UN2005/UN2401 2018 NOTES FOR LECTURE 12 **Last Updated 10/14/18**

Handouts: You need handouts 12-1, 12-2 & 12-3. Links to PDFs of the handouts are on the handout list. If you have problems linking to the PDFs, let Dr. M know. *Extra paper copies of the handouts are placed in the hall on the 7th floor of Mudd between 749 and 753C after the afternoon lecture.*

References to the texts are to Sadava 11th ed. & Becker 9th edition. References to 10th ed. of Sadava or 8th ed. of Becker are in parentheses **if** they are different.

For nice animations of the Meselson-Stahl experiment and DNA replication (including primers) go to http://highered.mcgraw-hill.com/sites/0072437316/student_view0/chapter14/animations.html#

A box with the animation should appear on the upper left when you click on the appropriate link. If nothing happens when you click on the link, the box may be *behind* the page you are looking at. Shrink the main page to see the animation. These animations (& your texts) may mention enzymes not discussed in class. You are ***not*** responsible for any extra details.

Note: There are many animations of the M-S experiment, DNA replication, etc. on the web. (The link to the one from Sadava is below in the section on DNA replication.) Please tell Dr. M if you find another one that seems especially helpful. Some of the good ones we have found are listed on the links page.

See notes of Lecture #11 for details of topics I to IV.

I. Structure of DNA – How DNA has info to do its 2 jobs (A & B were covered last time.)

A. Primary Structure of DNA – Nucleotides and how they are hooked up.

B. 3D DNA structure

1. Base Pairing -- See Recit. Prob. 6 to review base pairing.

2. What does the final structure look like? The famous double helix.

Note: The double helix structure was figured out in 1953 by Watson & Crick, using data from Rosalind Franklin and others. In 2003, 50 year later, there was a series of articles in the *NYTimes* commemorating the discovery of DNA structure. You can get all the articles in the series from the *NYTimes* archive.

C. Parallel or antiparallel? In double stranded (ds) DNA -- how are the two strands paired up?

1. Possibilities: The two chains of DNA could be parallel or anti-parallel as shown by pop bead model (demo) or diagram in IV-D from notes of lecture 11.

2. Result: The two chains are antiparallel -- see handout 10-3 (bottom panel) or white plastic model in class.

3. Significance: Whenever a single nucleic acid strand is used as template to make a partner, the partner must always be made so that the two strands will be antiparallel. This creates problems, as all new chains grow from their 5' end towards their 3' end, as will be explained below.

II. Additional Details on DNA Structure (FYI only) -- See topic VI of Lecture #11.

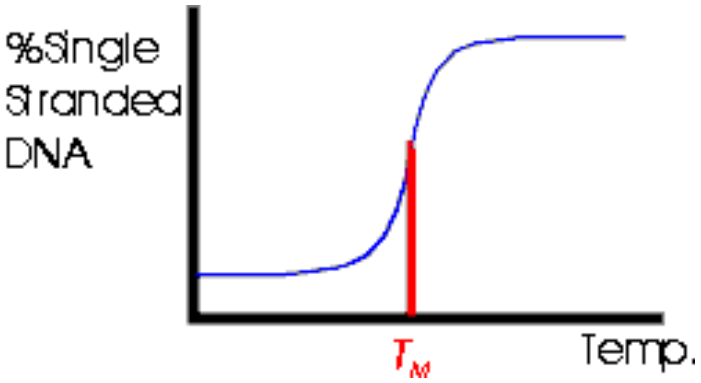
A. Base Stacking. (Will be demonstrated)
B. Super coiling.

III. "Non genetical" implications of DNA structure -- Denaturation, Renaturation & Hybridization. See notes of #11, topic VII for all details.

A. Denaturation

1. Double Stranded DNA can be separated into its single strands

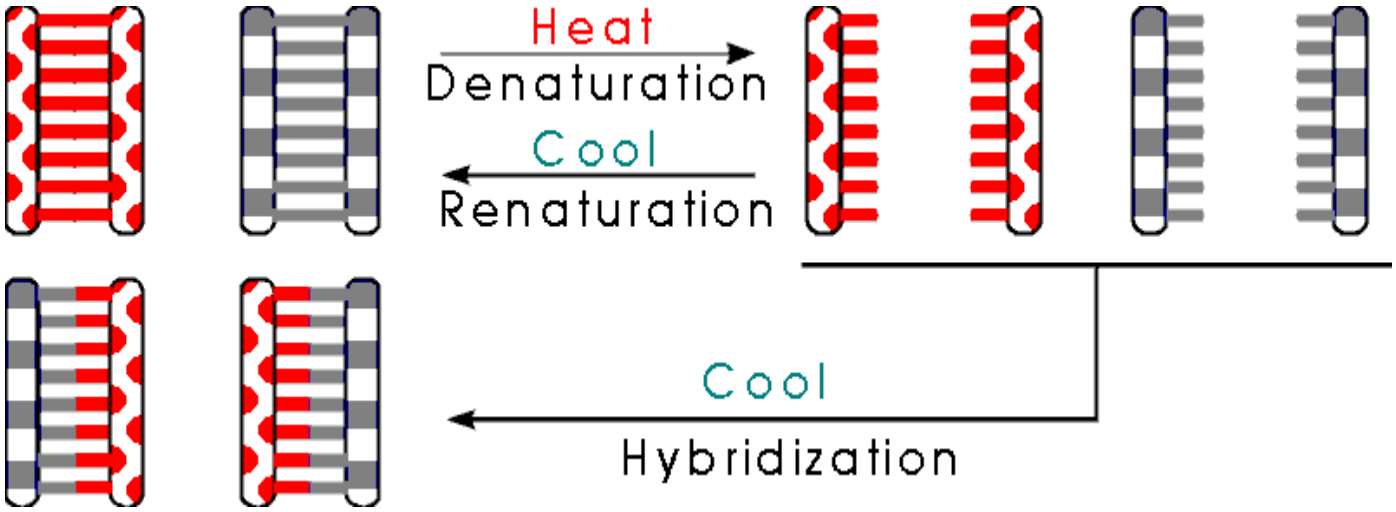
2. T_m -- Melting Temperature



To review denaturation, do Problem 6-4, parts A-C.

B. Renaturation
C. Hybridization

Some examples of the uses of hybridization will be discussed in class as they come up.



Note: In picture above, there is only one starting DNA molecule of each type (red or black). In a real experiment, there would be many 'red' molecules and many 'black molecules'.

To review renaturation/hybridization, try problem 6-4, part D.

Question to think about: How do you assay an enzyme? That is, how do you detect or measure the amount of an enzyme? How do detect or measure the amount of a nucleic acid?

IV. "Genetical Implications" -- The Meselson-Stahl experiment. Does DNA replicate as implied by the Watson-Crick Model?

A. Possible modes of DNA replication. See picture below & handout 12-1. Also See Sadava fig. 13.8 (13.9) or Becker 17-3 (19-3) .

1. Semi-conservative replication.

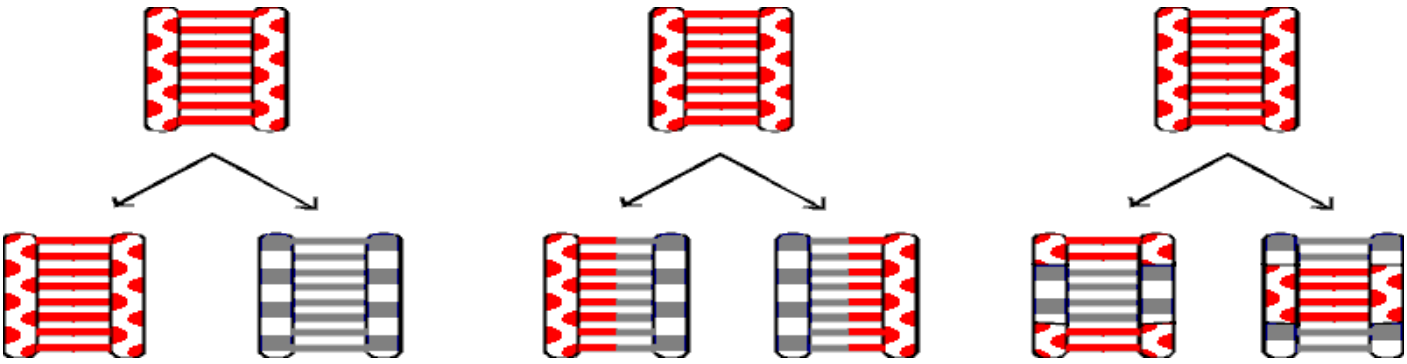
The obvious possibility is that DNA really comes apart into single strands and each strand then serves as template to re-create the missing strand (as implied by the Watson-Crick structure and discussed last time). In this case each of the 2 resulting daughter molecules has one old strand and one new strand; this is called semi-conservative replication. (See Becker fig.17-2 (19-2).)

2. Other possibilities -- conservative and dispersive replication.

There are (at least) 2 other possibilities:

a. Conservative replication. DNA could replicate without coming apart and the daughter molecules would then consist of one completely old molecule with 2 old strands, and one completely new molecule with 2 new strands.

b. Dispersive Replication. An alternative possibility is that pieces of old and new could be scrambled in some way so that each daughter molecule would have new sections and old sections in each individual strand. There are several variants on how this could happen, and all are called dispersive replication.



<Conservative> **<Semi Conservative>** **<Dispersive>**

B. The M-S experiment -- first generation -- how to rule out Conservative Replication

How do you find out which possibility is the correct one? The Meselson-Stahl experiment was done to settle this issue. (Meselson received the Lasker award for special achievement in medical science in 2004.) The experiment is explained in Becker, fig. 17-4(19-3), and in Sadava "Investigating Life'

p. 274 (fig. 13.10). There are many descriptions and animations of this experiment on the web. For the one from the authors of Sadava, go to http://www.life10e.com/at13.03.html. (Or see the link at the top of this lecture.)

The basic idea is to use density as a label to distinguish old and new pieces of DNA. M & S grew bacteria a long time in medium containing heavy N (^{15}N), so all the DNA was heavy. Then they grew the bacteria for 1 generation in medium containing light or ordinary N (^{14}N), so all new DNA strands or newly made sections should be light. Then they extracted the DNA from the cells and measured the density of the DNA. All the DNA was of an intermediate density in between heavy and light. This result is consistent with semi-conservative replication (and dispersive replication) and it rules out conservative replication -- if replication were conservative, then after 1 generation 1/2 the DNA (the "old" molecules) should be heavy and 1/2 (the "new" molecules) should be light.

C. Some Details of the Method -- **How to tell DNA's of different densities apart?**

The method M & S used is called equilibrium density centrifugation. See Sadava 'Working with Data' in section 13.3 or Becker, Fig. 4B -3 (Box 12A). Equilibrium density centrifugation separates molecules on the basis of differences in density.

How is this different from measuring S values using sedimentation velocity centrifugation, as described previously? Sed. vel. centrifugation separates molecules on the basis of differences in size, not density. A table summarizing the differences between the two methods is on handout 12-1.

D. M-S experiment, cont. -- How to rule out dispersive Replication

1. Method 1 (2nd generation).

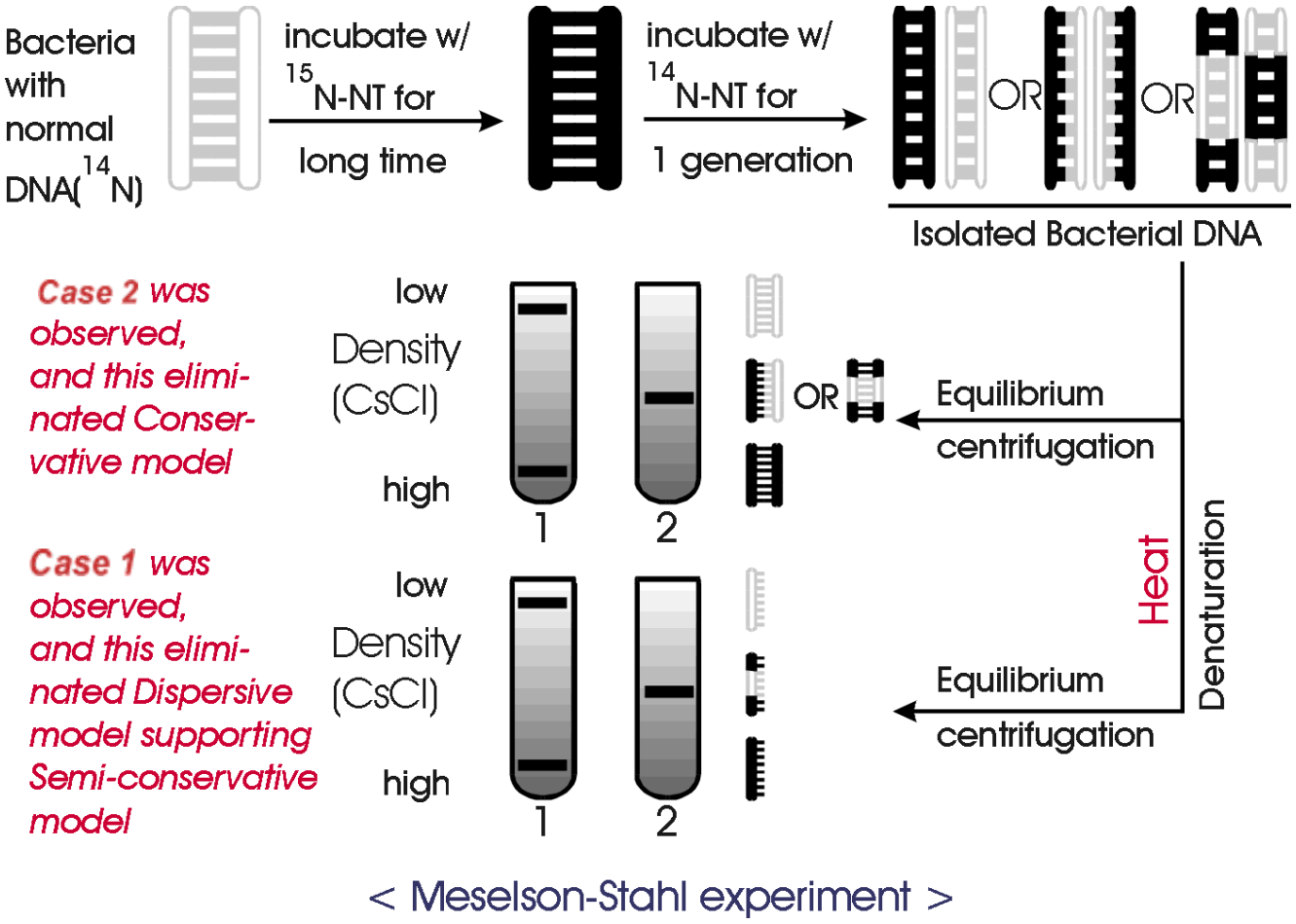
M & S grew the bacteria as above in medium containing heavy N, and then grew it for two generations (instead of one) in medium containing light N. Then they looked at the density of the resulting DNA -- what you get after **2** rounds of DNA replication. The result is in the texts and you should be sure you understand how the result rules out dispersive (and conservative) replication.

To check your understanding, consider the following: For semiconservative DNA replication, what do you expect after 2 generations? What should the double stranded DNA molecules look like (H-H? H-L), and what should the test tubes look like after centrifugation -- how many bands, and of what density?
(Try this at home if we don't get to it; also answer the same questions for conservative and dispersive DNA replication.)

2. Method 2 (Denaturation)

M & S did another experiment to rule out dispersive replication -- they took the hybrid DNA obtained after 1 generation of growth in light N and denatured it. They reasoned that if replication is semi-conservative, the denatured DNA should be 1/2 heavy and 1/2 light or 1/2 old strands and 1/2 new ones. If replication is dispersive, each individual single strand should be part heavy and part light, and so all the denatured DNA should be intermediate in density. The denatured DNA was 1/2 heavy and 1/2 light, ruling out dispersive replication and supporting semi-conservative.

Below is a diagram of the M-S experiment. Top line shows how bacteria were grown, and how DNA should look (in terms of old and new segments). The two cases below depict the results expected after centrifugation of native and denatured DNA. For a more detailed explanation, see legend under the diagram.



< Meselson-Stahl experiment >

Explanation of Diagram:

Line on top (first two arrows) refers to how bacterial are grown -- in medium with ^{15}N or ^{14}N. The isotope was NOT added to the DNA -- it was added to the growing bacteria, who then used the N to make new DNA. After the bacteria were grown, and had doubled their DNA, they were lysed (broken open), and the DNA was isolated from the cell extract.

The picture at the upper right shows how the isolated DNA would look if replication were conservative, semi-conservative, or dispersive respectively. These are the same 3 choices as the 3 shown on handout 12-1 where it shows 'DNA after one doubling.' However, on the handout, the possibilities are shown in a different order -- semi-conservative, conservative, and dispersive, respectively.

The second line in the diagram shows the results to be expected after equilibrium centrifugation of double stranded (native, not denatured) DNA.
The third line in the diagram shows the results to be expected after equilibrium centrifugation of single stranded (denatured) DNA.

(More explanation on next page.)

The shading in the test tubes represents a gradient of density -- higher near the bottom, lower near the top. The gradient is generated during centrifugation. (At the end of the experiment, there is a higher concentration of CsCl near the bottom, and a lower concentration near the top -- the concentration of CsCl determines the density of the solution in each part of the test tube.)

Case 1 = 2 bands in the centrifuge tube, meaning two types of DNA of different densities (so called Heavy and Light).
Case 2 = 1 band in the centrifuge tube, meaning only one type of DNA of intermediate density.

The red italic comments on the left indicate how the results rule various models of replication in or out. You should be able to figure that out from the results, and be able to explain it in your own words (without reading the comments).

To review the M.S. experiment and its implications, do problem 6-6, parts A & B. Then try 6-8 & 6-9.

V. More "Genetical" Implications of DNA structure, or how does DNA do job #2?

In other words, how does DNA determines its own nucleotide sequence? What are the major issues?

A. Is DNA self replicating? If you put DNA in a test tube with nucleotide monophosphates (deoxy AMP, TMP, etc.), the DNA should act as template and the nucleotides should get hooked up to make more DNA. But if you try this, you won' t get any new DNA made. Why not? Because you forgot the enzymes and the energy problem. DNA ***is*** self replicating in the sense that it determines its own order, but it is ***not*** self replicating in the sense that it can not replicate all by itself -- it requires enzymes (& energy) to do the job.

B. The 3 big issues: There are 3 issues to keep in mind when making a long nonrepeating molecule like DNA (or protein or RNA). They are:

1. *Order:* How do you line up the monomers in the right order? What is the template?
2. *Energy:* Where does the energy for synthesis come from?
3. *Enzymes:* What is the role of enzymes in this process?

C. How do order, energy & enzymes fit in? M-S experiment described above settles issue 1, but what about 2 & 3? How do the enzymes and energy come in? First let's focus on the energy, and then come back to the enzymes. (Any part of issues 2 & 3 not covered in Lecture #12 will be covered in Lecture #13.)

VI. DNA Replication -- Issue 2 -- Energy Considerations. (See Handout 12-2 for all Reactions & a summary diagram.)

A. Overview: To add a nucleotide to a growing chain, you will need to split some ATP. Why will you need an energy input, how much ATP will you need to split (per nucleotide added), and how will you couple the splitting of ATP to the synthesis of nucleic acids?

Short Answer: Costs 2ATP to add on one (mono)nucleotide. Why? Raw materials for chain growth are dXTPs, not dXMPs. -- 2 ATPs are used to convert XMP to XTP and then the 2 extra phosphate groups (from ATP) are removed when XMP is added .

Long Answer on next page!

Long Answer: Let's look at the individual reactions involved. (These are all diagrammed out on Handout 12-2.)

Important Notes:

(1). DNA vs RNA: If you are making RNA, the sugars are ribose, and the bases are A, G, C & U. If you are making DNA, the sugars are deoxyribose and the bases are A, G, C & T. In this case it is understood that all the sugars are deoxy, since we are discussing DNA synthesis. The basic process of chain growth is the same for DNA and RNA.

(2). You would need a DNA template to synthesize RNA or DNA -- you need the template to specify which nucleotide to add next. The template is not drawn in here (or on handout 12-2) because the focus of this section is on energy, but you should assume template is present.

B. Reaction 1 -- Synthesis or hydrolysis?

The obvious reaction for adding a nucleotide to a growing chain is **Reaction 1:** (all reactions refer to numbered reactions on handout 12-2).

Rxn 1: Chain n nucleotides long + nucleotide mono-phosphate → chain n+1 nucleotides long + H_2O. New nucleotide is added to 3' end of chain.

For example: AGC + TMP → AGCT + H_2O. (See handout for more detailed picture.)

Reaction 1 has a positive ΔG^o of about +7 kcal/mole, so it goes to the left, not the right. Cells contain an enzyme that catalyzes rxn. 1. That's because rxn. 1 is actually used (to the left) to break down polynucleotides that you eat → mononucleotides for building your own DNA. You have to break down ingested DNA so it doesn't transform **you** -- you want to recycle the nucleotides, but you want to destroy the foreign genetic information.

C. How to drive rxn. 1 to the right? Why we need rxn. 2

To drive rxn. 1 strongly to the right (and achieve synthesis, not breakdown), we need to break 2 "high energy" bonds, that is to knock 2 phosphates off an ATP or the equivalent. In other words, we want to couple the following reaction (rxn. 2) to rxn 1:

Rxn. 2 2 ATP + 2 H_2O → 2 ADP + 2 $\mathbf{P_i}$ ΔG^o = -14 kcal/mole

The $\mathbf{P_i}$ is written in bold to emphasize that it is a phosphate group, not a phosphorous atom.

D. How are reactions 1 & 2 coupled? The coupling is done in three steps (rxns 3 - 5). The individual reactions and a summary diagram showing the results on an energy scale are shown on Handout 12-2. The coupling of the three steps (rxns 3 - 5) occurs like so:

1.Step 1 -- Rxn 3. First the energy of ATP is used to convert TMP, CMP, GMP etc. to TTP, CTP, GTP (using the appropriate enzymes). For example, for TMP:

Rxn. 3 2 ATP + TMP → 2 ADP + TTP ΔG^o = 0 kcal/mole

2. Step 2 -- Rxn 4. Then CTP, GTP, etc. are used as starting materials, so knocking off the extra 2 phosphates can provide the energy to drive nucleotide synthesis. The 2 **P-P** bonds are broken in 2 steps. First the end **P-P** is removed as a unit and the nucleotide is added on as follows:

Rxn. 4: Polymerization catalyzed by DNA polymerase (See handout 12-2 for more detailed picture.)

For example: AGC + **TTP** → AGCT + **P-P$_i$** (inorganic pyrophosphate)

3. Step 3 -- Rxn 5. Then the **P-P$_i$** is hydrolyzed using pyrophosphatase as follows:

Rxn. 5 $\mathbf{P}$ - $\mathbf{P}_i$ + H_2O ---Pyrophosphatase---> 2 $\mathbf{P}_i$ ΔG^o = -7 Kcal/mole

ΔG^o for reactions 3 & 4 is about zero, and ΔG^o for rxn 5 is about -7 kcal/mole. By carrying out reactions 3 - 5, the cell uses the energy of ATP hydrolysis to drive polynucleotide synthesis. In other words, the cell couples reactions 1 & 2. The cell does not carry out rxns 1 & 2 separately -- instead it carries out reactions 3 + 4 + 5 which have the same net effect as reactions 1 + 2. (So what enzymes does the cell have? Does it have enzymes for reactions 1, 2, 3, 4, and 5?)

Rxn 5, which is catalyzed by the enzyme pyrophosphatase really "pulls" the entire synthesis of polynucleotides. (This is what Dr. C calls 'indirect coupling.') Rxn 5 is strongly to the right, so it removes one of the products of rxn 4, thus "pulling" rxn 4 to the right. (So ΔG for reaction 4 is <<0 even though ΔG^o is zero.) When rxn 4 goes to the right, it uses up the product of rxn 3, pulling it to the right too. So the whole combination, reactions 3 - 5, goes to the right.

E. Overall Result

To summarize: To end up with a chain of mononucleotides (For example: .....AMP- GMP- CMP or A-G-C), you start with nucleoside ***tri***phosphates (XTPs). Each time you add a new nucleotide to the 3' end, you knock two phosphates off the nucleotide to be added, and add a nucleoside ***mono***phosphate (XMP) to the growing chain.

5' .....AGC 3' + XTP ---> .....AGCX + 2 **P$_i$**. (See reactions 4 + 5 on handout)

Energy from breaking two phosphate-phosphate bonds (in the XTP) is enough to drive synthesis of one new phosphoester bond (between the CMP on the 3' end of the chain and the added XMP.) Net result = Reactions 4 + 5 on handout = formation of a phosphodiester bond connecting two nucleosides. (Note that it takes two steps, not one, to release the two **P$_i$**'s from the XTP.)

F . Summary Diagram. The relative free energy changes involved in all these reactions can be summarized by putting the components on an energy scale, as shown on Handout 12-2. (ATP hydrolysis does not belong on the same energy scale. See note 1.)

Important notes:

(1). In the summary diagram, reaction 3 is divided into two linked or coupled reactions -- hydrolysis of ATP (rxn 2, which is 'downhill') and phosphorylation of TMP (which is uphill). The two balance out, and the net change in free energy is zero. Both arrows are shown pointing in the same direction to indicate that the reactions are coupled (rxn2 provides the energy to drive phosphorylation of TMP). Both arrows point up, to indicate that the net effect is to convert TMP to TTP (which is uphill). It would be more accurate to draw the ATP hydrolysis reaction outside of the plane of the paper, since it is downhill not uphill.

(2). In the summary diagram, reactions 4 & 5 are combined.

To review the energy issues in DNA synthesis, try problem 6-7.

VII. DNA Replication -- Issue 3 -- Enzymes. (This topic is discussed at length in Sadava, section 13.3 & Becker, section 17.1)

A. How many enzymes? You need enzymes to carry out reactions 3-5 on handout 12-2 (or see notes above). This includes DNA polymerase (for rxn 4), pyrophosphatase (for rxn 5) and enzymes for rxn 3.

Note: We are deliberately ignoring the names of the enzymes for rxn 3. These are discussed in more advanced classes and in the texts.

B. How Many Polymerases?

An enzyme that catalyzes addition of nucleotides to a growing chain of DNA (rxn 4) is called DNA polymerase. There are multiple DNA polymerases, but we will not distinguish them, since they all work in the same direction -- by adding nucleotides to the 3' end). A separate enzyme is needed to catalyze reaction 5 (Pyrophosphatase).

C. How many other enzymes?

A very large number of additional enzymes and proteins are required to replicate DNA properly, since the DNA must be unwound, unfolded, etc. (and then rewound and refolded) in addition to being polymerized. (If you are interested, in unwinding proteins, see Becker figs. 17-14 (19-12); for a summary of all proteins involved see fig 17-15 (19-13.) It also turns out that polymerization (especially starting and stopping) is quite complicated. We will ignore most of the topological and enzymatic complications, and most of the proteins required to deal with them. (If you want a picture of the whole shebang, see Fig 17-16 in Becker (9th ed.)

Given DNA polymerase, pyrophosphatase, and the winding/unwinding enzymes (& whatever extra enzymes you need to start and stop), how many additional enzymes do you need? Given that the two chains of DNA are antiparallel, and DNA polymerases only add to the 3' end, how will you make the complements of both strands? You will need ligase, as explained below.

VIII. DNA polymerase & Ligase

A. Direction of Chain Growth

1. DNA polymerases all add one way -- There are multiple DNA polymerases, but we will not distinguish them here, since they all catalyze addition to the free 3' end of a growing chain.

a. How it works: DNA polymerase adds nucleotides to the 3' end of a growing chain as in rxn 4 on 12-2, so the new chain is made from its 5' end to its 3' end. DNA polymerase will not add nucleotides on the the 5' end, and neither will any other enzyme, so all new chains must be made 5' to 3'.

b. Rationale: Once an enzyme is designed to hold the 3' end of a nucleotide so the 5' end of the nucleotide can hook up the 3' end of the growing chain, then the same enzyme cannot hold the 5' end of a nucleotide, which has a different shape and chemical make up, and so the enzyme cannot catalyze additions to the 5' end of the growing chain.

2. All chains grow antiparallel to template.—see next page.

All nucleotide chains must be made 5' to 3' AND each new single strand must be antiparallel to the its template strand, so the resulting double stranded molecule will have antiparallel strands. See Sadava fig. 13.9 (13.11) or Becker fig. 19-7. For example:

Template		Daughter Molecules		Direction of Synthesis of New Strand
		5' A G C T T A G 3'	**(old)**	
5' A G C T T A G 3'	→	**3' T C G A A T C 5'**	**(new)**	←
3' T C G A A T C 5'	→	**5' A G C T T A G 3'**	**(new)**	→
		3' T C G A A T C 5'	**(old)**	

3. The two "new" strands in a double helix must grow in opposite directions.

- If bottom strand of template is used, the new strand must be made left to right (→)
- If top strand of template is used, the new strand must be made right to left (←)

- In both cases, the new strand is made from 5' to 3'.

- What if you want to use both strands of a double helix as templates simultaneously? Then you must make the complement to the top strand in one direction and the complement to the bottom strand in the other direction -- at the same time. This is the only way both strands can be made 5' to 3'. How is this possible? See below.

To review how DNA chains grow, try problems 6-6, part C, & 6-12 parts A-C . Other problems on this topic are 6-10 & 6-11.

B. Discontinuous Synthesis -- the Need for Ligase

How does replication work with a real DNA molecule that is millions of base pairs long? See Sadava fig. 13.14 (13.16) or Becker 19-9. This is diagrammed on handout 12-3. The steps and letters listed below refer to the top diagram on the handout.

1. *Step 1.* You don' t unwind the entire molecule and replicate each template strand separately. Instead you unwind a little of the double helix at a time, starting from one end, to give molecule A.

2. *Step 2.* Then you replicate the short denatured region as above, to get molecule B.

3. *Step 3.* Then you unwind a little more, to get molecule C.

4. *Step 4*. Now one of the new chains (the leading strand, which is the one being made on the left on the handout) can keep on growing 5' to 3' in a continuous manner while the new section of the other chain (the lagging strand, the one being made on the right) must be made 5' to 3' in a retrograde, discontinuous, fashion. This gives molecule D.

5. *Terminology*. The short, discontinuously synthesized fragments of about 100 - 1000 bases made in step 4 are known as Okazaki fragments after their discoverer. (See Becker 19-8.)

6. *Skip steps 5 & 6 (and molecules E & F) for now. Steps 5 & 6 involve primers and will be explained below.*

7. *Step 7.* If this retrograde or discontinuous synthesis continues, the new chain on the right (the lagging strand) will have breaks in it, so the cell has an enzyme called ligase that ligates or ties up the short fragments into one continuous chain. This gives molecule G.

8. *Repeat 3-7 (see molecules H to K).* These steps are repeated until the entire double helix is duplicated. The result is 2 complete molecules, as in L.

To review discontinuous synthesis, go over handout 12-3 (top) and/or figures in texts. Problems to do are listed below after we cover primers.

IX. Bi-directional Replication. (Bottom of handout 12-3).

A. How many replication forks per DNA? The more forks, the faster replication is. Most small genomes (such as bacterial and viral DNA's) are circular, and replicate bi-directionally -- 2 forks emanate from a single origin (starting point) as shown on the bottom of handout 12-3 or Sadava fig. 13.10A (13.12 A) or Becker 19-4. Longer DNA molecules are usually linear and often have multiple bidirectional origins of replication as shown in Sadava fig. 13.10B (13.12 B) or Becker fig. 19-5 -- this will be discussed later when we get to eukaryotes.

B. How does bi-directional Replication go? In the top picture on the handout you have one fork or zipper moving down the DNA. In the bottom picture, you have 2 zippers or forks. Both start from the same point (the dotted line = origin of DNA replication = ori) but one fork goes to the left and one fork goes to the right. The events at each fork are the same as those shown in the top of the handout, but the forks go left and right instead of down. At each fork you have unwinding, continuous synthesis on one strand and discontinuous synthesis & ligation on the other strand, just as before. If the DNA is circular, the right fork is really going clockwise and the left fork counterclockwise, and the 2 forks proceed until they meet in the middle of the molecule, approximately 180 degrees from where they started. (See Becker fig. 19-4.)

C. An Important Definition: Bidirectional replication means that there are 2 **forks** that move in opposite directions. It does NOT refer to the fact that the 2 DNA **strands** (leading and lagging strands) are made in opposite directions. That is called ***discontinuous*** synthesis, and it always happens at every fork whether there is one fork (unidirectional replication as in the top panel of handout 12-3) or two (bidirectional replication as on the bottom of the handout) starting at each origin.

To be sure you understand what is happening in the bottom picture, it is a good idea to write in all the 5' and 3' ends on the DNA's shown and also to number the Okazaki fragments at each fork to **show the order in** which they are made.

D. Topology. FYI only.

1. What Moves? In the pictures on the handouts and in many pictures in the texts, it looks like the DNA stays put and the enzymes move down the DNA. It is probably the other way around -- the enzymes stay put, and the DNA slides through them. This and many more details of DNA replication are drawn and explained in Sadava, section 13.3.

Topology, cont.

2. How Polymerase is Oriented: The polymerases making the leading and lagging strands are probably moving (or facing) effectively in the same direction, relative to the fork, because the template for the lagging strand is looped around. See Becker fig. 17-16 (19-14). (The leading and lagging strands are both being made in the 5' to 3' direction, anti-parallel to their respective templates.) The end of the animation (see link at start of lecture) called 'How nucleotides are added in DNA Replication' shows the looping.

To review bi-directional replication, see problem 6-13, part A.

X. Primers & Primase. (Top of handout 12-3. Steps 5 & 6)

A. The Starting Problem

If you put DNA polymerase, ligase, pyrophosphatase, dXTPs (dATP, dGTP, dTTP & dCTP) in a test tube (+ all unwinding enzymes) will you get DNA? No, because DNA polymerase can not start a new chain -- it can only add on to the 3' end of a pre-existing chain. (There are multiple DNA polymerases, but all have this property.) So how do new DNA strands get started? Using primer and primase.

B. The Solution *in vivo*

1. How Primase makes Primer -- see Sadava fig. 13.11 (13.13) or Becker Fig. 17-13 (19-11).

a. Primase: Primase is a type of RNA polymerase that uses nucleotide triphosphates to make a short RNA stretch (probably less than 20 bases long {Q&A}) complementary to the 3' end of the template (= 5' end of new strand). RNA polymerases (unlike DNA polymerases) can start new chains from scratch. RNA chains are made 5' to 3' in much the same way as DNA, using ribo-nucleoside triphosphates (containing U, not T) instead of deoxy-nucleoside triphosphates.

b. Primer: Short RNA stretch made by primase is called primer. On handout (12-3) of events at fork, RNA primer is represented by a dot. (In diagram below, primer is a red squiggly line.) Primase catalyzes synthesis of primer, and then DNA polymerase adds on the 3' end of the RNA primer.

2. How Primer is Removed & Replaced

The primer (the short RNA section) must be removed and replaced by DNA. The process is shown in steps 5 & 6 of handout 12-3 and in the diagram below. Some of the steps below may occur simultaneously, but are described separately to make the process clearer.

- Step 5: Primer is removed, leaving a gap between the 3' end of Okazaki fragment #2 and the 5' end of fragment #1, giving molecule E.
- Step 6: DNA polymerase adds on to the 3' end of fragment #2 to fill the gap, giving molecule F.
- Step 7: Ligase joins the loose ends of the lagging strand, giving molecule G.

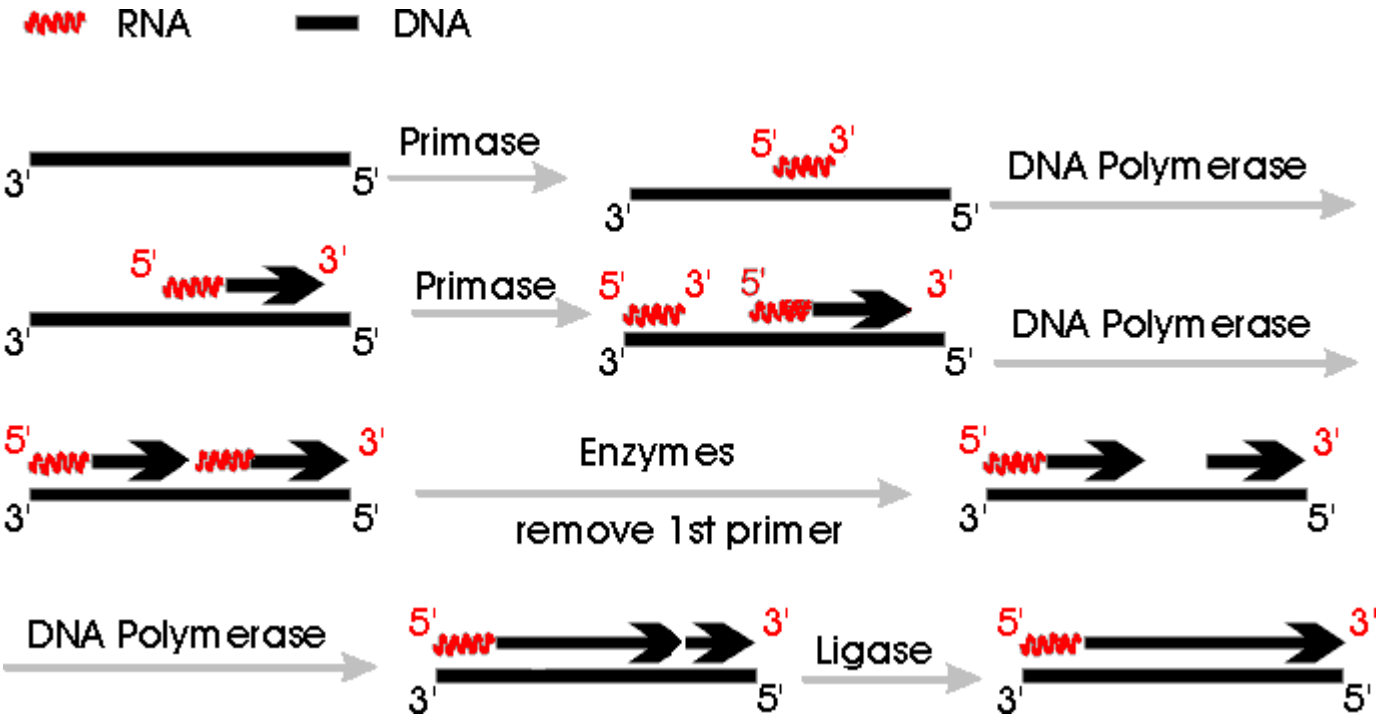
Removal of RNA primer (step 5) and filling of the gap with DNA (step 6) may occur at the same time, using two different catalytic parts of a single enzyme. The primary enzyme responsible is a DNA polymerase , although not necessarily the same one that adds to the 3' end of the regular growing chain. (Note that enzymes can have more than one catalytic activity.)

3. Summary Pictures of Use & Replacement of Primer

See Becker Fig 17-15 (19-13) or Sadava fig. 13.15 (13.17) or Picture Below. Note: Some of the pictures in the older editions of the texts don't have all the details right. Some of the figures imply that DNA can replace RNA primer without the need for a free 3' end for DNA polymerase to add on to. Other figures show ligase joining the Okazaki fragments at the wrong place. (See picture below or solution to problem 6-14, part B-3, for correct position of ligation. Note that the replication fork in problem 6-14 goes in the opposite direction from the fork in the picture below.)

In the picture below, which summarizes the process of primer synthesis and replacement, all arrows go 5' to 3'. Only one side of the replicating fork is shown -- the side carrying out synthesis of the lagging strand. The side carrying out continuous synthesis is omitted. Note that replication fork below goes *right to left -- DNA is unzipping from right to left.*

Function & Replacement of Primer; see also handout 12-3. This diagram = right arm of fork on top of 12-3. Note that the picture in Sadava has the template strand written 5' to 3', so new strand is growing right to left.



For animations of primer removal and other events at the replication fork, see the links given at the start of the previous lecture, or go to the links page.

To review primers, see problem 6-12, A-D.

Next time: *Wrap up of anything above we didn't get to. Then more on discontinuous Synthesis & Primers -- How do DNA chains get started? Then on to PCR, and RNA synthesis. What do you need RNA for, and how is RNA made? How do RNA and DNA synthesis compare?*