Chapter 4: How Cells Work

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Presentation Outline:

1 Introduction: Central Dogma
1 DNA Replication: Preserving and Propagating DNA
1 Transcription: Sending the Message
1 Translation: Message to Product (Proteins)
1 Regulation of Transcription and Enzyme Activity
The cell must control and regulate the biosynthesis of proteins, amino acids, lipids, etc. Chapter 4 outlines the major cellular processes for doing this, starting with the replication of DNA and ending in protein synthesis.

The Central Dogma

All life employs similar methods to store, express, and utilize the genetic information resident in DNA.

"Bioprocess Engineering: Basic Concepts
Shuler and Kargi, Prentice Hall, 2002
Elements of Genetic Information

Genetic information is stored on DNA strands in the chromosome as sequences of nucleotides.

4-letter alphabet in DNA
- A - adenine only H-bonds with T
- T - thymine U-uracil in RNA only H-bonds with A
- G - guanine only H-bonds with C
- C - cytosine only H-bonds with G

3-letter words "codons"
- Table 4.1
- each word codes for 1 amino acid
- \(4^3 = 64\) possible words

| TABLE 4.1 The Genetic Code: Correspondence between Codons and Amino Acids |
|----------------|----------------|----------------|----------------|
| First base    | U              | C              | A              | G              |
| U             | UUU phe†       | UCU ser        | UAU tyr        | UGU cys        |
|               | UUC phe        | UCC ser        | UAC tyr        | UGC cys        |
|               | UUA leu        | UCA ser        | UAA (none)\(^2\) | UGA (none)\(^2\) |
|               | UUG leu        | UCG ser        | UAG (none)\(^2\) | UGG try        |
| C             | CUU leu        | CCC pro        | CAC his        | CGG arg        |
|               | CUC leu        | CCC pro        | CAC his        | CGG arg        |
|               | CUA leu        | CCA pro        | CAA glu-N      | CGA arg        |
|               | CUG leu        | CGC pro        | CAG glu-N      | CGG arg        |
| A             | AUA leu        | ACC thr        | AAG asp-N      | AGU ser        |
|               | AUA leu        | ACC thr        | AAG asp-N      | AGU ser        |
|               | AUA leu        | ACA thr        | AAA lys        | AGA arg        |
|               | AUA leu        | ACC thr        | AAG lys        | AGG arg        |
| G             | GUU val        | GCU ala        | GAA glu        | GGA gly        |
|               | GUC val        | GCC ala        | GAC asp        | GGC gly        |
|               | GUA val        | GCA ala        | GAC asp        | GGC gly        |
|               | GUU val        | GCG ala        | GAG glu        | GGG gly        |

"Bioprocess Engineering: Basic Concepts, Shuler and Kargi, Prentice Hall, 2002"
DNA Replication: Major Steps

- **Unwind DNA double helix** - DNA gyrase
- **Original DNA (template) “read” in the 3’ → 5’ direction**
- **New DNA strand synthesized in the 5’ → 3’ direction**

DNA Replication (E. Coli), Figure 4.2

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.</td>
<td>Strands separate</td>
</tr>
<tr>
<td>b.</td>
<td>RNA primer made - RNA Polymerase</td>
</tr>
<tr>
<td>c.</td>
<td>DNA synthesized using DNA polymerase III</td>
</tr>
<tr>
<td>d.</td>
<td>RNA primer hydrolyzed - DNA Polymerase I</td>
</tr>
<tr>
<td>e.</td>
<td>DNA replaces RNA primer - DNA Polymerase I</td>
</tr>
</tbody>
</table>

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DNA Replication (E. Coli), Figure 4.3

DNA double helix
New “complimentary” DNA strand
Okazaki fragment
A closer view of the replication fork
DNA ligase

DNA Elongation Reaction

Release of energy

DNA nucleotide triphosphate
pyrophosphate

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Overview of Information Transfer from DNA to Proteins

Transcription Step, a → b

Translation Step, b → c

Figure 4.6

Transcription

Creating RNA from a DNA Template

Types of RNA
1. Messenger RNA, m-RNA, carries genetic information unstable, about 1 minute life time
2. Transfer RNA, t-RNA, carries one amino acid stable
3. Ribosomal RNA, r-RNA, 65% of ribosome stable
Messenger RNA Synthesis (Fig. 4.4)

One dominant $\sigma$ subunit for most genes on the DNA

Other $\sigma$ subunits become active under adverse conditions

Procaryotic Cells and m-RNA Synthesis

One promoter causes a polygenic m-RNA to be made. Polygenic means that more than one protein will be made from that m-RNA molecule.
Eucaryotic Cells and m-RNA Synthesis

- No polygenic m-RNA (1 protein per m-RNA)
- DNA genes contain “nonsense DNA” that do not code for protein biosynthesis
- The resulting m-RNA contains “introns” that must be spliced out by specific enzymes
- The presence of introns complicates eucaryotic gene transfer to procaryotes using Genetic Engineering
- Additional m-RNA processing -
  + methylated guanine nucleotide added to 5’ end
  + adenine nucleotides added to 3’ end

Figure 4.5

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Translation (Fig. 4.7)

Protein synthesis by Ribosomes using m-RNA as a template and t-RNA as amino acid carriers

a) Initiation - ribosome attaches to m-RNA at binding site on m-RNA at AUG codon on m-RNA → N-formylmethionine

b) Elongation of Protein - the anticodon portion of the t-RNA attaches to the m-RNA. A second t-RNA attaches.
c) Elongation of Protein - The amino acid from 1 is joined to the amino acid on 2.

d) Elongation of Protein - This process continues for other t-RNA amino acids.
Translation (cont.) (Fig. 4.7)

10-20 ribosomes at once

**e) Termination** - When the ribosome encounters a stop sequence on the m-RNA (3 codons: UAA, UAG, or UGA), it separates and releases the polypeptide.

Energy Requirements in Protein Synthesis

4 high energy phosphate bonds are required per amino acid (aa) added.

- 2 required to "charge" t-RNA
- 2 required to elongate the protein by 1 aa unit

\[ \text{GTP} \rightarrow \text{GDP} + \text{P}_i + 7.3 \text{ kcal/mole} \]

\[ (4)(7.3)= 29.2 \text{ kcal/mole aa} \]

Guanosine triphosphate

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Post Translational Processing of Proteins

Secretion through a membrane
20-25 amino acids clipped off

Other modifications (Eucaryotic proteins)
Phosphorylation - addition of phosphate
Glycosylation - addition of sugars

Important to consider in choosing a host organism for protein production

Metabolic Regulation

Genetic-Level Control - Which Proteins are Made?

Repression of Transcription (m-RNA)
an end product of enzyme activity or of the metabolic pathway (co-repressor) blocks m-RNA synthesis

Figure 4.9

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Metabolic Regulation

**Induction of Transcription (m-RNA)**

A substrate for a metabolic pathway accumulates and induces m-RNA synthesis

![Diagram](image)

**Figure 4.10**

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Modification to Repression/Induction

**Catabolic Repression:**

When multiple substrates (e.g. glucose and lactose) are available, the preferred one will be used up first (e.g. glucose)

**How?** The Lactose Operon, though it is induced by lactose, can not yield much m-RNA because the RNA Polymerase has a low affinity for binding to Promotor region of the operon. This binding affinity is under the control of glucose utilization through the accumulation of CAP (cyclic AMP Activating Protein).
Catabolic Repression:

CAP/c-AMP binds to RNA Polymerase and drastically increases the affinity of RNA Polymerase for the Promotor region of the Operon. Now Transcription can take place to create the m-RNA needed for protein synthesis and metabolism of lactose.

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Figure 4.11: Diauxic Growth

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Operon

A set of functionally related genes under the control of a single promoter-operator

Metabolic Pathway Control

After being made, enzyme activity is controlled by end products of a metabolic pathway

- Isozymes
- Concerted Feedback
- Sequential Feedback
- Cumulative Feedback

*Bioprocess Engineering: Basic Concepts
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L-Aspartic Acid Metabolic Pathway Control

What type of control is being exerted on L-lysine production?

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