BIO1140 Exam Study Guide

Unit 1: Chapter 1 - DNA & RNA Structure

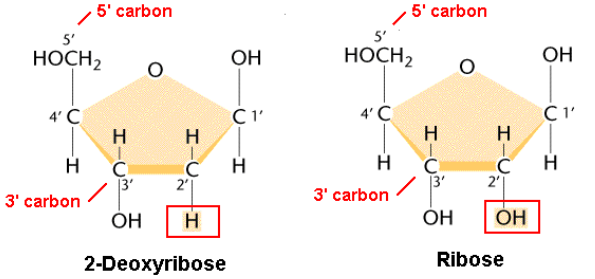
- **What is the structure of DNA and how do we know?**
- **Is DNA always the genetic material?**
- **Is the DNA structure always the same?**
- **Is the DNA structure in the cell relevant?**
- **Is the genome what we think it is?**
- **Without chromatin, would it be necessary to invent it? (next section)**

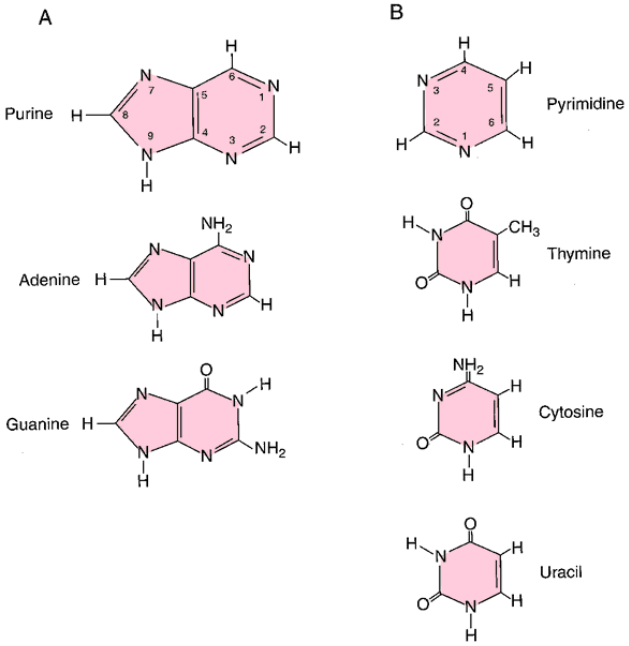
1. Structure of DNA:

[BASE (Purine/Pyrimidine) + Pentose Sugar (deoxyribose)] + Phosphate

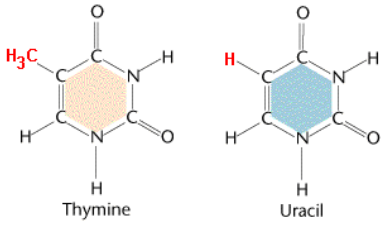
Nucleoside or Deoxyribonucleoside Nucleotide or Deoxyribonucleotide

2. Differences between DNA and RNA



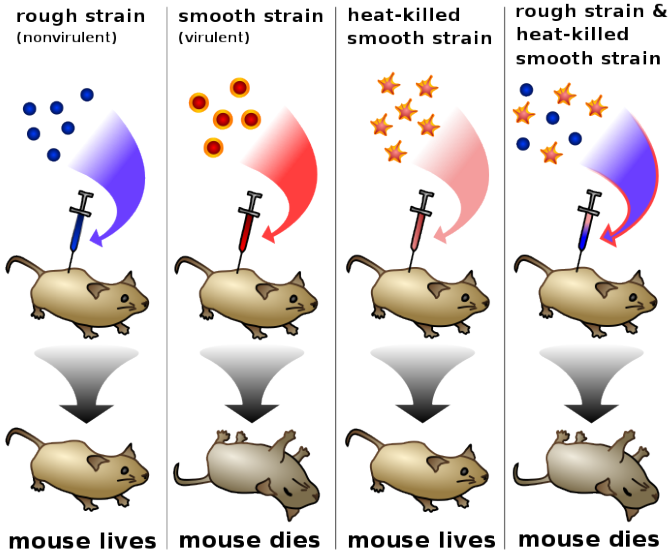


(Klug & Cummings 1997)



(Klug & Cummings 1997)

Griffith's Experiment



However – how does this relate to DNA specifically? How can we be sure that it is DNA that is the factor for the *transformation* property?

Avery-McLeod-McCarty Experiment

- Treatment with varying types of enzymes that degrades protein, RNA, and DNA.
- Found that only the enzyme degrading DNA resulted in *non-transforming* cells.
- Therefore – DNA must have something to do with virulence/*transformation*

*What is DNA? – What is the structure? – How does it transfer information?

Chargoff's Experiment

- Treatment of DNA with harsh chemicals breaking the component down to its constituent bases
    - Concentration of A=T, G=C

*SO? – What does this mean? How does it relate to *transformation* and heredity?

Hershey and Chase Experiment

- Experimented with bacteriophage T2 with labeled sulfur (found in capsid shell) and labeled phosphate (DNA)
- Progeny of labeled phosphate displayed radioactivity while sulfur did not – therefore DNA is the basis for heredity

*Still – what is the structure? How is this information passed on now that we have established it is the basis for heredity? **In addition, is this always true?**

3. What are the instances where DNA is NOT the hereditary information?

- Viruses have RNA as information
- Viroids have RNA as information

Fibre Diffraction Experiment (Rosalind Franklin and Raymond Gosling)

- Determined the helical structure of DNA by X-ray diffraction experiments.
- DNA exists as a right-handed spiral helix.
- The two strands are *anti-parallel*

4. Is DNA structure always the same, and is the structure important?

- Some DNA are circular, and others are linear, some are single stranded while others are double-stranded (identify which ones)

A-DNA	Z-DNA	B-DNA
- Compact - Occurs in dehydrated samples of DNA (for crystallographic experiments) - Deepening of major groove and shallower minor groove	- LEFT-handed - Higher GC content - Involved in transcription and binding proteins (torsional strain relief – supercoiling)	- Right-handed - "normal" DNA

- Can also form cruciform (binding upon itself), G-tetraplex, and DNA aptamers (enzymatic DNA)
- Structure determines function!

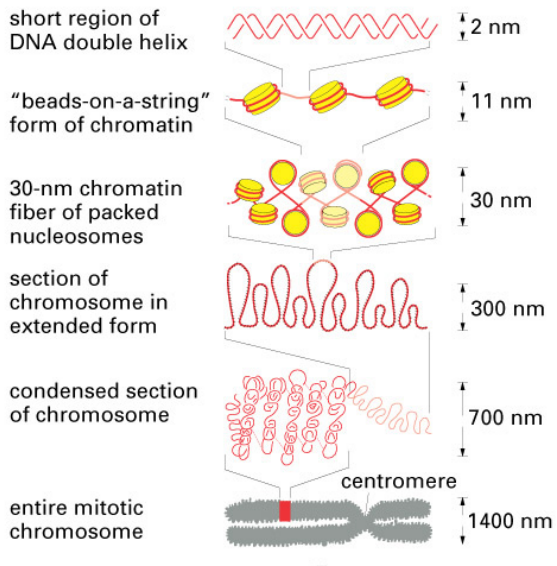
5. What is the genome like?

- Depending on the organism, it can be circular or linear dsDNA
- For eukaryotes – highly organized with histones and chromosomes – allows packaging of a large amount of information

6. RNA

- RNA is very diverse and highly organized in its structure to allow for correct function
- mRNA = transcript – for relaying information
- tRNA = requires binding recognition to mRNA while correct sequences to sequester an amino acid corresponding to the correct 3 base codon
- rRNA = ribozyme with enzymatic characteristics that binds to proteins and mRNA to allow translation to be possible

Unit 1: Chapter 2 – Chromatin



NET RESULT: EACH DNA MOLECULE HAS BEEN PACKAGED INTO A MITOTIC CHROMOSOME THAT IS 10,000-FOLD SHORTER THAN ITS EXTENDED LENGTH

Figure 5-24 Essential Cell Biology, 2/e. (© 2004 Garland Science)

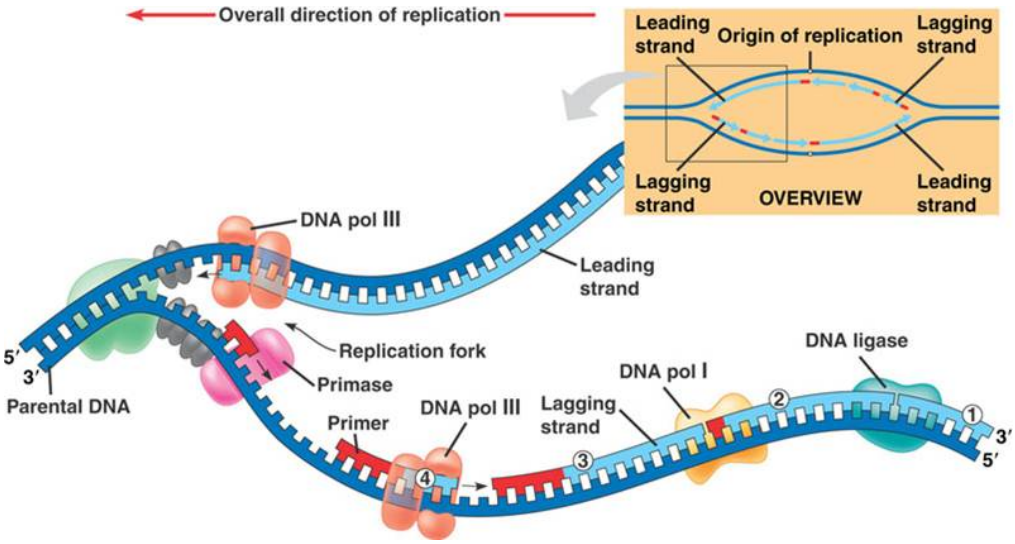
Nucleosome: ~200 bp of dsDNA plus 2 copies each of the four histones H2A, H2B, H3 and H4 plus one copy of H1. It includes the linker DNA.

Core Nucleosome: ~146bp of dsDNA plus 2 copies each of the four histones H2A, H2B, H3.

H1 – binds to nucleosome as well as the linker DNA – brings nucleosomes closer together and creates a compact *solenoid*

Heterochromatin	**Euchromatin**
▪ More highly condensed ▪ Genes are generally inactive ▪ DNA is more highly methylated. ▪ 15% or higher of genome ** ▪ Centromeric and telomeric regions ▪ Highly enriched in DNA repeats	▪ Less condensed, more dispersed ▪ Genes are generally active ▪ DNA is less highly methylated.
Alternate between the two through chromatin remodeling - **Acetylation** of lysine → acetyl groups are negatively charged which causes DNA to be loosely associated with histones → this loose structure allows it to become EUCHROMATIN because it provides easier access to transcriptional factors - **Methylation** → results in tighter packaging – turning the gene "off"	

Unit 2: Chapter 1 – Replication



1. Helicase untwist and separate template DNA strands from the origin of replication, break hydrogen bonds and moves along the direction of leading strand
2. SS binding proteins keep the unpaired template strands apart during replication
3. Gyrase releases any tension brought about by the unwinding of the DNA strands
4. Primase attaches RNA primer as soon as there is a single strand
5. DNA polymerase 3 catalyze the elongation of new DNA and attaches free flowing nucleosides on to the single strand
6. Leading strand is replicated continuously towards replication fork while lagging strand is replicated in okazaki fragments. The growing direction of the new DNA strand is always 5 to 3. Therefore leading strand always starts with 3 prime.
7. DNA polymerase 1 checks over the new replicated DNA strand and remove the RNA primer.
8. DNA ligase adds sugar phosphate backbone between the Okazaki fragments (fill in gaps)

Enzyme	Activity
Helicase	Unwinds DNA helix
Single-stranded binding proteins	Stabilize single-stranded DNA and prevent the two strands at the replication fork from reforming double-stranded DNA
Topoisomerase	Avoids twisting of the DNA ahead of the replication fork (in circular DNA) by cutting the DNA, turning the DNA on one side of the break in the direction opposite to that of the twisting force, and rejoining the two strands
Primase	Assembles RNA primers in the 5′ → 3′ direction to initiate a new DNA strand
DNA polymerase III	Main replication enzyme in *E. coli;* extends the RNA primer by adding DNA nucleotides to it
DNA polymerase I	*E. coli* enzyme that uses its 5′ → 3′ exonuclease activity to remove the RNA of the previously synthesized Okazaki fragment, and uses its 5′ → 3′ polymerization activity to replace the RNA nucleotides with DNA nucleotides
Sliding clamp	Tethers DNA polymerase III to the DNA template, making replication more efficient
DNA ligase	Seals nick left between adjacent bases after RNA primers replaced with DNA

REMEMBER THE DIFFERENT ENZYMES AND THEIR ACTIVITIES

To get faster replication – have more replication origin sites!

BACTERIA: Begins another replication cycle as soon as termination is reached

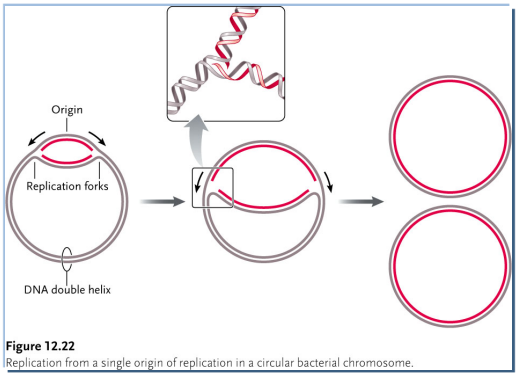
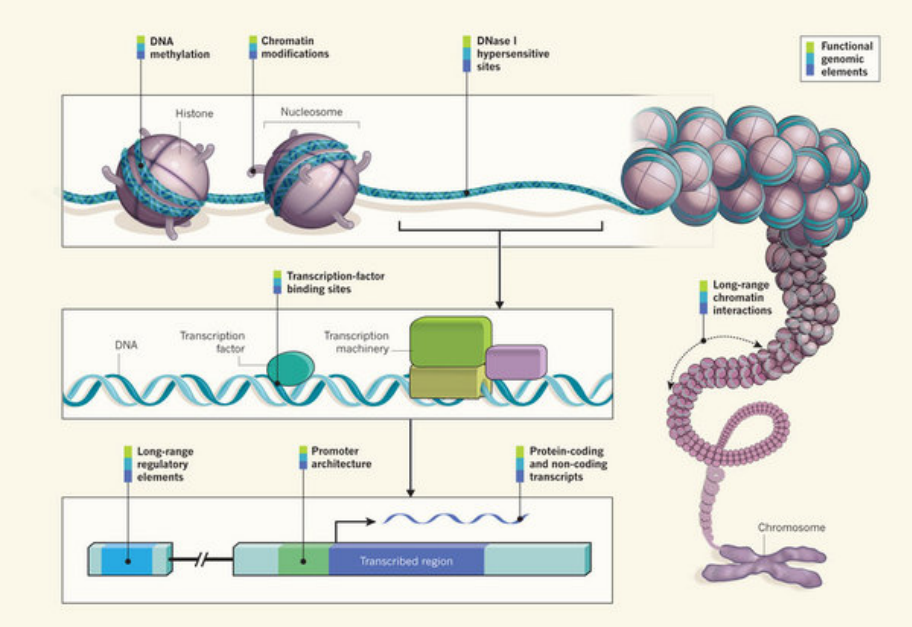


Figure 12.22
Replication from a single origin of replication in a circular bacterial chromosome.

REPAIR:
DNA Polymerase III – proofreading while it adds bases (excises the wrong strand and replaces with correct bases)
Repair enzymes POST-Replication proofreads the strand – if there is a mis-match, an entire section is excised and replaced by DNA polymerase I

Unit 2: Chapter 2 – Transcription



Terminology:

- RNA polymerase I transcribes rRNA in the nucleolus (part of the nucleus)
- RNA polymerase II transcribes mRNA and most snRNAs
- RNA polymerase III transcribes tRNA, 5S rRNA, some snRNAs and scRNAs

- Promoter: **Control sequence initiates transcription**
- Transcription unit: **Portion of gene that is copied into RNA**
- Terminator: **Signals the end of transcription of a gene**

Pre-initiation:

1. Transcription factors bind to the TATA box near the promoter region
2. Recruits other TFs and RNA polymerase = transcription initiation complex
3. DNA is unwound

Initiation:

1. Synthesis begins
2. 5'→3' direction
3. Promoter clearance (about 23 nt before RNA pol is secure on the DNA – loses its tendency to slip away and prematurely release RNA)

Elongation:

1. RNA polymerase synthesizes RNA transcript according to DNA template (antisense strand as template)

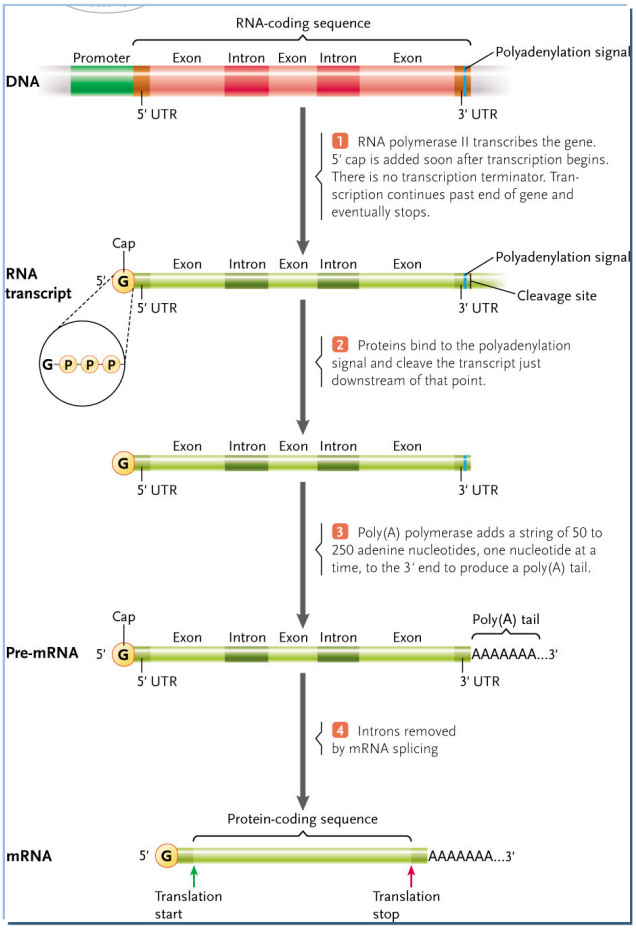
Termination:

1. Depending on eukaryote/prokaryote – will have different termination signals
2. But in any case, the RNA is cleaved and polyadenylated at the 3' end

3. Rho-dependent termination
    a. Termination (rho) factor stops DNA synthesis at specific rates (binds early on and travels faster down the RNA transcript than RNA polymerase travels down DNA therefore catching up to RNA pol eventually and cause it to dissociate)
4. Intrinsic (Rho-independent)
    a. Stops at GC rich sequence that leads to dissociation

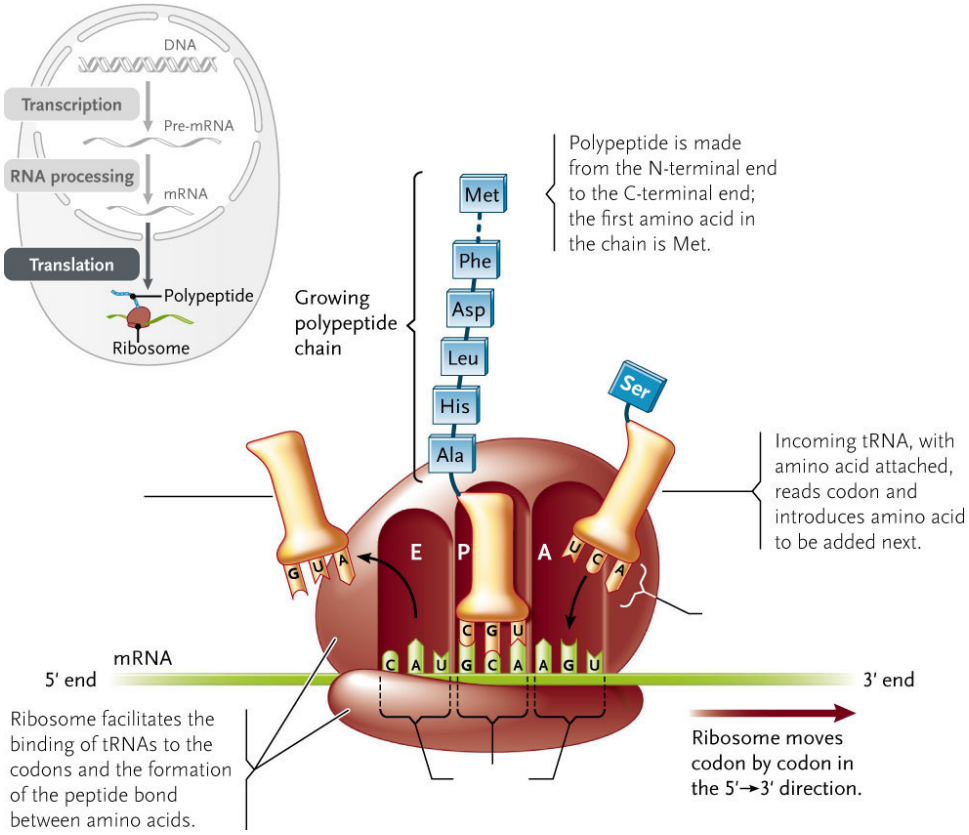
RNA Processing:

1. RNA Cleavage
    a. rRNA – cleaved into respective active mature rRNA transcripts
2. RNA Addition
    a. Non-template regions retain extra nucleotides (as in tRNA)
3. **RNA splicing**
    a. Excise the introns from exons and ligate resulting exons together
    b. snRNA complexes = spliceosomes
    c. Different combination of splicing results in different mRNA transcripts and ultimately different proteins
        i. *Alternative splicing*
4. **mRNA Capping**
    a. Attach a 7meG cap to the guanosine at the 5'-end
    b. Allows more efficient translation (recognized by enzymes in the cytosol)
5. **Polyadenylation**
    a. Add about 50-250 adenine nucleotides
    b. Protect the end from degradation
6. Nucleoside Modification
7. RNA Editing
    a. C→ uracil
    b. A→ inosine (analogous to G)



Unit 2: Chapter 3 – Translation

A site: the site where the charged aminoacyl tRNA enters the complex (except for the first aminoacyl tRNA, Met-tRNAMet or fMet-tRNAfMet, which enters at the P site).
P site: the site of synthesis where the peptidyl tRNA is formed in the ribosome.
E site: exit site where the now uncharged tRNA leaves after the amino acid has been added to the growing peptide chain.



Genetic Code:

- 3 bases = 1 codon to allow 3^4 = 81 possible combinations with ATCG
- Start Codon → AUG (specifies for methionine)
- Sense Codon → 61 codons specifying 21 amino acids
    - Redundant to mitigate mutation
- Stop Codon → UAA, UAG, UGA

tRNA

- Contains the Anti-codon that is complementary to the codon on the mRNA
- **Wobble hypothesis**
    - Third base in the codon is "wobbly" paired meaning that it is not necessary to be EXACT to incur the right amino acid attachment
- Aminoacyl-tRNA = tRNA bound to amino acid
    - Specificity determined by anticodon and other bases as well as other enzymes

rRNA

- Prokaryotes
  - Small subunit = 30S
  - Large subunit = 50S
    - Together = 70S
- Eukaryotes
  - Small = 40S
  - Large = 60S
    - Together = 80S

TRANSLATION (can be simultaneous – polysomes)

Initiation

- Initiator tRNA (Met-tRNA) with GTP binds to small subunit
- Complex binds to 5'-cap of mRNA and scans the sequence till it reaches the AUG (start codon)
- Large subunit binds – hydrolyzes GTP – and completes initiation

Elongation

- Aminoacyl-tRNA enter at A site → Peptidyl transferases catalyze formation of peptide bond and cleaves tRNA at P site → movement to next codon (tRNA from P→E and released, new peptidyl-tRNA moves from A→P) → A site open to accept a new aminoacyl-tRNA
- **Where does GTP fit into all of this?**

Termination

- A site reaches a stop codon
- Release factor/termination factor binds to A
- Polypeptide chain is released from P site

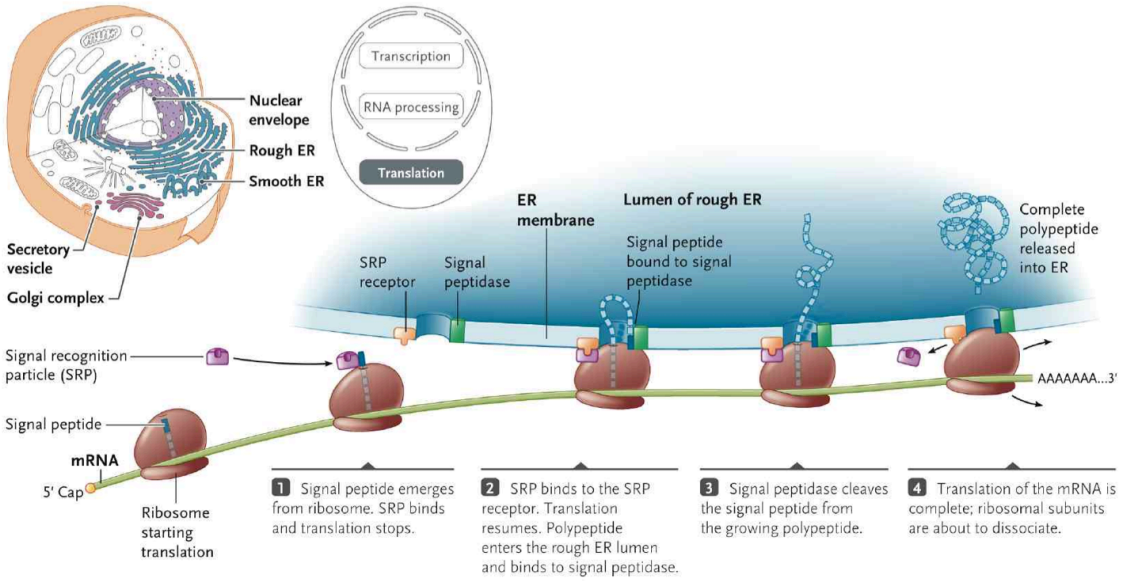
Unit 2: Chapter 4 – Protein Targeting

1. Protein trafficking or sorting or targeting-what is it and why is it important??
    - Protein Targeting = mechanisms to transport proteins to the correct destination intra- or extra-cellularly
    - This is important because all cells contain membrane bound compartments – and correct measures need to be taken to ensure the right protein gets to the right place!

Steps to protein targeting:

1. Translation
2. Interaction with receptor and unfolding (requires a receptor and signal peptide – about 20-50 aa's long (hydrophobic core) recognized by the signal recognition particle)
3. Translocation (signal peptide is cleaved from the protein peptide)
4. Refolding within new space

Transport to ER = first step to further protein transportation (exported in this manner if no additional information is present)



2. Proteins do not function without context. They interact with other macromolecules and within the correct context (part of the cell).

3. With the exception of proteins made in organelles, all proteins are translated in the cytoplasm. How to they get to their correct destination? How do they cross membranes? What are these different destinations and what are the signals or tags (targeting information) that direct the proteins to them?

- In the Lumen
    - Glycosylation
    - Retention

- Next Steps
    - Lysosome
    - Golgi
    - Vacuole
    - Nucleus (through NLS)
        - Transportation through budding vesicles

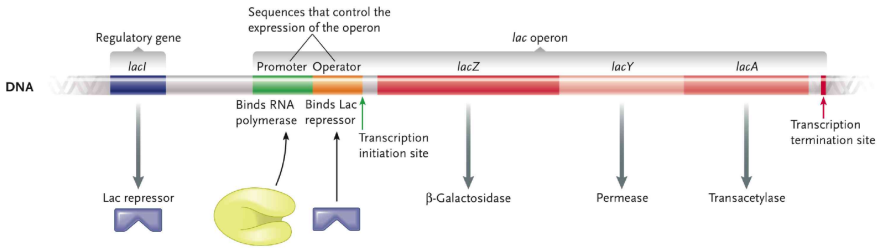
Target Organelle	Usual Signal Location within Protein	Signal Removal
Endoplasmic reticulum	N-terminal	(+)
Mitochondrion (into matrix)	N-terminal	(+)
Chloroplast (into stroma)	N-terminal	(+)
Peroxisome	C-terminal	(−)
Nucleus	Internal	(−)
Vacuole	C-terminal or N-terminal (if via ER and Golgi)	(+)

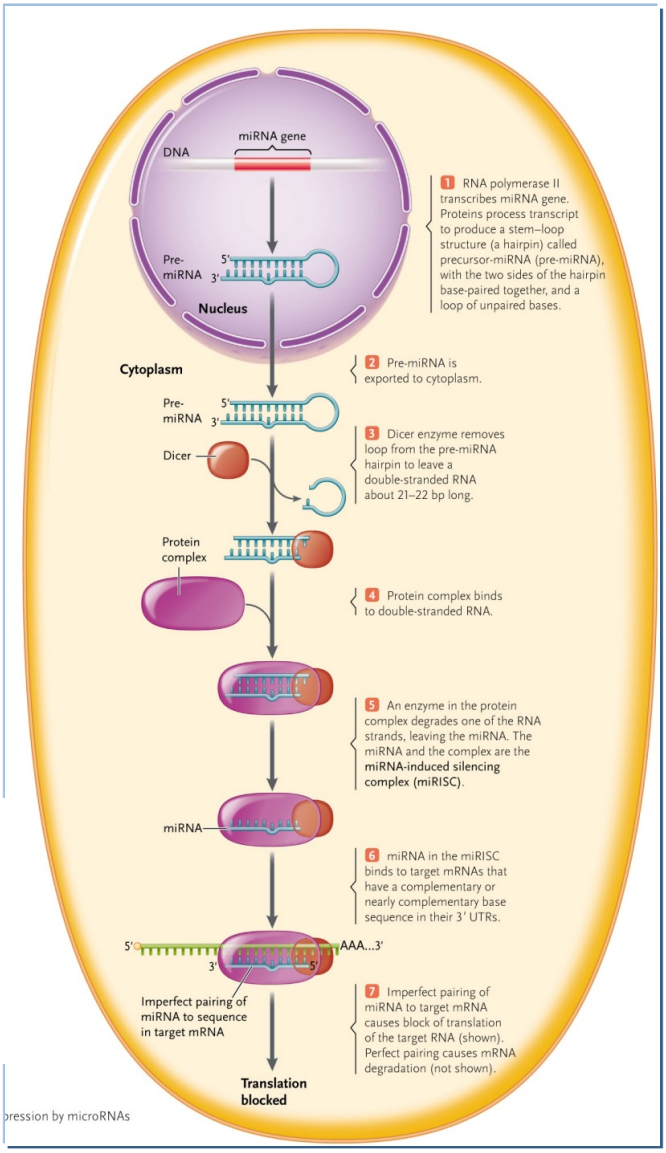
Unit 2: Chapter 5 – Regulation

Regulation may occur at:

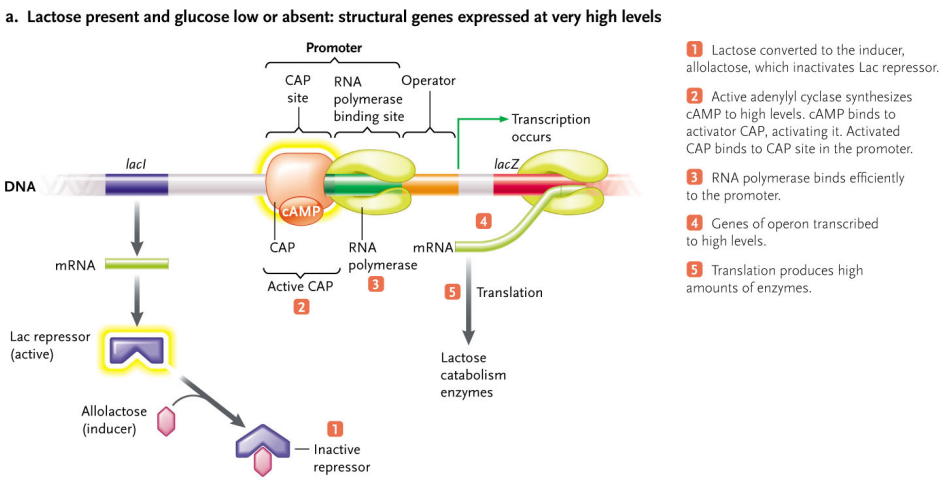
- Transcription
- Post-transcriptional – regulate mRNA availability (processing/breakdown – miRNA/siRNA)
- Translation – rate of protein synthesis
- Post-translational – controls availability of functional proteins

1. Transcriptional Regulation
   - TATA Box – controls gene expression by binding the TFs and polymerase
   - Repressor/Activator domains – bind repressor and activator proteins
   - Eg. *Lac* operon



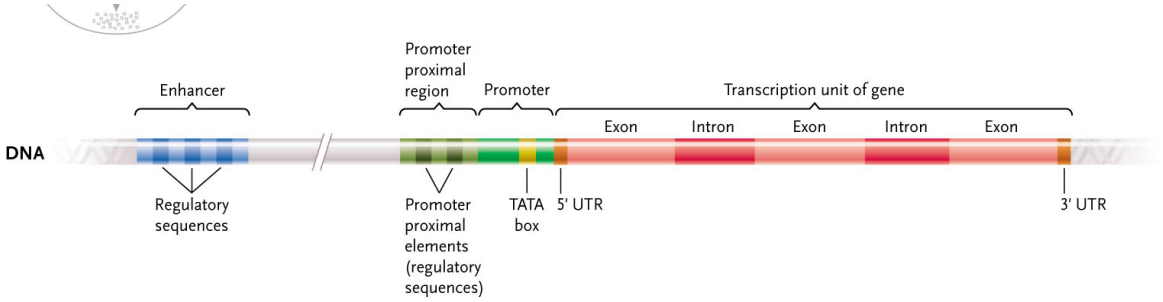


   - **UNDERSTAND THIS and all its constituent parts**
   - It is an inducible operator because it is inducible by specific molecules in the environment
   - Think of it logically
     - The *lac* operon is designed to express proteins that break down GALACTOSE
     - If there is no galactose in the environment – there will be no need to break down galactose! – it would be a waste of energy!
     - Likewise, galactose takes MORE energy to break it down than does glucose, so even if there is galactose – the organism will PREFERENTIALLY break down glucose
     - Therefore, the ABSENCE of glucose and the PRESENCE of galactose will result in the induction of the *lac* operon
   - Specifically
     - Decrease in glucose = high cAMP levels → binds to CAP = activator
     - BUT – lac repressor is always expressed and inhibits the gene
     - Therefore to ALLEVIATE this repression – galactose in the surroundings must bind to it and release it from the promoter → turns the gene on



In Eukaryotes

- Chromatin plays a huge role
- Hetero- vs Euchromatin
- Methylation and acetylation



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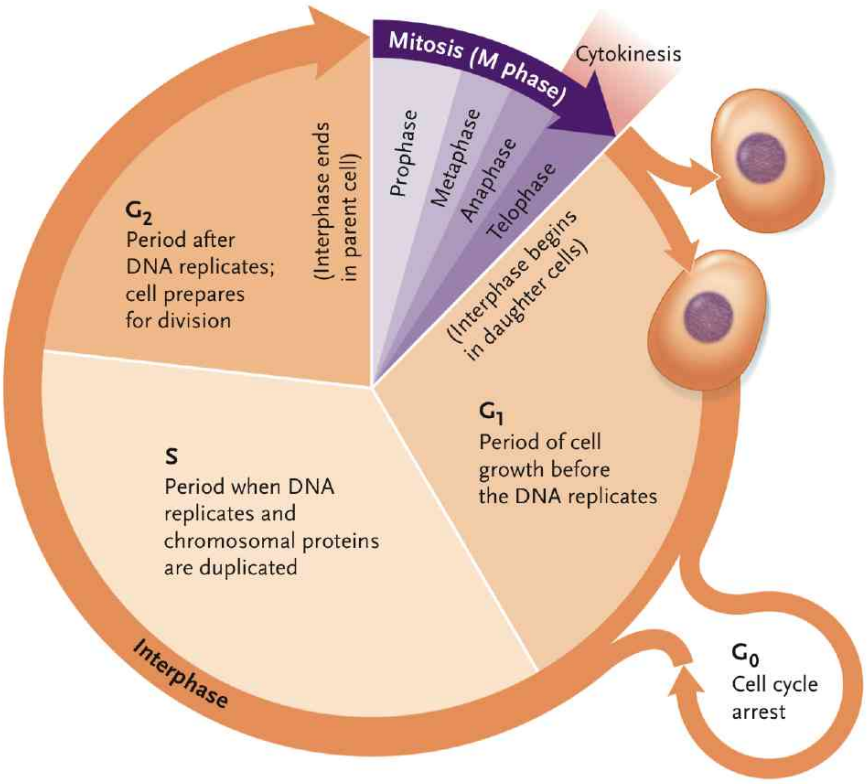
- Activators and repressors will bind to the regulatory sequences which will either directly act on the promoter, or indirectly recruit other factors that changes chromatin structure
- *Genomic imprinting:*
    - Permanent silencing of maternal/paternal allele
    - Inherited methylation

Can you answer these questions?

1. **What is a gene?**
2. **What is a transcript?**
3. **How does transcription work?**
4. **How does a cell regulation transcription?**
5. **How does regulation respond to external factors?**

Unit 3: Cell Cycle



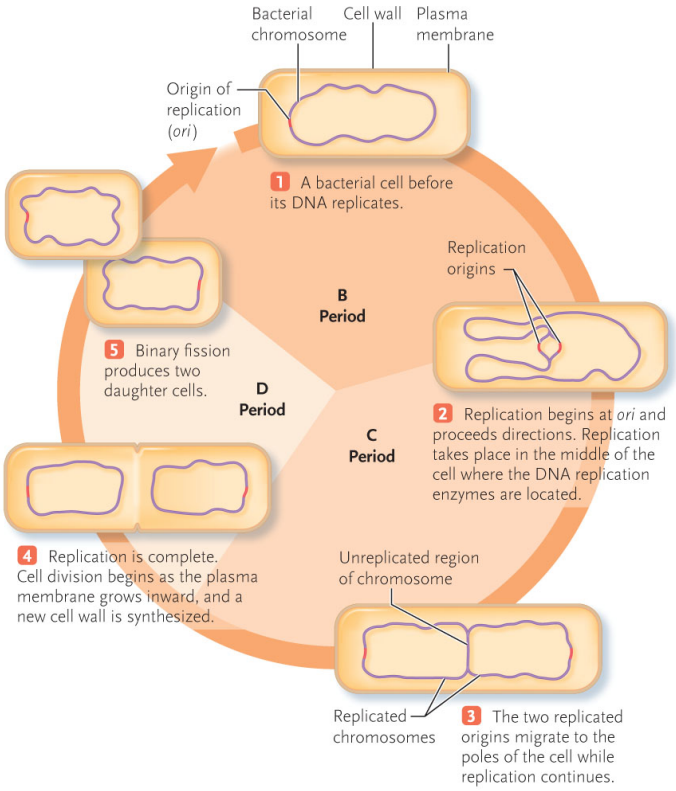
Interphase (G1-S-G2) → Mitosis (Prophase, Prometaphase, Metaphase, Anaphase, Telophase)→ Cytokinesis → Interphase

PHASE		Activity
Interphase	G0	Resting phase – cells stop dividing
	G1	Metabolically active – grows – but does not replicate
	S	DNA replication
	G2	Growth continues – proteins synthesized in preparation for mitosis

Cyclins and cyclin-dependent kinases = internal controls that directly regulate cell division
- cyclin B/A + CDK1 (adds phosphate groups and activated when bound to a cyclin) = Maturation promoting factor
- addition of phosphate groups = major regulatory step
- Controlled by internal and external factors
- The binding of specific cyclins to its corresponding CDK = checkpoint and determines what stage the cell is at based on the specific phosphorylation activities of that cyclin-CDK complex

P27 = inhibitor of cyclin dependent kinases (CDK) → because CDK is largely active during replication and DNA synthesis – the degradation of p27 is stark at these times (through ubiquitination or proteolysis)

Prokaryotes:



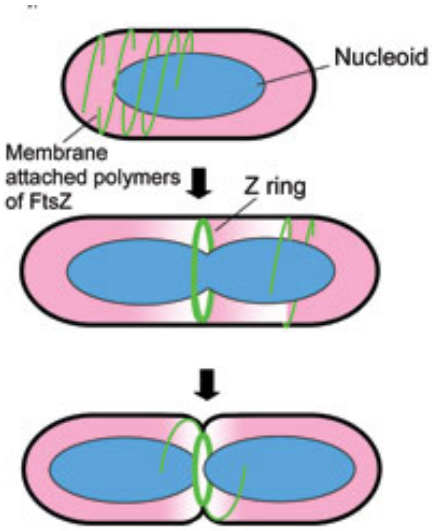


Figure 8.3
The bacterial cell cycle. During the B period, from birth to the initiation of DNA replication, the cell grows in size. The chromosome is replicated and the resulting daughter chromosomes move to opposite ends during the C period. Then the cell divides by binary fission during the D period. In very fast growing cultures, the B period may be nonexistent; cells may be born with chromosomes that are already partly replicated!