Chapter 4: How Cells Work

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Presentation Outline:

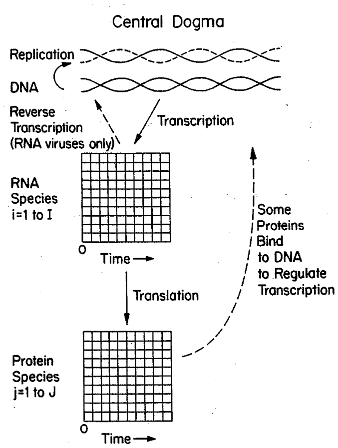
- Introduction : Central Dogma
- DNA Replication: Preserving and Propagating DNA
- Transcription: Sending the Message
- Translation: Message to Product (Proteins)
- Regulation of Transcription and Enzyme Activity

Introduction

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

Central Dogma



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

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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*

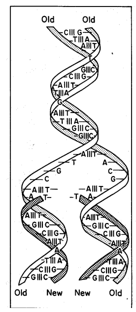

Figure 2.17. of DNA, sho replication b pairing. (Wit Brock, D. W gan, *Biology* ed., Prentice NJ, 1984, p

3-letter words "codons"

- *Table 4.1*
- *each word codes for 1 amino acid*
- *4^3 = 64 possible words*

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Genetic Code: Codons in RNA and Amino Acids

TABLE 4.1 The Genetic Code: Correspondence between Codons and Amino Acids

First base	Second bases: U		C		A		G	
U	UUU	phe[a]	UCU	ser	UAU	tyr	UGU	cys
	UUC	phe	UCC	ser	UAC	tyr	UGC	cys
	UUA	leu	UCA	ser	UAA	(none)[b]	UGA	(none)[b]
	UUG	leu	UCG	ser	UAG	(none)[b]	UGG	try
C	CUU	leu	CCU	pro	CAU	his	CGU	arg
	CUC	leu	CCC	pro	CAC	his	CGC	arg
	CUA	leu	CCA	pro	CAA	glu-N	CGA	arg
	CUG	leu	CCG	pro	CAG	glu-N	CGG	arg
A	AUU	ileu	ACU	thr	AAU	asp-N	AGU	ser
	AUC	ileu	ACC	thr	AAC	asp-N	AGC	ser
	AUA	ileu	ACA	thr	AAA	lys	AGA	arg
	AUG	met	ACG	thr	AAG	lys	AGG	arg
G	GUU	val	GCU	ala	GAU	asp	GGU	gly
	GUC	val	GCC	ala	GAC	asp	GGC	gly
	GUA	val	GCA	ala	GAA	glu	GGA	gly
	GUG	val	GCG	ala	GAG	glu	GGG	gly

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DNA Replication : Major Steps

- *Unwind DNA double helix - DNA girase*
- *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

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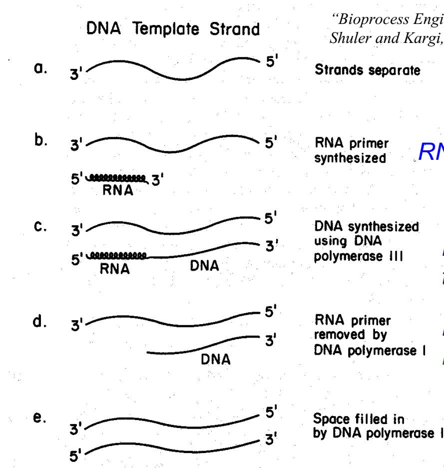


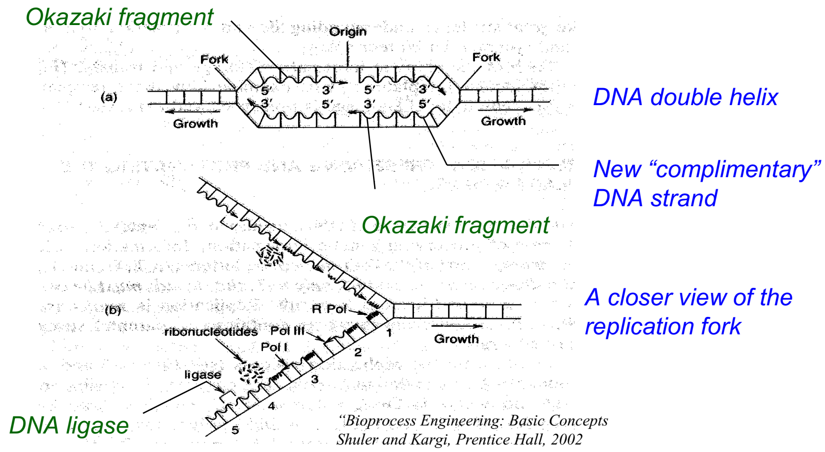
RNA primer made - RNA Polymerase

DNA nucleotides added to RNA primer - DNA Polymerase III

RNA primer hydrolyzed - DNA Polymerase I

DNA replaces RNA primer - DNA Polymerase I

DNA Replication (*E. Coli*), Figure 4.3



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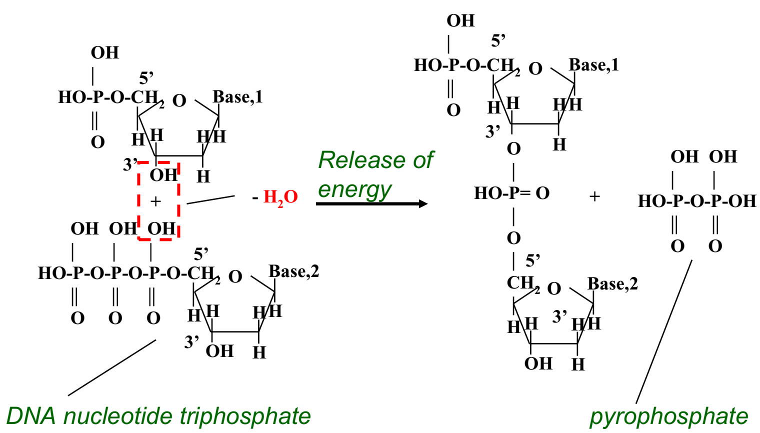
DNA Elongation Reaction



Overview of Information Transfer from DNA to Proteins

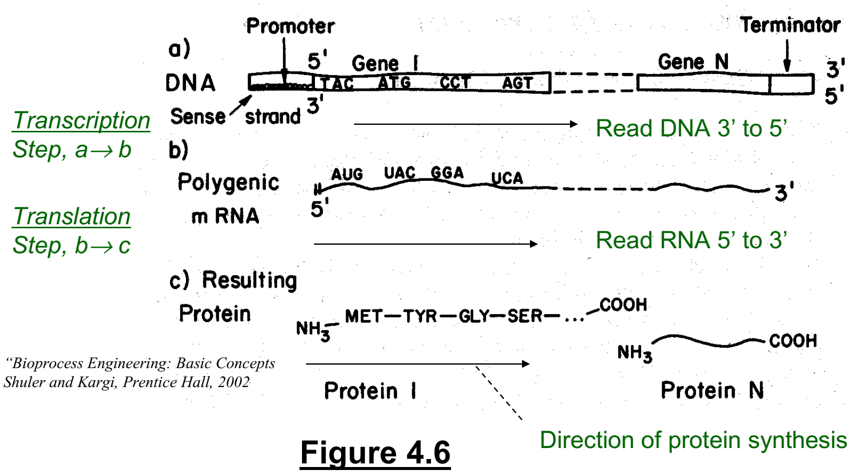


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Figure 4.6

Transcription

Creating RNA from a DNA Template

Types of RNA

1. Messenger RNA, m-RNA, carries genetic information
unstable, about 1 minute life time

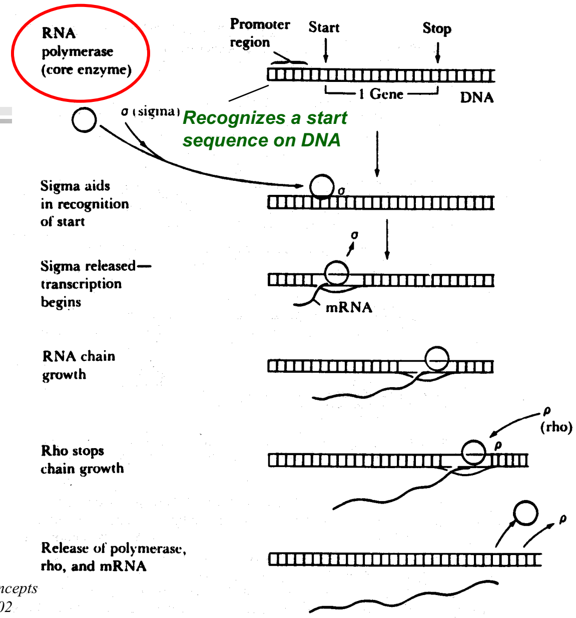
2. Transfer RNA, t-RNA, carries one amino acid
stable

3. Ribosomal RNA, r-RNA, 65% of ribosome
stable

Messenger RNA Synthesis (Fig. 4.4)

One dominant σ subunit for most genes on the DNA

Other σ subunits become active under adverse conditions



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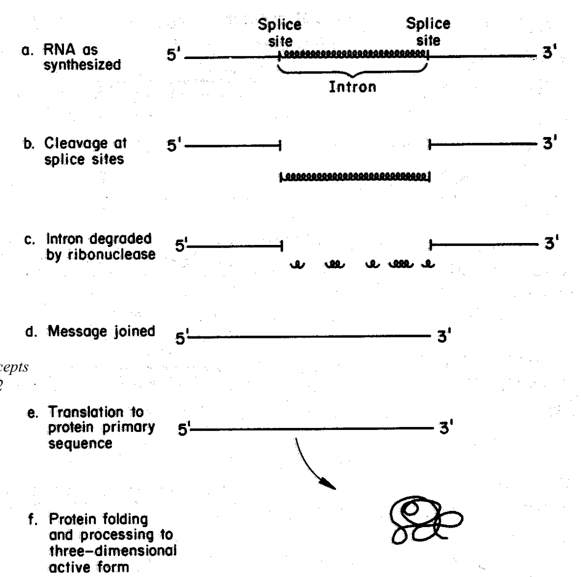
Procaryotic Cells and m-RNA Synthesis

One promotor 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*

Eucaryotic Cells and m-RNA Synthesis



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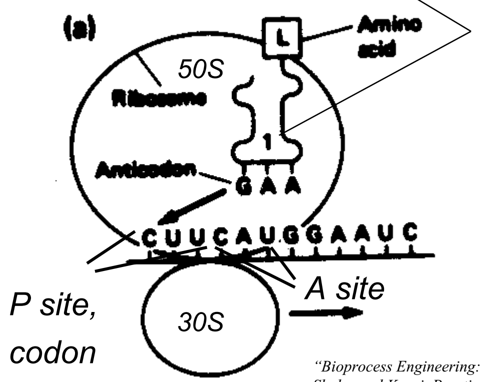
<u>Figure 4.5</u>

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

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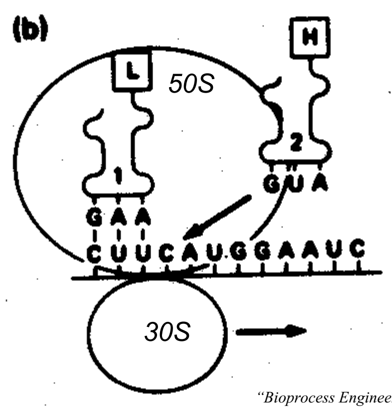
Translation (cont.) (Fig. 4.7)



b) Elongation of Protein - the anticodon portion of the t-RNA attaches to the m-RNA. A second t-RNA attaches.

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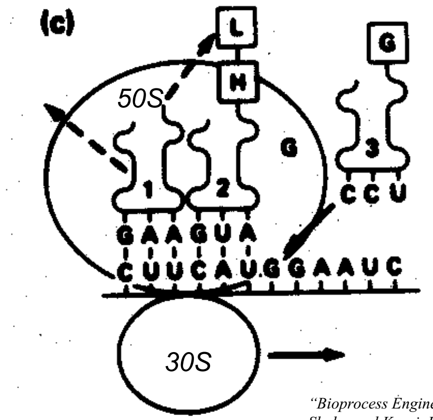
Translation (cont.) (Fig. 4.7)



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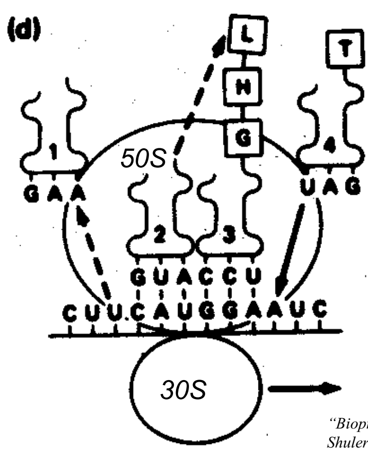
c) <u>Elongation of Protein</u> - The <u>amino acid</u> from 1 is joined to the amino acid on 2.

Translation (cont.) (Fig. 4.7)



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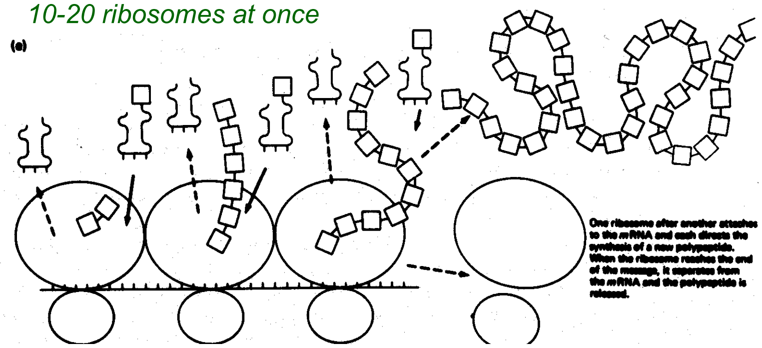
d) <u>Elongation of Protein</u> - This process continues for other t-RNA amino acids.

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, UA□G, or UGA), it separates and releases the polygenic peptide.

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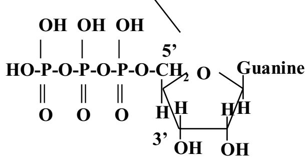
Energy Requirements in Protein Synthesis

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

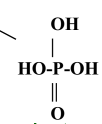
2 required to "charge" t-RNA
2 required to elongate the protein by 1 aa unit

$$GTP \rightarrow GDP + P_i + 7.3 \text{ kcal/mole}$$

(4)(7.3)= 29.2 kcal / mole aa



Guanosine triphosphate



Phosphate

Post Translational Processing of Proteins

Secretion through a membrane

20-25 amino acids clipped off

Other modifications (Eucaryotic proteins)

Phosphorlylation - addition of phosphate
Glycosylation - addition of sugars

Important to consider in choosing a host organism for protein production

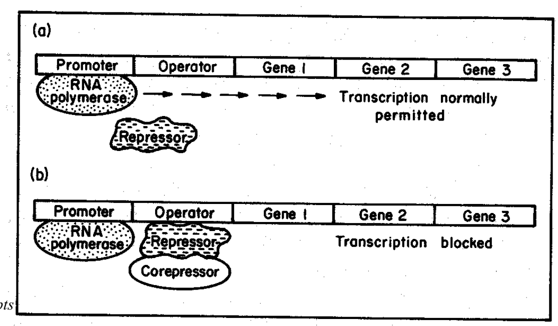
Metabolic Regulation

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

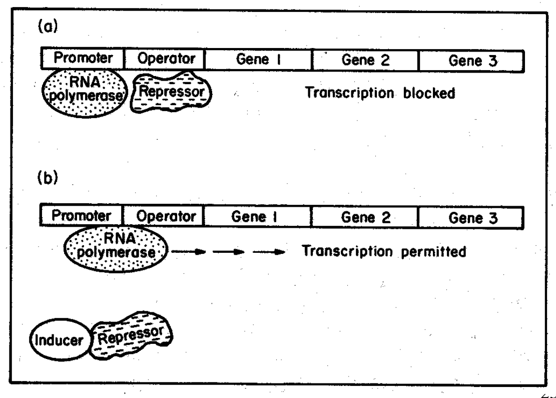


Figure 4.10

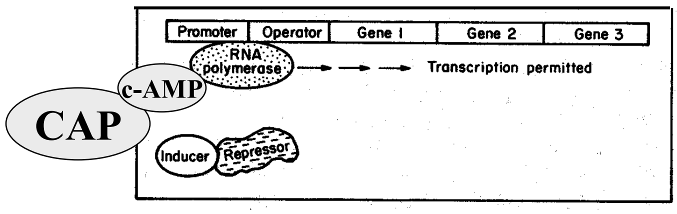
Modification to Repression/Induction

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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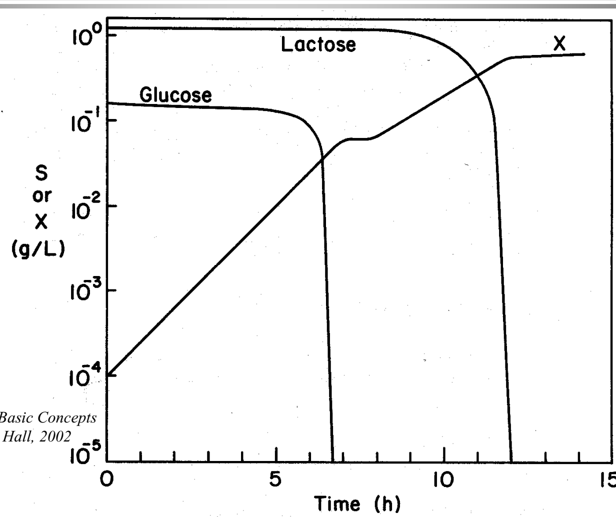
Figure 4.11: Diauxic Growth



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Operon

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

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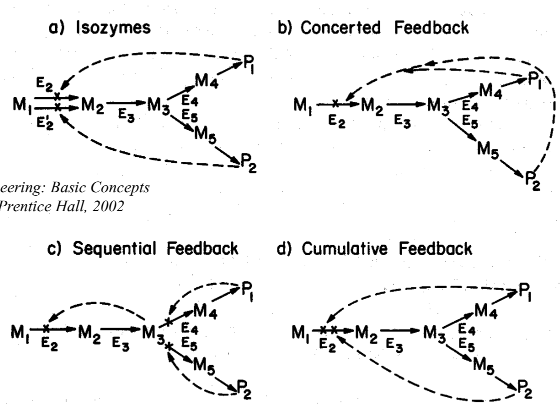
Metabolic Pathway Control

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



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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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