymes and a ligase, having foreign DNA as its central portion, with lambda DNA at each end; carries β-galactosidase gene; packaged into lambda phage particles; useful for cloning large DNA inserts</td>
</tr>
<tr>
<td>Virus</td>
<td>Lambda 1059</td>
<td><em>BamHI</em></td>
<td>Will carry large DNA fragments (8–21 kb); recombinant can grow on <em>E. coli</em> lysogenic for P2 phage, whereas vector cannot</td>
</tr>
<tr>
<td>Virus</td>
<td>M13</td>
<td><em>EcoRI</em></td>
<td>Single-stranded DNA virus; useful in studies employing single-stranded DNA insert and in producing DNA fragments for sequencing</td>
</tr>
<tr>
<td>Plasmid</td>
<td>Ti</td>
<td><em>SmaI</em>, <em>HpaI</em></td>
<td>Maize plasmid</td>
</tr>
</tbody>
</table>

DNA Cloning Vector

2. The pBR322 plasmid

Major elements of a plasmid:
1) Multiple clone sites (MCS)
2) Resistance gene
3) Origin of replication
DNA Cloning Vector

3. Recombinant plasmid construction & cloning

Transformation & selection
4. Cosmid plasmid & cloning

a. Map of the cosmid pWEB

b. the cosmid cloning procedure using the pWEB-TNC
Summary

• Major regulation modes
  – Inhibiting enzymes
  – Modification of enzymes
  – Protein processing
• Regulation of transcription
  – DNA binding proteins
  – Common structures
  – DNA structure and binding sites
  – Negative and positive control
  – Two-component systems
• DNA modification enzymes
• DNA clone vectors (plasmid & cosmid).