Notes 03/31

Monday, March 31, 2008
10:58 AM

- Polynucleotides
  - Found in DNA and RNA
  - Precursors of polynucleotides

- Biological roles of nucleotides
  a) Energy "currency" (ATP and GTP) - coupling hydrolysis to thermodynamically unfavorable reactions (>10^8)

$$X \rightarrow Y \quad \Delta G >> 0$$
$$K_{eq} \; \frac{Y}{X} << 1$$
$$\Delta G' = -RT \ln K_{eq}'$$

$$X + ATP \xrightarrow{P_i} Y + ADP \quad \Delta G << 0$$

  b) Carriers
     - carbohydrate phospholipids

$$\text{eg UDP-Glu} + \text{Glycogen}_n \xrightarrow{\text{UDP}} \text{Glycogen}_{n+1} \quad (\alpha 1\text{-}4)$$

$$\text{eg CDP-diacylglycerol} \rightarrow \text{cardiolipin}, \text{phosphotidyl}, \text{phosphotidylglycerol}$$

  c) Nucleotide cofactors
     - NAD, NADP, FMN, FAD, coenzyme A

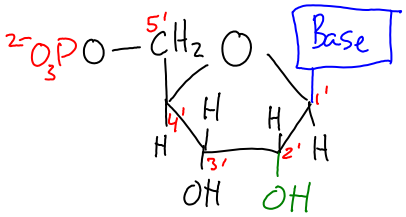
  d) Metabolic regulators
     - NTP, NDP's, NMP's (noncyclic)
     - cNMP's (cyclic amp,gmp... second messengers)
     - Allosteric effectors

  e) Precursors of RNA and DNA
     - Future lectures will focus on this

  f) "Catalytic" nuceotides
     - (ribozymes)
     - Act as nucleophiles to perform transesterification reactions

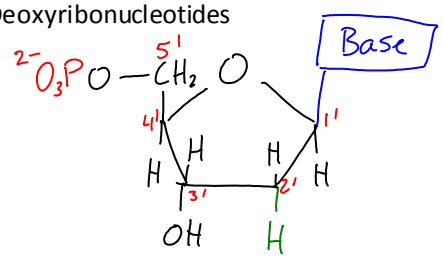
- Structure of Nuceltides
  - 3 components
    - Base (purine or pyrimidine)
    - Pentose sugar
    - Phosphate

- Ribonucleotides

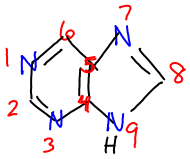


  - 1' carbon is attached to base
  - Glycosydic linkage - bond of base to sugar

- Deoxyribonucleotides

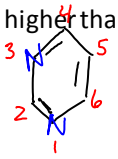


- Base components
  - Purine
    - Aromatic
    - Planar
    - Weak base
    - Low H_2O solubility
    - Absorb light in UV range



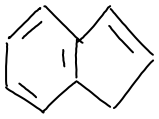
  - Know # positioning
  - 9 position joins to sugar

  - Pyrimidine
    - Planar aromatic
    - Low H_2O solubility but higher than purine
    - Absorb UV light



  - 1 position attach to sugar

- Bases in RNA+DNA
  - 6-amino purine



  - Nucleoside is nucleotide without phosphate

Problem Set 1

Monday, March 31, 2008
11:00 AM

1. Draw the structures of the following:
   a. 5-methylcytosine
   b. 5-bromodeoxyuridine
   c. 1,3,7 trimethylxanthine
   d. 2-methoxyadenosine monophosphate
   e. Dideoxythymidine
   f. N^6 methyladenine
   g. O^4 methylthymidine

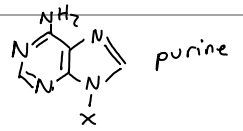
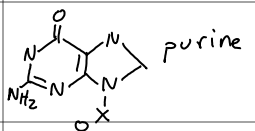
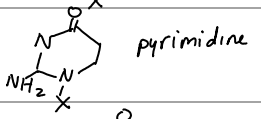
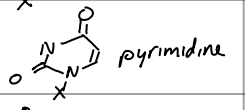
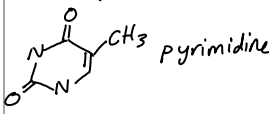
Notes 04/02

Wednesday, April 02, 2008
10:59 AM

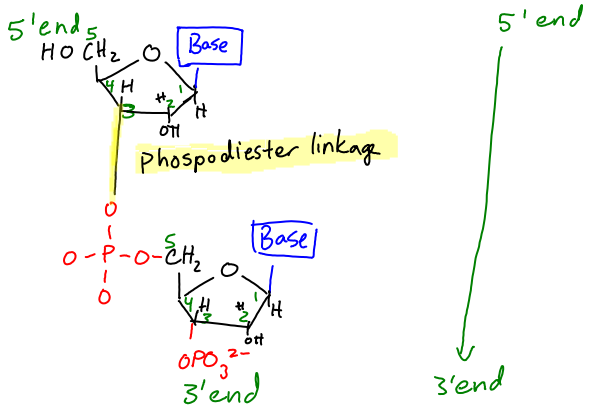
Office Hours:

McEntee	Wed. 2-3pm	Geology 4607
Benson Thurs/Fri	Thurs/Fri 12-1pm	Geology 4607
Angie	Wed 1-2pm	Geology 4607

- Numbering and naming conventions for bases
  - When attached to sugar without phosphate it is nucleoside
  - When attached to sugar with phosphate it is nucleotide

Base (no sugar or phosphate)	Nucleoside	Nucleotide	Structure
Adenine Ade A	Adenosine Ado A	Adenylic acid Adenosine monophosphate AMP	
Guanine Gua G	Guanosine Guo G	Guanylic acid Guanosine monophosphate GMP	
Cytosine Cyt C	Cytidine Cyd C	Cytidylic acid Cytosine monophosphate CMP	
Uracil Ura U	Uridine Urd U	Uridylic acid Uridine monophosphate UMP	
Tymidine Thy T	Deoxythimidine dThd dT	Deoxythymidic acid	

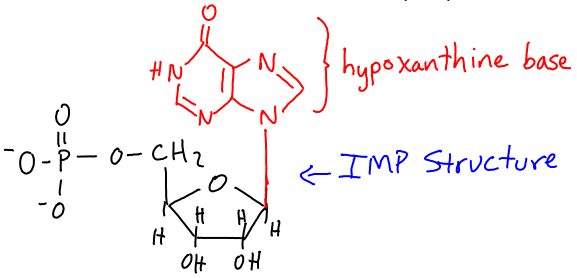
- Attachment of nucleotides.
  - Attachment of of 3' hydroxyl to 5' of other nucleotide. (Phosphodiester linkage)
  - Stereochemistry - base is aromatic/planar. Sugar atoms are in another plane perpendicular to the bases.
  - Attachment made from condensation



- Differences between DNA and RNA
  - Uracil instead of thyamine.
  - Ribose sugar instead of deoxyribose
    - The hydroxyl group effects stereochemistry and prevents flexibility of RNA chain
    - The hydroxyl group also effects the stability. Under basic conditions (pH 9 or >), OH is nucleophile

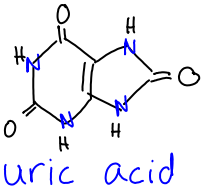
and attacks phosphate to form new covalent bond
    - This determines the lifetime of RNA. Bacteria for example have enzymes to create lifetime of 3 minutes for RNA. Humans is about 24hours.
    - DNA is a stable molecule because it lacks this hydroxyl group.

- De novo Synthesis of Purines
    a. Evolutionary conserved pathways
        - Different organisms though diverse, follow similar pathways with same intermediates.
    b. Expensive synthesize
        - Costs a lot of energy to make new purines from scratch
    c. Enzyme activities highly regulated
        - Activated more during cell division
        - Enzyme regulation in allosteric fashion
    d. NOT synthesized at free bases but as nucleotides
        - This serves to keep bases soluble in aqueous polar environment since the free base alone is not soluble.
    e. AMP, GMP -> derrived from common intermediate (IMP)

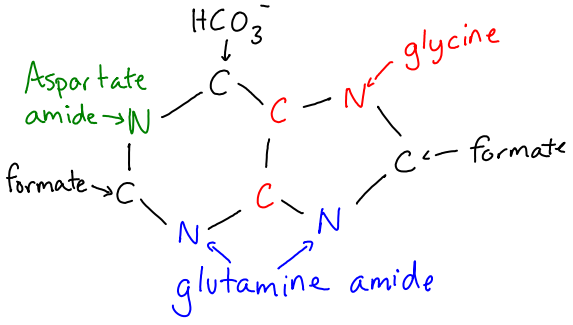


    a. Pathway enzymes are target for clinical intervention
        - Cancer chemotherapy
            - Target enzymes or steps in de novo pathway
        - Autoimmune and organ transplantation
        - Antimicrobials (antibiotics)
            - Target bacteria enzymes that carry out purine synthesis without effecting the host

    - John Buchanan
        - Used metabolic labeling - using radioactive isotopes to discover metabolic pathways
        - He studied metabolic pathway of uric acid.
        - Discovered the source of synthesis of uric acid.



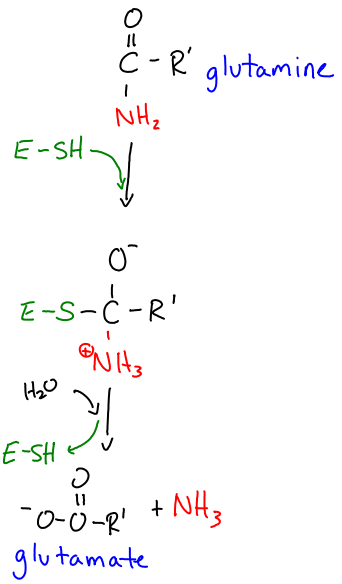
Ucic acid is in bird poop.



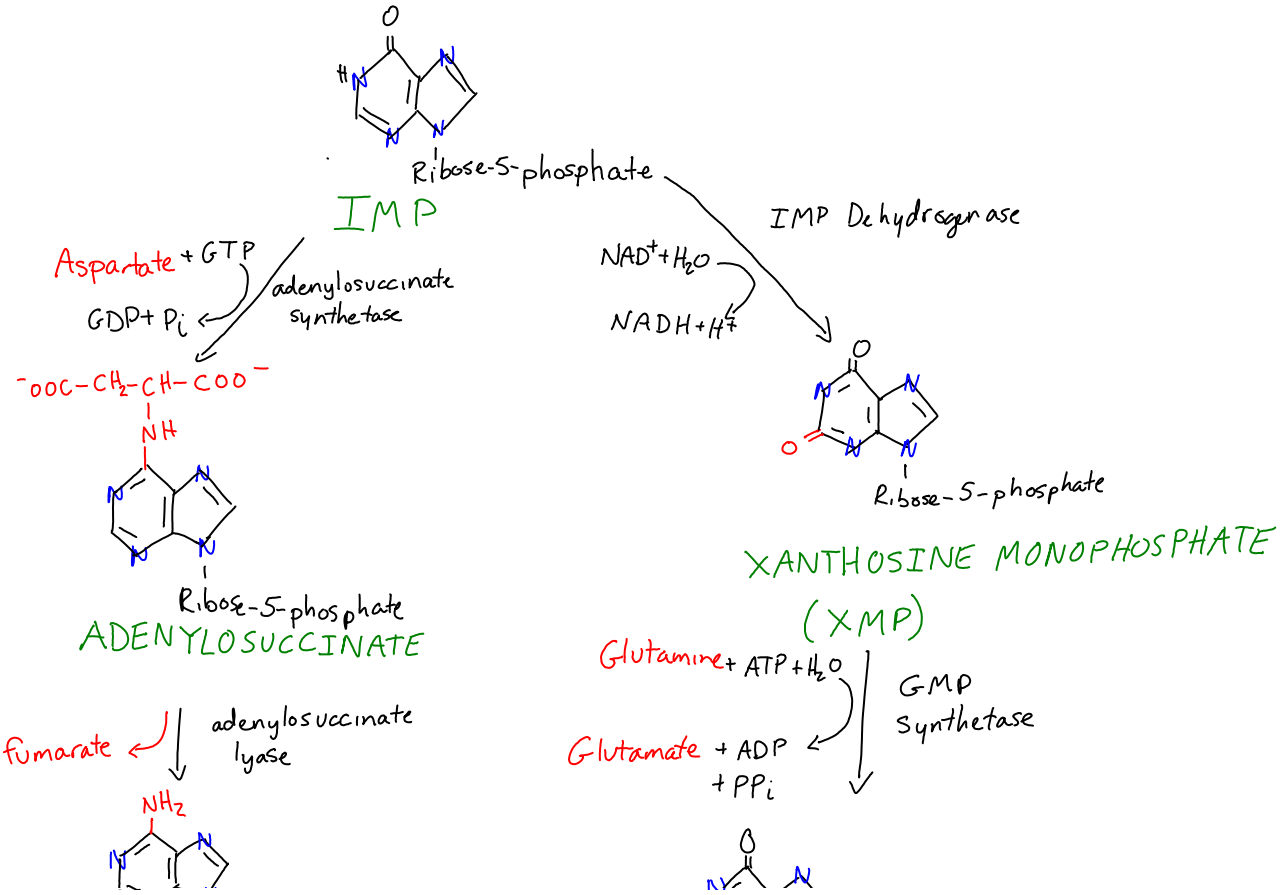
    - Do not need to know all enzymes and intermediates of nucleotide synthesis
        - Purine biosynthesis
            - Ribose-5-phosphate is starting.
            - ATP used to add diphosphate to 1' position

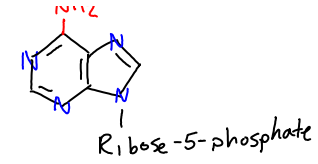
- Glutamate provides amide to replace diphosphate. This is first committed step and establishes stereochemistry of purine.
- Amide performs Nu attack on Gly
- Addition of one carbon by THF (carrier of 1 C)
- Conversion of carbonyl oxygen of glycine to imino group
- Etc.. See textbook. pg 1071

- Glutamine is often a source of amine addition in purine synthesis

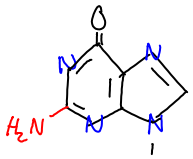


- IMP to AMP or GMP





AMP



GMP

- Conversion of AMP (GMP) to ATP (GTP)

a) AMP + ATP ⇌ (adenylate kinase) 2ADP

(ATP from oxidative phosphorylation)

GMP + ATP ⇌ (guanylate kinase) GDP + ADP

Base specific, sugar non specific (rAMP,
Nucleoside diphosphate kinase

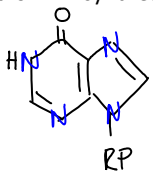
b) GDP + ATP ⇌ GTP + ADP

Base nonspecific, sugar nonspecific

Notes 04/04

Friday, April 04, 2008
11:02 AM

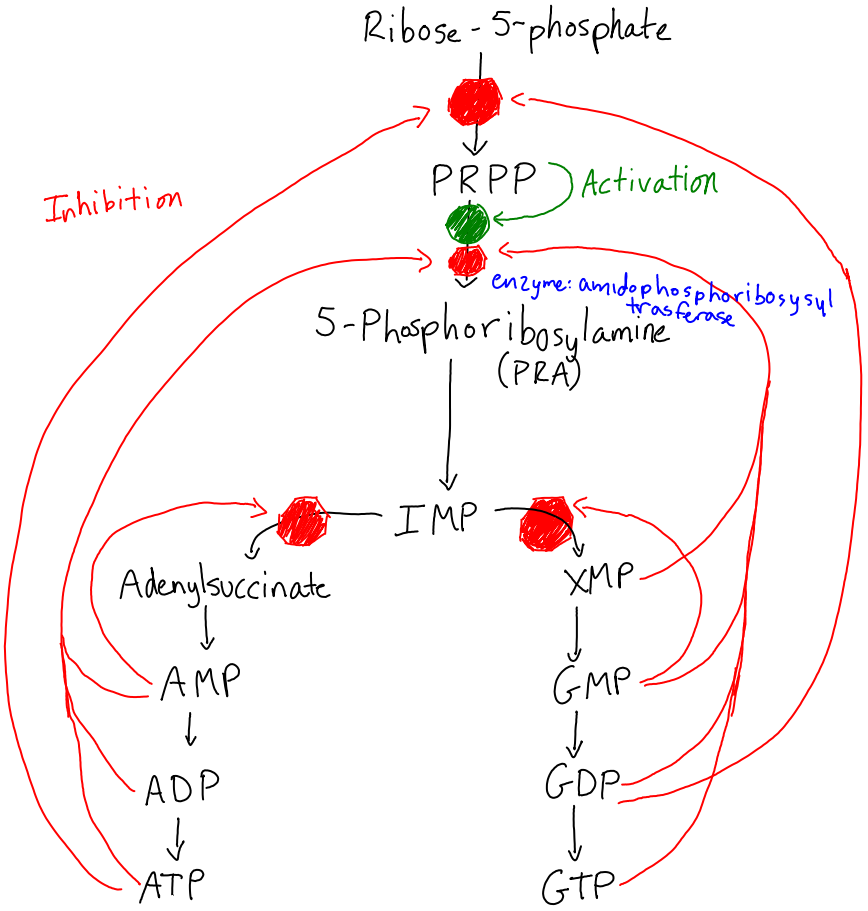
Energy cost for IMP synthesis



.	ATP -> ADP
a) Directl yin pathway	+7
b) Glu -> Gln (x2)	+2
c) Fumerate -> ASP	-3
d) Regenerate Gly	+8
e) N^{10}-THF -> N^{10}-THF-CHO (x2)	+10
TOTAL	+24

- ATP source is from oxidative phosphorylation

- Regulation of nucleotide synthesis.

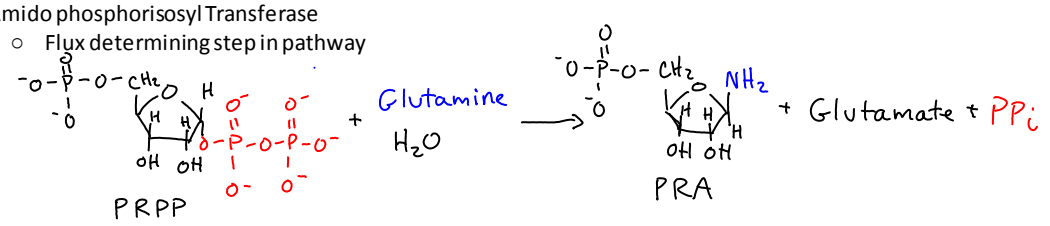


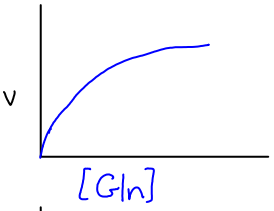
ATP and GTP are allosteric enzymes

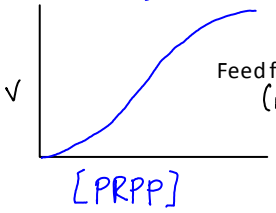
Feed forward activation. When PRPP levels are high, this drives amino transferase enzyme

this drives amino transferase enzyme.
Transferase for PRA synthesis is allosterically stimulated by PRPP (feed forward)

- Amido phosphorisosyl Transferase
  - Flux determining step in pathway







Feed forward.
(not Mechalis Menten Kinetics)
sigmoidal

- In cells that are proliferating have a greater demand for nucleotides than resting cells.
- Early antibiotics blocked purine synthesis… this prevented bacteria from growing.

- Sulfa drugs inhibit production of folate which impacts purine synthesis in bactera. Does not effect humans because our folate is dietary and we do not make folate.

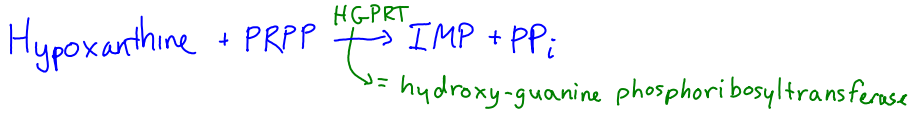
- Inhibitors of DeNovo purine Biosynthesis

Sulfa drugs	Block folate biosynthesis in microorganisms N^{10}-THF deficiency (no effect on human cells)
Azaserine (Acivicin)	Inhibits purine denovo synthesis. It was later discovered to be a potent mutagen. Suicide inhibitor of amido transferase and other enzymes that release activated NH_4^+ from Gln
Mycophenolic Acid	Fungus product that blocks IMP dehydrogenase enzyme.

- Salvage pathway
  - Recovering bases from breakdown of nucleic acids

- Salvage of Purines
  - Breakdown of RNA (intacellular)

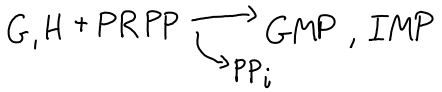
(Ade = Adenine)



NB post mitotic cells use salvage more than donovo synthesis
Neuronal cells -> salvage is major pathway for nucleotide biosynthesis.

There are individuals that lack HGRPT so they have disorder called Lesch -Nyans syndrome

- Defect in purine salvage
  - Lesch-Nyhans syndrome
    - x-limed (sex link)
    - Mental retardation
    - Spasticitiy
    - Self mutilation
    - Urate accumulation. Urate is insoluble and will rise to kidney stones and accumulates in joints. (Gout)
    - Loss of HGRPT

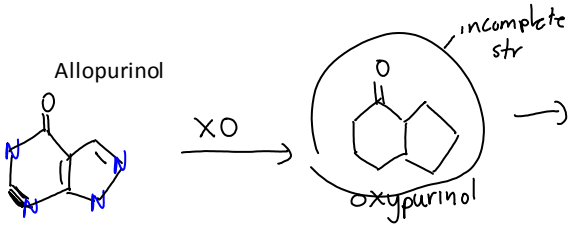


- In lesch-Nyhans

HGRPT -> accumulation PRPP
[PRPP] increase -> feed forward activates amide transferase
-> increase adenelate (amp) and guanelate (gmp) production

- Degradation of purines (figure in textbook) Fig 28-23 pg. 1093
  - Uric acid is end product of degradation

- Inhibitor of Xanthine Oxidase (treatment of Gout)



Remains bound to Xanthine oxidase (reduced) and cannot oxidize....

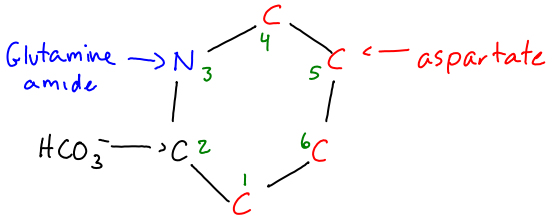
Notes 04/07

Monday, April 07, 2008
11:02 AM

Notes 0407

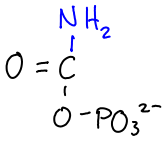
Audio recording started: 11:02 AM Monday, April 07, 2008

- Pyrimidine pathway
  - metabolic labeling by John Buchanan



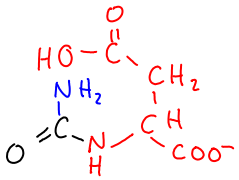
  - 6 step Pathway (see book pg 1077) for UMP synthesis
    - Ring structure is synthesized prior to ribose-5-phosphate moity

1. **2ATP + HCO_3^- + glutamine + H_2O**
   Enzyme: Carbamoyl phosphate synthetase II
   → 2ADP + glutamate + P_i



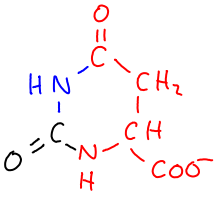
Carbanoyl phosphate

2. **Aspartate**
   P_i
   Enzyme: aspartate transcarbomoylase (ATCase)



Cabonyl aspartate

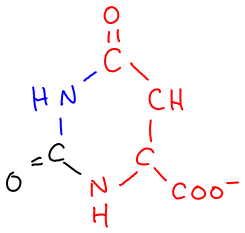
3. H_2O
   Enzyme: dihydroorotase



Dihydroorotate

4. Quinone → Reduced quinone

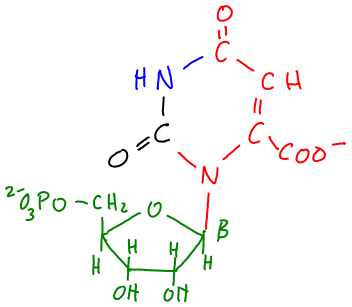
Enzyme: dihydroorotate dehydrogenase



Orotate

5. PRPP → PP_i

Enzyme: Orotate phosphoribosyl transferase

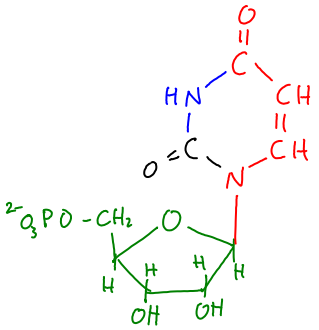


Orotidine-5'-monophosphate (OMP)

6. CO_2

Enzyme: OMP decarboxylase

Step 6 enzyme increases reactivity by 2×10^{23} making most catalystically proficient enzyme known

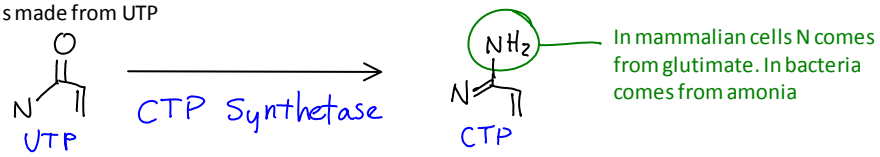


Uridine monophosphate (UMP)

UMP + ATP ⇌ (uridate kinase) UDP + ADP

UDP + ATP ⇌ (nucleotide diphosphate) UTP + ADP

CTP is made from UTP



In mammalian cells N comes from glutimate. In bacteria comes from amonia

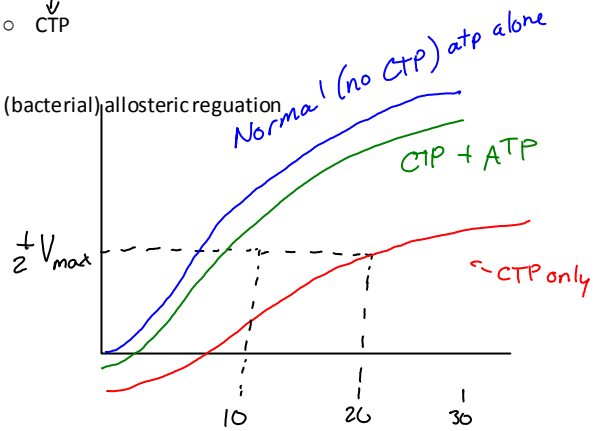
- Regulation of pyrimidine biosynthesis
  - Different from bacteria and animal

1. HCO_3^- + Glutamine + ATP
   ↓ Mammals reg here
2. Carbomyl phosphate
   ↓ bacteria reg here
3. Carbomyl aspartate
   ↓
4. Dihydroorate
5. Orotate
   ↓ PRPP
6. OMP
   - ↓
   - UMP
   - ↓
   - UDP
   - ↓
   - UTP
   - ↓
   - CTP

Bacteria step 2 regulation: allosteric stimulation of ATCase by ATP and inhibition by CTP or UTP

In animals, ATCase is not regulatory. Carbamoyl phosphate synthetase II is inhibited by UDP and UTP and activated by ATP and PRPP

ATCase (bacterial) allosteric reguation

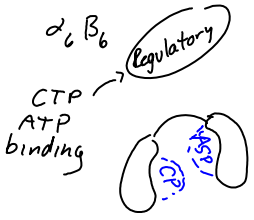


$K_{0.5}$ = 12 mM $K_{0.5}$ = 23 mM

[Carbamoyl Aspartate] (mM)

T $\overset{ATP}{\underset{CTP}{\rightleftharpoons}}$ $R_{CATALYTIC}$

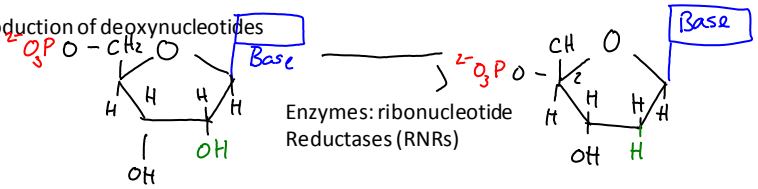
ATP, CTP bind same allosteric site (approx 60 Å from catalystic site)



ATP binding -> conformation of catalystic subunit changes -> favors rxn
CTP binding -> comformation change inhibits rxn

- CHANNELING
  - Applies t both pathways (purine and pyrimidine): intermediates are labile compounds (easily degraded - will hydrolize and decompose in solution). These pathways are optimized to maximize program by minimizing contact with aqueous solution. So each step channels to next active site in a process called channeling.
  - In mammalian cells (pyrimidine synthesis of UMP):
    - First 3 steps of pyrimidine pathway in mammalian cells occur on one protein enzyme in cytoplasm
    - Step 4: mitochondrial membrane (FMN and coenzyme Q used to reduce and take H_2 off of dihydroorotate)
    - Next steps located in cytoplasm on same contiguous polypeptide chain
  - In bacterial cells (pyrimidine synthesis of UMP):
    - Intermediates are the same and susceptible to breakdown
    - Do not find channeling in bacteria though

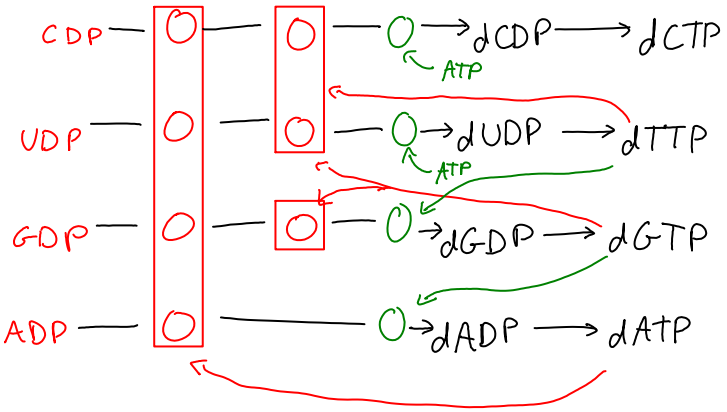
- Salvage of pyrimidine
  - Uricil base and cyto(dine)? are carried out by phosphoribosil transferase? Enzyme
- Production of deoxynucleotides



Ribonucleotides deoxyribonucleotides

  - 3 classes of these enzymes (read about in book)
    - Different classes differ by substrates (NDP or NTP), cofactors they employ, and the way they obtain reducing equivalents
    - Class I and II RNRs in prokaryotes. Class I in eukaryotes. Class III in prokaryotes grow anaerobically. Class I needs O_2
  - Mechanism (free radical) see textbook. Do not need to know mechanism
    - 3' extraction of hydrogen via free radical exchange to 3' position
    - 2' OH becomes positive OH_2
    - Net result 2' position is reduced and free radical is regenerated in enzyme
    - If free radical in enzyme is abolished then enzyme doesn't work
      - Compound: hydroxyurea which will quench free radical

- The control network for regulation of deoxyribonucleotide biosynthesis by ribonucleotide reducatase
  - 2 enzyme states, inactive and active
  - Activity site, specificity site (allosteric site) for what nucleotide, catalystic site (where reduction takes place)
  - Activity site:
    - ATP (active) and dAPT (inactive) when bound
  - Specificity site
    - What nucleotides are reduced
    - Find:
      - ATP binds, TTP binds, and GTP binds
  - Catalystic site
    - Bind ribonucleotide as substrates
  - When ATP is bound to activity site and specificity site, CDP and UDP will be made



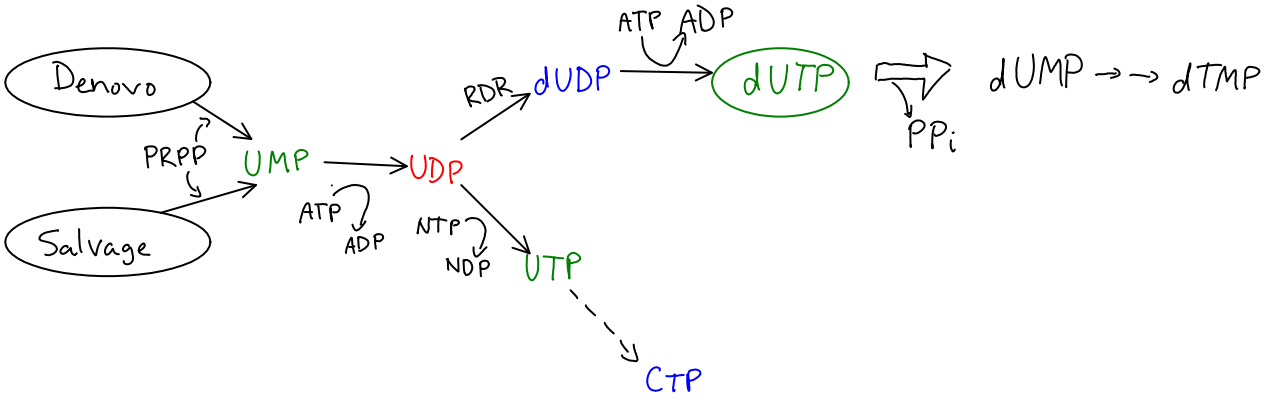
What would happen if you administer high concentration of thymidine to cells. High amounts of TTP

Notes 04/09

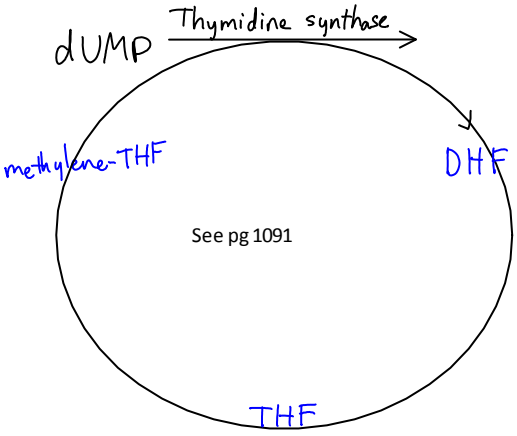
Wednesday, April 09, 2008
11:04 AM

Notes 0409

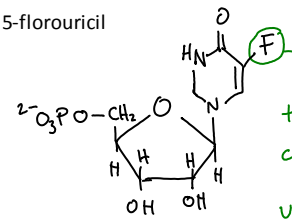
Audio recording started: 11:04 AM Wednesday, April 09, 2008



dUMP + N,N^{10} Methylenetetrahydrofolate ⟶ dTMP + dihydrofolate
↳(cofactor sorta but does not regenerate so it is really substrate)
↳ mechanism in textbook



5-florouricil



no proton to extract so ternary complex is formed but cannot resolve & kills the enzyme
Suicide inhibitor
used as chemotherapeutic agent

Methyltrexate (antitumor agent) - binds to active site of dihydrofolate reductase about 1000x stronger

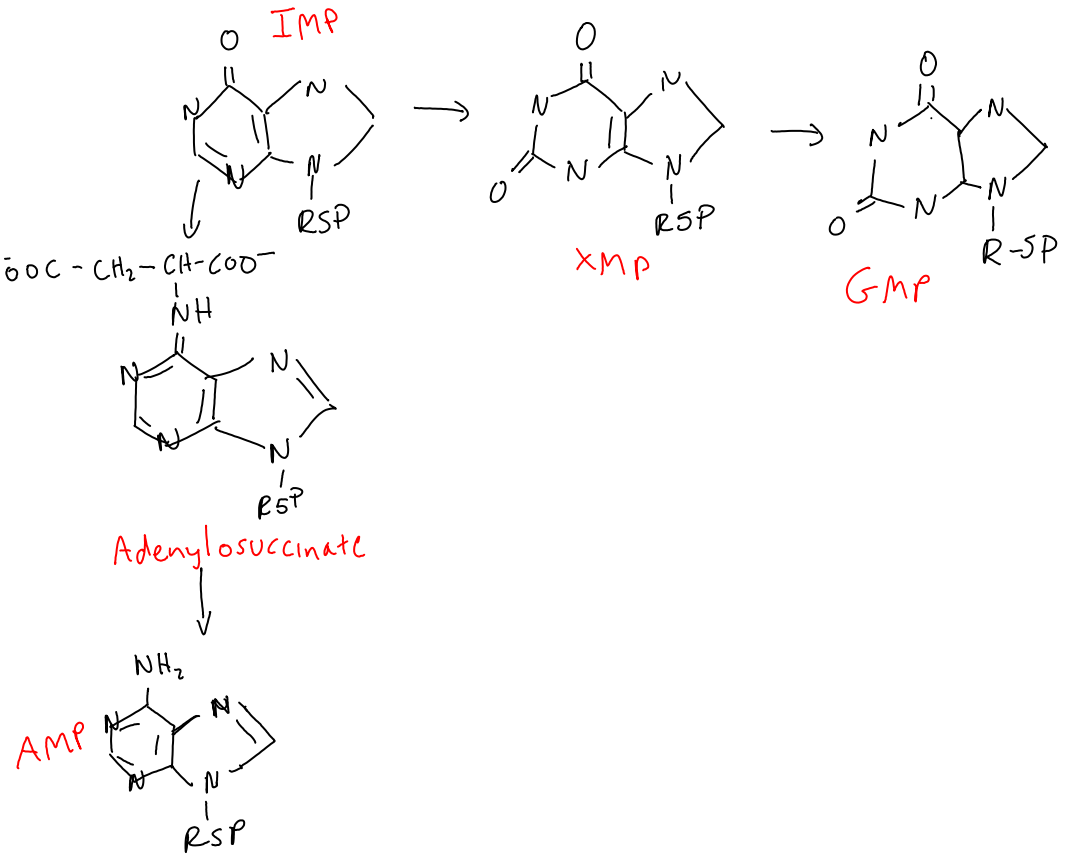
than normal substrate. Blocks reductase so THF production is blocked
Aminopterine - also blocks DHR
Trimethoprim - bacterial

DNA structure and properties

- B form DNA
- Chargaff rules
- Watson and crick

Discussion Week 2

Thursday, April 10, 2008
11:01 AM



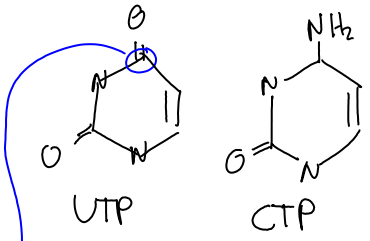
Problem set I

2. APRT + Adenine -> AMP
   HGPRT + Hypoxanthine -> AMP
   HGPRT + Guanine -> GMP

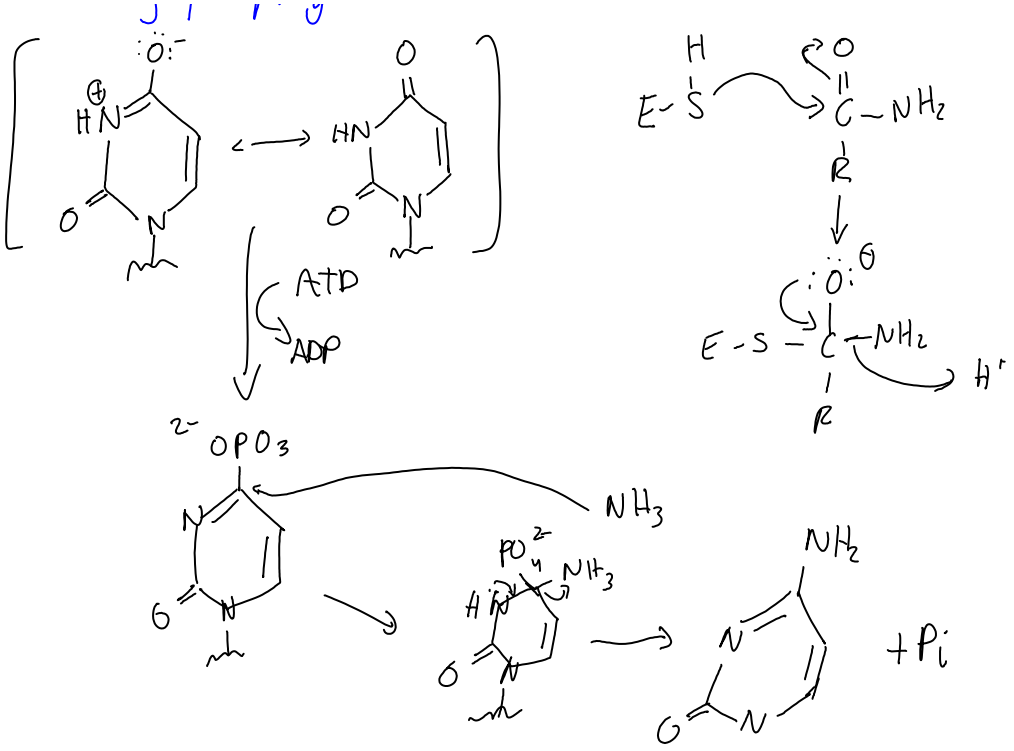
Can survive	Cannot survive
Adenine	Guaning + hyp
Ade + hyp	Guanine
Ade + guan	hypoxanthine

Convert AMP to GMP

3. ATP + glutamine + H_2O + UTP -(citrate synthetase)--> ADP + glutamate + CTP



activated by phosphorylation.



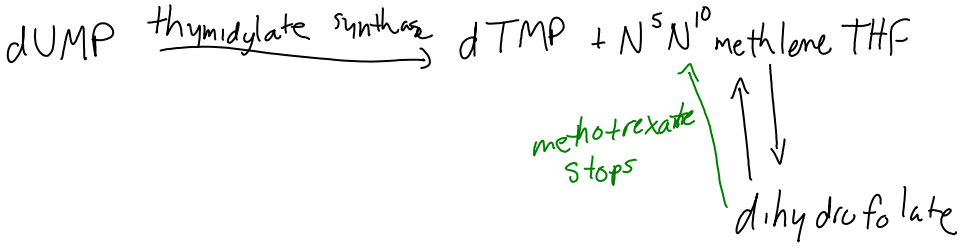
Inhibitor: acivicin - glutamine analog that reacts with CTP Synthetase to inhibit. Suicide inhibitor
Acylserine

4. Deoxyadenosine dAMP
   dAMP if high concentration than others are inhibited
   dCMP
   dGMP
   dTMP

   Higher levels can increase chance of mutation by incorperating adenine over correct nucleotide

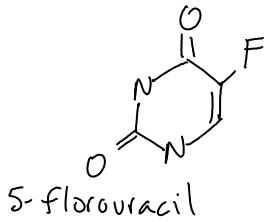
5. There are 2 types of carbamoyl phosphate synthetase (1 and 2). Bacteria only has 1 form that carries out several functions. If this 1 type is inhibited then it will also stop arganine synthesis. Mammals have 2 types of carbamoyl phosphate synthetase so inhibiting type II will not affect arginine biosynthesis. Step one requires ATP so it is best for mammals to regulate at step 1 to conserve energy

6. .



THF is also used in de novo synthesis of purines which explains why methotrexate effects purine synthesis.

7. 5-florouracil inhibits 1st step because no H on 5 position which kills the enzyme.



5-florouracil

Both 5-floroulacil and methotrexate inhibit dTMP synthesis

By using both it gives more effective inhibition of production of dTMP.

8. Page:
   - dUTP
   - dCTP
   - dGTP
   - dATP

   Inhibit dTTP because phage doesn't need it. An inhibitor of thymidylate synthease

Notes 04/11

Friday, April 11, 2008
11:01 AM

Notes 0411

Audio recording started: 11:01 AM Friday, April 11, 2008

- **B-form DNA by Watson and Crick. Based on:**
  1. Chargaff's Rules: Molar ratios of A/T and C/T
  2. Jerry Donahue: enolate form of guanine was not predominate form. It is keto form
  3. X-ray diffraction data of DNA fibers
     - 2 fold rotation symmetry

- **DNA has multiple conformations**
  1. **B-form DNA (most common)**
     - Comes from lab notebook B
     - G-C pair bond angles are extremely similar (the same) as A-T so they can be interchanged without changing helical parameters.
     - Only 6 member rings of purine/pyrimidine interact in base pair hydrogen bonds
     - Glycosidic bonds are not 180^0 apart on helix so they create minor and major grooves.
     - 10bp per helical twist ($360/10=36^0$ per bp)
     - 3.4A per bp so 34A per helical twist
     - 20A in diameter
     - Right handed
     - Bp are perpendicular to helix axis
     - Water stabilizes B-form to form a spine of hydration through binding minor groove.

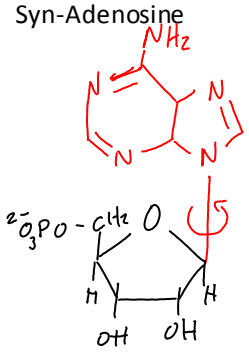
  2. **A-form DNA**
     - Comes from lab notebook A
     - Structurally convertible from A to B or B to A
     - A form is observed when B form DNA is placed in less polar solution. Also known as lowering the humidity
     - Wider helix
     - Pitch is different. 11bp/turn
     - Right handed
     - Bp are no longer perpendicular to helix axis
     - Less of difference between major and minor grooves
     - Channel down center of A DNA.
     - Spores of bacteria and fungi are starved of nitrogen and undergo complete halt of metabolism. They have their DNA in A-form while suspended. No transcription takes place and in inert stage.
       - This also protects itself from UV-light
         - Pyrimidines very sensitive to photochemical damage and in A-form their different configuration protects form damage.

  3. **Z-form DNA**
     - Left handed
     - Zig zag of nucleotides cutting into sugar phosphate bands.
     - Major and minor groove is much less noticable
     - Created antibodies that react with Z-DNA and not other forms to test if Z-DNA is found in biological systens

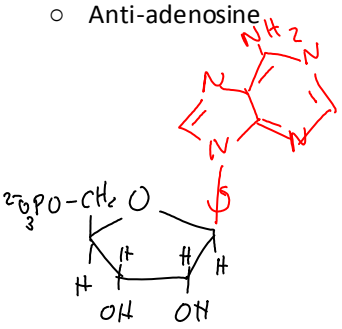
        - Z-DNA is involved in transcription. Relive tortional stress as a result of overwinding of DNA molecule.

    - Through all these structural changes, the base pairing does not change. It is only changes in sugar phosphate backbone.

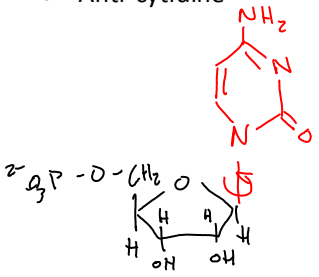
- Rotation of base and sugar along glycosidic bond.
    - Syn-Adenosine



    - Anti-adenosine



    - Anti-cytidine



    - Purines are syn or anti
    - Pyrimidines only anti easily formed, due to pyrimidine's C2 steric interaction
    - In DNA, they are anti configuration except in Z-DNA

- Conformations of sugar
    - Puckering - 3' or 2' carbon is pushed out of plane of the ring.
        - When pushed up in direction of base and sugar's C5 it is called Endo
        - When pushed down in opposite direction of base and sugar's C5 called Exo
    - Puckering matters greatly because it effects phosphate position due to OH on 2' or 3' interaction. This then can change overall helical parameters.
    - Table 28-1 Structural features of ideal A,B, and Z DNA (see book)

- DNA forms and structural features Table 29-1 pg 1109

	A-DNA	B-DNA	Z-DNA

Helical sense	Right	Right	Left
Diameter	26 A	20 A	18 A
Base pairs per helical turn	11.6	10	12(6dimers)
Helical twist per base pair	31^0	36^0	for pyrimidine-purine steps; 51^0 for purine-pyrmidine steps
Helical pitch (rise per turn)	34A	34A	44A
Helical rise per base pair	2.9A	3.4A	7.4A per dimer
Base tilt normal to the helix axis	20^0	6^0	7^0
Major groove	Narrow and deep	Wide and deep	Flat
Minor groove	Wide and shallow	Narrow and deep	Narrow and deep
Sugar pucker	C3'-endo	C2'-endo	C2'-endo for pyrimidines; C3'-endo for purines
Glycosidic bond	Anti	Anti	Anti for pyrimidines; syn for purines

Notes 04/14

Monday, April 14, 2008
10:53 AM

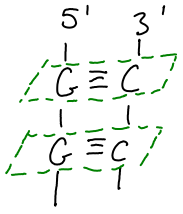
Notes 0414

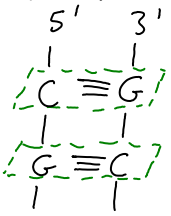
Audio recording started: 11:02 AM Monday, April 14, 2008

- Alternative DNA structures
  - Dramatically alter DNA structures via sugar phosphate backbone, the base pairing is still in tact.
  - A-DNA - water content is lowered.
    - Increased resistance to UV-radiation damage
    - Also found in active site of enzymes such as DNA polymerase where water content is low
    - Right handed
  - Z-DNA
    - Crystals of certain DNA molecules
    - Patients with Lupus produced antibodies to Z-DNA
    - Z-DNA is associated with transcriptionally active portions of genome.
    - Left handed
    - B-form to Z-form by increasing salt content or to put on hydrophobic bases.
    - Zig-zag of phosphate groups
    - Zig-Zag results by rotation around glycosidic bonds for purines.
      - B-DNA has purines/pyrimidines in anti configuration
      - Z-DNA has purines in syn configuration. Pyrimidines still in anti configuration (impossible for pyrimidine to be in syn configuration with glycosidic bond). Pyrimidine have nucleoside.
      - Z-DNA is sequence dependent and makes Z conformation sterically possible by purine, pyrimidine, purine alternation of sequence pattern.

- Stability of DNA molecule
  - Base pair H-bonds do **not** play major role in thermodynamic stability of DNA but rather play major role in specificity of base pairing
  - Stacking interaction (hydrophobic) between base pairs is primary energetic stabilization of DNA
  - In nonpolar solvent DNA is less stable due to weakening of stacking interactions

b) Base stacking of adjacent base pairs along helix axis (overlap of aromatic bases)

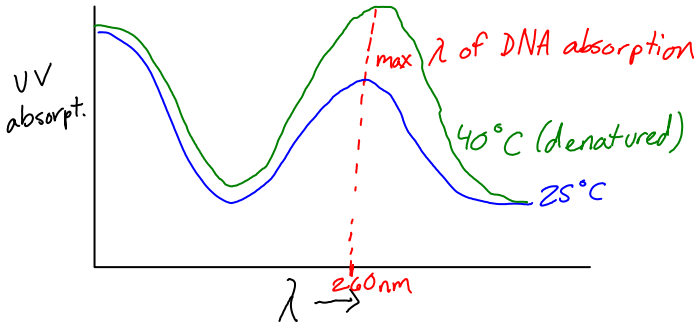




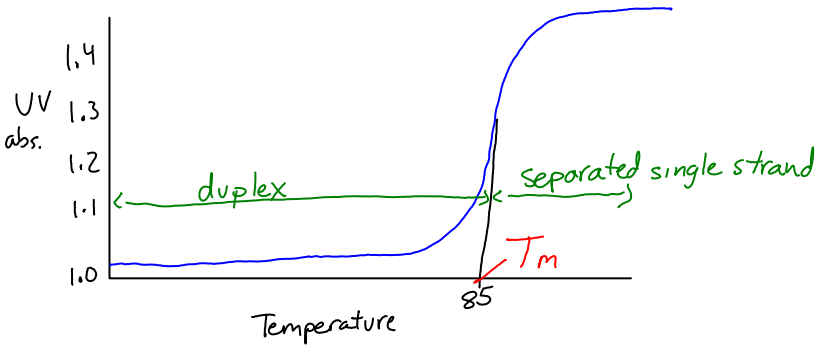
$\Delta G = -5$ kcal/mol
more overlap

$\Delta G = -3.2$ kcal/mol
less overlap

- Experimentally following stability
  - Purines/pyrimidines are aromatic and absorb UV light



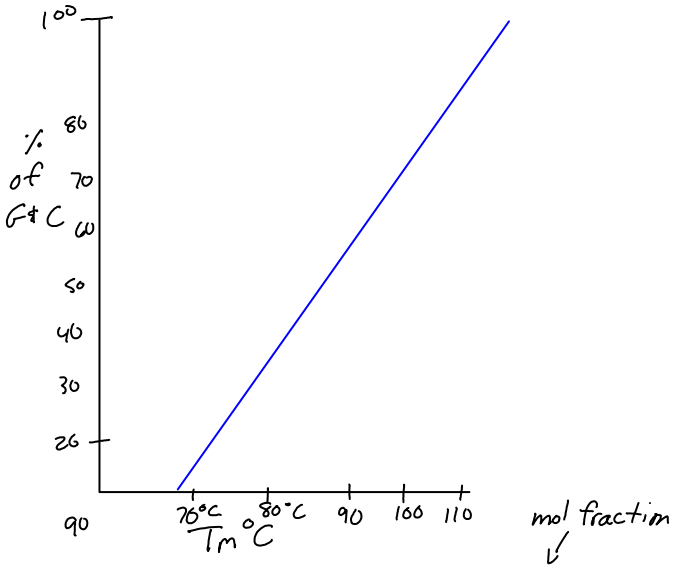
- **Hyperchromicity** - increase in intensity absorption accompanying DNA transition from double stranded (25^0C) to single stranded (40^0C)
- 260nm is still max wavelength absorbed by DNA (single stranded or double stranded)



T_m = 1/2[DNA] denatured

Determined by:

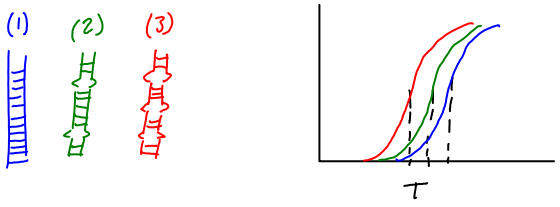
- Solvent composition - polarity
- GC content of DNA (extent of overlap more so than G C triple H-bond)
- Salt concentration - shields electrostatic repulsions
- pH - groups participating in H-Bonding are titratable protons
- DNA topology



$T_m = 69.3^0 + 0.41(G+C)$ don't memorize ->will be given if tested

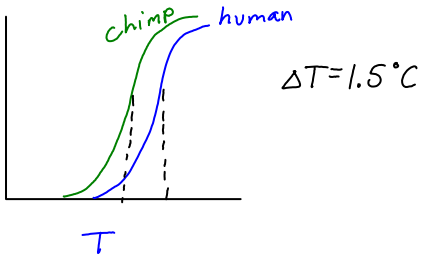
Melting temperature of DNA hybrids containing mismatchnes

- Melting temperature changes depending on number of mismatches

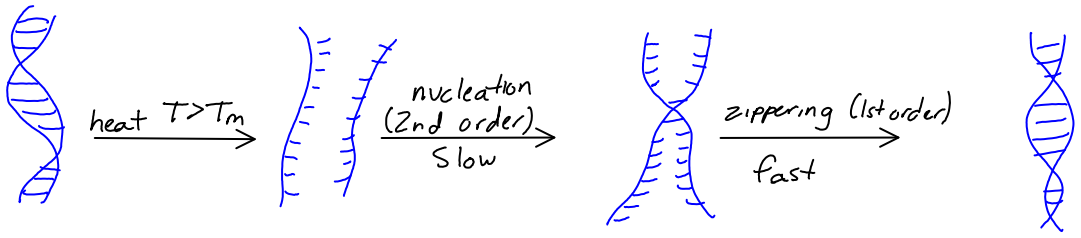


T_m lowered about 1^0C for each 1% mismatch

- To compare 2 species for homology can sequence or just calculate difference in T_m



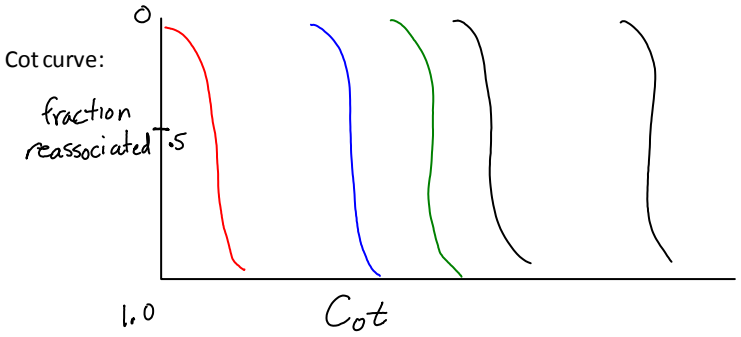
- Renaturation (annealing)



  - Overall rate of hybrid is 2nd order
  - Nucleation involves small number of base pairs (20ish)

Rate eq. $-\frac{dC}{dt} = k_2 C \cdot C = k_2 C^2$, C = conc of single strands

Solve $\frac{C(t)}{C_0} = \frac{1}{1+k_2 C_0 t}$ at

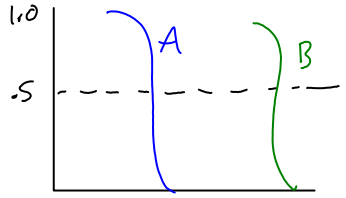


As complexity (# of sequences) increases, the longer it takes to find its partner

$$\frac{Cot_{1/2}(A)}{} = \frac{L_1(A)}{}$$ (L = length)

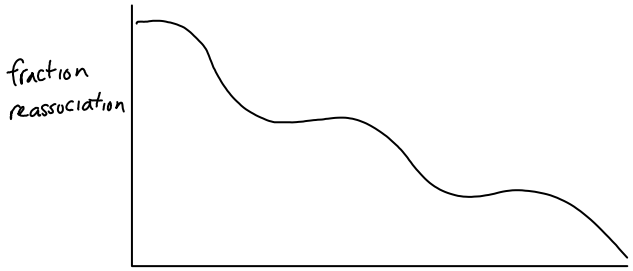
$Cot_{1/2}$ (B) L (B)

- How do you determine the size of a genome? Cot curve



B has greater kinetic complexity than A.
For simple genomes kinetic comp. directly related to sequence complexity

- Complex Cot Curve
  - For eukaryotic organisms, multiple component re-association curves



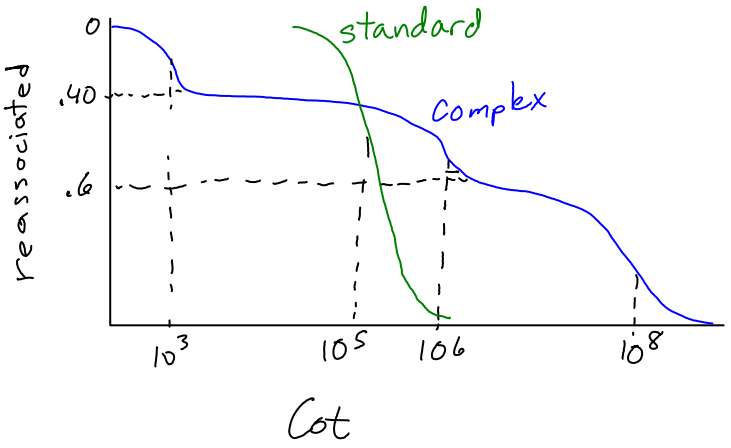
Notes 04/16

Wednesday, April 16, 2008
10:57 AM

Notes 0416

Audio recording started: 11:02 AM Wednesday, April 16, 2008

- Denaturation
  - Longer sequence of DNA, the longer it takes to renaturate
  - Higher eukaryotic organisms have a complex cot curve:



Standard $Cot_{1/2} = 10^5$
"size (unique sequence) = 3×10^6 bp

Unknown $Cot_{1/2} = 10^8$

$$\frac{Cot_{1/2}(known)}{Cot\ 1/2\ (unknown)} = \frac{10^5}{10^8} = \frac{3 \times 10^6\ bp}{x}$$

$x = 3 \times 10^9$ bp = "kinetic complexity"
= sequence (unique)

Total genome size = $(.4)(L_1) = 3 \times 10^9$ bp
$L_1 = 7.5 \times 10^9$ bp

To determine kinetic complexity (sequence complexity) of moderately repetitive fraction:

$$\frac{10^6}{10^8} = \frac{x}{3 \times 10^9} \qquad x = 3 \times 10^7\ bp$$

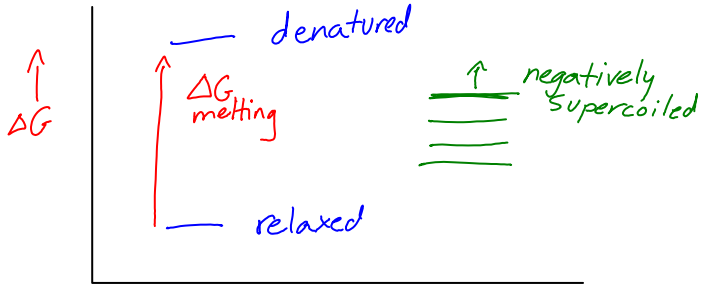
How much DNA is moderately repetitive fraction?

$$(.2) \times 7.5\times10^{9} b_p = 1.5\times10^{9} b_p$$

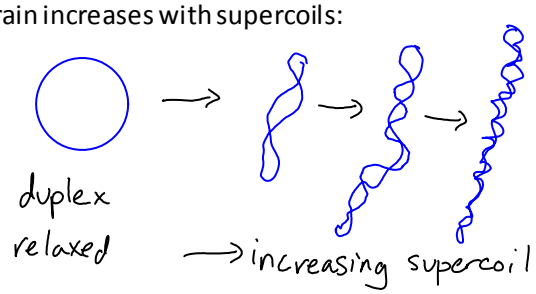
$$\frac{1.5\times10^{9} b_p}{3\times10^{7} b_p} \sim 50$$

This analysis allows you to determine genome size, number of repeated sequences, and number of copies of these.

- DNA Topology
  - Super coiling
    - Figure 28-33 Electron micrographs of circular duplex DNAs with various amounts of supercoiling.
      - Plasmids is common topological state for DNA
      - DNA supercoiling altered using enzymes that carry out supercoiling reaction
      - Relaxed DNA (no supercoiling)
      - Coiling - cross over each other to create nodes. Nodes are proportional to number of coils
      - Covalent structure of DNA is unaltered in coiling.
      - Supercoiling is high energy strained state (tortional strain) for DNA molecule and requires energy input (enzymes)
      - Reason for tortional strain:
        1. Phosphate groups on sugar-phosphate backbone on highly coiled have high electrostatic repulsion that is unfavorable.
        2. Entropy increases at relaxed state. Entropy is lower in coiled state.
      - When isolate DNA from bacteria, it is supercoiled
      - In supercoiling, there are 2 possibilities of right/left handed (clockwise/counterclockwise) wrapping.
        - Negative supercoiling favors separation of duplex:



      - Strain increases with supercoils:



$$\Delta G \simeq \text{constant} \ (\Delta L)^2$$

(supercoil strain)

- Types of supercoiling: (figure 28-37)
    1. Plectonemic supercoiling (AKA interwound) - DNA is by itself in solution
    2. Toroidal - DNA is wrapped around a center core (histone or enzymes etc)
    - □ Left handed toroidal and right handed interwound are equivalent topologically
    - □ Right handed toroidal and left handed interwound are also equivalent topolgically
        - Mathematically explained by linking number
            - ◇ $L_1 = T + W$
                - ▶ T = twist number (# of helical turns). # bp/bpperturn Bform
                    - ▸ Bform #bp/+10 (+ right handed)
                    - ▸ Aform #bp/+11 (+ right handed)
                    - ▸ Zform #bp/-12 (negative because left handed)
                - ▶ Writh value is measure of suprecoiling
                    - ▸ Right handed toriodal: +
                    - ▸ Left handed toroidal: -
                    - ▸ Left handed interwound: +
                    - ▸ Right handed interwound: -
            - ◇ ΔL = 0 if DNA intact and no covalent disruption

L	T	W
10	10	0 (relaxed)

So molecule has 100bp

Disrupt

L	T	W
10	9	+1

L	T	W
10	8	+2

Notes 04/18

Friday, April 18, 2008
11:02 AM

Review Session Wed 6:30

- Supercoiling
  - Interwound (wrapped around self)
  - Toroidal (wrapped around a histone core)

  - DNA is more compact with supercoiling
  - Negative supercoiling - makes easier to separate 2 strands of helix (right handed interwound or left handed toroidal).
    - Replication or transcription
  - Linking number L_1 - Strand crosses another strand
  - L=T+W
    - T=twist, number of helical turns
      - Zdna gets negative value since it is left handed

  - Most organisms are negatively supercoiled.
    - Exceptions are hot springs bacteria - they are positively supercoiled.
  - Supercoil density = W/T (.04-.07)

- Ethidium Bromide - binds to duplex DNA
  - Planar aromatic
  - Binds between stack pair of DNA (interplation)
  - Mutagen is more toxic
- Chlorophin - same but less toxic

  These drugs interact to reduce twist value

- Experimentally finding the supercoiling
  - Move through gradient and more relaxed moves slower
  - Can add ethhidium brominde will increase W

Notes 04/23

Wednesday, April 23, 2008
10:57 AM

Notes 0423

Audio recording started: 11:01 AM Wednesday, April 23, 2008

1) Review session tonight 6:30pm young 2200
2) Office hors today 1:30-3PM geology 4607
3) Email q's
4) Text reading through pg 1144

Midterm 1

Q1-6

1. T/F
2) Identify
3) Nucleotide structure/repl
4) DNA renaturation
5) Pathway inhibitors
6) Topology/topoisomerases

- DNA Replication
  - General properties
    1. Semiconservative replication
    2. Bidirectional
    3. Simidiscontinuous

    Prokaryotes replication rate - 1000nt/sec
    Eukaryotic replication rate - 50nt/sec

  - Experiments involving general properties of replication
    1. Semiconservative replication - mes__- and staw
       - Grew bacteria in medium with heavy isotope of nitrogen
       - Density labeling experiment
       - N^{15} ammonium sulfate
       - Figure 20/23 (mcgraw-hill)

       - Semiconservate model and dispursive model

       - Semiconservative experiment for eukaryotic organisms: Herbert taylor with plants (couldn't use same procedure because eukaryotic cells died in prokaryotic dense nitrogen medium)

       - Figure 20.11 McGraw-Hill

Notes 04/21

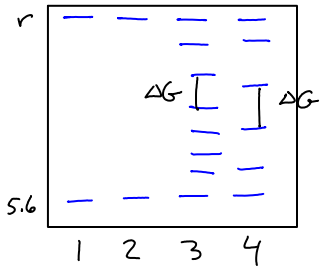
Monday, April 21, 2008
11:03 AM

Midterm 1. coveres through Wed.

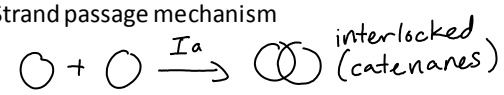
Review session Wed evening: 6:30 @ young room 2200

- DNA topology
  - DNA molecules that are not circular can have supercoiling properties of circular if it has something such as a protein on end constricting untwisting.
  - Chromosome of prokaryotes are circular.
  - Enzymes in cell can alter covalent structure of DNA (topoisomerases)
  - Topoisomerases will covalently disrupt DNA to change linking number (reduce tortional strain by nicking DNA and rejoining together). This allows to reduce strain caused when replication occurs.
    - Some enzymes are specific for negative while others for positive supercoils
  - One enzyme can introduce strain to DNA - called DNA girase
-

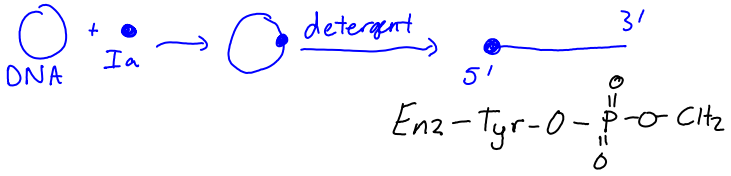
Lane 1	Supercoiled DNA and relaxed DNA
Lane 2	Relaxed DNA incubated with type 1b topoisomerase
Lane 3	Supercoiled DNA incubated with Tye 1b topoisomerase
Lane 4	Supercoiled DNA incubated with type 2 topoisomerase



  - Type 1
    a) ΔL = 1
    b) Transient single strand break
  - Type 1a
    - Strand passage mechanism

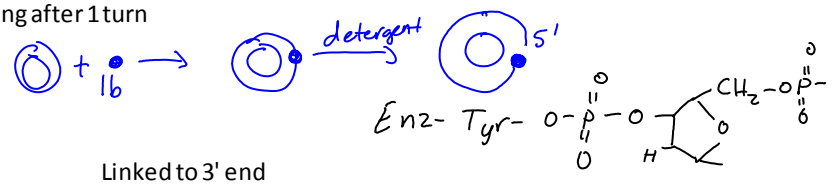


    - Covalent attachment to 5' end of DNA strand



    - Topo 1a only relaxes negative supercoils

  - Type 1B
    - controlled rotation of one strand around the other
    - Resealing after 1 turn

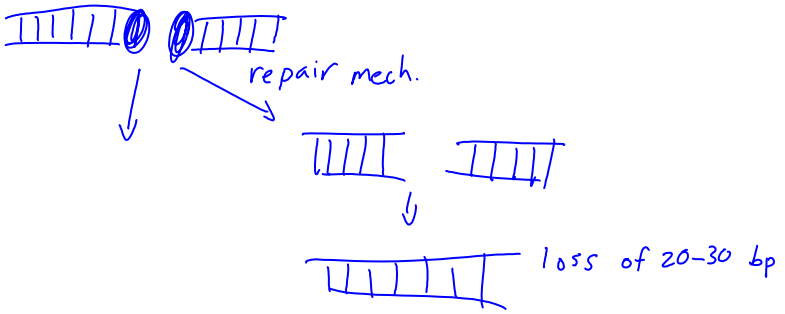


Linked to 3' end

    - Topo 1b relaxes both positive and negative supercoils.
    - NOT found in E. coli

- Type II (ΔL=2)
  - Mechanism: Transient cutting of both DNA strands
    Covalent attachment to 5' ends
    Strand passage
    Replication of DNA
  - ATP used to drive protein through catalytic site
- DNA girase is responsible for negative supercoiling found in bacterial chromosome.
  - Drugs that prevent girase.
- Other enzymes that reseal after cutting by topoisomerases
- Class of antibiotics that interfere with resealing step. Cells treated with antibiotic, DNA of chromosome becomes fragmented.

- Inhibitors of topoisomerases
DNA Gyrase inhibitors (antibiotics)	Novobiocin (coumarin family antibiotics), ciprofloxacin (quinolone)
Type II eukaryotic (anti tumor agents)	Doxorubicin (adriamyein), etoposide
    - Mechanism (excluding novobiocin)
    - Type 2 inhibitor problem: have high probability of giving 2nd unrelated tumor that appears 5-10yrs later



Inhibitors

Thursday, April 24, 2008
11:15 AM

Aminopterin - inhibits DHF reductase so THF recycling affected

Hydroxyurea - stops free radical reaction radical required in RNR

Allopurinol - inhibits xanthine oxidase

Azaserine - gln analog (substrate used in purine biosynthesis) - inhibit dinovo purine synthesis (cannot make ump, cmp, amp, gmp)

Methotrexate - interferes with recycling of THF by inhibiting DHF reductase. So affects TMP synthesis and purine synthesis

5-florouracil - inhibits TMP synthesis

Novobiocin (coumarin) - DNA Gyrase inhibititor

Cipofloxacin (quinolone) - DNA Gyrase inhibitor

Doxorubicin - topoII isomerase inhibitor in eukaryotes

Etoposide - topoII isomerase inhibitor in eukaryotes

After midterm 1

Rifampicin -RNA polymerase inhibitor

Notes 04/28

Monday, April 28, 2008
10:58 AM

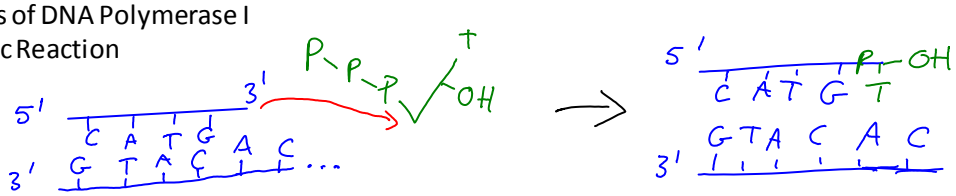
Notes 0428

Audio recording started: 11:02 AM Monday, April 28, 2008

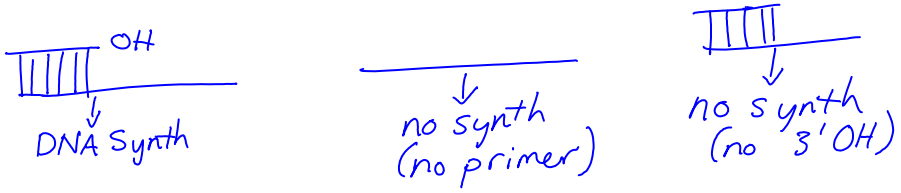
DNA Replication (eukaryotic & prokaryotic)

- DNA Polymerase I
  - Found in E.Coli
  - Doesn't do DNA replication but does lagging strand synthesis (not continuous strand)

- Properties of DNA Polymerase I
  - Basic Reaction

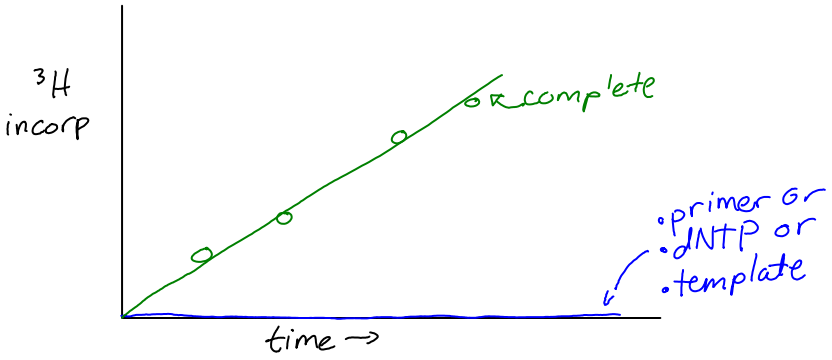


  - Enzymatic Properties of DNA Polymerases
    1) Template Dependent
       (DNA Template -> DNA polymerase)
       (RNA Template -> Reverse Transcriptase)
    2) Primer Depending (3'OH)
       (RNA or DNA)



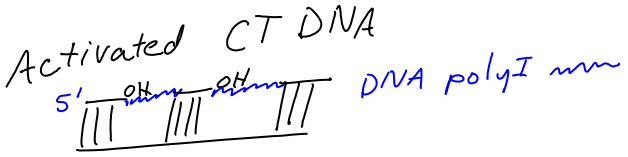
    - Nucleoside triphosphate is linked to 5' end
    - Why aren't 3' NTP used for DNA synthesis?

  - 80% of DNA polymerase activity in e.coli is Polymerase I
    Assay ^{3}H-dTTP + polymerase +DNA:primer

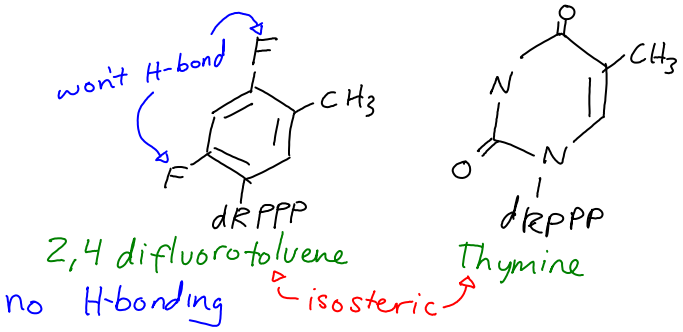


    - Incorporation -> 10% acid precipitable (big precipitate)

- Incorporation -> 10% acid preciptable (big precipitate)



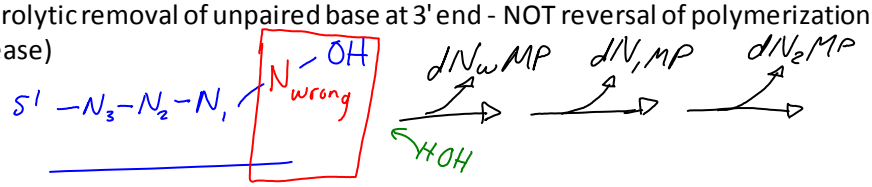
- Fidelity - Accuracy of Synthesis
  - E.coli: Overall mutation rate (in vivo) approx 10^{-9} - 10^{-10} errors/bp
    1 replication error/10^3 gen.
  - Polymerase error rate (in vitro) approx 10^{-6} errors/bp or more
  - 2 sequential steps involved in fidelity
    1. Base selection
       a) Geometry of incoming nucleotide (W-C geometry)
       b) H-bonding between template and nucleotide NOT important



Both incorporate, so H-bonding is NOT important. Geometry of active site and chosen nucleotide is most important.

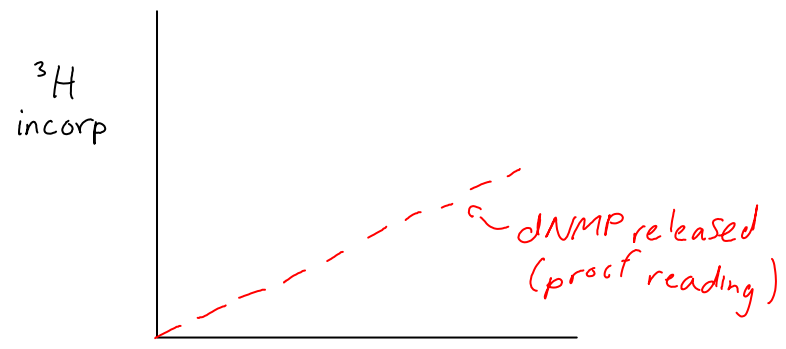
- Polymerase changes template structure because water is removed from minor groove for 3-4 nucleotides so these 3-4 nucleotides becomes A-form DNA.

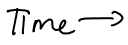
2. Proofreading (editing)
   a) 3'-5' exonuclease
   b) Hydrolytic removal of unpaired base at 3' end - NOT reversal of polymerization (PP_i release)



3' END unravels -> removal of adjacent NTS where dsDNA -> single strand for editing

T_4 DNA Polymerase (gp43)
Very active 3' exo





Approx 20% of nucleotides that get incorporated are later removed by proof reading.

Notes 04/23

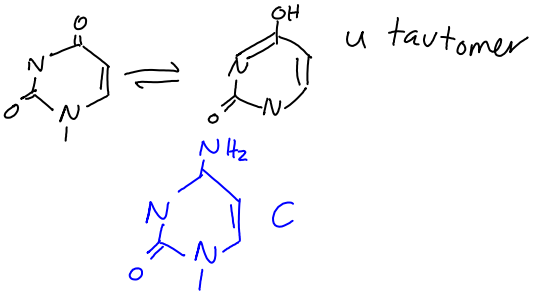
Wednesday, April 30, 2008
10:59 AM

Notes 0423

Audio recording started: 11:01 AM Wednesday, April 30, 2008

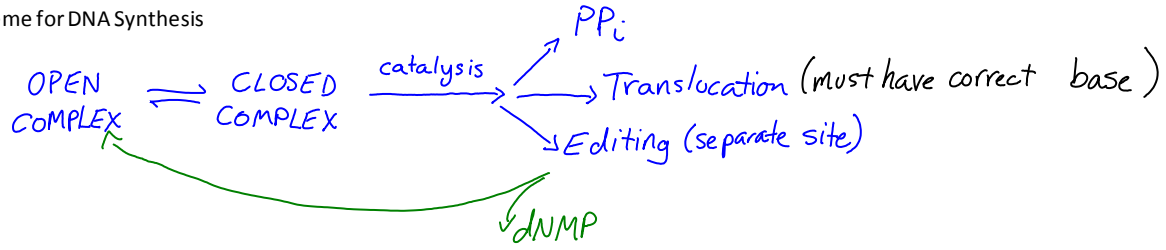
Emphasis in reading
- Basic properties of polymerases
- 2 enzymatic issues (speed/accuracy)
- Discussion of different replicating systems. Read & be responsible for it but lecture will not cover

DNA Polymerase I
- Base selection
  - Geometrical constraints (not H-bond… hbond only 1%)
  - When there is purine in template strand, only pyrimidine can enter
  - Implications of Geometrical Selection Model:
    - Most frequent type of mistake will be A:C or G:T NOT A:G or C:T
    - Predict A:T -> G:C or G:C -> A:T mutations are most frequent
    - 5' position of T not contracted by polymerase -> explains why
  - Active site… looked at 5' position of pyrimidines -> Does not appear to have major steric straints. Can add long fatty acid chains to 5' (or cholesterol) and it can still be incorporated, so 5' position is not contacted by polymerase. This is position that thymidine is distincted from uracil. 5' position of thymidine has no sterric affect of incorperation.
    - **5' Position of T not contacted by polymerase -> explains why dUTP hydrolyzed in synthetic pathway of dUTP -> substrate of DNA polymerase**
    - Tautomerization: (keto most prevalent)



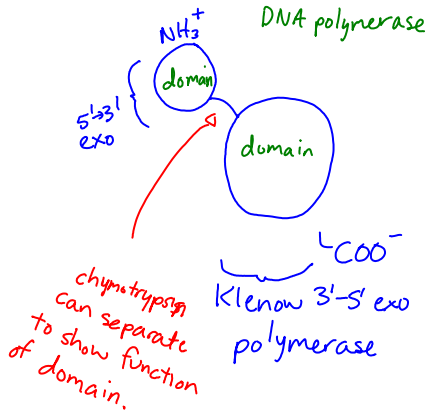
- Reducing amount of dUTP in cell to avoid this mutation.

- Kinetic Scheme for DNA Synthesis



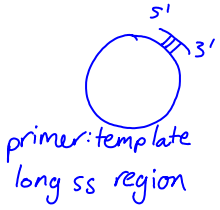
a) Base selection (OPEN)
   -> conformational change
b) Catalysis, PPi release

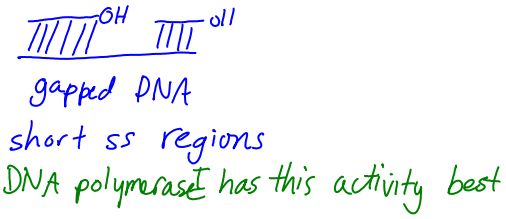
  - DNA Polymerase Activities lacking 3'->5' exo
    - Mammalian DNA Pol α
    - HIV Reverse Transcriptase
    - TAQ Polymerase (PCR)
  - Enzymatic Activities of DNA Polymerase I
    - 5'->3' DNA polymerase
    - 3'->5' Exonuclease (ss)
    - 5'->3' Exonuclease (ds) (Nick Translation) ← only essential



Amber mutation - changes code of one nucleotide to make stop codon. Karon's mutant stops DNA polymerase synthesis between domains and only 5'->3' exo activity exists.

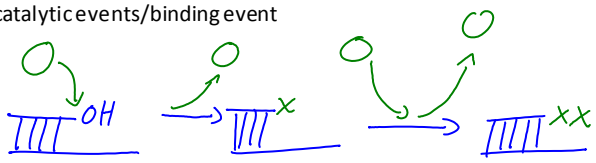
- ....Of DNA during replicatoin
  a) Too slow
  b) Pol I: mutants (<3% of polymerase activity) ->grow okay



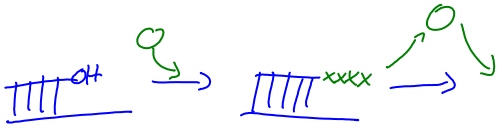


- DNA Polymerase III - another subunit - has 3'-5' exonuclease

- Processivity - # catalytic events/binding event



Example: each binding - one nucleotide added. Low processivity



Each binding n nucleotides added

Fore replicative polymerases

Notes 05/02

Friday, May 02, 2008
11:08 AM

Notes 0502

Audio recording started: 11:08 AM Friday, May 02, 2008

- Subunit Structure of Pol III, Pol III*, Pol III HE
  - **Pol III**

α
ε
θ
τ

core substituent
α → 5'-3' polymerization
ε → 3'→5' exonuclease
τ → dimerizes

  - **Pol III*** = Pol III + $\gamma\delta$
    Core subunits + ($\gamma\delta$) - complex

$\Psi\delta = (\gamma\delta\delta'\chi\Psi)$ ATP dcp: clamp loader

  - **Pol III HE** = Pol III + $\partial\delta+\beta$

Pol III* + β β = sliding clamp, dimeric, encircles DNA
HE > 900 kDa

Beta

Pol III core complex
↓
loading of beta clamp
↓
Dissociation of clamp loader
↓
Exchange of beta clamp pol III
↓
Processive DNA synthesis
↓
Dissociation of pol III

+ ADP + Pi

Dissociation of pol III

↓

Exchange of beta clamp to gamma complex

↓

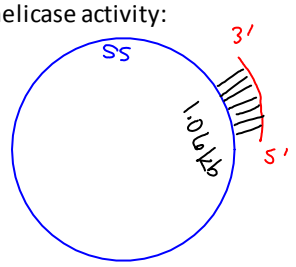
Dissociation and recycling

- All DNA Pol require primers. How are primers set down on DNA?
- Other enzymatic activities needed for complete replication
  - Priming of DNA synthesis
    - E.coli chromosome -> RNA synthesized by primase (NOT RNA polymerase)
    - Average size 10-12 nt

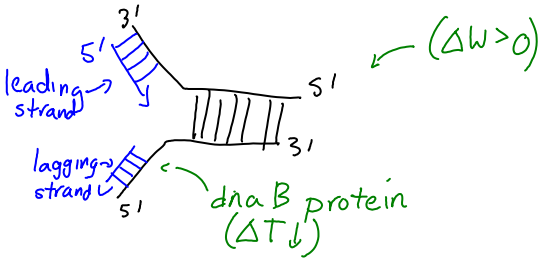
primase - dnaG- Initiates at 5' GA

    - Primase interacts with dnaB
    - Protein: helicase
    - Helicase needed for strand unwinding

    - Assay for helicase activity:



- DNA B -> hexameric ATPase moves along lagging strand template in 5'->3' direction



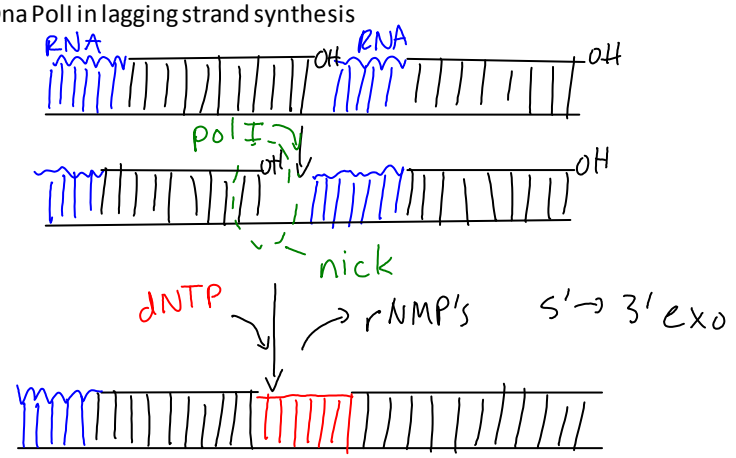
  - dnaB -> unwinds DNA DNA strands coupled to ATP hydrolysis
  - Note: to allow priming by dnaG....

- Enzymes needed fo rE.coli Dna replication

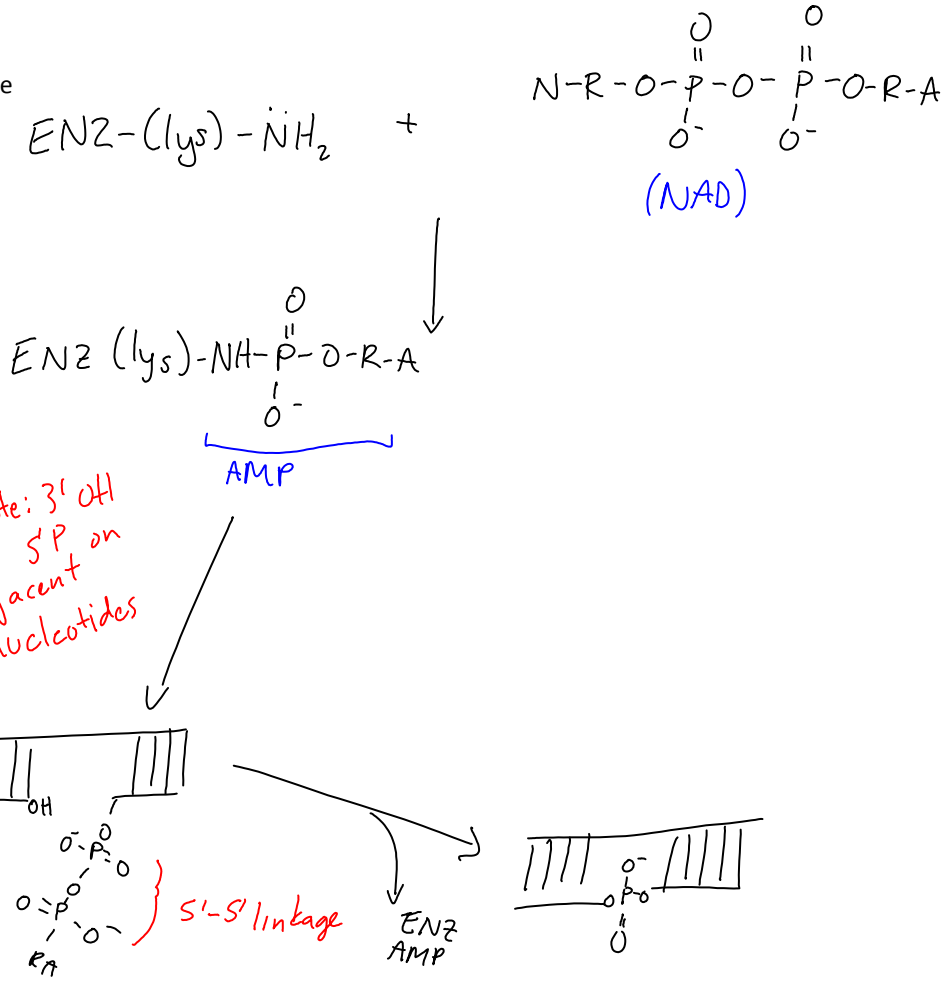
Name	Function	Leading	Lagging
dnaB	Helicase		5'->3'
dnaG	Primase	1x	OF priming

Pol III HE	Polymerase	Yes	Yes
SSB	DNA Binding protein	Yes	Yes
Dna Pol I	Polymerase	1x	OF
Dna ligase	OF joining	1x	Yes

- Role of Dna PolI in lagging strand synthesis

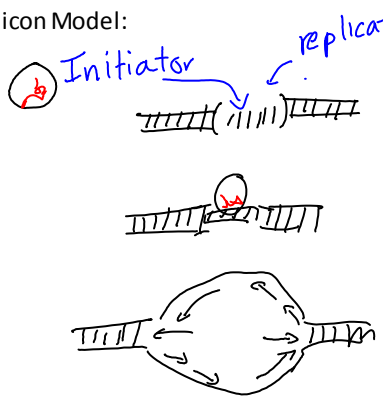


- DNA Ligase



- Figure 29.16 from Garrett/Grisham Biochemistry
  - Trombone model

- Replicon Model:



Notes 05/05

Monday, May 05, 2008
11:03 AM

- Origin replication
  - In bacteria 1 copy per chromosome. Highly conserved (concentrated?)

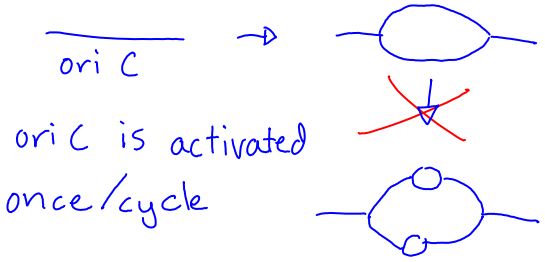
- TATC/GATC modification site for methylatoin
  - Expect to have 1 for every 250bp by just probability of combinations
  - Actually find about 10-11 for every 250bp

- Initiator Protein is DNA A Protein

- Origin replication steps
  - DNA must be negatively supercoiled (underwound). If not underwound it will not bind
  - Toroidal wrapping around Dna A multimer -> induces unwinding
  - Open complex - local unwinding of A-T rich region
  - Recruitment - Dna C p -> chaperone delivers Dna B $(DnaB)_6(DnaC)_6$ ATP hydrolyzed to release dnaC
    - DnaC is an escort protein to deliver DnaB
    - DnaB associates with primase to prime dna synthesis
    - One strand becomes leading and one lagging strand. Suggestion that RNA polymrease is associated with this complex, but not proved

- SSB prevents DNA from forming duplex and prevents degradation of the single strand dna.

- GATC has regulatory function
  - Once replication starts, why aren't there multiple initiations? Mechanism to prevent replication from starting immediately in bacteria -> process called sequesteration



hemi methylated

fully methylated

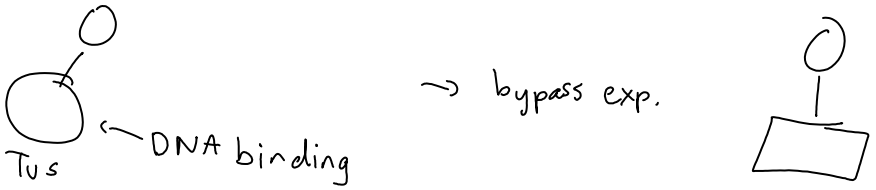
N_6 methylation

- Ori C region replicated → [-GATC-/-CTAG-]$_{11}$ (me)

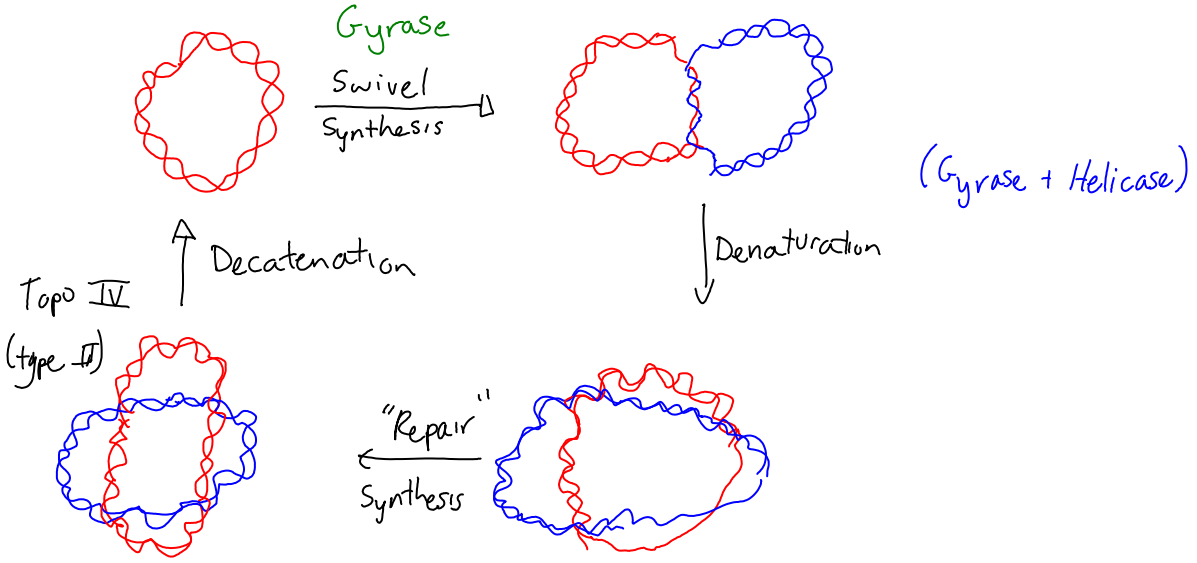
  Associate with seq A -> membrane attachment; delays methylation of newly synthesized strand by approx 10-15min
  (only hemi methylated DNA is sequenced)
  Seq A - cells -> No delay in replication oriC region

- If increase methylase, it would shorten time between replication

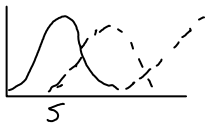
- Termination region
  - Single origin of replication and region that have terminator sites (redundant sites). There is no obligatory termination site for E coli. Can delete all termination sites and replication continues just fine.



- Chromosomes as we complete the replication phase
  - DNA synthesis



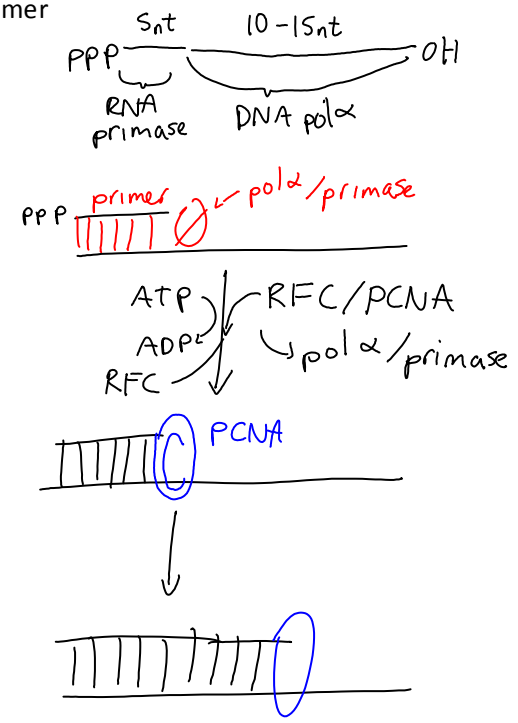
- Replicaiton - Eurkaryotes



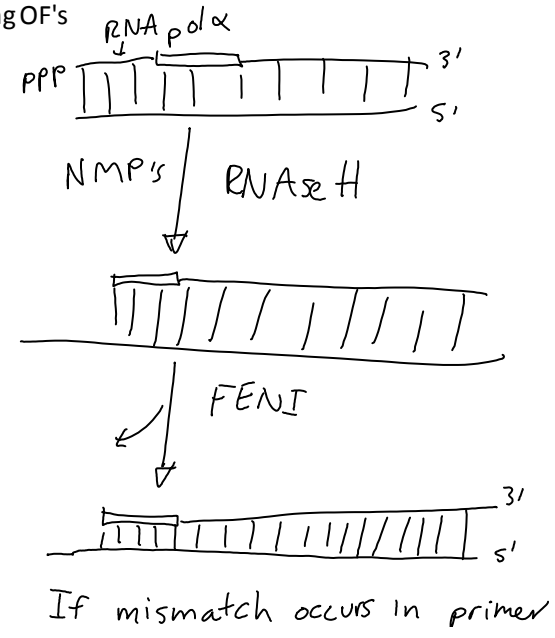
Function	E.coli	Eukaryotes

Priming	Primase	Poly α/ primase
Leading/lagging strand synthesis	Pol III* HE	Pol δ
Processivity factor	Beta protein?	PCNA (proliferating cell nuclear antigen)
Clamp loading	γδ complex	RFC
ss Binding	Single strand DNA binding protien (SSB)	RPA

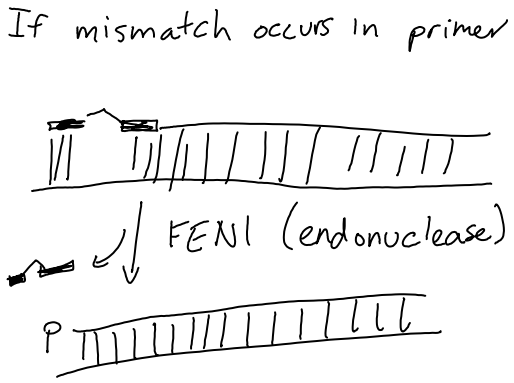
- Elongation - Eukaryotes
  - Polα/primase -> no 3' exo (pencil w/out eraser)
    - primer



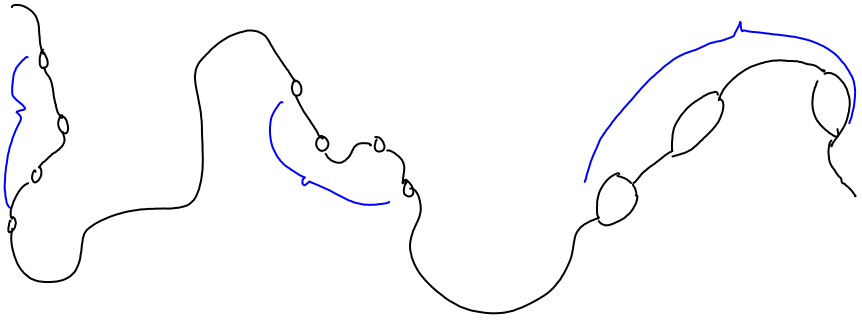
Processing OF's



If mismatch occurs in primer



- Origins of DNA synthesis of eukaryotic cells
  - Electron microscopy



  - Replication origin - clusters
    Distance between origins 10-50 kb apart
    Distance separating clusters > 300kb
    Coordinated activation of origins within cluster
  - Multiple origins of replication -> replicon is area of DNA replcation from 1 origin of replication

Grades

Monday, April 28, 2008
11:50 AM

Mean on Q1 + m1 -> 100
Medium 102 pts
Total points assigned 150pts

Notes 05/07

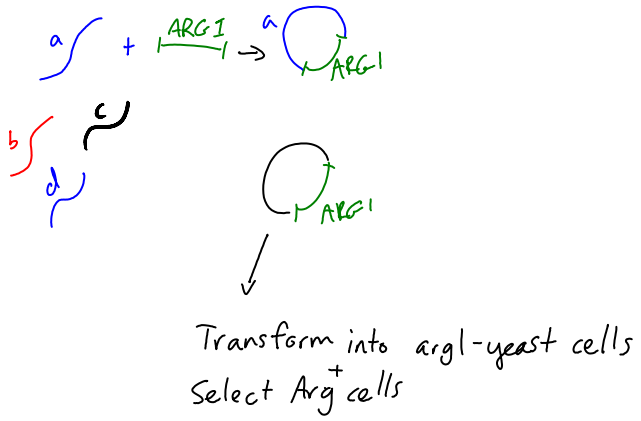
Wednesday, May 07, 2008
11:05 AM

Simple Eukaryotic Origin

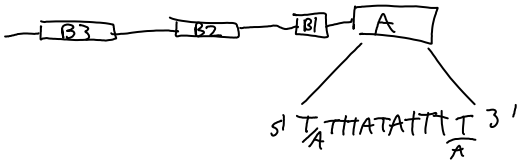
Yeast - Saccharmyeas cervisiaz (haploid)

Cloning:

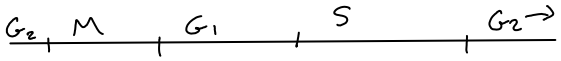
1. Cut yeast DNA into short fragments
2. Covalently attach a selectable marker



- Properties of Autominously replicating sequences (ARS)
  - Approx 400 different ARS in yeast
  - Minimal sequence <70bp
  - Conserved element AC
  - Replicates in S phase
  - Requires replication proteins
  - Replication initiates in ARS
  - Bidirectoinal, semiconservative

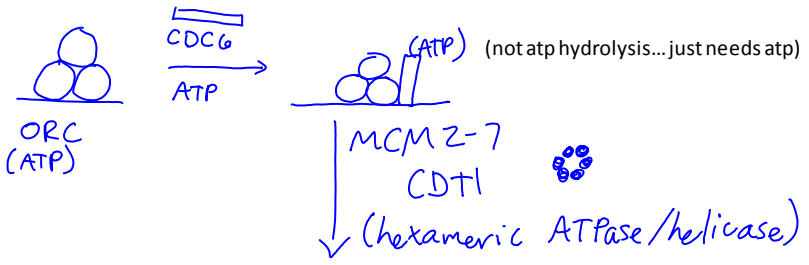


- Activation of origin (yeast)

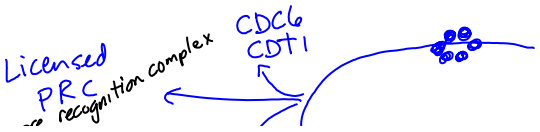


Pre RC (replication complex) assembly

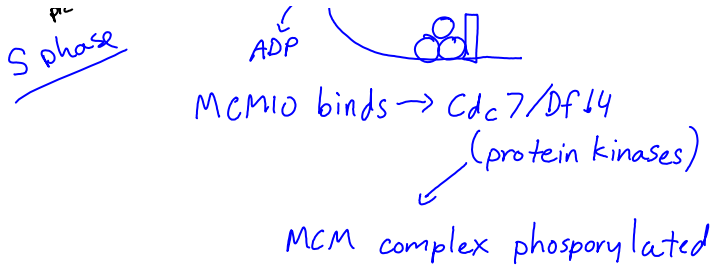
ORC (origin recognition complex) - 6 proteins ORC1, ORC2... bind to ORi region



(not atp hydrolysis... just needs atp)



Cdc6/cdt1 analogous dnaC
Mcm's analogous dnaB (helicase)

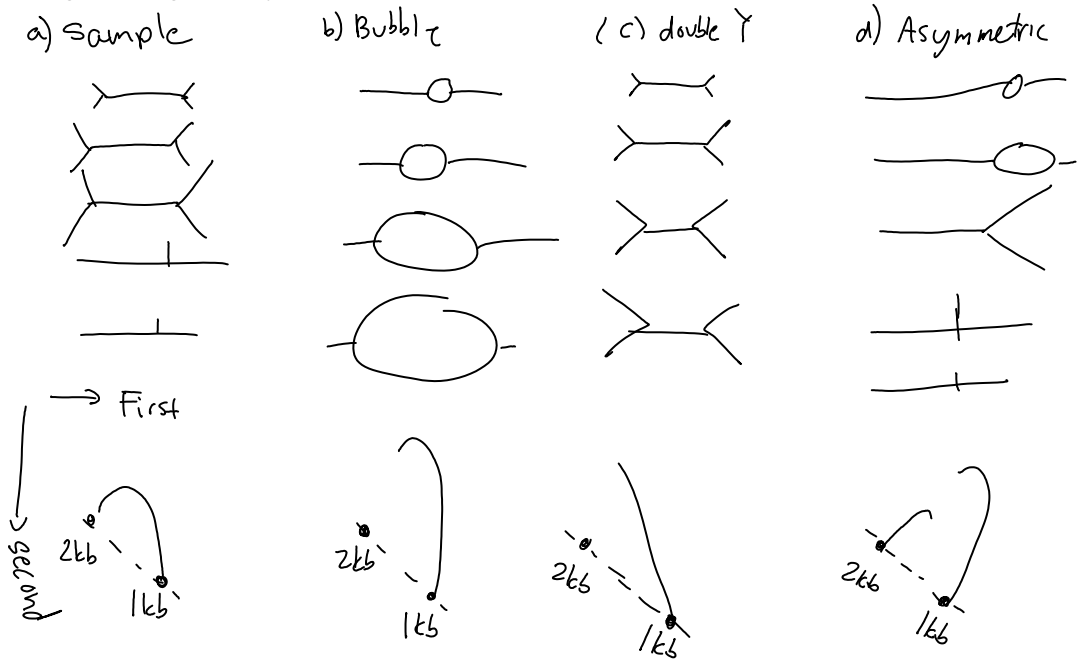


- Functionally analogous yeast vs e.coli

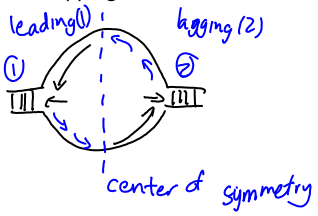
Eukaryotes	Prokaryotes
ORC (1-6)	Dna A
MCM (2-7)	DnaB
Cdc6/Cdt1	DnaC

Licensed PRC (G1)
Ddk → phosphorylation MCMs

- Figure 21.9 mcgraw hill (higher eukaryotic)



- Okazoki fragment mapping
  - Physical properties
    - 2d gel analysis of genomic OF mapping



Chapter 30 Text Notes

Thursday, May 08, 2008
1:39 PM

Enzymes of Replication

- Pol I (DNA polymerase I) - couples deoxynucloeside triphosphate son DNA templates in a reactio nthat occurs through the nucleophilic attack of the growing Dna chain's 3'-OH group on the alpha-phosphoryl of an incoming nucleoside triphosphate. The reaction is driven by the resulting elimination of Pp_i and is subsequent hydrolysis by inorganic pyrophosphatase.
  - Can act as 3' -> 5' exonuclease
  - Can act as 5' -> 3' exonuclease

Notes 05/12

Monday, May 12, 2008
11:03 AM

Notes 0512

Audio recording started: 11:04 AM Monday, May 12, 2008

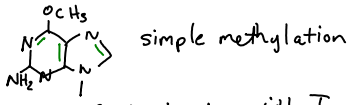
Midterm II - rescheduled to may 21

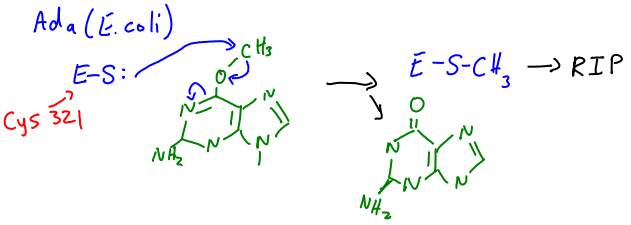
Types of Repair Systems

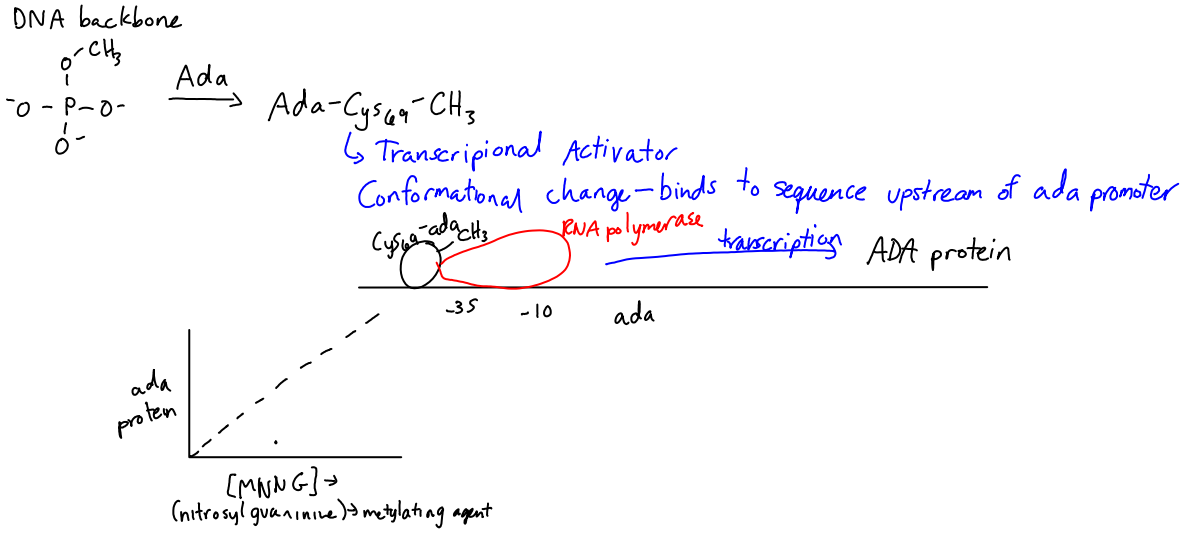
1. Direct repair - direct reversal of damanaged base/site
   a. Photoreactivatoin (UV dimer) - not available for mammals
   b. Alkyl transferase methylation
      - O_6 of guanine most preferred for methylation
      - O_7 of guanine and O_4 on thymine also possible
      - Methyltransferase - exists in eukaryotic and prokaryotic

2. Excision Repair
   a. Base excision repair (BER)

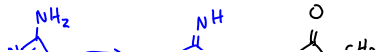
- **Alkyl Transferase**



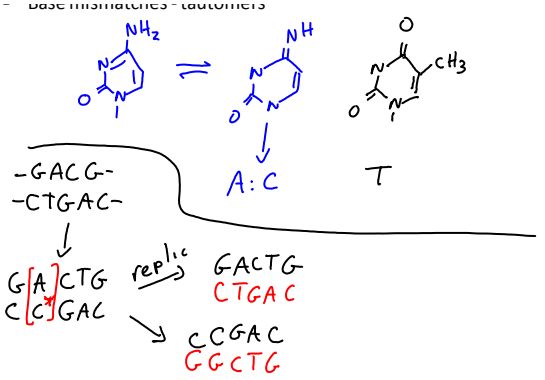




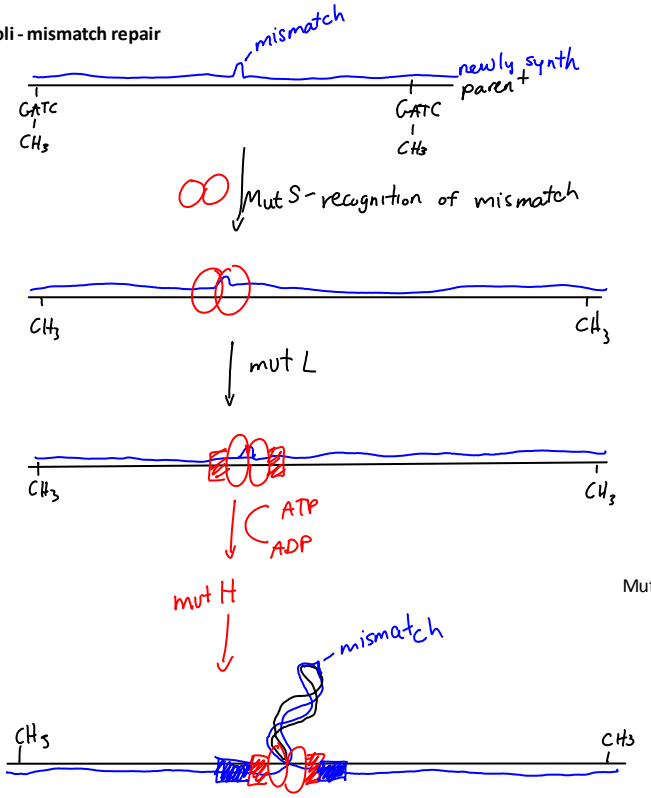
- DNA mismatch repair
  - **Replication Errors**
    - Base mismatches - tautomers



- Base mismatches - tautomers

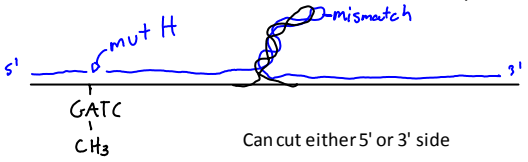


- **E.coli - mismatch repair**



Mut H only cuts nonmethylated strand

- At hemi methylated GATC / CTAG (me)
  - Mut H -> endonuclease -> cuts 5' to GATC on unmethylated strand



Can cut either 5' or 3' side

- Mut H cuts at first GATC sequence encountered 5' or 3' side of lesion.
- Cut locatd up to 1-2kb from mismatch!
- DNA exonuclease removes nucleotides through mismatch site.

  - Humans have 8-12 Mut S homologs while E.coli has 1
  - Human mut S descriminate between new and original stands is not through methylated GATC. Not understood fully what they do use to select.
    - Defect in mut S in rapidly replicating tissue such as colon

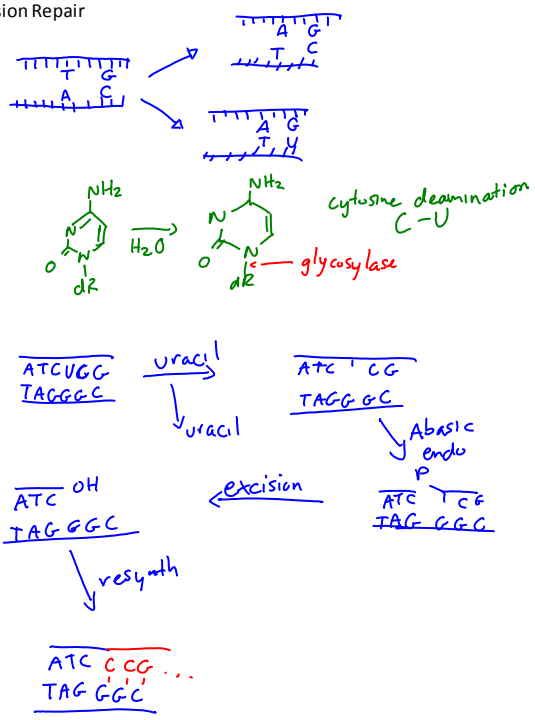
- Base Excision Repair
  - Several variates, depending on nature of damage, nature of glycosylase, and nature of DNA polymerase

Glycosylases cleave glycosidic bond

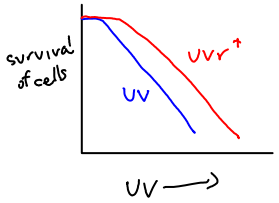
- All have in common the following steps:
  1. Removal of incorrect base by an appropriate DNA N-glycosylase to create an AP site.
     - AP is a basic site P for purimidemic or purinic
  2. An AP endonuclease nicks on the 5'-side of the AP site to generate a 3'-OH terminus
  3. Extensoin of the 3'-OH terminus by a DNA polymerase

Creates AP site
AP endonucelase cleaves deoxyribose
Repair by dna pol and ligase

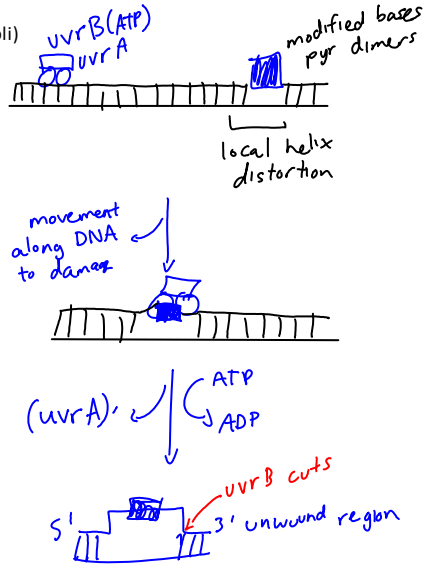
- Base Excision Repair



- NER (E.coli) - recognizes helical distortions rather than specific addnot
  - UV photodimers
  - Benzpyrene adducts
  - Variety of genotoxic adducts
  - Oxidative damage



- NER (e.coli)



- Xeroderma pigmentosum
  - Autosomal recessive
    Cancer susceptibility increases skin cancer > 2000fold
    Stomach, intestine, lungs greater 20-fold
  - 8 complemntation groups
    - XPA, XPB, … XPG (XPV)
  - Legion recogition XPC (XPA)
  - Unwinding at lesion XPB/XPD
  - Cutting of damage strand: XPG (3') XPF/ERCCI (5')
  - Excision repair coupled to transcription
  - Transcribed regions of chromosomes repaired 5x faster than non transcripted

Notes 05/14

Wednesday, May 14, 2008
10:57 AM

Notes 0514

Audio recording started: 11:00 AM Wednesday, May 14, 2008

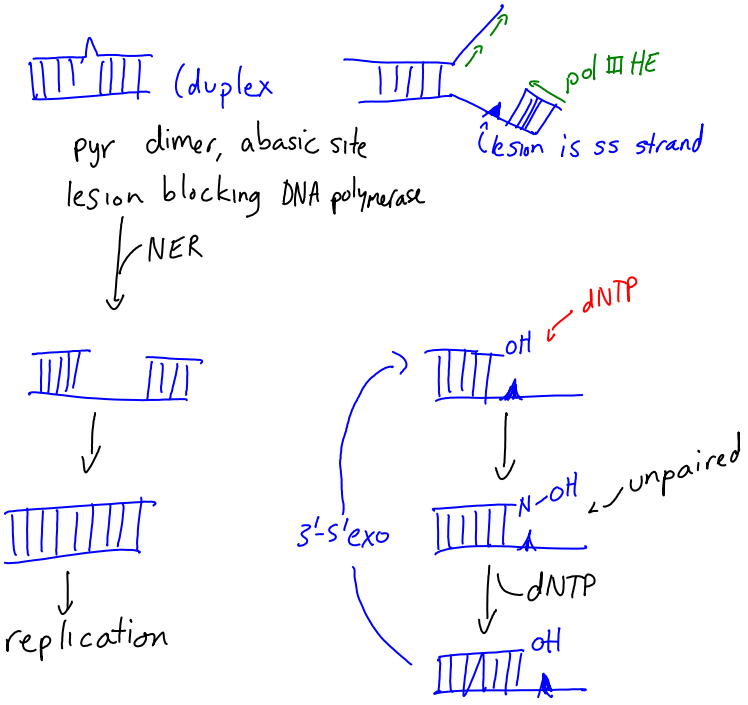
1) Take home quiz 2 due Friday end of lecture
   - 2 problems (1 bonus)

2) Review session for midterm 2.
   - Mon 6:30pm
   - Tues 11am-noon discussion

3) Midterm
   - 100pts closed book
   - 25pts take home problem (like quiz)
   - Material covers through Monday's lecture

- Last time: repair pathways in prokaryotic and eukaryotic cells
  - These repair mechanisms usually occurs when DNA is in duplex
  - There is possibility that damage occurs while cells replicating and damage is at or near replication fork (ss dna)
    - Normal excision pathways cannot repair these ss cases

- Bypass (error prone) synthesis (TLS)

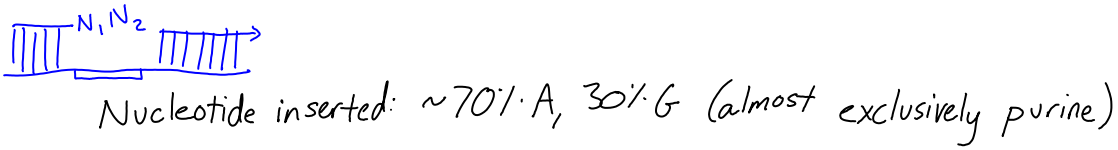


- Pol V $(umuC)(umud')_2$ - only expressed when there is damage
  - Requires 3'OH
  - Uses 5' dNTP's
  - No 3'->5' exo
  - Template dependent
    - At lesion site it abandons template dependence
  - Non processive (approx 7nt)
  - Inserts nucleotide opposite lesion
  - NOT template directed

- NOT template directed

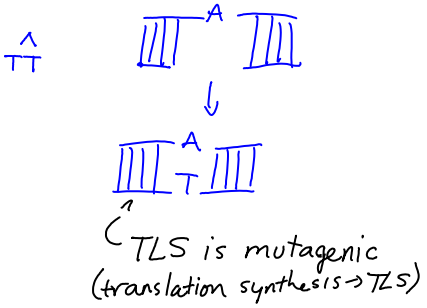
Extends chain beyond legion site
NOTE: N NOT. We base paired with lesion
Still has legion but is **now duplex DNA and then can be repaired by nucleotide excision repair**
Cost of repair is that the sequence is changed

- At dimers - 2 nucelotides incorporated without pairing



NOTE DAMAGE NOT REMOVED AFTER SYNTHESIS

Why A? -> most common type of photo damage:



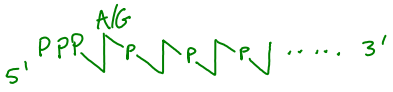
This is prokaryotic... eukaryotic is similar

- New topic - TRANSCRIPTION
  - 2 sections discussed
    1) Mechanism
    2) Gene regulatory schemes

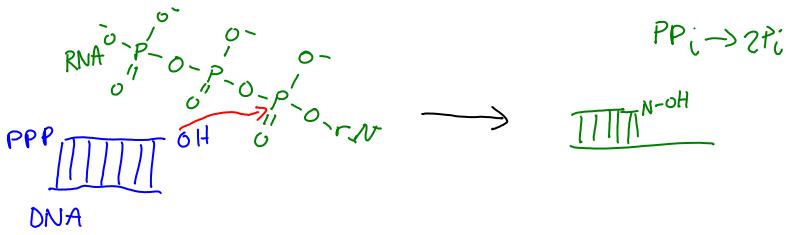
- Transcription
  - Copying DNA sequence int oRNA
  - DNA->RNA ->Protein
  - Prokaryotic transcription
    - Only certain regions of chromosomes are transcribed
    - Amount of RNA made from each of these regions varies (approx 1000-fold)
    - Amount of transcription can be affected by cellular/environmental signals
    - RNA is unstable - varies (1-3min) severe (days)

- Classes of RNA
  - rRNA - stable abundant (approx 40%)
  - mRNA - unstable (1-2min), variable size, amount
  - tRNA - small stable

  - All RNA species transcribed by single DNA dependent RNA Polymerase
  - General properties of RNA polymerase
    1) 5' rNTP's
    2) Template dependent
    3) Initiates RNA chain (de novo - ability to put down first nucleotide in chain)

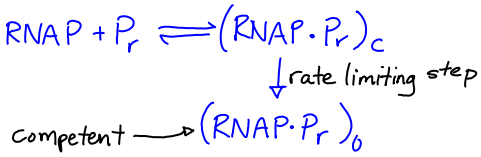


4) Nucleotides added at 3' end of chain (5'-> 3' synthesis)

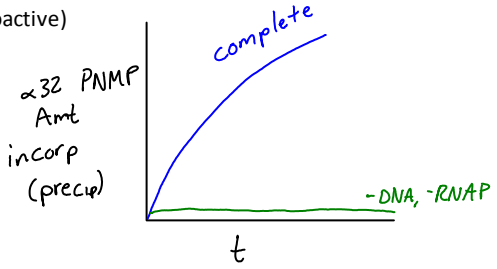


- Transcription Process
  1) Initiation - binding of RNA polymerase to DNA sites (promoters) -> conversion to competent form
     - Key step, most regulated
  2) Elongation - extension of RNA chain
  3) Termination - completing of RNA, release of RNA polymerase

- Initiation



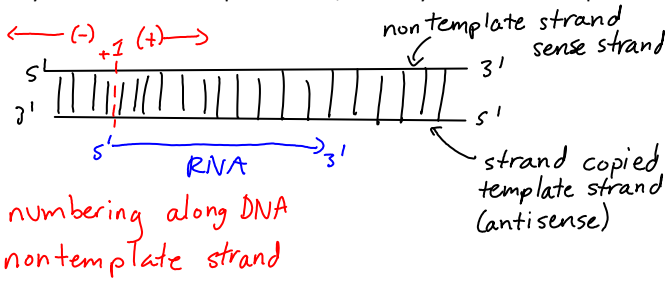
- Assay of RNA Polymerase Activity
  - RNAP
  - rNTP's (radioactive)
  - DNA (DS)
  - Buffer



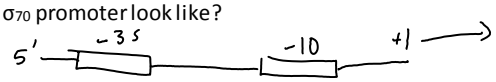
RNA Polymerase subunit structure

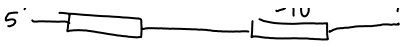
2 forms	460kd
$\alpha_2 \beta\beta'\mu$	$\alpha_2 \beta\beta'\omega\sigma$
core	holoenzyme (HE)
nonspecific	specific transcription

- Core Polymerase - Rna synthesis is nonspecific - initiates at DNA nicks - binds DNA relatively weakly
- Holoenzyme - Rna synthesis initiates at specific sites, RN aPolymerase HE binds promoters tightly



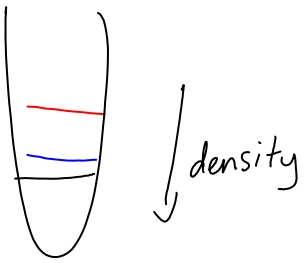
- σ factor -> specificity factor
- σ_{70} most common σ (5-8 others)
- HE (σ_{70}) -> binds to specific DNA
- Sites-promoters (σ_{70} other σ's recognize other sequences)
- What does σ_{70} promoter look like?





Discussion Week 7

Thursday, May 15, 2008
11:05 AM

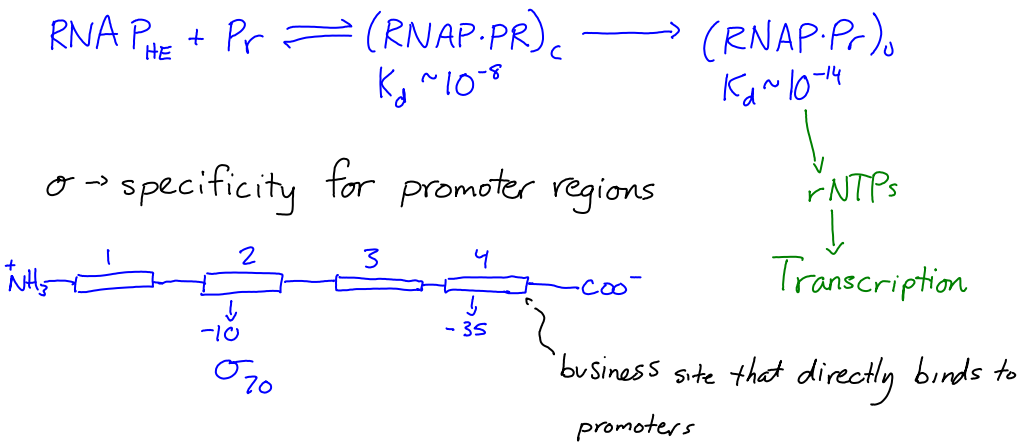
4. 

Notes 05/16

Friday, May 16, 2008
11:02 AM

Notes 0516

Audio recording started: 11:02 AM Friday, May 16, 2008



- σ_{70} most common
- HE(σ_{70}) -> binds to specific DNA sites - promotors (σ_{70}; other σ's recognize other sequences)
- What does a σ_{70} promoter look like?

-10 (Pribnow) $T_{77}\ A_{76}\ T_{60}\ A_{61}\ A_{56}\ T_{82}$

77% of 298 promoters

-35 region $T_{69}\ T_{79}\ G_{61}\ A_{56}\ C_{54}\ A_{54}$

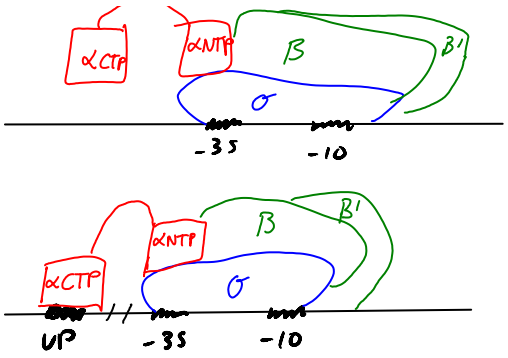
Spacing between -35 and -10 is important and if isn't conserved transcription doesn't occur.
Sequence between these regions doesn't matter.

- Statically preferred nucleotide at each position
  - Most promoters - 2 or more base changes from consensus
  - **UP** mutations -> basic change that produces a sequence more closely similar to consensus
  - **DOWN** mutation -> base change producing a sequence that is further from consensus

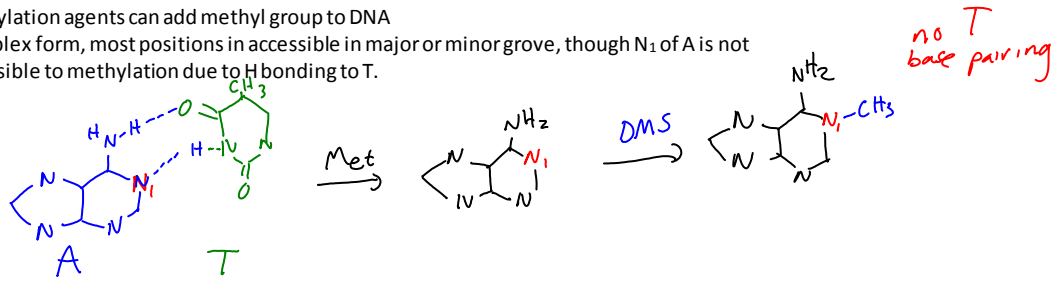
	-35		-10
Consensus	TTGACA	-N_{17}-	TATAAT
trp	TTGACA	-N_{17}-	TTAACT
lac	TTTACA	-N_{17}-	TATGTT

UP element - upstream of -35 (-40 to -60) for some highly expressed genes
UP element is a very AT rich element
UP element is not bond by σ

- Alpha subunit with UP element creates stable closed complex and more efficient conversion to open complex.



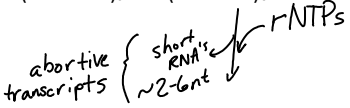
- DNA Methylation
  - Methylation agents can add methyl group to DNA
  - In duplex form, most positions in accessible in major or minor grove, though N_1 of A is not accessible to methylation due to H bonding to T.



- Function of other RNAP subunits

β, β' – DNA binding
(rifamycin resistance β) → antibiotic binds β & blocks RNA polymerase
β – catalytic center for Nucleotide addition
α – DNA contact

- RNAP + P_r -----> $(RNAPxPr)_c$ ----> $(RNAPxP_r)_o$

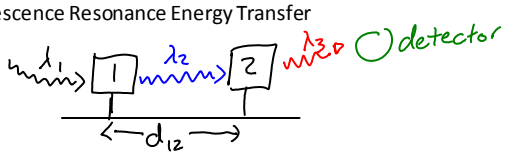


>>1 abortive transcript / bound RNAP → promoter clearance → elongation

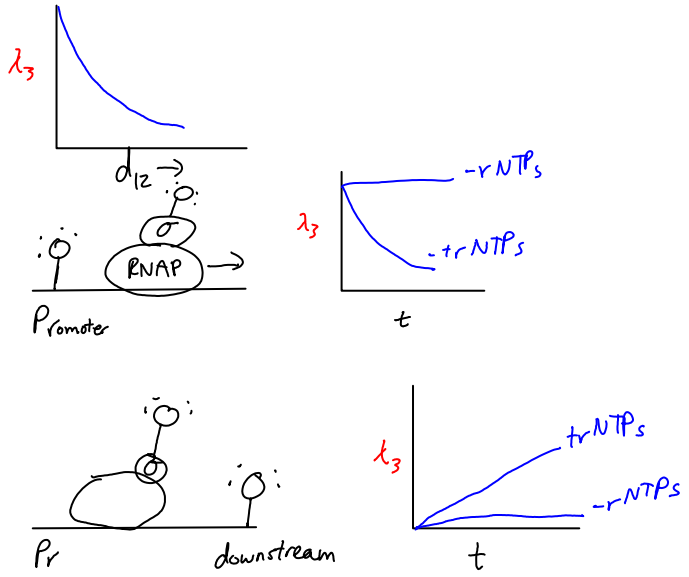
σ

Σ only neded until RNAP clears promoter -> release from HE... or is it?

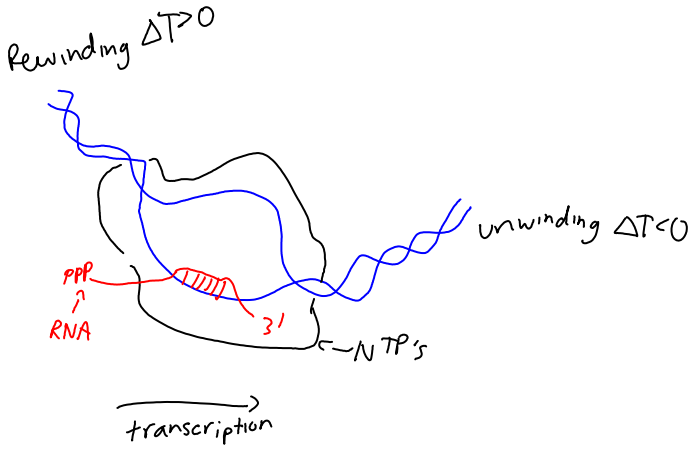
- Fluorescence Resonance Energy Transfer

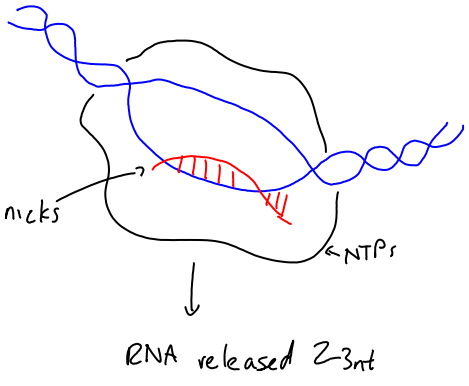


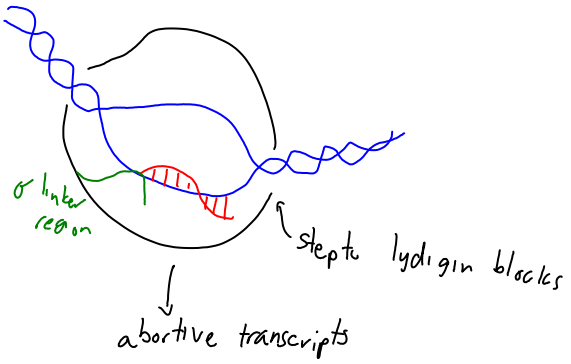
Measuring wavelength change from 1 to 2. Distance between 1 and 2 changes wavelength change dramatically



Sigma is loosely associated with RNA polymerase for elongation but is not tightly associated as it was in beginning.







Notes 05/19

Monday, May 19, 2008
11:04 AM

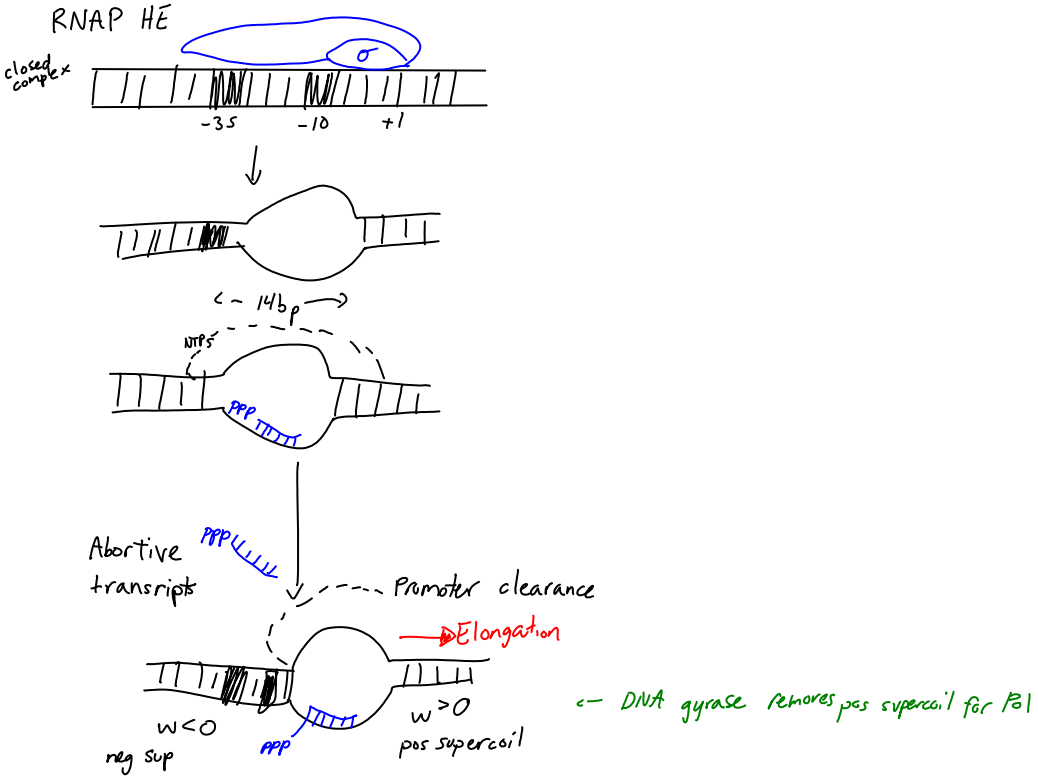
Notes 0519

Audio recording started: 11:05 AM Monday, May 19, 2008

1. Handout PSII answers, 2-d gel figures
2) Review session tonight 6:30 -> 10:00pm young 2200

Test questions
1) T/F
2) Identify
3) DNA Replication - Elongation
4) DNA Repair/ DNA Repl
5) Initiation of Repli
6) TLS Translation Synthesis (take home)

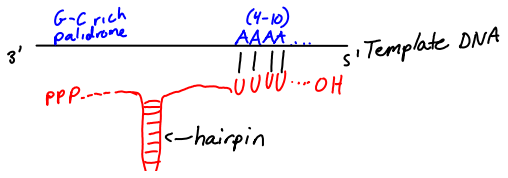
- Inhibitor of initiation: RIFF
- Binding of Rna pol to promotor:



RNA polymerase is processive (remains bound to template) (unlike DNA polymerase)
It will transcribe large regions of RNA and then pause so we can observe many transcriptional intermediates

- Transcription Termination
  - Two types
    1) Rho independent approx 50%
    2) Rho dependent approx 50%

- RHO independent:

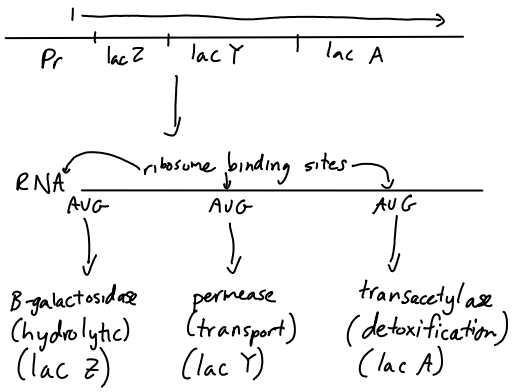


- RNA polymerase pauses at A stretch
- G:C hairpin forms
- Destabilize A:U pairs (weak)
- RNA released
- Experiments
  - Make a weaker G:C (I:C... hypoxanthine and cytosine which is weaker bond). Observe if this causes A:U bonds to not be weakened as much.
  - Results reduce efficacy of termination
  - Other method is strengthening A:U pair, RNA polymerase continues.
  - So, thermodynamic balance between G/C hairpin weakening AU bonds

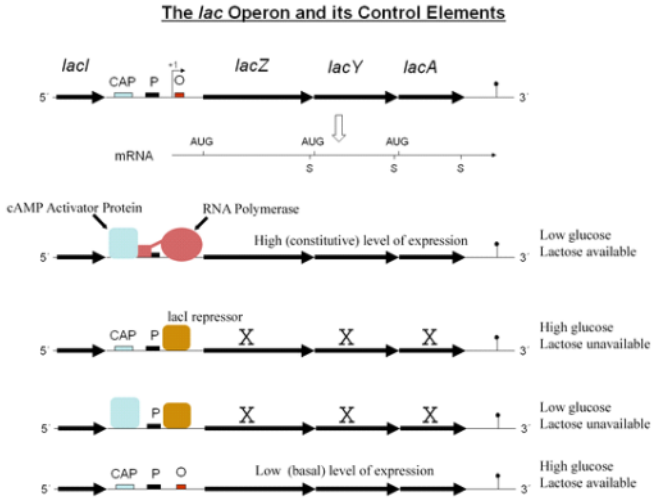
- Rho - factor
  - Hexameric helicase - unwinds RNA:RNA, RNA:DNA
  - ATP -> ADP + P_i
  - A lot like DNA B protein
  - Rho dependent terminator
    a) Hairpin (DNA palindromic, does not need to be G:C rich)
    b) No template 'A' stretch
    c) 5' upstream sequence in RNA
       Rut site - (for roh utilization site)... site where roh will assemble on RNA
       C-rich, little 2^0 structure

    Rho factor binds to RNA at rut site
    translocates along RNA (5'->3')
    Unwinds RNA:DNA hybrid; RNA release (mechanism is not known)
    3' end of RNA vary approx 50nt

- Regulation expression of genes
- Lac operon - offshoot of studying fermentation of bacteria in wine
  - Lactose (Lac) operon - set of 3 genes on e.coli chromosome transcribed as a unit



Lac Z^- -> no beta-galactosidase (lac^-)
Lac Y^- -> no permease - no uptake of lactose into cell (Lac^-)



lac operon in detail

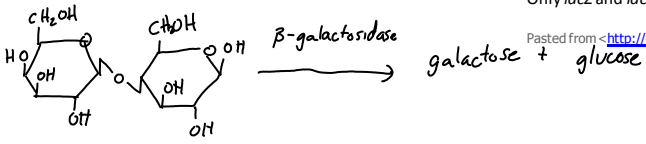
Pasted from <http://en.wikipedia.org/wiki/Lac_operon>

lacZ encodes β-galactosidase (LacZ), an intracellular enzyme that cleaves the disaccharide lactose into glucose and galactose.
- *lacY* encodes β-galactoside permease (LacY), a membrane-bound transport protein that pumps lactose into the cell.
- *lacA* encodes β-galactoside transacetylase (LacA), an enzyme that transfers an acetyl group from acetyl-CoA to β-galactosides.

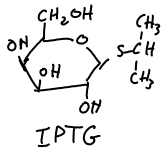
Only *lacZ* and *lacY* appear to be necessary for lactose catabolism.

Pasted from <http://en.wikipedia.org/wiki/Lac_operon>

- Lactose (beta 1-4 between lactose and glucose)



Natural inducer: allolactose (beta 1-6) galxglu
Gratuitous inducer IPTG (isopropyl-beta-D thiogalactoside



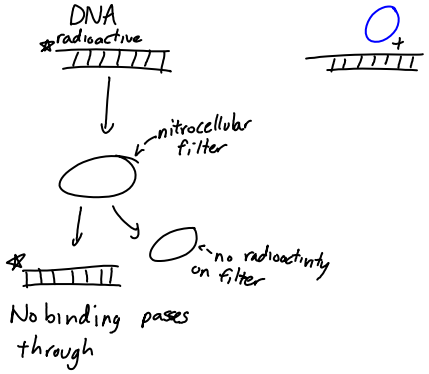
Isopropyl-β-D-thio-galactoside (IPTG) is frequently used as an inducer of the *lac* operon for physiological work.[1] IPTG binds to repressor and inactivates it, but is not a substrate for β-galactosidase. One advantage of IPTG for *in vivo* studies is that since it cannot be metabolized by *E. coli* its concentration remains constant and the rate of expression of *lac p/o*-controlled genes, is not a variable in the experiment. In addition, IPTG is transported efficiently independent of whether the *lacY* gene is functional.

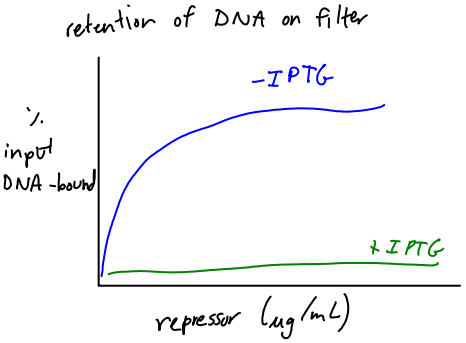
Allolactose is an isomer of lactose and is the inducer of the lac operon. Lactose is galactose-(β1->4)-glucose, whereas allolactose is galactose-(β1->6)-glucose. Lactose is converted to allolactose by β-galactosidase in an alternative reaction to the hydrolytic one. A physiological experiment which demonstrates the role of LacZ in production of the

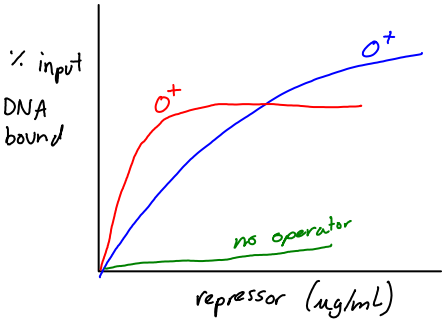
- Lac Operon
  - Identified and created by Jacob + Monod
  - Trans acting regulator - repressor
  - Cis - acting regulation (site) - operator
  - Repressor mutants
    - Lac I - > repressor gene

- LacI -> repressor gene
  - I^- -> nonfunctional repressor (can't bind to operator tightly)
  - I^-/I^+ --> "I^-" dominant (I^{-d}) - there are cases I^- is dominant
  - Lac I^S -> cannot be induced
    - I^S/I^+ -> "I^S"
  - Genetics -> repressor acts as a multimer (I_4)

- Studying binding of lac operon
  - Filter binding Assay - DNA binding







- Operator site has dyad symmetry

- Mutations affecting repressor binding
  - Tighter binding -> greater 2-fold symmetry *
  - Weaker binding -> reduces symmetry *
  - O_c mutations -> reduce binding 10-30 fold

DNA	Repressor	Repressor + IPTG
Operator	$2x10^{13}$	$2x10^{10}$
Non operator	$2x10^6$	$2x10^6$
% bound	76%	<3%

  - Ecoli contains approx $4x10^6$ non operator sites

"true" inducer in *E. coli* cells is the observation that a null mutant of *lacZ* can still produce LacY permease when grown with IPTG but not when grown with lactose. The explanation is that processing of lactose to allolactose (catalyzed by β-galactosidase) is needed to produce the inducer inside the cell.

Pasted from <http://en.wikipedia.org/wiki/Lac_operon>

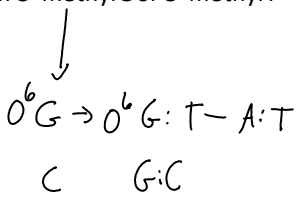
Midterm 2 Review Session

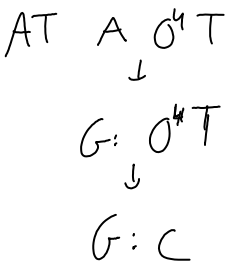
Monday, May 19, 2008
6:59 PM

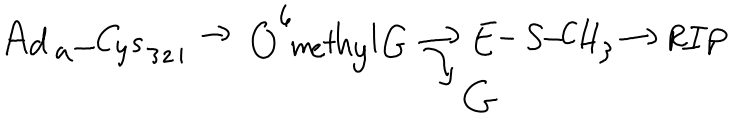
DNA Repair:

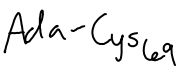
1) Direct repair - "ada" O^6 methyl G
   - Methylation is the damage
2) Methyl directed mismatch repair (N^6 methyl)
   - Methylation marks parental from newly synthesized to repair new synth)
3) BER
4) NER
5) Translation Synthesis

1) Direct repair - ada O^6 methyl G
   - Electrophylic addition
   - Ada - adaptive response
   - Source of methyl: O^6methylG or O^4methylT

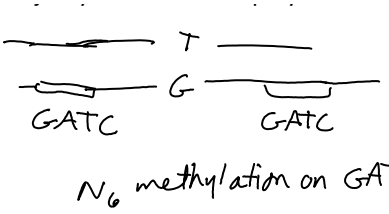








2. Methyl directed mismatch repair
   - Deals with replication errors
   - Major system to correct polymerase errors due to factors such as tautonomerization



N_6 methylation on GATC on A

This distinguishes parental strand from template to compare sequence

Dam methylase
SecA

3. BER Base Excision Repair - specific enzyme for each base
    Uracil N glycosylase - removes U from DNA
        How does U get in dna?
        - □ C-> U (G:U)
        - □ dUTP concentration (A:U)

            Glycosylases (whole family of glycolases for each base) cleave glycosidic bond between sugar and base. Do not disrupt sugar phosphate backbone, so base needs to leave
            Base is removed to create abasic site (no base) by flipping outside of helix
            NO ATP requirement
            Incision at abasic site leaving 3' OH and 10-20 nt, dna pol1 fills in

4. NER - nucleotide excision repair. ONLY DS DNA
    - High capacity, low specificity system
    - Broad specificity - remove dimers, abnormal bases (modifie, oxidized, damaged)
    - Recognizes helical distortion
    - Major repair of UV damage
    - Repair of benzpyrine, aflotoxin, and many carcinogens (doesn't work on methylation damage)
    - $(uvrA)_2$ recognizes lesion site
    - Works together with (uvrB), uvrb piggy backs on uvra
    - When problem is found, uvra is released and uvrb unwinds dna sort of like a helicase
    - Unwinding of dna by uvrb uses ATP
    - Uvrc binds to urvb, and they make site (uvrb on 3', uvrc on 5' side) and fragment leaves
    - polI repairs

5. TLS translation synthesis
    - Pol V under tight regulation so isn't produced much
        - Multisubunit of 2 parts $(umuC)(umuD')_2$
        - Doesn't have 3'->5' exonuclese
        - Extends nucleotide chain past dimer to make DS dna
        - Pol V is non processive
        - Inserts 2 NT over dimer… generally A residues and does not look at template for insertion base
        - Pyrimidine dimer still need to be get rid of, but pol V put dimer in duplex complex. Then NER comes and removes dimer and copies strand
        - Mutational consequence to repair… potential mutagenesis at dimer site is a consequence of TLS mechanism

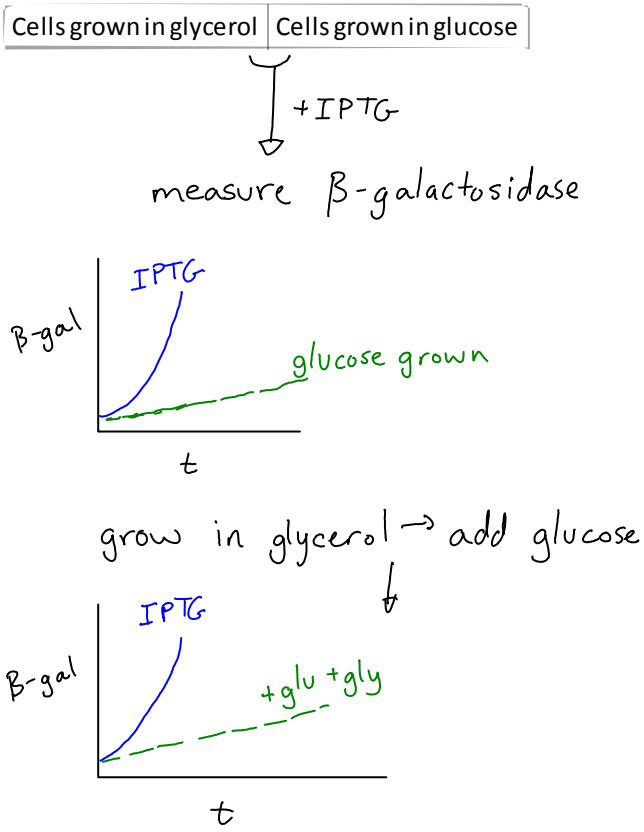
- Continuing repair mechanisms:
- Glycolases can also work on SS DNA. DNA polIII cannot work on abasic site (ss) but PolV can/does

Notes 05/23

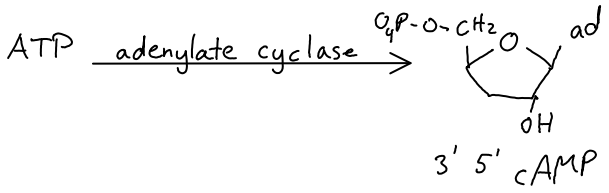
Friday, May 23, 2008
11:20 AM

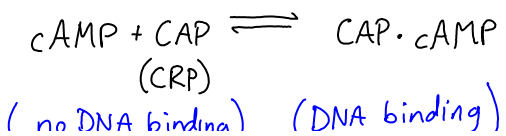
Notes 0523

Audio recording started: 11:20 AM Friday, May 23, 2008



- Catabolite Repression
  - Reduced expression of Lac operon in presence of glucose
  - Approx 200 genes in E. coli catabolite sensitive
  - Global regulation of gene expression through cAMP levels

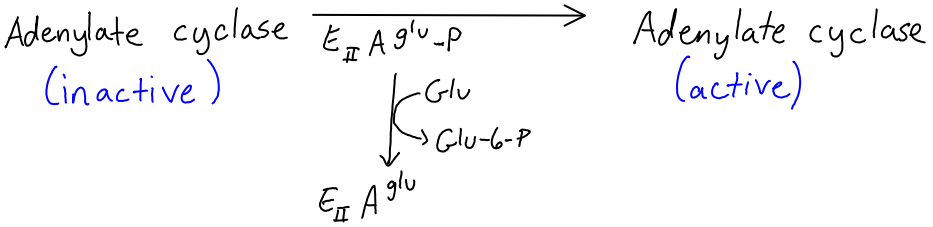




(CRP)

(no DNA binding) (DNA binding)

- Pathway for cAMP synthesis



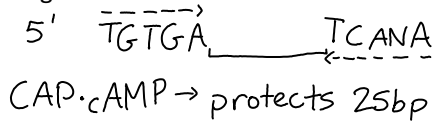
- RNAP interacts with CRP(cAMP)
  a) Cosedimenation
  b) Covalent crosslinking

  What subunit of RNAP interacts with CAP(cAMP)? - α subunit

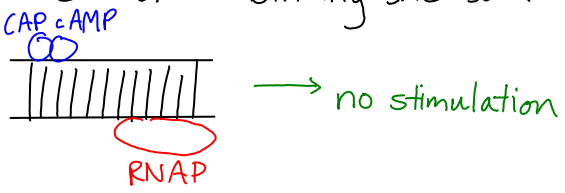
  At lac CTD of α subunit needed for contacting CAP·cAMP bound at CAP site
  At other sites N-terminal domain of α contacts CAP·cAMP (cap site overlaps promoter at these sites)

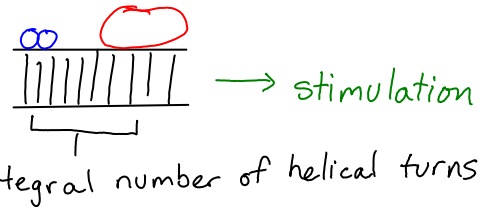
CAP(CRP) -> dimer (210aa)

CAP binding site



At lac CAP·cAMP binding site - same side of helix as RNAP binds





In summary:

- When lactose is absent then there is very little Lac enzyme production (the operator has LacI

bound to it).

- When lactose is present but a preferred carbon source (like glucose) is also present then a small amount of enzyme is produced (LacI is not bound to the operator).
- When lactose is the favored carbon source (for example in the absence of glucose) cAMP -CAP binds upstream of the promoter at a specific site. This bends the DNA around the protein which creates tension, and allows the RNA polymerase to bind to the promoter and Lac enzyme production is maximized. The DNA is not easily unwound under normal conditions, without the bound CAP, as the DNA contains a large number of the nucleotides which have 2 hydrogen bonds between them, needing more energy to part them

Pasted from <http://en.wikipedia.org/wiki/Lac_operon>

Notes 05/28

Wednesday, May 28, 2008
11:07 AM

Notes 0528

Audio recording started: 11:07 AM Wednesday, May 28, 2008

$$RNAP + P_r \overset{K_B}{\rightleftharpoons} (RNAP \cdot P_r)_c \overset{k_2}{\rightleftharpoons} (RNAP \cdot P_r)_o$$

CAP · cAMP → K_B ↑
lac I (repressor) → K_B ↓

CAPxcAMP binds DNA (same side RNAP)
CAPxcAMP contacts RNA polymerase (αCTD)
CAPxcAMP bends DNA (about 90^0)

Figure 7.21

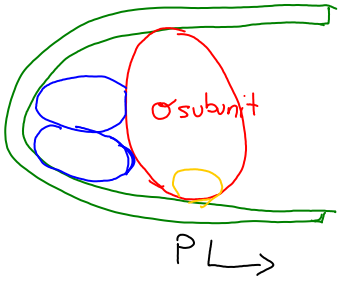
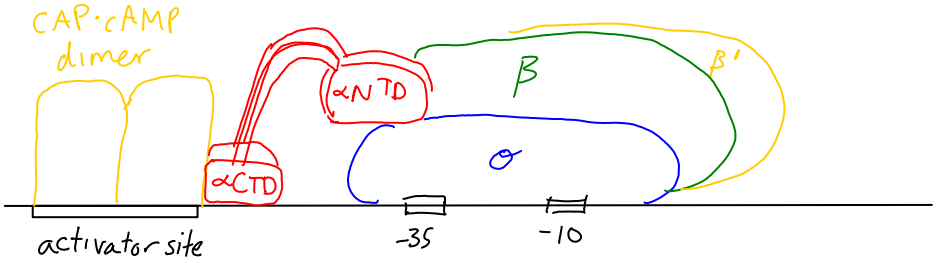
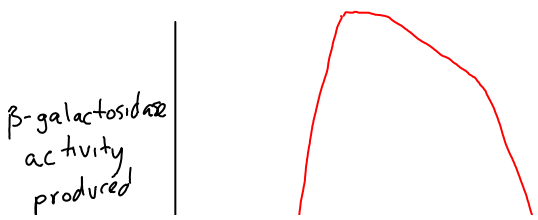
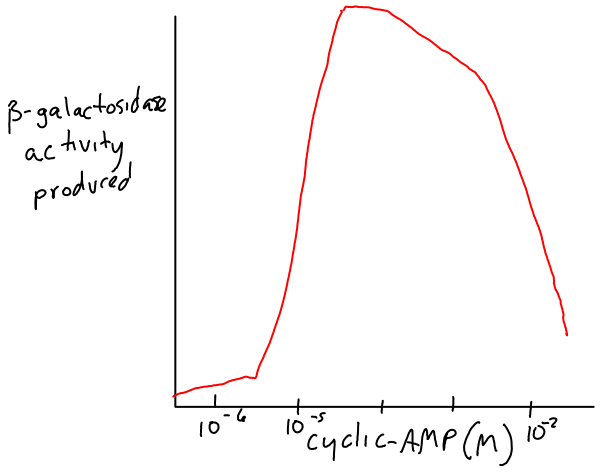


Figure 7.17



- Looking in vitro of lacZ expression as function as cAMP concentration:





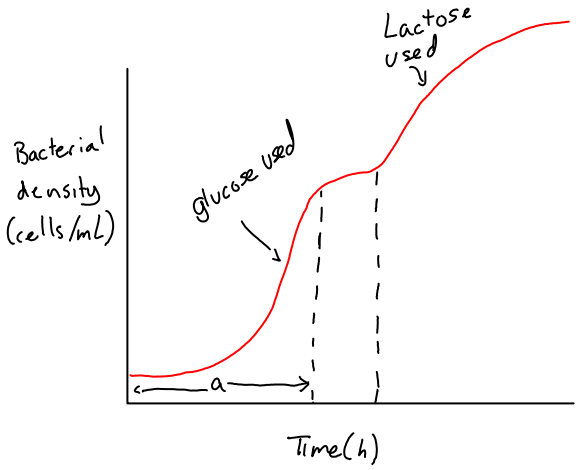
-CAP-cAMP	P_1	UV5
αNT	46	797
α256	53	766
α235	51	760

+CAP+cAMP	P_1	UV5
αNT	**625**	748
α256	62	723
α235	45	643

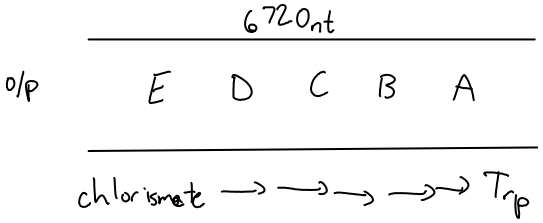
αNT = RNA Polymerase (normal)
α256, α235 prevent CAPxcAMP binding
P_1 = normal Lac promoter
UV5 = consensus promoter

- Looking for difference in activity between normal and mutant in the presence of CAPxcAMP

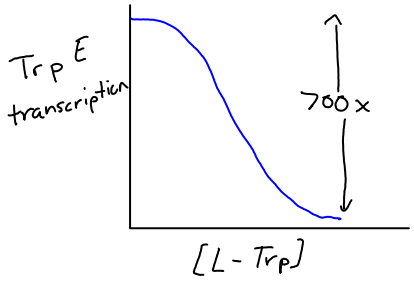
- Diauxic Growth Experiment
  - Diauxic growth curve
  - Glucose+catabolite "repressible" (lactose)
  - Glucose consumed 1st (a)



- Trp Operon (needed for biosynthesis of Trp) (Lac needed for catabolic breakdown of lactose)



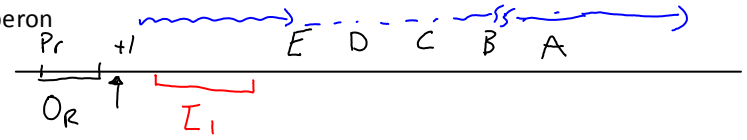
- Anabolic operon -> biosyntehsis of essential metabolite
- Transcription controlled by L-Trp

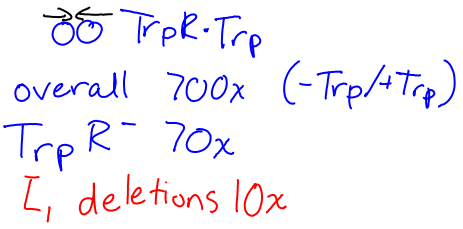


- Trp R -> repressor < Trp / aroH

- Trp R
  a) Low tryptophan: no repression
  b) High tryptophan: repression

- Trp Operon



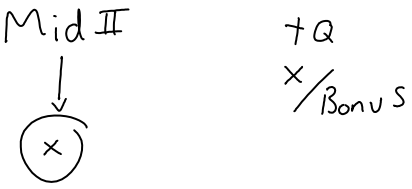


Transcript length
+Trp approx 170 nt
-Trp >6700nt

- 2 RNA secondary structure.
  - Self complimentary regions (forms hairpins)
  - Two continuous Trp codons

Since in prokaryotes the ribosomes begin translating the mRNA as soon as the RNA polymerase has moved farther down the DNA sequence, upstream translation occurs simultaneously with transcription of downstream genes. So, as soon as the polymerase has created the mRNA for the leader sequence, it is being translated. When the ribosome reaches the double-trp codons, if enough trp is present, the ribosome will not be delayed, and will continue translating until it reaches the stop codon and falls off the leader transcript. A hairpin will then form in the mRNA transcript (remember, still attached to RNA polymerase on other end) between regions 1-2, and 3-4, which destabilizes the RNA polymerase and halts transcription of the rest of the operon, thus preventing production of trp. On the other hand, if there is little or no trp available, the ribosome will be delayed or stopped on the double -trp, and a hairpin will form between regions 2-3 of the mRNA instead. This does not destabilize the polymerase, so transcription and translation occur. Similar mechanism regulates the synthesis of histidine, phenylalanine and threonine.

Grades:



Entire course grade distribution

Median = 188

Total = 300 possible (not including bonus)

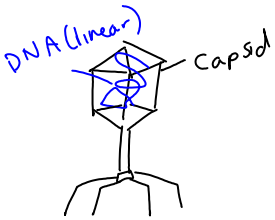
B or better 170 or above

Notes 05/30

Friday, May 30, 2008
11:04 AM

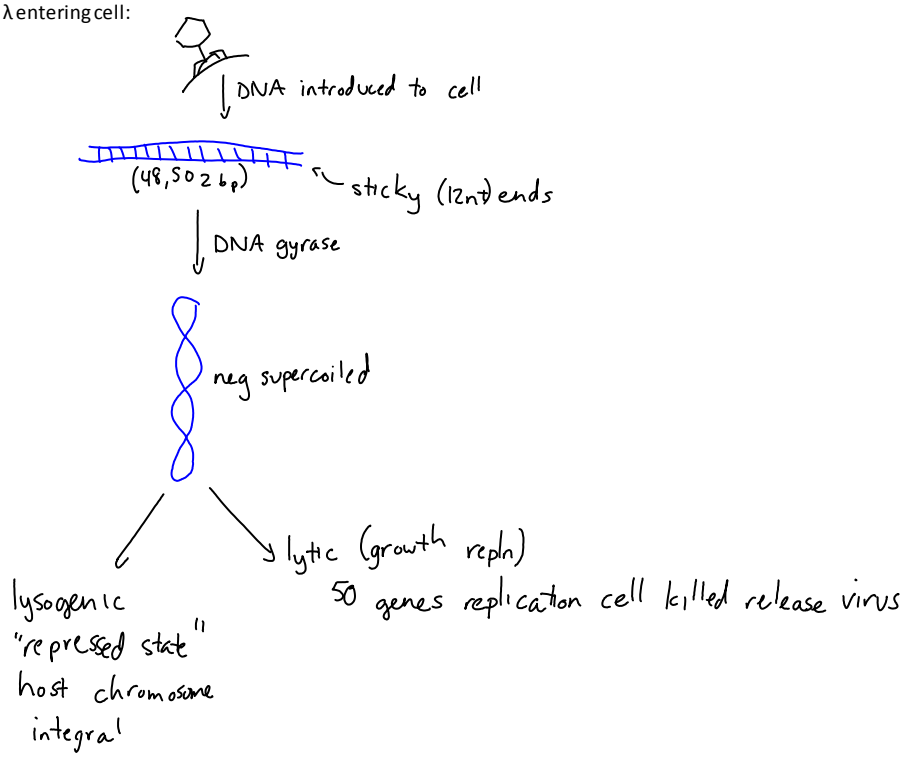
Notes 0530

Audio recording started: 11:04 AM Friday, May 30, 2008

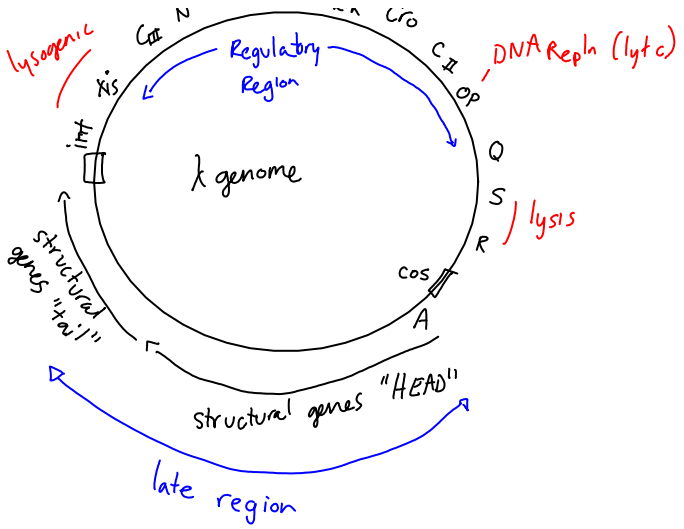


- λ phage
  a) Negative regulation
     - λ cro
     - λ cI
  b) Strong promoters P_L, P_R
  c) Weak promoters PRM, PRE
  d) Proteins transforming weak promoters into strong promoters (c_{II})
  e) Terminators (roh dep. Roh ind)
  f) Defeating terminators
     - "antiterminator"
     - (pN) (pQ)
  g) RNAP vs Repressors
  h) Environmental Signals (cAMP/glu)
  i) Cooperative protein binding Rated - "R" for repressor

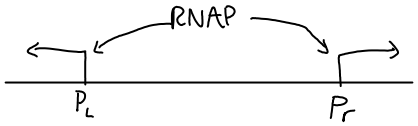
- λ entering cell:



- λ genome

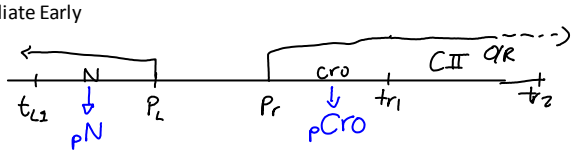


- Immediate early transcription



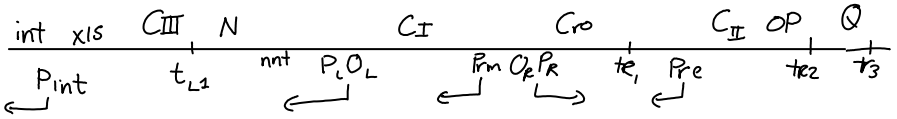
  - P_R, P_L strong promoter - RNAP
  - Binding - divergent transcription

- Immediate Early



t_{L1}, t_{R1} → rho dependent terminators
pN → antiterminator
pCro → repressor

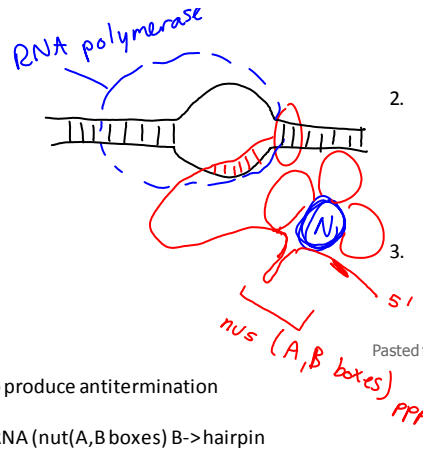
Also P_r' $t_{R'}$ transcript "no gene"



pN -> binds RNA and RNA polymerase

This occurs without the N protein interacting with the DNA; the protein instead binds to the freshly transcribed mRNA. Nut sites contain 3 conserved "box's", only BoxB of which is essential.

1. The boxB RNA sequences are located close to the 5' end of



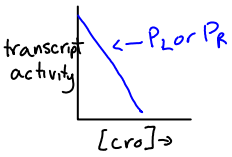
the pL and pR transcripts. When transcribed, each sequence forms a hairpin loop structure that the N protein can bind to.

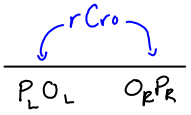
2. N protein binds to boxB in each transcript, and contacts the transcribing RNA polymerase via RNA looping. The N-RNAP complex is stabilized by subsequent binding of several host Nus (N utilisation substance) proteins (which include transcription termination/antitermination factors and, bizarrely, a ribosome subunit).
3. The entire complex (including the bound *Nut* site on the mRNA) continues transcription, and can skip through termination sequences.

Pasted from <http://en.wikipedia.org/wiki/Lambda_phage>

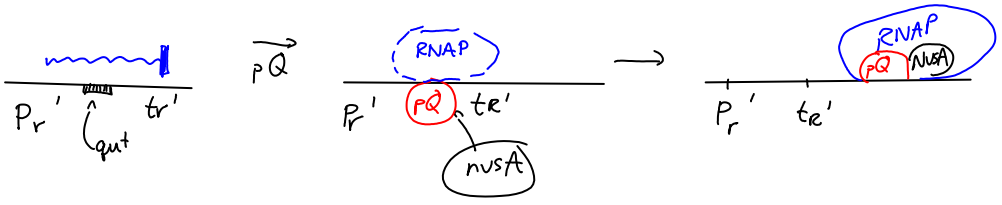
NusA,B,G
sio
Bind with pN to produce antitermination complex
- Contact RNA (nut(A,B boxes) B->hairpin
- Processive complex ->stays with

- pN -> pOpP pQ (antiterminator)
  - Cro->repressor (O_R, O_L)



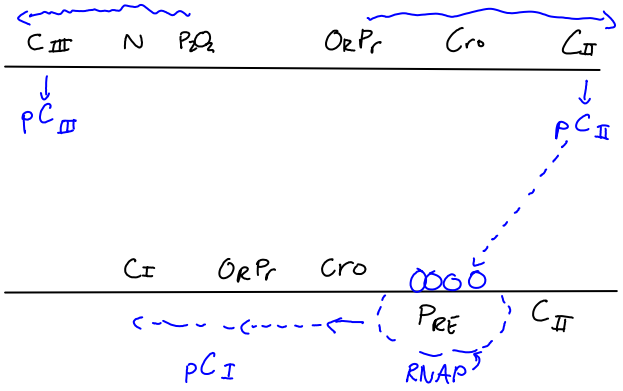


O,P ->replication (dnaA,C homologs)
Late transcription =
pQ-> acts at t_R' binds DNA



LATE GENES HEAD, TAIL PROTEIN VIRION

- Lysogenic Pathway - delayed early

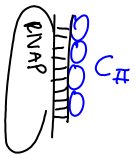


- C_{II} is transcriptional activator
  - Highly unstable and half life of 30minutes
- C_{III} is inhibitor of protease that brakes down C_{II}

pC_{II} ->needed for RNAP binding to pRE

ACGCAAACAAACG
TTGACA consensus
Big difference from consensus

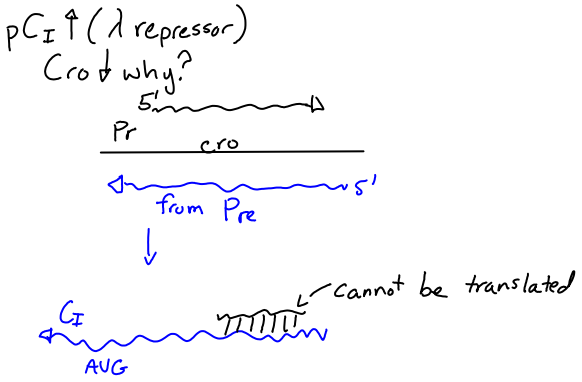
C_{II} binds to P_{RE} with RNA polymerase

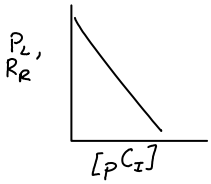


C_{II} binds on opposite faces of DNA

- rC_{III} -> interferes with HflAB protease
  - pC_{III} -> hflAB decrease so pC_{II} -> more stable
  - High cAMP -> low HflAB
  - Low cAMP -> high HflAB
  - In glucose (low cAMP) -> C_{II} unable, λ prefers lytic pathway
  - In poor carbon source (starvation) λ prefers lysogenic pathway

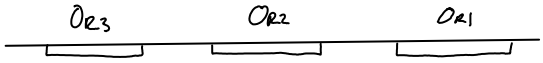
- pC_{II} -> increased transcription form P_{RE}





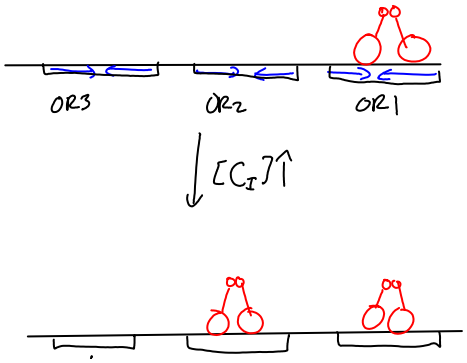
As C_I synthesis increases, P_L, P_R decrease
How can this lead to lysogeny?

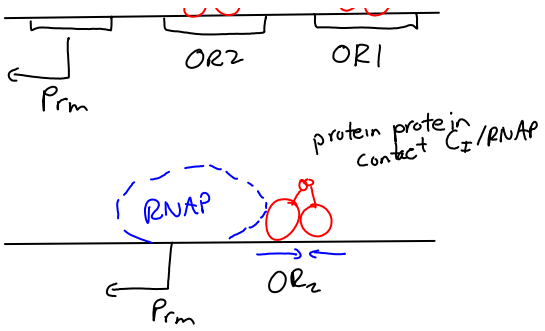
- Operators O_r and O_L
  - O_R -> contains 3 operators



Sequences are different
Binding affinities are different
C_I: $O_{R1} > O_{R2} > O_{R3}$ order of binding
Cro: $O_{R3} > O_{R2} = O_{R1}$

- pC_I binds each operator as dimer





N-terminal domain of C_I contacts sigma subunit of RNAP

$$RNAP + P_{rm} \rightleftharpoons (RNAP \cdot P_{rm})_c \longrightarrow open$$

C_I stimulates ~10 fold

1. Bacteriophage Lambda binds to the target *E. coli* cell, the J protein in the tail tip interacting with the lamB gene product of E. coli, a porin molecule which is part of the maltose operon.
2. The linear phage genome is injected past the cell outer membrane.
3. The DNA passes through a separate sugar transport protein (ptsG) in the inner membrane, and immediately circularises using the *cos* sites, 12-base G-C rich cohesive "sticky ends". The single-stranded nicks are ligated by host DNA ligase.
4. Host DNA gyrase puts negative supercoils in the circular chromosome, causing A-T rich regions to unwind and drive transcription.
5. Transcription starts from the constitutive P_L, P_R and $P_{R'}$ promoters producing the 'immediate early' transcripts. Initially these express the *N* and *cro* genes, producing N, Cro and a short inactive protein.
6. Cro binds to *OR3* preventing access to the P_{RM} promoter preventing expression of the *cI* gene. N binds to the two *Nut* (N utilisation) sites, one in the *N* gene in the P_L reading frame, and one in the *cro* gene in the P_R reading frame.
7. The N protein is an antiterminator, and functions to extend the reading frames that it is bound to. When RNA polymerase transcribes these regions, it recruits the N and forms a complex with several host Nus proteins. This complex skips through most termination codons. The extended transcripts (the 'late early' transcripts) include the *N* and *cro* genes along with *cII* and *cIII* genes, and *xis*, *int*, *OP* and *Q* genes discussed later.
8. The cIII protein acts to protect the cII protein from proteolysis by FtsH (a membrane-bound essential E. coli protease) by acting as a competitive inhibitor. This inhibition can induce a bacteriostatic state, which favours lysogeny. cIII also directly stabilises the cII protein[2]. On initial infection, the stability of cII determines the lifestyle of the phage; stable cII will lead to the lysogenic pathway, whereas if cII is degraded the phage will go into the lytic pathway. Low temperature, starvation of the cells and high multiplicity of infection (MOI) are known to favor lysogeny (see later discussion).

Pasted from <http://en.wikipedia.org/wiki/Lambda_phage>

Notes 06/02

Monday, June 02, 2008
11:06 AM

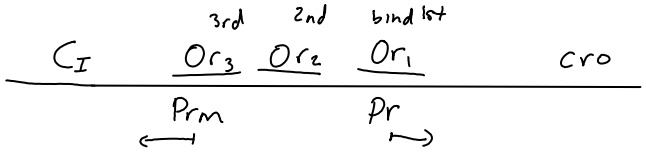
Lytic
- Cro increased (no C_I)
- O_P (DNA replication)
- Q late transcription

Lysogenic
- C_{II} increased (cro decreased C_I increased)
- C_I increased (P_i, P_I off; Prm on)

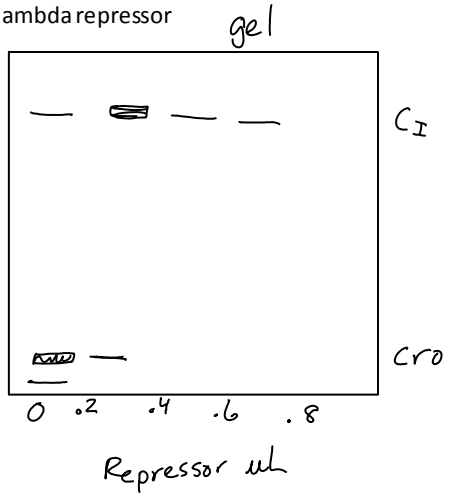
Choice between lytic and lysogenic
- Favor C_{II} increase (High cAMP -> (poor carbon source starvation)

pC_{II} -> inactivated by HflAB protease
- High multiplicity of infection
- C_{II} increased -> lysogeny

Experiment: biding of repressors and effects of Prm and Pr promoters

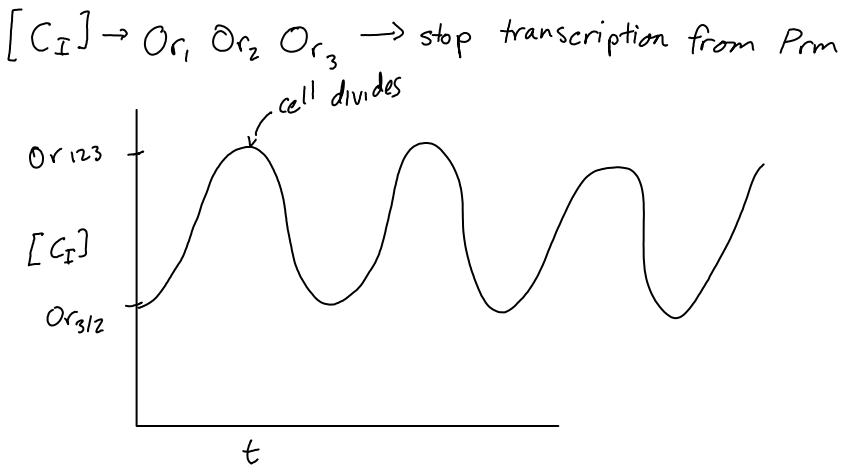


Adding increasing amounts of lambda repressor



RNAP + Prm $\rightleftharpoons$ $(RNAxPrm)_c$ -> open

C_I stimulates ~10x



[C_I] will drop after cell division -> Or1/Or2 -> Prm stimulation

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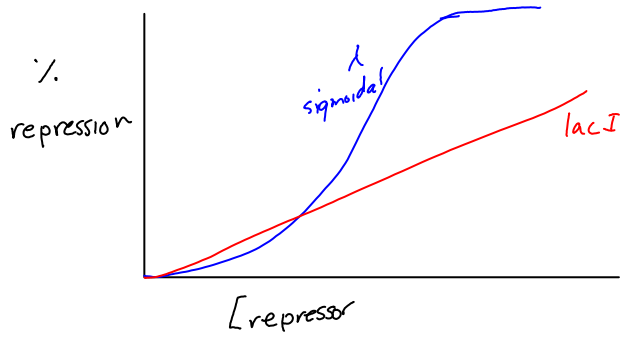
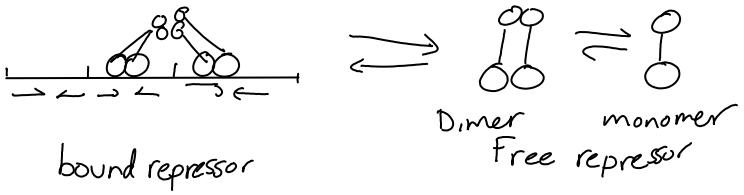
pCI N terminal domain - DNA binding RNA poly contact
C terminal domain - monomer·monomer, dimer·dimer (pairwise)



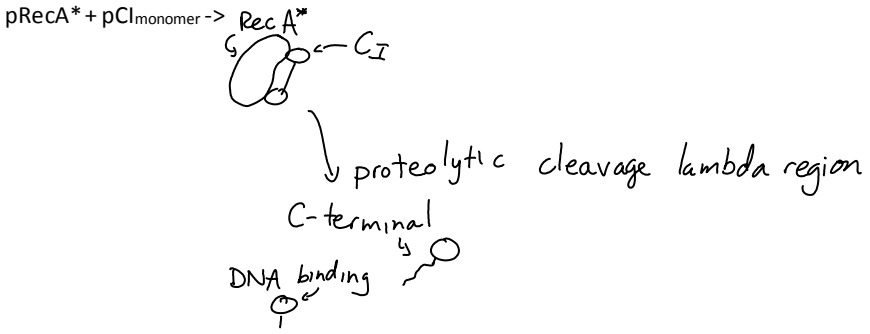
Cooperative binding (pairwise) -> small ahanges in [C_I] -> large changes

---

Lysogenic Induction



UV -> DNA Damage -> pRecA*
pRecA -> acivation (reversible)



The active site for proteolytic cleavage inactivation of pCI is located in the C terminus of pCI!

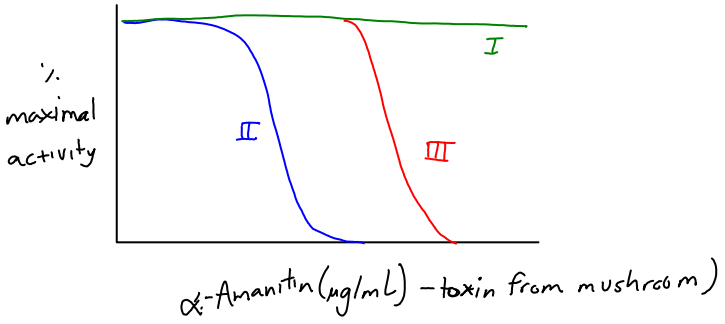
Eukaryotic Transcription

Multiple RNA polymerases unlike prokaryotes

RNA polymerase	Cellular RNAs synthesized	Mature RNA (vertebrate)
I	Lage rRNA precursor	28S, 18S, and 5.8S rRNAs
II	hnRNAs (heterogeneous nuclear) snRNAs	mRNAs snRNAs
III	5S rRNA precursor tRNA precursors U6 snRNA (precursor?) 7SL RNA (precursor?) 7SK RNA (precursor?)	5S rRNA tRNAs U6 snRNA 7SL Rna 7SK RNA

Will look only at RNA polymerase II

Different types can be distinguished many ways… one is sensitivity to toxins



RNA Polymerase II has about 12 subunits

5-7 subunits shared by all polymerases

Then I, II, and III have other very distinct subunits

RNA Pol II have homologous subunits to beta', beta and alpha.

One subunit of RNA pol II is phosphorylated (Rpb1)

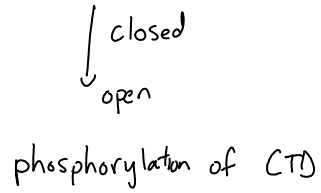
RPB1 subunit

- C-terminal domain (CTD)
  $(PTSPSYS)_{52}$ -> mammals

Phosphorylation is essential to go

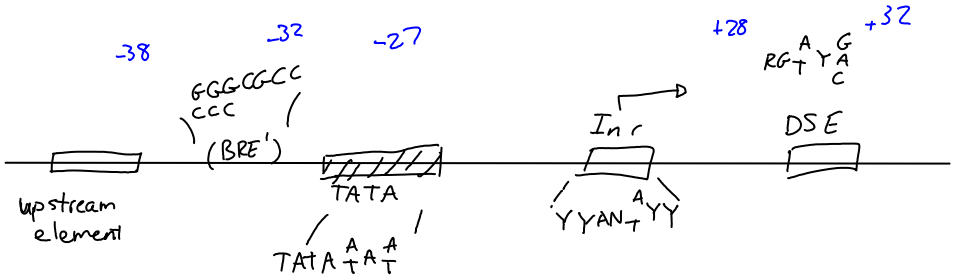
$(PTSPSYS)_{26}$ -> yeast
Sites for phosphorylation by TFIIH kinase
RNAP must not be phosphorylated in order to assemble into PIC

from closed to complex to open



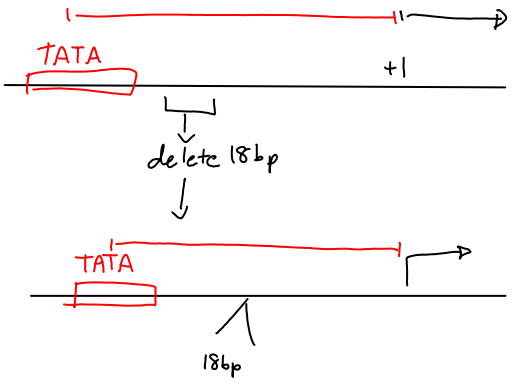
Typical Elements in Eukaryotic Promoters

- Multiple transcriptional elements



1) TATA box centered at -27
2) Inr initiation - start site of transcription
3) DSE down stream element

TATA - determines start for transcription



Start of transcription more downstream by approx 18bp

ie: spacing between TATA end transcription start same. Level of transcription unchanged*
NOTE: approx 50% genes (regulated) contain TATA. Others contain combination of other promoter elements

RNA Pol II is unable to recognize promoters
TF2D is one of several transcription factors (15 subunits... 1 called TBP). TBP binds to TATA box

Look at positions of H bonding in major and minor grove:

- H bond acceptors and donors in major and minor groove differ in distance from helix axis
- Major groove contact gives much information
- Minor groove just has hydrogen bond acceptor on AT base pair and symmetry... so protein that contacts minor grove can see symmetries that the major grove cannot provide.

- So major grove BP distinction, in minor groove symmetry distinction (doesn't care exact base but symmetry A:T or T:A not distinguished)
- TATA box is AT rich but exact order doesn't matter because TBP binds to TATA box from minor groove.

Notes 06/04

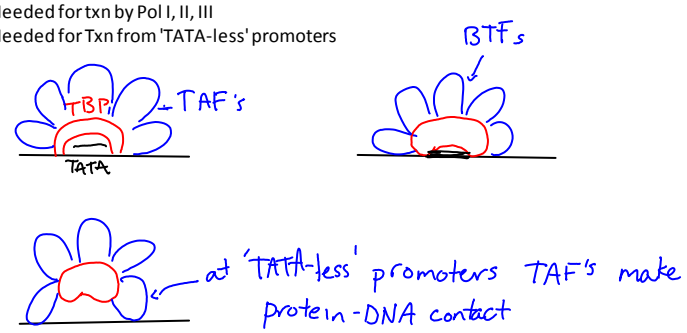
Wednesday, June 04, 2008
11:04 AM

Final:

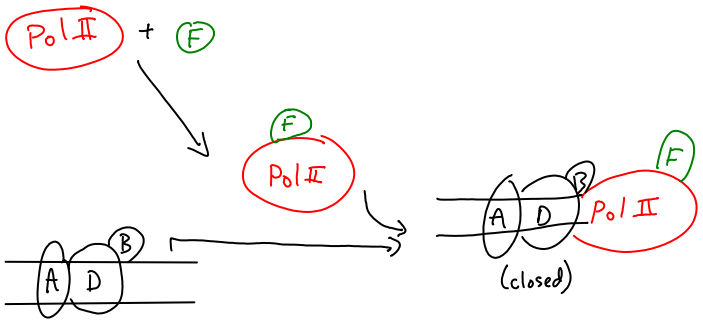
1) T/F
2) Identify
3) Lac
4) Trp (attentuation)
5) Lambda
6) Eukaryotic Txn
7) Eukaryotic Gene Reg
8) Splicing
9) Bonus

TFIID = TBP + TAF's

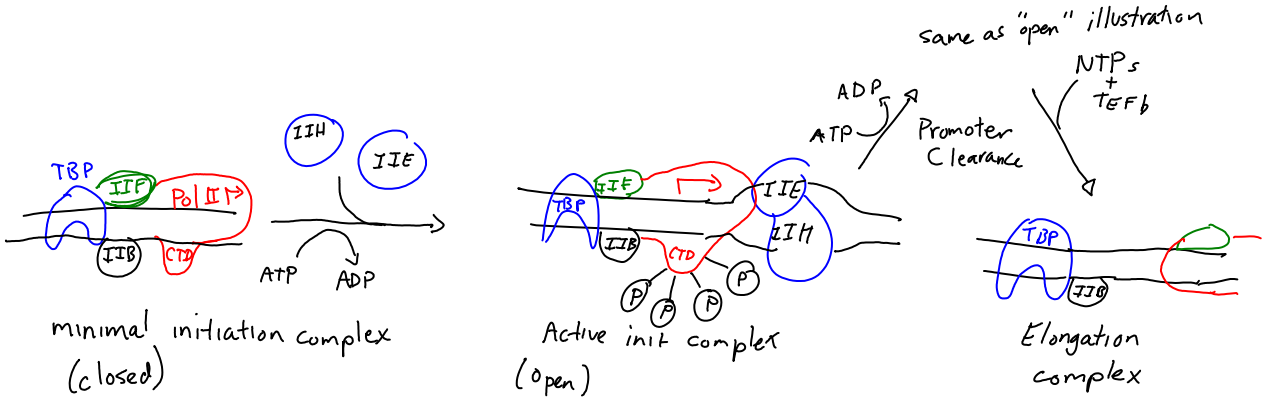
- TBP
  - Highly conserved
  - Binds TATA minor groove
  - Establishes start site for txn
  - Needed for txn by Pol I, II, III
  - Needed for Txn from 'TATA-less' promoters



- **Basal transcription Factors (BTF)**

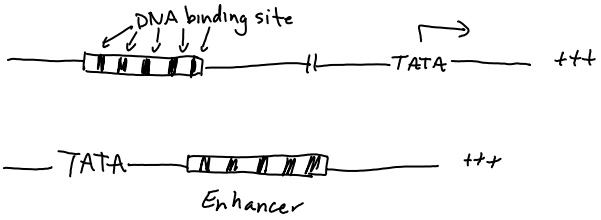


- **Transcription Initiation**



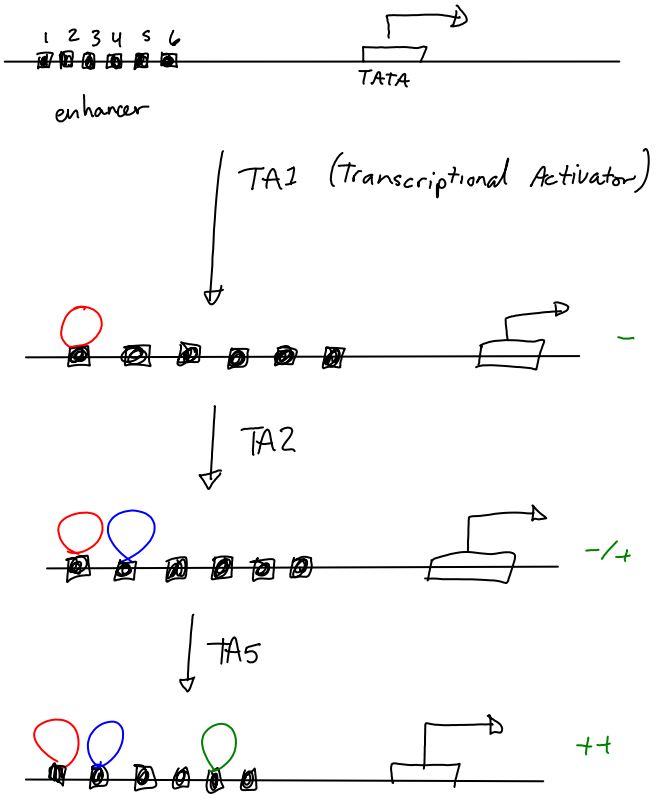
- **Transcriptional Activators**
  - Determine the frequency of transcription initiation
  - Activators contacted basal transcription factors OR
  - Work through co-activators (mediators)
  - Contact proteins that alter chromatin structure.
  - Bind DNA in a sequence specific manner; Enhancer
    - Dna sequence to which transcriptional activator binds - enhancer
    - Bind upstream (or downstream) of promoter
    - Work in cis (generally) - Promoter and enhancer reside on same DNA molecule

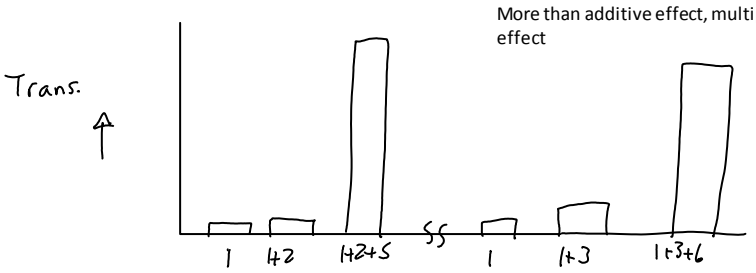
- **Enhancers**
  - Contain binding sites for transcription activators
  - Multiple sites in close proximity



- Enhancers
  - Work in cis on promoter
  - Acts through bound transcription activators to increase rate of PIC complex bound at promoter
- Enhancers do not
  - Alter structure of DNA template (A form B form etc)
  - Provide entry site for RNA polymerase (entry site for RNA Pol to promoter, not enhancer)
  - Localize promoter to cellular compartment
- In vitro - effects of enhancer can be bypassed by increasing concentration of RNA polymerase II.
  - Increase concentration of basal factors, no enhancers are needed. (can't do this in cells but can in vitro)

- Transcription of a Gene:

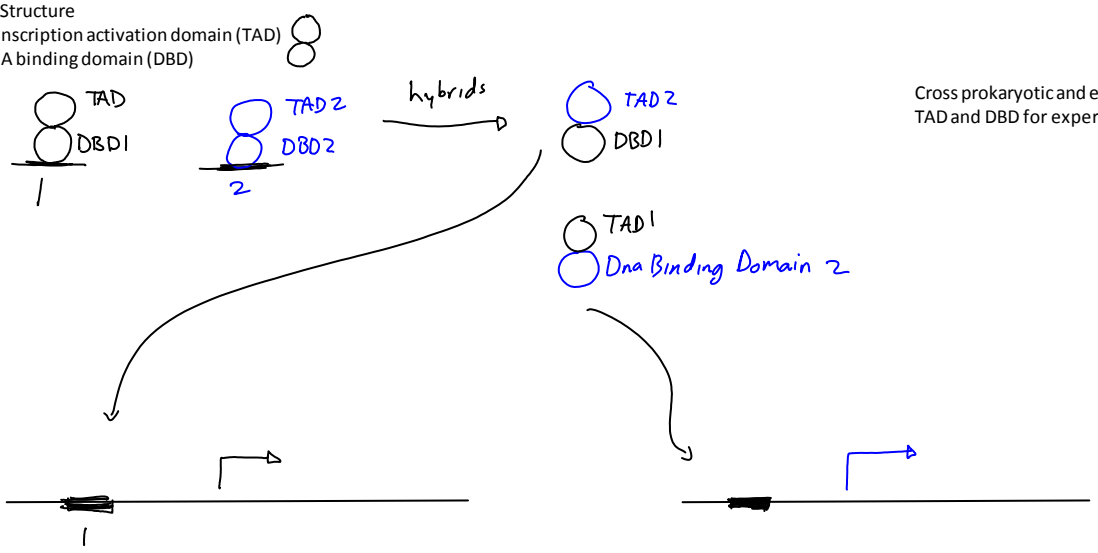




1) Transcriptional synergy
   1,2,5 >> 1+2 > 1

2) Combinatorial control
   Gene regulation by combination of different transcriptional activators

- Transcriptional Activators
  - Modular Structure
    - Transcription activation domain (TAD)
    - DNA binding domain (DBD)



Cross prokaryotic and eykaryotic TAD and DBD for experiments

- TAD - no well defined 3-D structure
  a) Glutamine rich region
  b) Proline rich region
  c) Acidic - most general/common (acidic amino acids, placed on surface of TAD)

GALY -> yeast TA needed for transcription of GAL1 gene

Increase negative charge (acidic residues) of Tad -> increase transcriptional activator activity

Net charge	Units of b-gal
-7	700
-8	970
-9	1628
-10	2490

- Transcriptional activator motifs:

- Helix turn helix motif
  - 2 small alpha helices. Helix 2 is pushed into major groove of DNA and amino acid side chains make contact with BS. Helix 1 will make contact with sugar phosphate backbone.
  - Found in prokaryotic regulatory elements
    - Lac repressor
    - Trp repressor
    - Lambda repressor
    - Cro
    - Cap

        - Sigma factor
    - Something similar but not same is used in eukaryotic cells.
        - 3 helix system (homeodomain)

- Zinc Finger transcriptional activators
    - Eukaryotic (few prokaryote ones) - several
    - Tetrahedrally coordinated from zinc atom
    - Each zinc finger helices have Arg contacting G bases
    - Unlike helix turn helix motif, zinc finger only contacts bases on 1 strand of DNA.
    - All zinc fingers in same polypeptide chain

- Other transcription factors
    - Mediator (co--activator) approx 20 subunits
        - No DNA binding
        - Stimulates only in presence of transcription activator

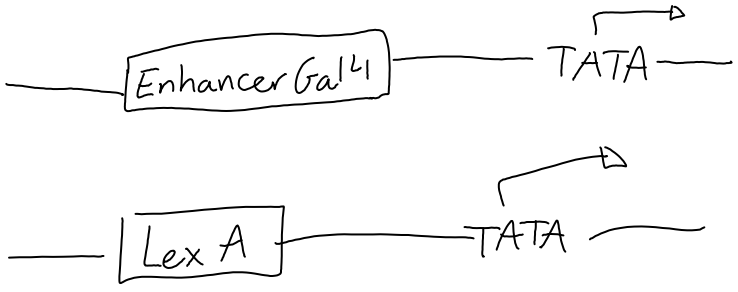
PIC = pre initiation complex

CRSP, NAT
ARC/DRIP
TRAP/SMCC

    - Architectural Proteins (in addition to mediators)
        - DNA binding required - alter DNA shape (binding, looping)
        - Eg. HMG$^{A/G}$ Do not activate transcription by themselves
        - Some show no binding specificity (minor groove) but LEF1: AT rich binding site -minor groove and tissue specific (lymphoid)

Discussion Week 10

Thursday, June 05, 2008
10:55 AM



Lex A: dbd/tad
Gal 4: dbd/tad

Chimeric proteins LexAdbd/Gal4TAD (and inverse)

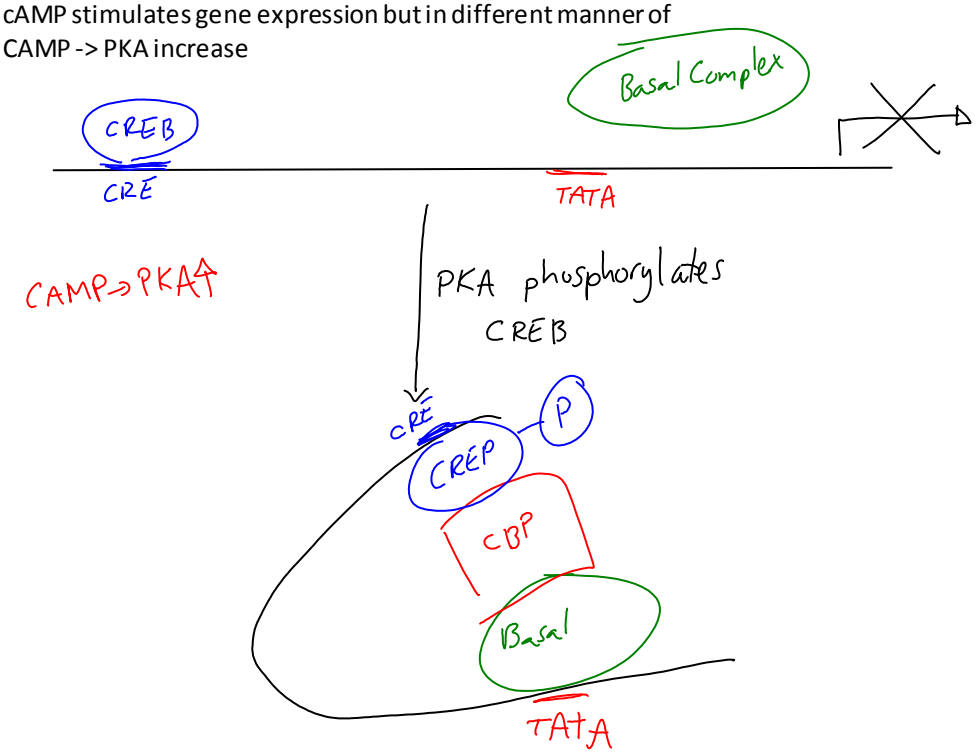
I^+ = repressor, wild type
O^c = constitutive operator - repressor cannot bind well

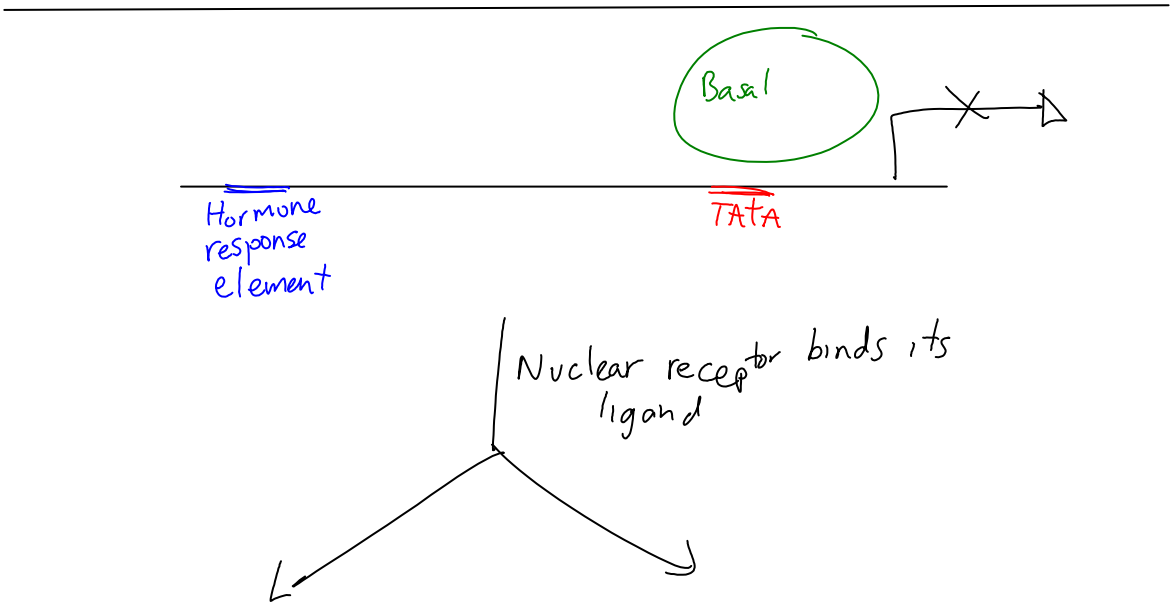
Notes 06/06

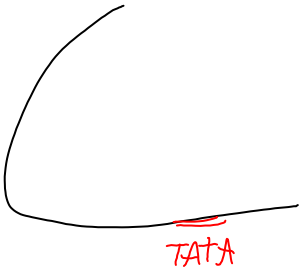
Friday, June 06, 2008
11:01 AM

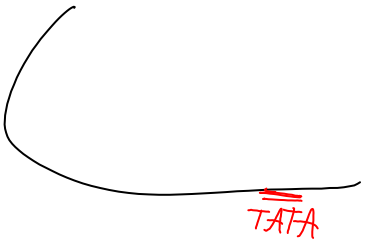
Final Exam June 9 Monday 11:30-2:30 Humanities 135
Review Session -> sat june 7th, young 2200 2pm-6pm

- Regulation of cAMP is eukaryotic cells
  - cAMP stimulates gene expression but in different manner of
  - CAMP -> PKA increase





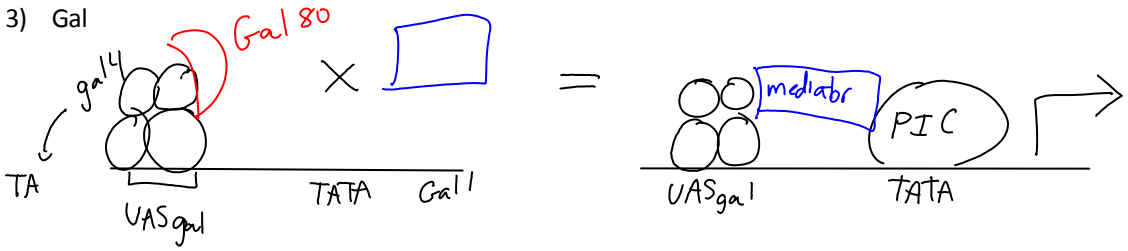


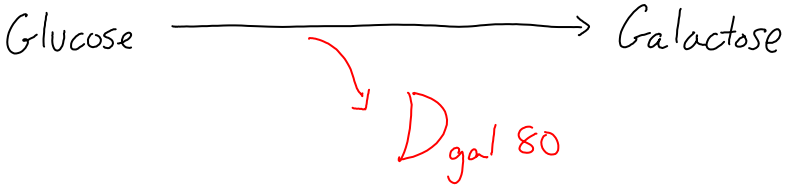


In summary binding of ligand or covalent modification effects transcription

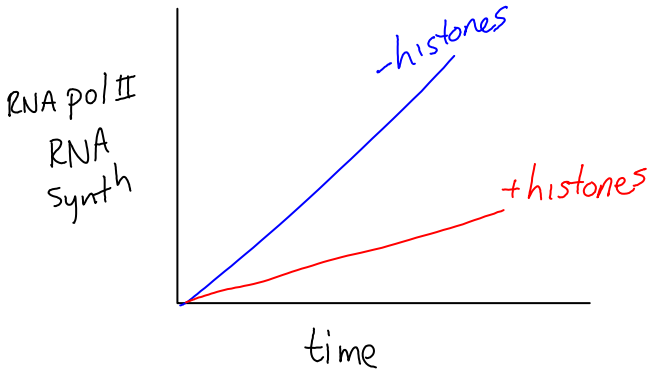
Other wais of controlling transcription activators

1) Control localization - noncovalent
   a. Association with proteins in cytoplasm -> release (HTO)
   b. Sumolation - covalent modification -> sequesters in nucleus.. TA sequestered in cellular compartments
2) Proteolysis (won't go through) polyubiquitination
3) Gal

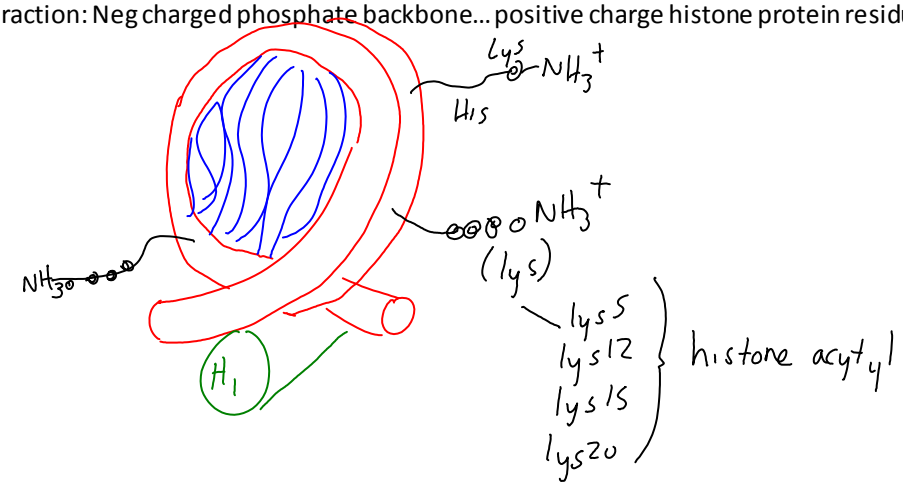


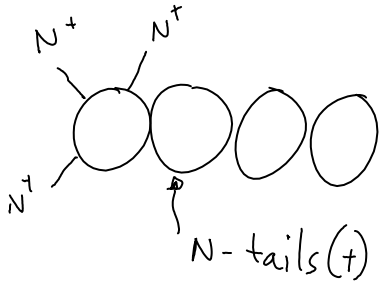


- Eukarytoic DNA template -> chromatin (approx 50% DNA, 50% protein)
  - Protein - histones (90%)
  - Histones inhibit transcritpion



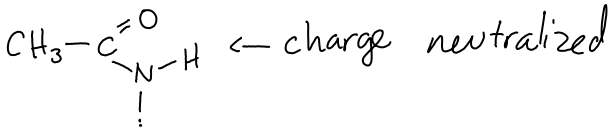
- When histones bind to DNA, creates histone octamer. Form disk shaped structure. Wound in left handed toroidal.
  - Interaction: Neg charged phosphate backbone... positive charge histone protein residues





Nucleosome "crosslinking" due to N-tails (electrostatic)
Acetylation - histone + acetyle CoA - Ac-histone
Specific positions acetylate!

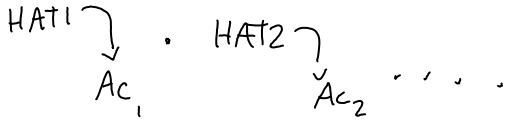
N-terminal tails



Acetylation reduces electrostatic charge so that more fluidity to DNA -> encourages transcription

- Histone Acetylation
  - Reduces charge of histone Lys (tail) weakens nuclesome-nucleosome
  - Specific binding sites for proteins containing BROMO DOMAINS (module binds acetyl -Lys)

  - Enzyme Acetylating Histones
    - HAT - Histone Acetyl Transferase (HAT's contain bromo domain)
    - HAT's associated with transcriptional activatoin
      - (mediator contains HAT activity)
      - TAF's contain HAT activity
    - Pattern of histone acetylation changes during transcription



- Methylation doesn't neutralize charge as well stabilizes nucleosome-nucleosome... discourages transcription

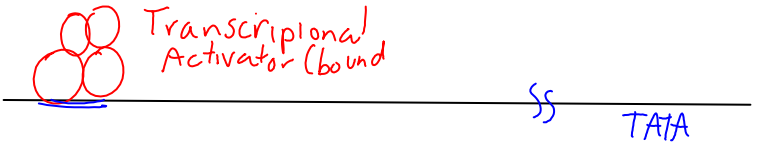
- Step after Acetylation is **chromatin remodeling**
  - Repositioning of nucleosomes

octamers are not removed

  - Large complexes 2.0×10^6 dal
    - ATP dependent
  - Disrupt Hitsone-DNA contacts (approx 14/octamer) -> reposition nucleosomes
    - SLUI/SNF
    - ISNI
    - INO80
    - NuRD

} different remodeling complex cells

- Hypothetical Gene Activation



recruits mediator containing HAT (eg GCN5)

Acetylatoin of lys8 (H_4), Lys 9 (H_3) lys 14(H_3) - chromatin containing TATA

SwI/SNF binds to acetylated histone (lys8)

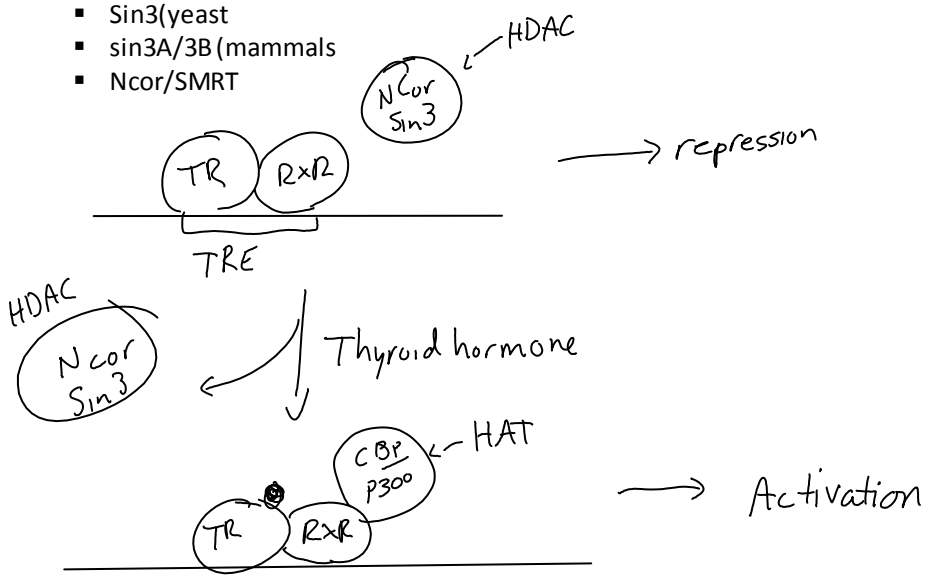
ATP → ADP

Remodel chromatin - displace histones from TATA

TFIID ($TAF_{II}250$) -> BINDS AcH_3 (Lys9/14)

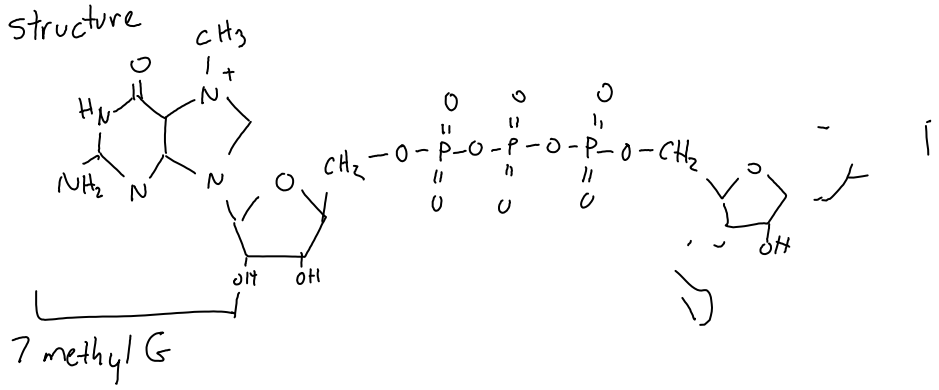
Transcription

- Histone Acetylation - increase transcription
- Histone Deacetylation -> Inhibits transcritpion (HDAC's)
  - Enzymatic removal of Ac groups
  - Recruitment of HDAC's -> repression
  - Co-repressors (Sin3, Sin3A/B, NCOR/SMRT) -> recruit HDAC's -> shut off transcription
    - Called co-respressor because they are recruited to promoter region
    - In prokaryotic repressor is mediated by binding to DNA
    - In eukaryotic repressor is recruited (co-respressor)
- Histone Deacetylation associated with repression of transcription
  - Co-repressors - HDAC's
    - Sin3(yeast
    - sin3A/3B (mammals
    - Ncor/SMRT



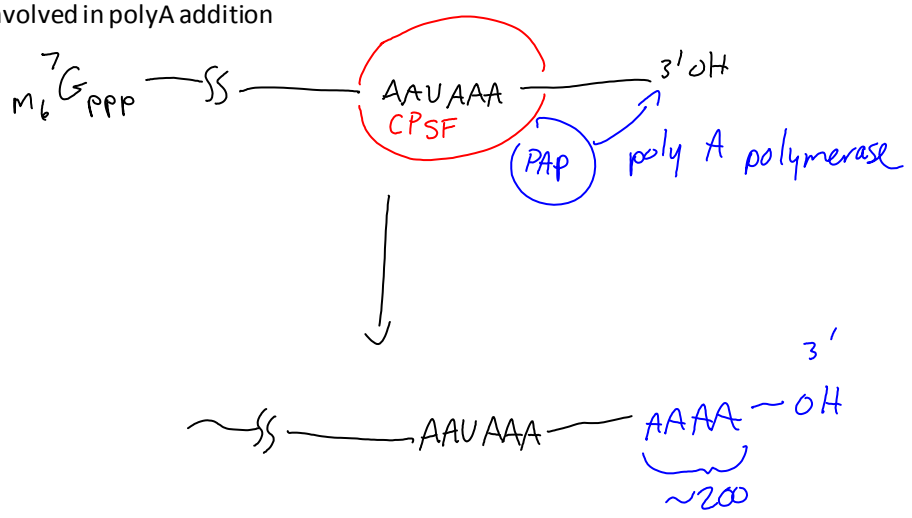
- Co-transcriptional processing of RNA
  - Capping of 5' end
  - 3' end formation and polyA addition
  - Removal of noncoding regions (splicing)
    - All 3 of these occur as transcription occurs… not after.
  - Enzymes needed for their covalent modifications recruited by CTD of RNA polII (phosphorylated)

- Capping function(s)… happens very early approx 40nt long
  a) Protect RNA from degradation
  b) Enhance translation of mRNA
  c) Enhance export to cytoplasm
  d) Enhance splicing efficiency of 5' most intron



- Poly A Adenylation
  - Functions of polyA
    - Protects from degradation
    - Increase translation
      - Poly A binding protein (ribosomes)
      - Synergistic with CAP)
    - Poly A added to RNA in nucleus during transcription
    - PolyA addition in cytoplasm by distinct polyA polymerase
    - Average length of nuclear polyA addition at 3' end approx 200nt

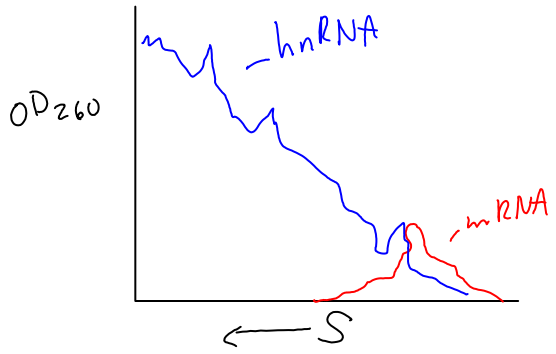
- Steps involved in polyA addition



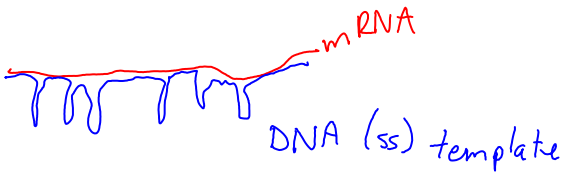
RNA Polymerase II continues tarnscription for 200-300 nt -> released from …..

- Splicing
  - Product of transcription by RNA pol II is hnRNA
    - hnRNA >>mRNA

(nucleus) (cytoplasm)



Electron microscopy



	mRNA	hnRNA
Exons	Yes	Yes
Introns	No	Yes

  - Exons -> coding (<300nt)
  - Introns -> noncoding (3500nt->2.4x10^6nt)

  - Removal of intron sequences from hnRNA while transcription is in progress (RNA Pol II)
  - (NB RNAs synthesized by RNA pol I and RNA pol III -> spliced)

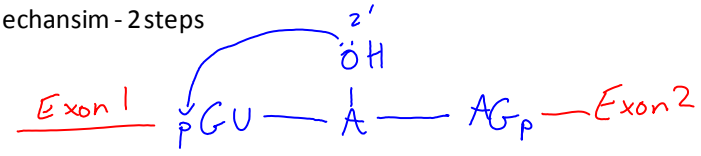
- Reactions of splicing

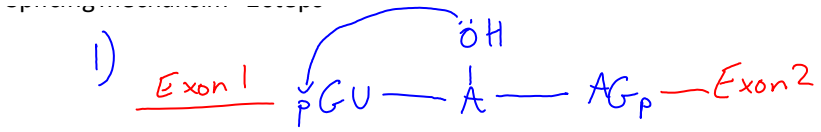
5'— GU.... YNCURAC-Y_nNAG - —

In yeast

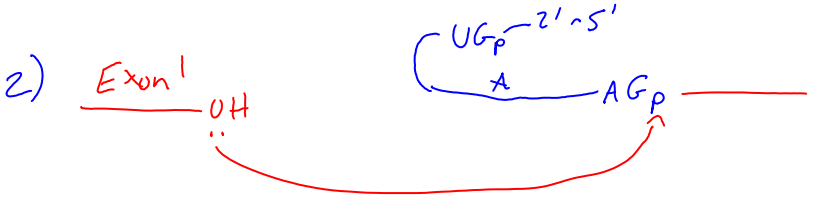
— GU... UACUAAC-YAG —

Splicing mechansim - 2 steps





2' OH of branche A -> nucleophilic atack on P 5' to GU (transesterification)



2nd nucleophilic attack of 3' OH of exon 1 at P 3' to AG at downstream intron - exon 2 junction

- Reaction occurs in spiceosome structure

- Spliceosome consists of Rna + protein
  - Approx 50% of spliceosome mass
    - snRNP's (small nuclear ribonuclear proteins
    - SnRNP's -> RNA + protein

- SnRNA's - act by base pairing with sequences in hnRNA
  - U1 -> base pairs with 5' splice site

  - U2 required to align to branch point junction
  - U4/U6 -> interacts with U2 to stabilize hybrid
  - U5 participates alignment of exon 1 to exon 2. interacts with 5'+3' exons

- Effects of defects in snRNAs

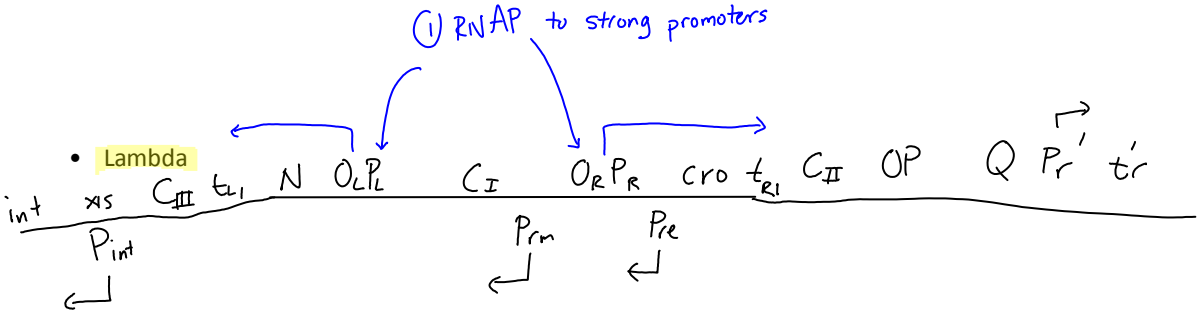
.	Trans ester 1	Trans ester 2
U1	NO	NO
U2	No	No
U4/U6	No	No
U5	Yes	NO

Final Review

Saturday, June 07, 2008
2:02 PM

1. T/F (14 questions)
2. Identify
3. Lac operon
4. Trp
5. Lambda
6. Eukaryotic transcription
7. Eukaryotic gene regulation
8. Splicing

Bonus: everything



- Lambda

Immediate early synthesis -> N and cro.
- pN is antiterminator
- Cro is repressor

- Lytic cycle - cells are growing in rich nutrient media (glucose)
- Lysogenic cycle - low carbon source

- Lytic pCro is produced more.
  - pCro binds to O_rP_r (Or3>Or2=Or1) Binding of Or3 prevents C1 gene being synthesized
  - N protein is antiterminator for tr1 and tl1... antiterminate action synthesizes C_{II} and C_{III}. With high glucose hflab is increased which degrades pCII.
    - OP and Q protein produced by passing tr1 (antiterminator N allows this).
      - OP -> DNA synthesis. OP (dnaA/dnaC)
      - pQ ->bind Pr' DNA and allow to extend past t'r to make late genes of lambda

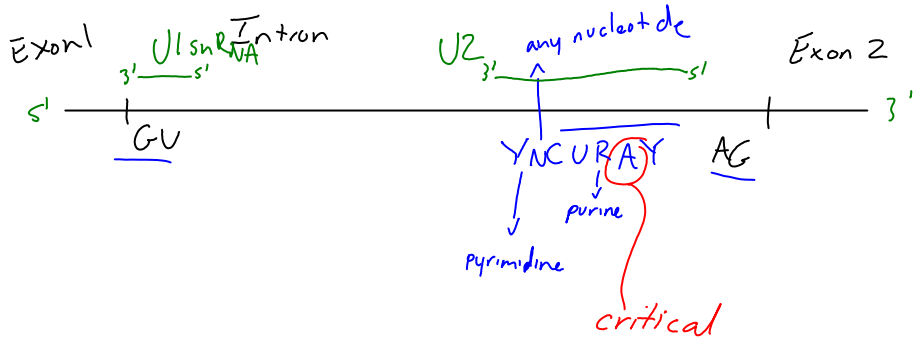
- Lysogenic
  - Low glucose, high cAMP, low HflAB, high pC_{II} (pC_{III} helps stabilize pC_{II})
  - C_{II} binds Pre to allow RNA Pol II transcribe to pC_I. Inhibition of Cro synthesis by antisense.
  - pC_I binds to Or1>Or2>Or3 cooperatively.
  - pC_I prevents open RNAP + Pr open complex

  - P_{int} uses C_{II} protein

- C_{II} blocks O_R and O_L

- Cells exposed to UV, cytotoxic agents (carcinigens in humans, mutagens in bacterial) -> generally block replication or transcription
- Lysogenic induction stimulated by UV and cytotixic agents

RecA -> RecA* (* from uv damage)
RecA* binds monomer of C1 protein and breaks in 1/2
Now OR and OL cannot be repressed... due to c1 binding affinity has been decreased
Virus then goes lytic

- Slicing
  - 

Responsible for basic spicing sequence of heterogeneous nuclear RNAs.
This is cotranscriptional in cell... in vitro, can uncouple spicing and transcription.

snRNP's (small nuclear RNAs) = snRNA's + proteins
snRNAs:
U1
U2
U4/U6
U5

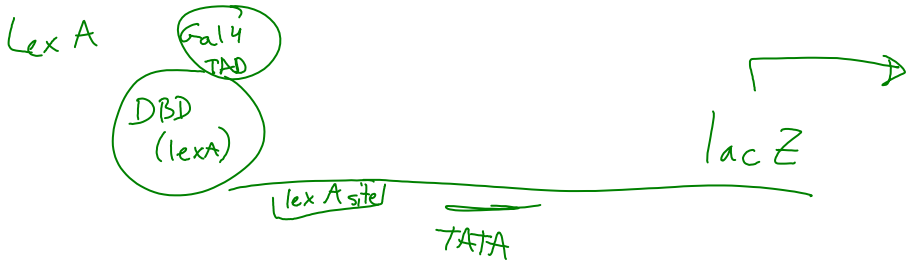
- U1, U2 show sequence homology with branch point regions Base pairing is important factor in U1 and U2 RNA transcript interaction...
- U1 is responsible for recognition of GU via base pair.
- U2 is responsible for recognition of YNCURAY via base pair
- U4/U6 don't interact directly with RNA but interact with U2. Form complex aligned to BP with branch junction (indirect involvement)
- U5 - no sequence homology with exon 1 and exon 2 but responsible for joining of exon 1 to exon 2.

Problem: gel, deduce structure of original RNA … which intermediates are likely to occur under certain circumstances. What are intermediates… intermediates if you did the following?

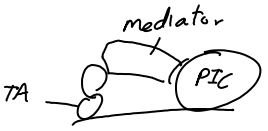
Low agarose gel behaves like linear (lariate structure)

High agarose gel travels less in gell (lariate structure)

- Eukaryotic Transcription
  - Need to know steps required to activate or bind polII to DNA.
  - TATA box (not equivalent to -10 AT rich in prokaryotes) - TATA box is at -25 or -27
  - TFIIB (TBP portion) interact with TATA box.
    - Sigma of e.coli prokaryote use major groove contact from sigma in -35 and -10
    - TBP uses minor groove so not as base specific (T and A are interchangeable) because A and T are symmetrical.
  -



Know difference between transcriptional activators, DBD, and TAF



.	DNA Binding	Contact PIC
Trans Activators	+	+
Co-activators/mediators	-	+
Architectural proteins HMG^{AB} LEF1	+	-

Transcriptional activator recruits RNA polII and basal transcription factors (glutamine rich, proline rich, and acidic transcriptional activators recruit). In vitro can put high concentration of Rna polII and basal transcription factors, so that won't require trans act.

- Rejected problem:

  3 genes

  1) AGTTCGAACT
  2) AGTTCGTTCT
  3) TGATCGATCT

1) Has perfect dyad symmetry

.	A+B+	A+B-	A-B+	A-B-
1.	+	+	+	-
2.	+	-	+	-

3.	+	-	+	-

Explain results:

A binds major groove

B binds minor groove