

Chapter 12

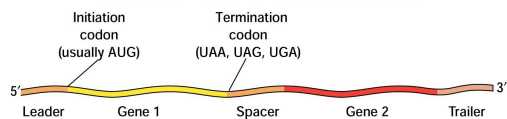
Genes: Expression and Regulation

DNA Transcription or RNA Synthesis

- produces three types of RNA
 - tRNA
 - carries amino acids during protein synthesis
 - rRNA
 - component of ribosomes
 - mRNA
 - directs protein synthesis

Transcription in Procaryotes

- polygenic mRNA
 - contains directions for > 1 polypeptides



Transcription in procaryotes...

- catalyzed by a single RNA polymerase
 - large multi-subunit
 - in *E. coli*
 - core enzyme = $\alpha_2\beta\beta'$
 - holoenzyme = core enzyme + sigma factor (directs core enzyme to promoter)

mRNA transcription

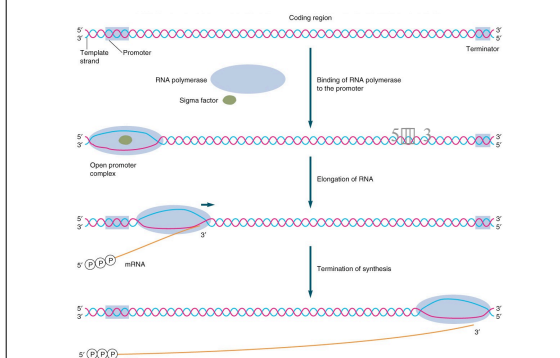
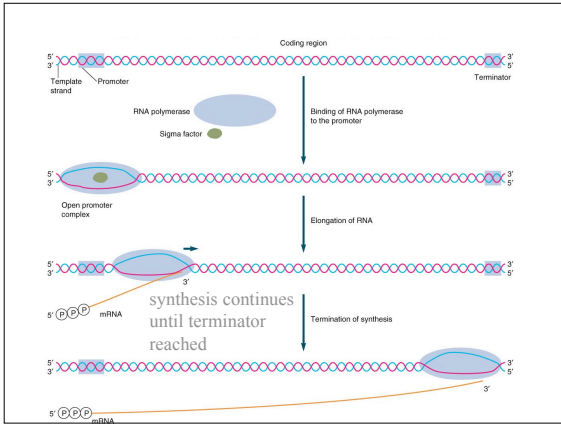


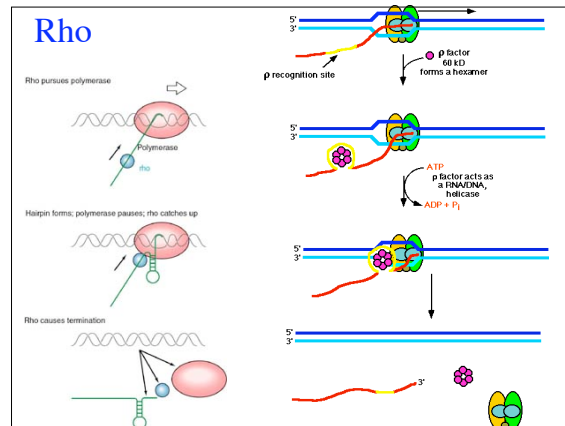
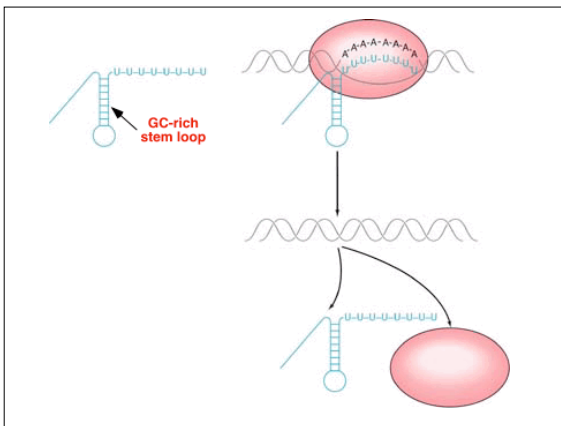
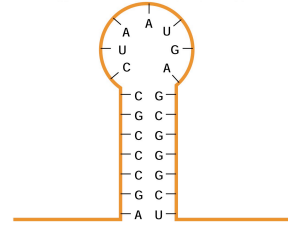
Table 12.1 RNA Bases Coded for by DNA

DNA Base	Purine or Pyrimidine Incorporated into RNA
Adenine	Uracil
Guanine	Cytosine
Cytosine	Guanine
Thymine	Adenine



Prokaryotic terminators

- two types
 - hairpin + 6 uridines
 - rho factor-dependent
 - lack polyU and often lack hairpin



Transcription in Eucaryotes

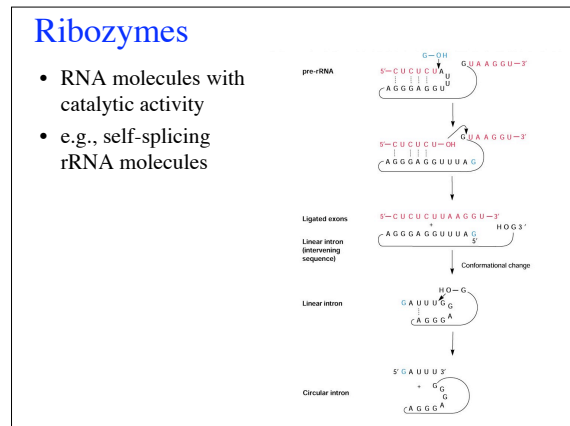
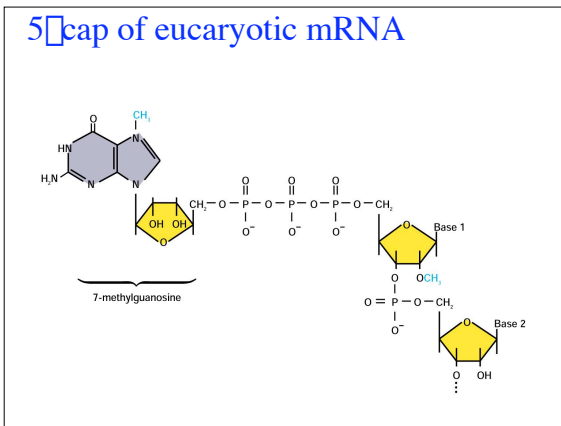
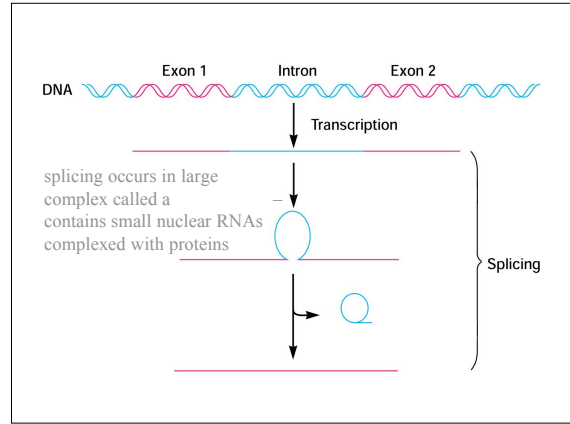
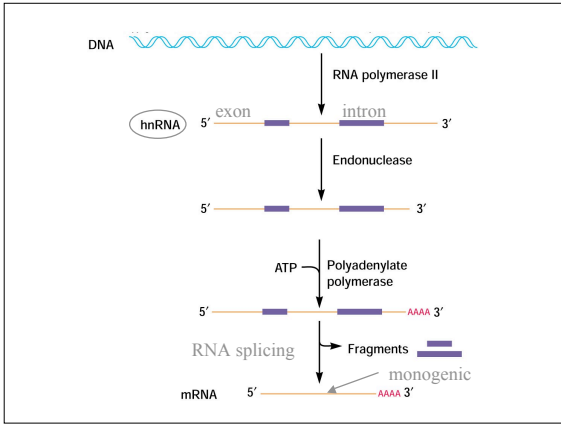
Table 12.2 Eucaryotic RNA Polymerases

Enzyme	Location	Product
RNA polymerase I	Nucleolus	rRNA (5.8S, 18S, 28S)
RNA polymerase II	Chromatin, nuclear matrix	mRNA
RNA polymerase III	Chromatin, nuclear matrix	tRNA, 5S rRNA

- monogenic mRNA

Transcription in eucaryotes...

- promoters contain three common elements
 - TATA box ~ 30 bases before transcription start
 - CAAT box ~ 75 bases before transcription start
 - GC box ~ 90 bases before transcription start

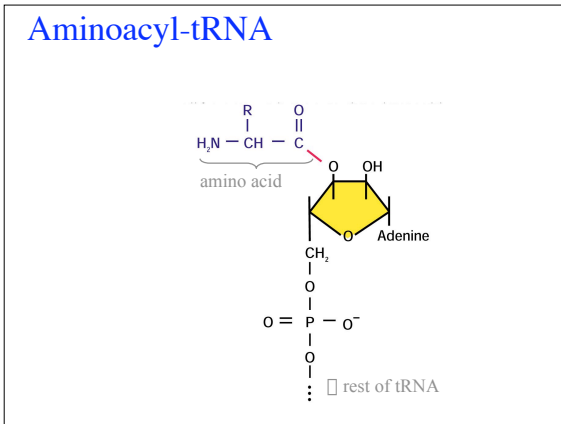
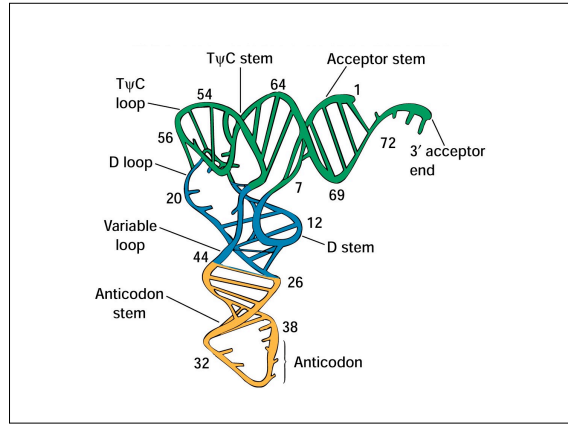
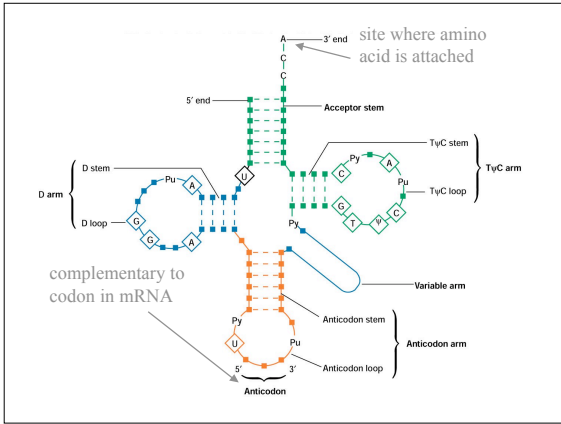


Protein Synthesis

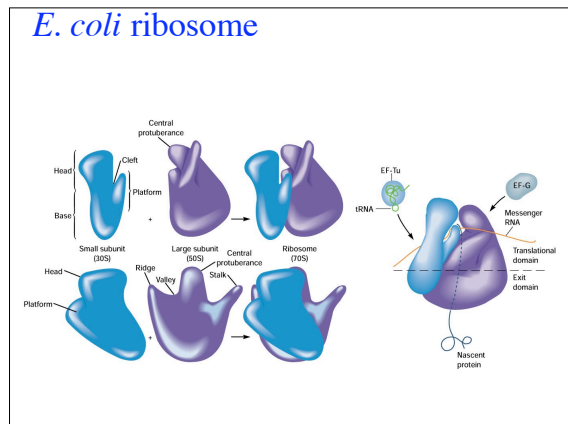
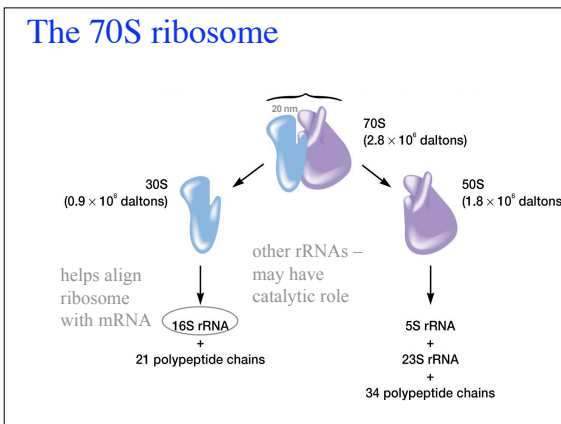
- translation
 - synthesis of polypeptide directed by sequence of nucleotides in mRNA
- direction of synthesis N terminal → C-terminal
- ribosome
 - site of translation
 - polyribosome – complex of mRNA with several ribosomes

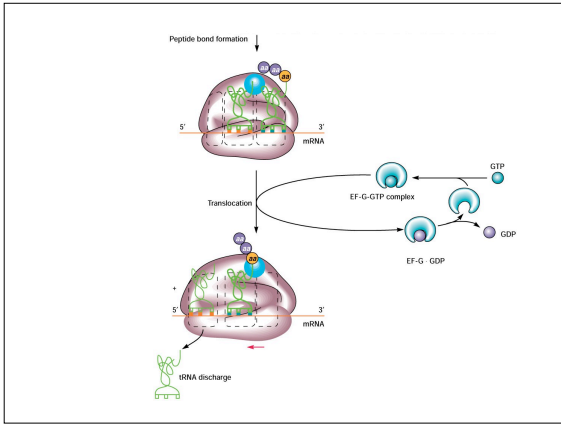
Transfer RNA and Amino Acid Activation

- attachment of amino acid to tRNA
- catalyzed by aminoacyl-tRNA synthetases
 - at least 20
 - each specific for single amino acid and for all the tRNAs to which each may be properly attached (cognate tRNAs)



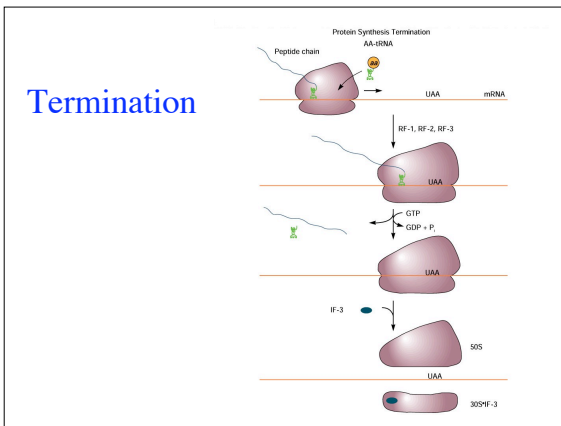
- ### The Ribosome
- prokaryotes
 - 70S ribosomes = 30S + 50S subunits
 - eukaryotes
 - 80S ribosomes = 40S + 60S subunits
 - mitochondrial and chloroplast ribosomes resemble prokaryotic ribosomes





Termination of Protein Synthesis

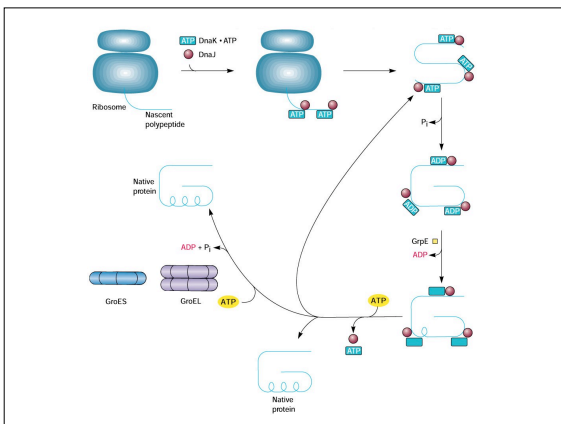
- takes place at any one of three codons
 - nonsense (stop) codons – UAA, UAG, and UGA
- release factors (RFs)
 - aid in recognition of stop codons



Termination

Protein Folding and Molecular Chaperones

- molecular chaperones
 - proteins that aid the folding of nascent polypeptides
 - protect cells from thermal damage
 - e.g., heat-shock proteins
 - aid in transport of proteins across membranes

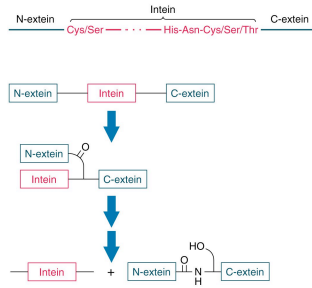


Protein folding – eucaryotes versus procaryotes

- domains
 - structurally independent regions of polypeptide
 - separated from each other by less structured portions of polypeptide
- in eucaryotes
 - domains fold independently right after being synthesized
 - molecular chaperones not as important
- in procaryotes
 - polypeptide does not fold until after synthesis of entire polypeptide
 - molecular chaperones play important role

Protein Splicing

- removal of part of polypeptide before folding
- inteins – removed portion
- exteins – portions that remain in protein



Regulation of mRNA Synthesis

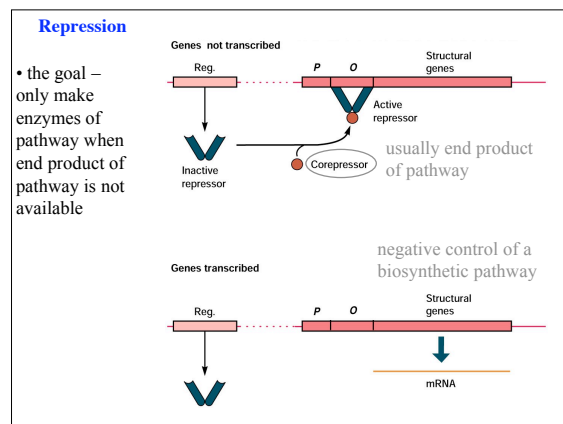
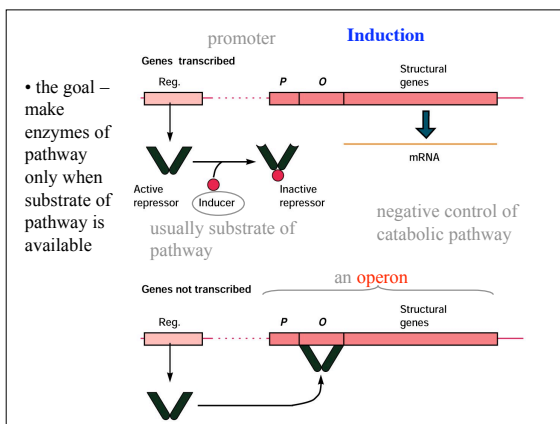
- regulation of gene expression
- conserves energy and raw materials
- maintains balance between the amounts of various cell proteins
- allows organism to adapt to long-term environmental change

Induction and Repression

- inducible enzyme
 - level increases in presence of inducer
 - small molecule, usually substrate of catabolic pathway in which enzyme functions
- repressible enzyme
 - level decreases in presence of corepressor
 - usually end product of biosynthetic pathway in which the enzyme functions

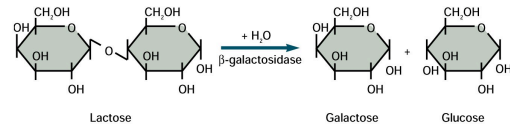
Negative Control

- presence of regulatory protein (repressor) at regulatory site (operator) decreases mRNA synthesis
- repressor proteins
 - exist in active and inactive forms
 - inducers and corepressors alter activity of repressor

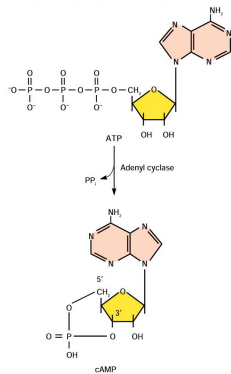


Positive Control

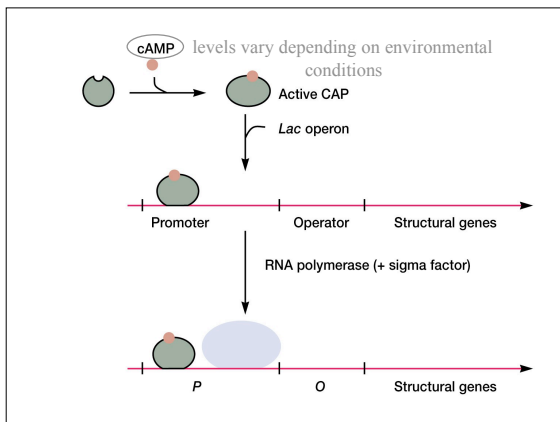
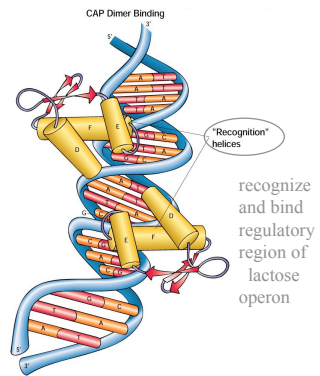
- presence of a regulatory protein (activator protein) at a regulatory region promotes transcription
- e.g., lactose operon
 - regulated by catabolite activator protein (CAP) and cyclic AMP (cAMP)
 - CAP also called cyclic AMP receptor protein (CRP)



cAMP

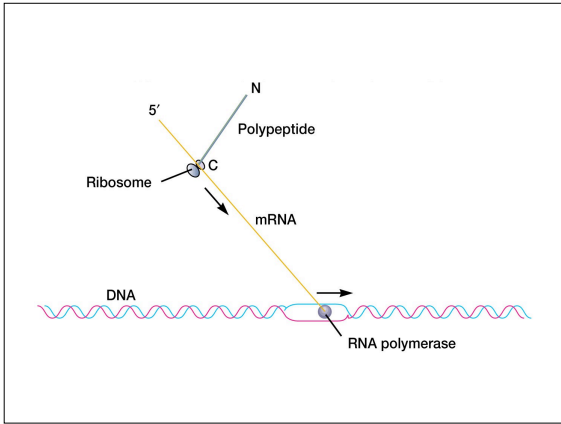


CAP



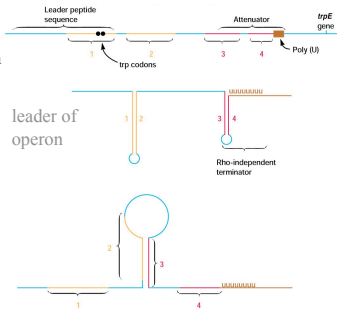
Attenuation

- regulation of transcription by the behavior of ribosomes
- observed in bacteria, where transcription and translation are tightly coupled
 - translation of a mRNA can occur as the mRNA is being synthesized

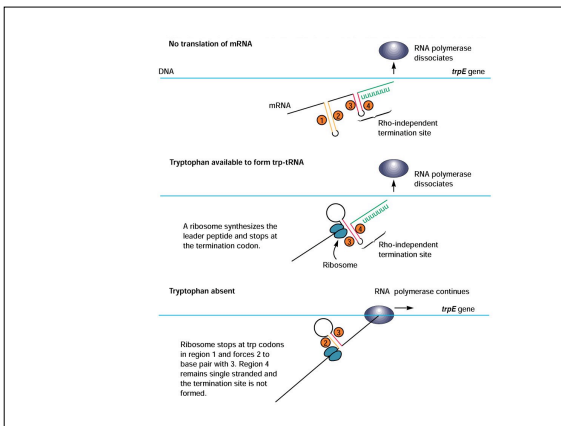


e.g., the tryptophan operon

- operon that encodes enzymes for the tryptophan biosynthetic pathway



- the goal – terminate transcription if tryptophan is available

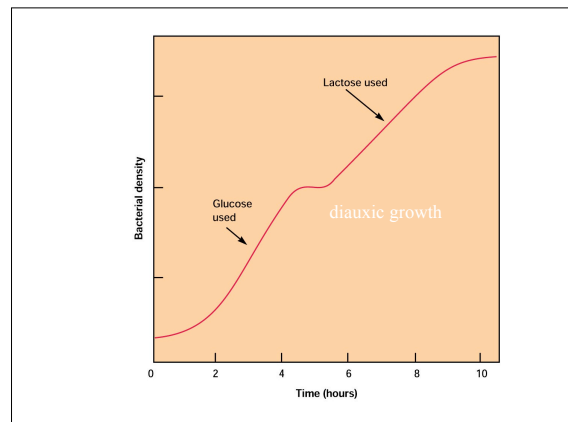


Global Regulatory Systems

- affect many genes and pathways simultaneously
- **regulon**
 - collection of genes or operons controlled by a common regulatory protein

Catabolite Repression

- occurs when operon is under control of catabolite other than initial substrate of pathway
- allows preferential use of one carbon source over another when both are available in environment
- e.g., catabolite repression of lactose and other operons by glucose
 - glucose decreases cAMP levels, thereby blocking CAP binding and decreasing mRNA synthesis

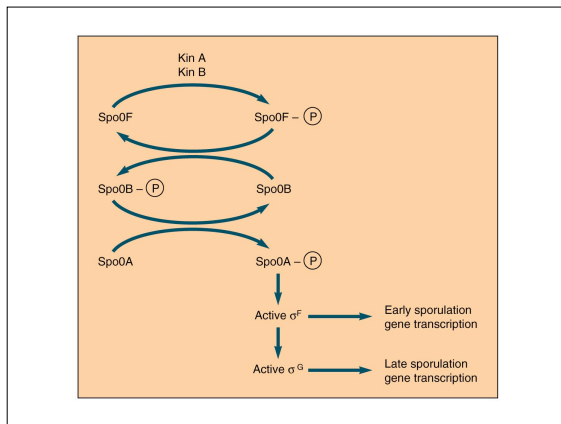


Regulation by Sigma Factors and Control of Sporulation

- different sigma factors recognize different promoters
- substitution of sigma factors changes gene expression of many genes and operons

e.g., *Bacillus subtilis* sporulation sigma factors

- synthesized only as cell switches from vegetative growth to sporulation
- lead to transcription of sporulation-related genes



Antisense RNA and the Control of Porin Proteins

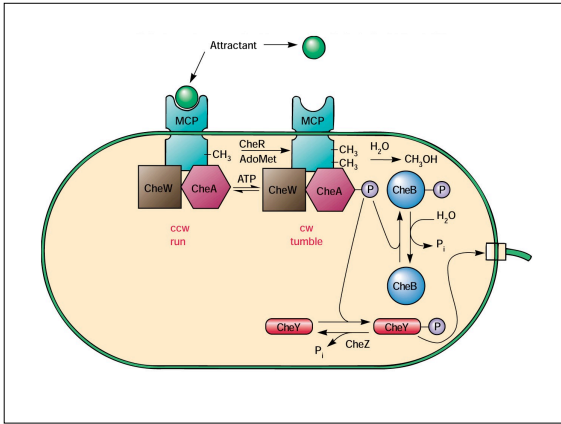
- antisense RNA
 - has sequence complementary to some RNA component necessary for gene expression (target RNA)
 - binds target RNA and blocks its function

e.g., porin proteins

- porin proteins
 - outer membrane proteins
- different porins produced under different conditions
 - *E. coli* OmpC porin made when in intestine
 - OmpF porin made when in dilute environment
- micF antisense RNA binds OmpF RNA and blocks its translation when bacterium in intestines

Two-Component Phosphorelay Systems

- transfer of phosphoryl groups control gene transcription and protein activity
- e.g., sporulation in *B. subtilis*
- e.g., chemotaxis in *E. coli*

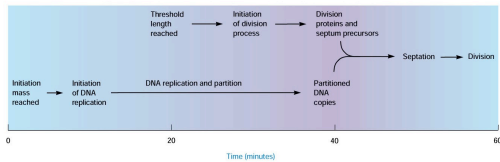


Control of the Cell Cycle

- cell cycle
 - complete sequence of events extending from formation of a new cell through next division
 - requires that DNA replication and cell division be tightly coordinated
- precise mechanisms of control are not known

Cell cycle control in *E. coli*

- two separate control pathways
 - sensitive to cell mass
 - sensitive to cell length



Effect of growth rate

- slow growth rate
 - DNA replicated then septation begins
- rapid growth rate
 - DNA replicated and new round of DNA replication begins before septation begins

