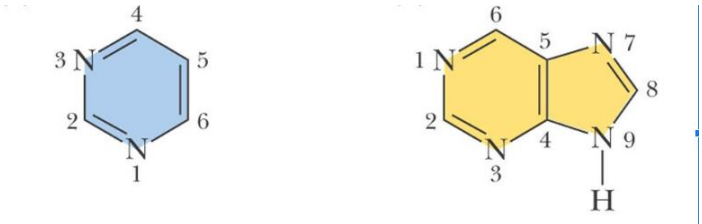
Unit 2 Exam Study Guide

DNA Structure and Organization

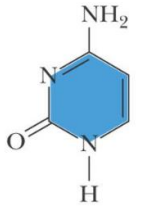
Nitro Bases

- Insol in h2o bc arom

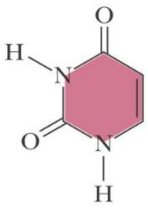


The pyrimidine ring

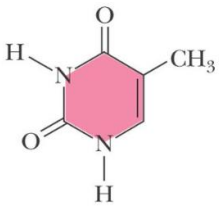
The purine ring system



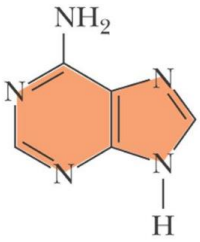
Cytosine
(2-oxy-4-amino
pyrimidine)



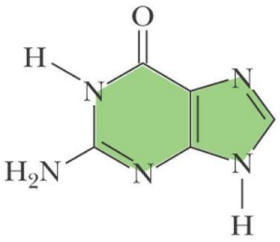
Uracil
(2-oxy-4-oxy
pyrimidine)



Thymine
(2-oxy-4-oxy
5-methyl pyrimidine)



Adenine
(6-amino purine)



Guanine
(2-amino-6-oxy purine)

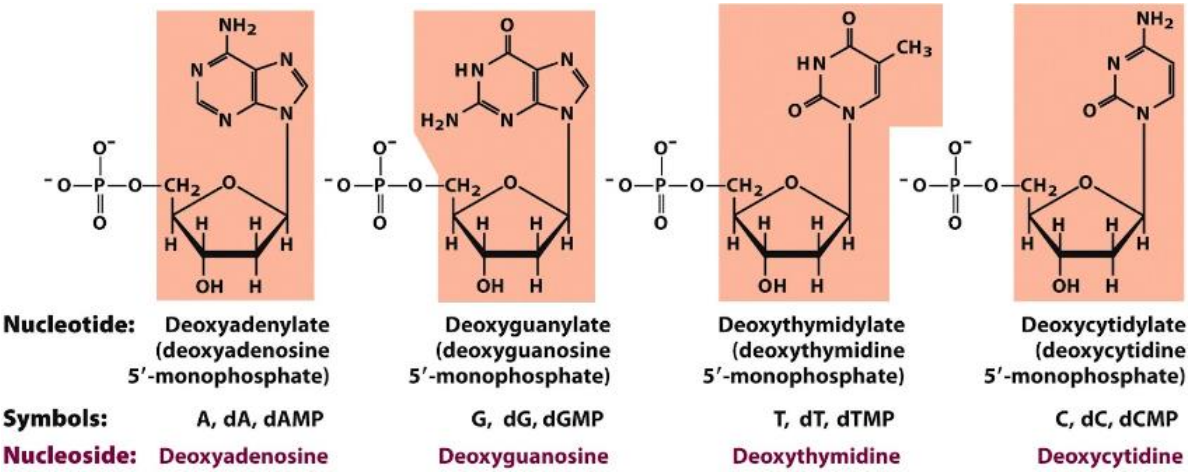
Nucleoside

- 9'N of pur or 1'N of pyr + glyco bond + 1'C deoxy/ribose sugar
- Sugar addition makes molec more water sol
- DNA more stable since deoxy; OH at 2' (on ribose) has effect on 2ndry struc & hydrolysis
- Syn conf favored by pur more than pyr bc steric hind
- Common ribonucleosides (ribose+nitro base): CYTOdine, URidine, ADENosine, GUANosine → pyr get -dine while pur get -sine

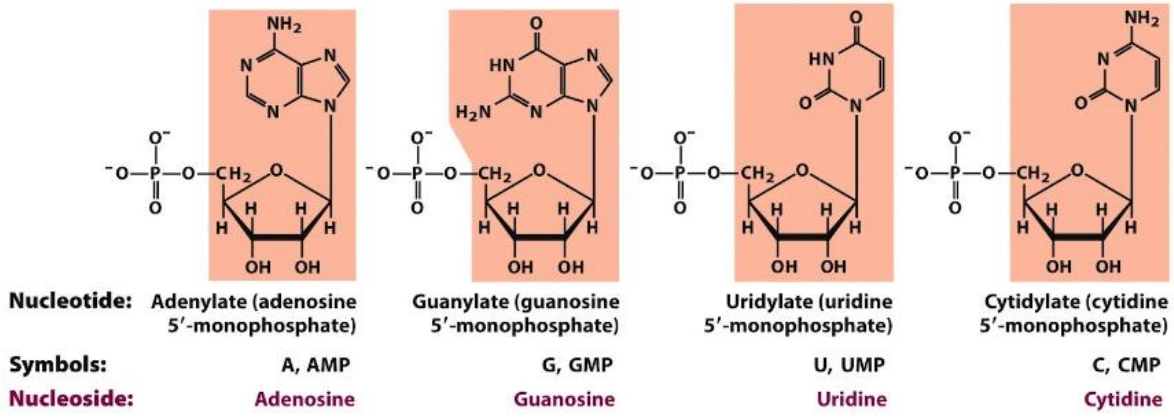
Nucleotide

- 5'C Nucleoside + esterification bond + phosphate
- DNA nuc = deoxyribonucleotides; RNA nuc = ribonucleotides

1) Deoxyribonuc Types:



2) Ribonuc Types:



3) Other Nuc Types
   - Used as epigenetic marker (pro: mark own DNA to degrade foreign; eu: mark which genes should be active)
   - DNA Only
     a) 5-Methylcytidine: found in eu/ bac
     b) N6-Methyladenosine: found in only bac
   - RNA Only
     a) Inosine: (adenosine w/o amine) gives rich gen code and found in wobble pos in anticodon
     b) Pseudouridine: helps fold rRNA, stabilize tRNA, found more in eu than eubac

Nucleoside 5' Triphosphates

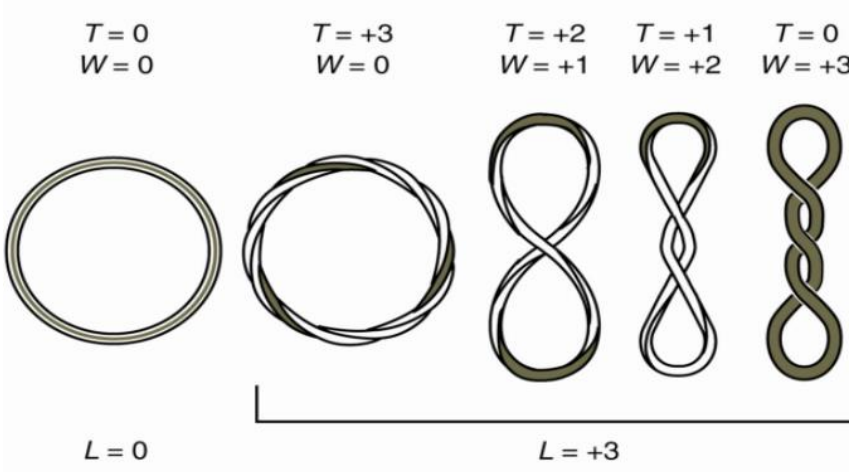
- Types: ATP (energy), CTP (lipid syn), UTP (carb met), GTP (prot syn)

- Function: E carriers; E stored in phosphoric bonds (explains E tranf in ADP to ATP)
- Major bchem rxn of nuc: pyro/phosphoryl group transf (ADP → ATP, GDP → GTP, etc)
- Cyclic nucleotides: sig molec/ regulators of cell met of all cells (cAMP, cGMP etc)
- Bases make nuc unique and can be used as recognition units; not involved in the biochem of met

Nucleic Acids

- Cov bond via phosphodiester link 3'C → 5'C

1) Primary Struc → Sequencing
    a) Chain Termination Method (F. Sanger)
    - DNA pol adds artificial primer and adds nuc to ssDNA
    - Last one will be dideoxynuc to terminate strand
    - Similar to PCR
    b) Base-Specific Chemical Cleavage (Maxam & Gilbert)
    - Using chem agent, cleave strand at certain places of ssDNA with P-32 at end
    - Produces fragments that are read in electrophoresis based on ACTG
2) Secondary Struc: reassociation is inversely prop to genome complexity (the more complex the organism, the less time it would take to anneal after denaturing)
3) Tertiary Struc
    - Duplex DNA (dsDNAO = 10bp / turn)
    - Linking Number (L) = Twist (T)+ Writhe (W).
    - Twist = helical turns, writhe = # times double helix crosses over itself
    Ex of pos supercoiling:

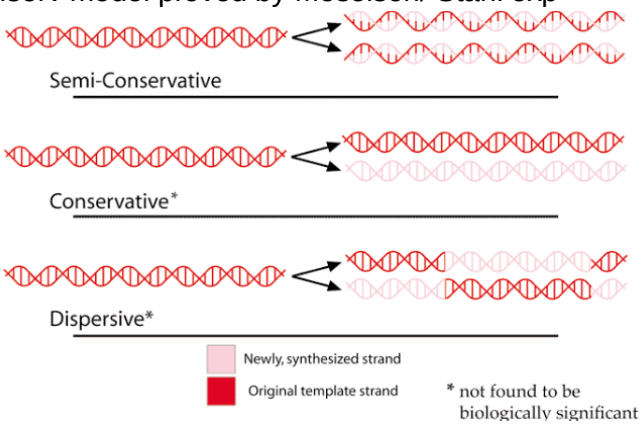


    - DNA gyrase creates neg supercoiling so easy unwind → A-subunit of gyrase cuts dna, holds ends, and ligates to form new supercoil

DNA Replication

Models

- Semiconserv model proved by Meselson/ Stahl exp



Elongation

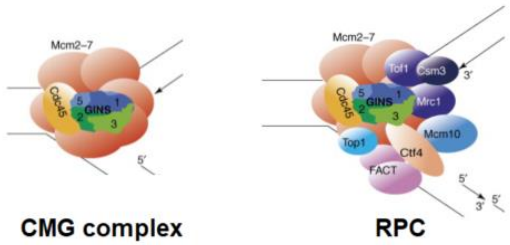
- Primase lays primer, gyrase undos supercoil, helicase unwinds, SSB prot attached
- DNA pol 3 adds new nuc: dNTP w/ 3 PO4 undergoes nuc attack on 3'OH to rid 2 PO4
  - ➔ Pol 3 subunits: alpha (polymerase), epsilon (3' exonuclease), theta (holoenzyme assembly)
  - ➔ Beta-subunit acts as a sliding clamp holds all subunits together
- Pol 1 (5' exonuclease) in pro only and is used for repair and primer removal
- DNA ligase glues 2gthr nicks made by pol 1 via phosphodiester linkage

Replication Experiments

- Rep is slow (50 nuc/sec) and activates in clusters 30k-300k bp apart (not all at same t) to create rep bubble pairs
- High condensed rep late, low rep early
- Exp to identify rep origins in yeast → yeast DNA seq called ARS (autonomously replicating sequence)

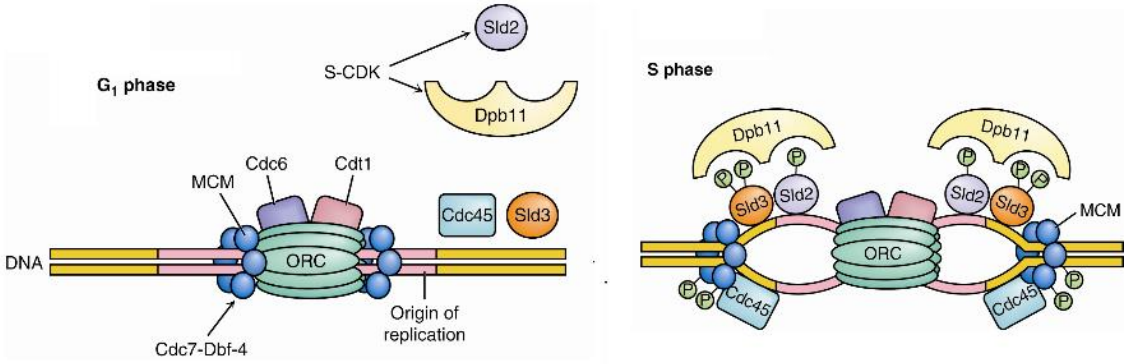
Origin Replication Complex

- Pre-replication complex (pre-RC) assembled via addition of Cdc6, Cdt1, MCM and ends in post-RC state
- *At rep fork of yeast,* **C**dc45 + **M**CM + **G**INS form **CMG** complex
- CMG complex + other factors = RPC (replisome progression complex)



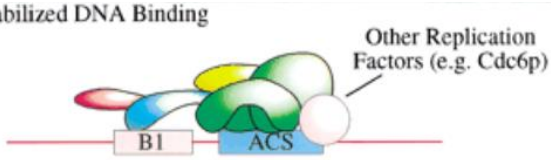
CMG complex **RPC**

- Overall Process:
  a) ORC binds to origin and recruits Cdc6, MCM (rep licensing factor), and Cdt1
  b) Ppl (phosphorylation) mediated by S-CDK
  c) Cdc7-Dbf4 trigger the switch from G1 to S phase
  d) Ppl of Sld2 and Sld3 leads to recruit pol to origins
  e) Ppl of MCM serve as helicase activ



- Mechanisms that may control ORC chrom binding sites:

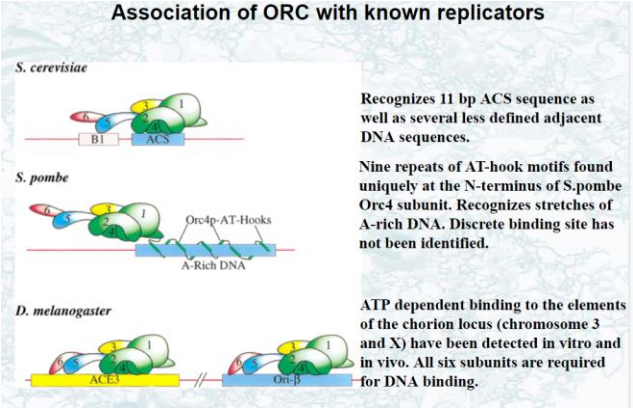
A. Stabilized DNA Binding



B. Recruitment

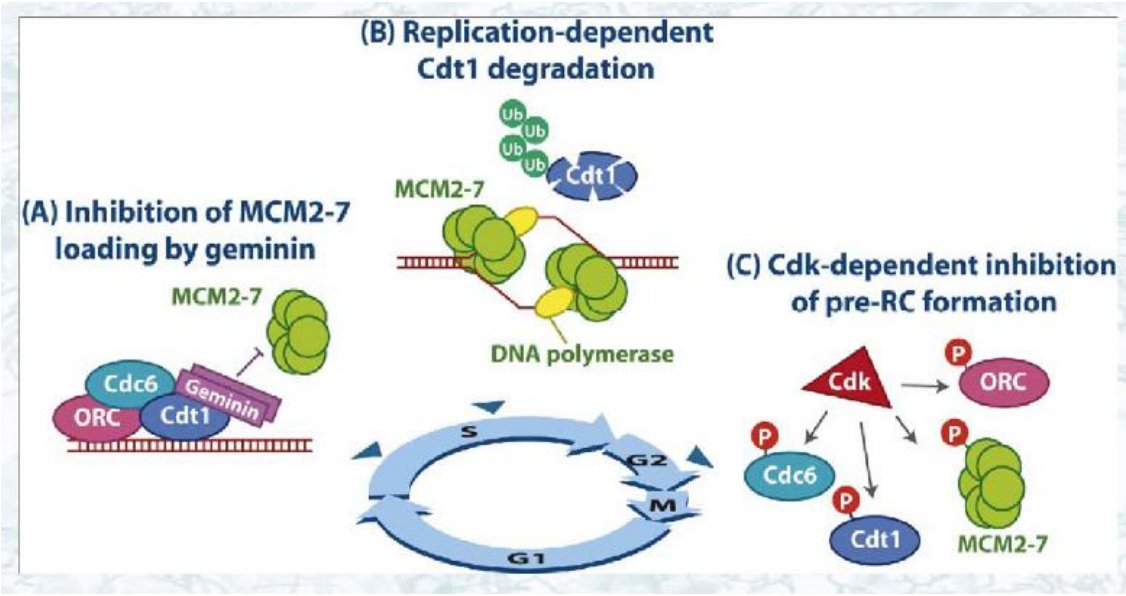
C. Local Chromatin Structure

- Rev this slide



Re-Replication Prevention

- Prevention by pre-RC Formation:
  a) Inhibitory complex between Cdt1 and geminin
     ➔ Geminin is dna inhibitor that appears in S phase and disappears in M phase)
     ➔ Geminin prevents MCM from forming in pre-RC
  b) Cdt1 degradation in S phase by Skp2
  c) High activity of cdk in G2/ M phases inactivates and degrades Cdc6, Cdt1, MCM2-7, and ORC



- Prevention by CDK-Mediation:
  a) Ppl of Cdc6 and Cdt1 renders them unstable/ degrades them
  b) MCM mod by cdk inactivates it
  c) ORC ppl inhibits rep

DNA Mutation, Repair, and Recombination

Homologous Recombination

The Holliday model for homologous recombination.

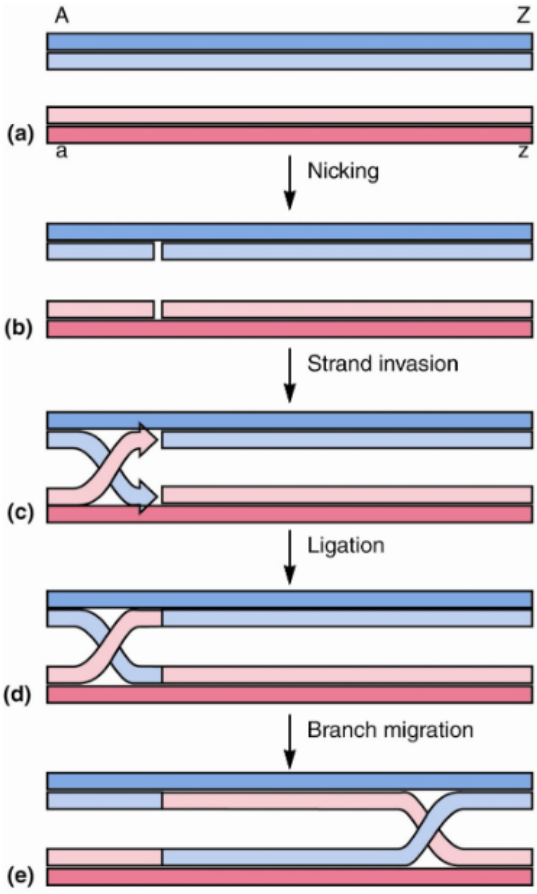
(A) Two homologous DNA duplexes are aligned – synapsis.

(B) Recombination begins with the introduction of single-stranded nicks at homologous sites on two chromosomes

(C) Strand invasion occurs through partial unwinding and base-pairing with the intact strand in the other duplex

(D) Free ends from different duplexes are ligated resulting in cross-stranded intermediate – Holliday junction

(E) Branches can migrate by unwinding and rewinding of two duplexes



- Bacterial
  - RecBCD: initiates recombo, helicase (D)/ nuclease (B)activ, c chi site
  - RecA: strand invasion and homologous pairing
- Eu
  - RuvA tetramer: helicase, branch migr, fits into Holliday jxn
  - RuvB hexameric rings: helicase, branch migr, assemble on DNA sides and acts as motor
  - RuvC resolvase: nuclease activ, branch migration

Transposons

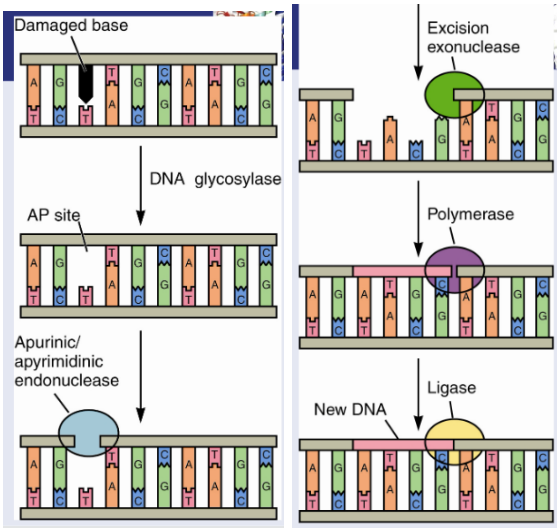
- Class I (retrotransposons)
  - copy/paste
  - DNa→RNA→DNA via reverse transcriptase
  - 40% of human genome
- Class II (DNA transposons)

- cut/paste
- No RNA intermediate
- Uses many transposon enzymes

DNA Repairs

- 150 genes involved

1) Double strand break (ds DNA)
    - Nonhom DNA: break in dsDNA (looks like =) → Ku 70/80 holds ends → kinase, enzymes, and ligase fix break
    - Homo: break in dsDNA (looks like __--) → D-loop like RecA in homo recombo → either noncross or crossover recombo
2) Direct reversal
3) Mismatch repair (ss DNA)
4) Base excision repair (ss DNA):
    a) Dna glycosylases remove damaged base (creating an apurinic/apyrimidinic or "AP" site)
    b) AP endonuclease cleaves backbone
    c) Exonuclease removes AP site/ several nuc
    d) Exp repaired by DNA pol/ ligase



5) Nuc excision repair (ss DNA):
    - Used instead of base excision if repair region too large
    - Lesion can be like pyr dimer (UV light bonds nuc, so use light to break them)
    - Uses exinucleases which break backbone in 2 places
        - Bacteria: ABC exinuclease (UvrA, UvrB, UvrC) hydrolyzes 5th bond on 3' and 8th bond on 5' and removes ~12 nuc
        - Eu: exinuclease hydrolyzes 6th bond on 3' and 22nd bond on 5 and removes ~28 nuc