UN2005/UN2401 '18 Lecture #13 -- Wrap Up of DNA Synthesis; PCR; What is RNA & What is it Good For? Intro to: How is RNA Made?

Handouts: 12-3 -- DNA Replication - Details at Fork
13-A -- Diagram of PCR; articles about PCR (article side is in paper only)
13-B -- DNA synthesis vs RNA synthesis Compared (This will probably be covered in #14)

Paper copies of all handouts are provided in class. Extra copies are placed in the boxes in the hall on the 7th floor of Mudd after the evening lecture. PDF copies of handouts are posted on the handouts page, usually the night before the lecture.

For all corrections in notes, current and previous editions of the problem book, etc., see the corrections page. If you find any errors, or dead links, don't hesitate to email Dr. M.

I. DNA replication, cont. The Big Issues

A. The 3 big issues

1. Order of monomers/information

2. Energy

3. Enzymes

B. The Results so Far, and Issues Remaining

1. Order of monomers/information -- You need a DNA template, and replication is semi-conservative. See Lecture #12, and handout 12-1.

2. Energy -- You need ATP. See Handout 12-2.

3. Enzymes -- Many enzymes are needed to replicate DNA. See below (& handout 12-3).

II. DNA Replication -- Issue 3 -- Enzymes

A. How many enzymes so far? You need enzymes to carry out reactions 3-5 on handout 12-2 (or see notes of lecture 12). This includes DNA polymerase (for rxn 4 – adding on a nucleotide & releasing PP_i), pyrophosphatase (for rxn 5 – hydrolyzing PP_i to $2P_i$) and enzymes for rxn 3 (converting XMPs to XTPs) .

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 other enzymes?

You will definitely need ligase for reasons explained below. 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.)

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

III. Discontinuous Synthesis & 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 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..

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.

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.

IV. 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 (13.12A) 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 counter clockwise, 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.

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 #12) called 'How nucleotides are added in DNA Replication' shows the looping.

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

V. Primers & Primase. (Top of handout 12-3. Steps 5 & 6, molecules E and F)

A. The Starting Problem

If you put DNA polymerase, ligase, pyrophosphatase, dXTPs (dATP, dGTP, dTTP & dCTP) in a test tube (+ all unwinding enzymes) and a DNA template, will you get more DNA made? No, because DNA polymerase cannot 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.

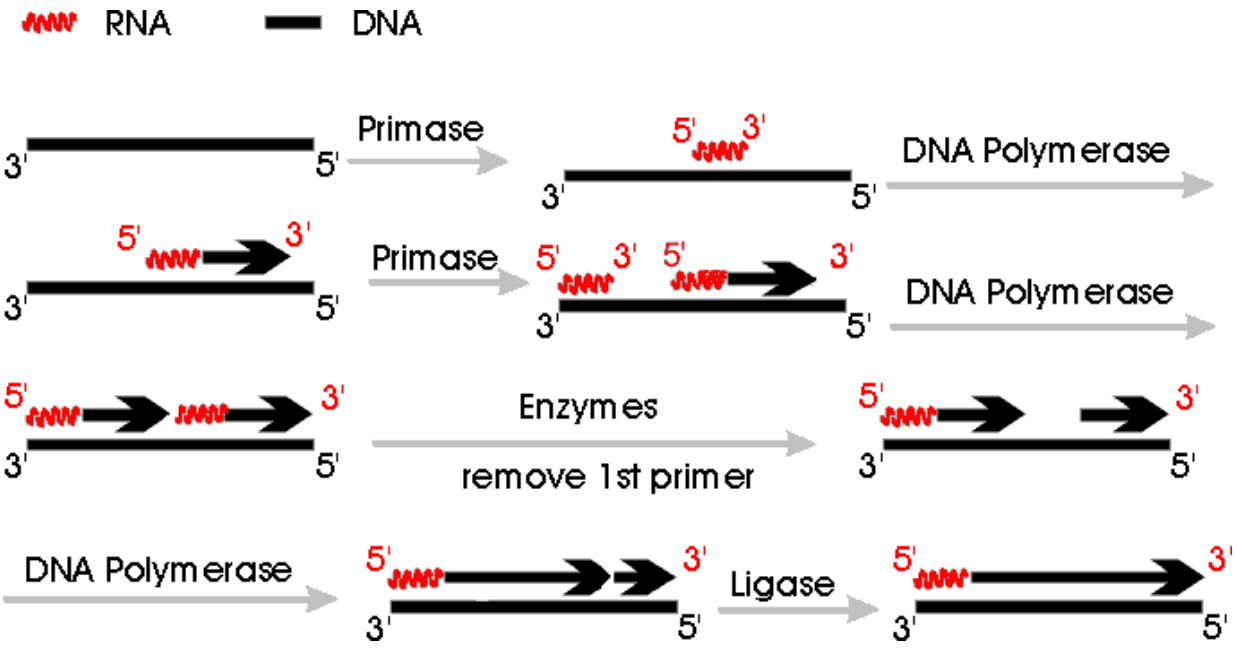
- Step 5: Primer is removed, leaving a gap between the 3' end of Okazaki fragment #2 and the 5' end of fragment #1, giving molecule E.
- Step 6: DNA polymerase adds on to the 3' end of fragment #2 to fill the gap, giving molecule F.
- Step 7: Ligase joins the loose ends of the lagging strand, giving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

Removal or displacement 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 or more than one enzyme. The enzyme responsible for filling the gap is a DNA polymerase, although not necessarily the same one that adds to the 3' end of the regular growing chain.

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.*

Funct. & Replacement of Primer; see also Handout 12-3. This diagram = right arm of fork on top of 12-3.



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.

C. Catalytic Activities of DNA polymerase -- How is RNA primer removed?

1. DNA polymerases are complex enzymes. DNA polymerases have multiple subunits (peptide chains) and multiple enzymatic activities. The different enzymatic activities may be catalyzed by different subunits of the same enzyme or by different enzymes. In this class, we are lumping all the DNA polymerases together and treating them as a single enzyme. In more advanced classes the properties of the different DNA polymerases will be distinguished.

2. How many Catalytic Activities per DNA polymerase?

(1). All DNA polymerases catalyze addition to 3' end of a pre-existing nucleotide chain. One XMP is added to the 3' end of the growing chain, using dXTP and releasing PPi.

(2) Some DNA polymerases have additional catalytic activities either 5' to 3' exonuclease and/or 3' to 5' exonuclease. (See 'Terminology' below.)

Note FYI: A DNA polymerase is required for removal of RNA primer. A DNA polymerase may actually degrade the primer, using it's 5' to 3' exonuclease, or the enzyme may simply act as a polymerase to extend the 3' end of one Okazaki fragment, displacing the RNA primer of the neighboring fragment as it goes. In that case, an additional nuclease would be needed to degrade the RNA 'flap'.

3. Terminology: The ability to remove nucleotides one at a time from the end of a chain is called exonuclease activity. (exo = from the exterior or end). There are two types of exonuclease:

a. 3' to 5' exo. The enzymatic ability of DNA polymerase that removes nucleotides one at a time from the 3' end of a chain is called 3' to 5' exonuclease activity. It has a role in increasing the accuracy of DNA replication called 'proof reading' which will be explained next time.

b. 5' to 3' exo. The enzymatic activity of DNA polymerase that removes nucleotides one at a time from the 5' end of a poly nucleotide chain (not from the 3' end) is called 5' to 3' exonuclease activity. It may be used to remove primer.

To review the multiple properties of DNA polymerases, see problem 6-14.

Note: The 2015 Nobel Prizes in Chemistry were awarded to 3 scientists who studied DNA repair. These repair mechanisms fix up DNA if the DNA is damaged by outside agents, or if mismatches slip by the proof reading function. For details, and their acceptance speeches, see the Nobel Prize Site.

D. The Ending Problem -- a Biological consequence (in eukaryotes) of the need for primers.

1. The "loose end" Problem

There is no easy way to replace the primer on the left end of the new strand (in picture above); also see Becker fig. 17-18 (19-15) or Sadava 13.17A (13.19A). The RNA can be removed, but no DNA can be made to fill in the gap.

2. Solutions

a. Small DNA's (& most prokaryotic chromosomes) are generally circular, which circumvents this problem.

b. Linear DNA's -- Telomeres & telomerase.

- Linear chromosomes (the norm in eukaryotes) do tend to get shorter with each replication -- in the next go round, chain that has just been made will be template, and it is shorter than the original by the length of the primer. (See Becker fig. 17-18 (19-15) or Sadava 13.17A (13.19A).

- How organisms with linear chromosomes avoid the consequences: the DNA molecules in the chromosomes have special repeated sequences (called telomeres) on the ends. These repeats are gradually lost, and the chromosomes do get shorter with each replication, but it doesn't usually matter because the sections that are lost (telomeric repeats) do not contain genetic (coding) information.

- In some cells, the lost repeats can be replaced by an enzyme called telomerase. More details will be discussed next term when we focus on eukaryotes.

The 2009 Nobel Prize in Medical Science was awarded to the investigators who identified telomeres and telomerase. Go to the official Nobel Prize home page for links to descriptions of all the awards in chemistry and in medical science. Many of these prizes were awarded for discoveries covered in this course.

More on telomerase (FYI only): See Sadava fig. 13.17 (13.19) or Becker 17-19 (19-16) if you are interested in how the lost repeats are replaced using telomerase. (Note: in Purves' picture in the 6th edition, the wrong strand is "too short." The 3' end should be longer than the 5' end, not the reverse.) The lack of telomerase may be what limits normal somatic (body) cells of mammals to a finite life span of 50-60 divisions. Germ cells, that produce eggs and sperm, make telomerase, so a new generation always starts out with full length telomeres. For an animation of how telomerase works, see http://faculty.plattsburgh.edu/donald.slish/Telomerase.html. (Note that this animation is for eukaryotes; in this case there are two different DNA polymerase for the leading and lagging strands. Both grow chains in the 5' to 3' direction.)

VI. PCR (Polymerase Chain Reaction) A Practical *in vitro* Application of the need for Primers.

The inventor, Kary Mullis, received the Nobel prize in 1993. For his acceptance speech, biography, etc. see the Nobel Prize official site. For uses of the technique, see PCR articles on class handout

A. Why Bother? To get amplification. Uses small number of starting molecules & produces large number of copies of target sequence. You need amplification to get enough target DNA to hybridize to a probe, to clone, to analyze, etc.

B. Basic Idea -- you decide where copying starts and stops.

- DNA synthesis will not start without a primer.

- In a living cell (*in vivo*) primase -- a type of RNA polymerase -- makes the necessary ***RNA*** primer. Then DNA polymerase can take over, adding on to the 3' end of the primer.

- In a test tube (*in vitro*) you can omit primase and use an oligonucleotide (short polynucleotide, usually ***DNA***) as primer (= prefab ***DNA*** primer) to force replication to begin wherever you want.

- The primer you add will hybridize to its complementary sequence, wherever that happens to be (not necessarily at the end of the DNA) and DNA polymerase will add on to the 3' end of the primer, thereby starting elongation of a chain from wherever the primer is.

- By using two different primers, complementary to the two strands of the DNA, you restrict exponential copying to the area between the primers, as explained below in detail.

C. Special Features of PCR (as vs. regular DNA synthesis)

1. No replication fork or discontinuous synthesis. Note that the entire template molecule is denatured (or 'unzipped') completely before each cycle, so the complement to each strand can be made separately and continuously. There is no replication fork and thus no discontinuous synthesis here.

2. Preformed DNA primer. Primase is absent, so no RNA primers are made. Oligonucleotides of DNA (***not RNA***) are added instead to act as primers.

3. Special Polymerase. The DNA polymerase used in this procedure is a special heat-resistant one (called Taq polymerase) that is not denatured when the temperature is raised to separate the two strands of the DNA. This special polymerase was isolated from bacteria that live in a hot spring. (What is the advantage of using heat-resistant polymerase?)

D. Steps of PCR -- see PCR handout (12A), Sadava fig. 13.19 (13.21), and/or Becker Key Technique on p. 476 (Box 19 A). For an animation, go to
http://www.dnalc.org/resources/animations/pcr.html
The site listed above (The Dolan DNA Learning Center) has many good features you may want to check out. There is a list of additional animations on PCR, DNA replication, etc. at
https://www.dna.utah.edu/PCR_Animation_Links.htm
Please let Dr. M know if you find any of these sites (or any others) particularly useful.

1. *First Cycle*: You take your template (A) and denature it. (Step 1 = denaturation; results in B.) Then you add primers (one to each strand) to the denatured DNA and cool the mixture. When you cool the mix down, each oligonucleotide primer hybridizes to its complement. (Step 2 = hybridization to primer; results in D*.) Under the conditions used, the two long strands of template do not renature to each other. Then the DNA polymerase adds on to the 3' end of primer until it reaches the end of the template strand. (Step 3 = elongation; results in E.) This completes the first cycle (ends at E). The new strands you just made (dashed on handout in E) include the target sequence, plus some extra DNA on their 3' ends. (This "extra" corresponds to the sequence between the target area and the 5' end of the **template** strand.)

*Note: There is no (C) on the handout to avoid confusion with Watson (W) and Crick (C) strands.

2. Second Cycle: Same procedure as before in cycle 1. You heat the DNA to denature it (step 4 = step 1), and add more of the same primers as before (step 5 = step 2). Then you allow DNA polymerase to add on to the 3' ends of the primers (step 6 = step 3). This completes the second cycle (ends at H). On the handout, only the fate of the new strands made in cycle two is shown after F. The old strands simultaneously go through another cycle just like the one above (steps 2 & 3), but this is

not shown on the handout. The ***new*** strands you made in cycle 2 (shorter strand of each molecule of H) include only the target sequence.

3. Third Cycle: Same procedure as before in cycles 1 & 2. You heat the DNA again to denature it (step 7), add primers (step 8) and allow DNA polymerase to add to the primers (step 9. This completes the 3rd cycle (ends at K). On the handout, only the fate of the new strands made in cycle two is shown after I. (The fate of the complementary strands, left over from the previous cycle, is to repeat steps 5 & 6.) At the end of this cycle, you finally have double-stranded DNA molecules the length of the target sequence (see K).

Note that primers are complementary to sequences in the middle of the original chain, but that after two cycles the parts beyond the primers are no longer copied. {Q&A}.

4. Additional Cycles: Same procedure as in previous cycles (repeat of steps 1-3). After each cycle you heat the reaction mixture to denature the DNA, and then you cool the mixture down to start the next cycle. In each cycle, primer sticks to the appropriate spot (its complement) and polymerase starts at the 3' end of the primer and goes to the end of the template.

5. How reaction is actually carried out. All components (template and excess of heat resistant polymerase, primers & dXTP's) are present from the very beginning. The mixture is heated and cooled repeatedly to end and start subsequent cycles. You don't have to add primers, polymerase, etc. to start each cycle.

6. How many different primers? New molecules of primer are used in each round. However, the primer molecules used in each round have the same sequences as the ones used in all the previous rounds. The primers are not reused -- new primers (with the same sequences as before) are needed for each cycle. You need only two types (sequences) of primer, but you need many molecules of each, just as you need many molecules of dATP, dTTP, etc.

7. Identification of Product. The products of the PCR reaction are usually identified by their lengths, which are determined by gel electrophoresis without SDS. (Why no SDS needed? Think about it.) Gels are used that separate DNA molecules on the basis of their molecular weights (which depends on chain length). Hybridization to labeled probes is often used to detect the positions of the bands of DNA on the gel. (More on this later.) An animation of DNA gel electrophoresis is at http://www.dnalc.org/resources/animations/gelelectrophoresis.html.

To review the PCR technique, see problem 6-13, part C, and 6-15.

For an animation of PCR and links to animations of other DNA techniques, see the urls listed above or go to the links page.

E. Uses/Advantages of PCR

1. *Amplification: Allows you to get enough DNA to analyze chemically, hybridize to a probe, use for IDs etc.*

The beauty of this scheme (PCR) is that the desired (target) sequence is copied exponentially and the other parts of the original DNA are copied linearly. So after a few cycles you have lots of copies of the target sequence (and not much of anything else). *To convince yourself of this, see the answer to problem 6-13, part C-2.* To use this technique and make many copies of the target sequence all you need (in theory) is ONE starting DNA molecule (and appropriate primers). Given current technology, you need 10-50 starting DNA molecules. You can use the multiple copies for many different purposes

such as characterization and/or identification as explained below. Before PCR, you couldn't get enough DNA to do chemical tests, so you couldn't compare different DNA samples.

2. *Detection -- Can be Used to see if a particular target DNA (or RNA) is present or not.*

You can add primers to a sample that you suspect contains some particular target DNA, such as HIV DNA, or DNA from genetically modified corn, or DNA from pond water. The primers are complementary to a sequence found only in the target DNA -- the one you are testing for. (In the cases mentioned, the primers would be complementary to a sequence in HIV DNA, or to a sequence added to ordinary corn DNA by genetic engineering methods to make the special corn, or to a DNA sequence unique to American bullfrogs -- see article.) Then you see if polymerase can make DNA. If no target DNA is present, primers will have nothing to hybridize to, so polymerase will have nothing to add on to, and no copies of DNA will be made. So if you *don't* get multiple copies, it indicates there was nothing to copy -- your target DNA was not there. If you *do* get multiple copies, your target DNA was in the sample.

Notes: (1) The standard HIV screening test is not for HIV itself or for HIV DNA but for antibodies to proteins of HIV. (PCR is used as a backup to confirm a positive result with the standard screening test, or to measure the actual levels of HIV.)

(2). Why would you test for genetically modified corn? StarLink corn is a type of genetically modified corn that was approved for animal feed, but not for human use. In spite of attempts to keep it separate, it turned up in many human foods. StarLink corn is probably harmless to humans, but no one wanted to take any chances, so StarLink corn was withdrawn from the market. Although there is no evidence that food derived from StarLink corn or any other GMO is harmful to humans, many people prefer to avoid GMOs, and testing for the modified DNA is the only way to tell if StarLink corn (or any other genetically modified food) is present in a mixture or not.

3. *Forensics -- Can be Used for identification -- DNA fingerprinting*

a. Basic idea: PCR can be used to copy specific sections of the DNA from different samples -- for example, from DNA left at the scene of a crime and from DNA from a suspect. The sections of amplified DNA can then be compared to see if they match or not (in length, sequence, etc.). The sections that are compared are highly variable ones that probably don't carry any information and are merely spacers in the DNA. If enough sections are checked, you can determine (to a very high degree of certainty) whether the two DNA samples came from the same person or not. DNA testing can be used to identify the guilty (inclusions) and to clear the innocent (exclusions). Alec Jeffreys, who first came up with the idea of using DNA testing for identifications, received a Lasker award in '05. For details on all Lasker awards, by year or awardee, see the Lasker site.

b. Examples: See article from the San Francisco Chronicle of 10/19/99. (Similar articles are reported in the press constantly. For another example, see link below.) Note: you'll need to go to the SFChronicle web site itself if you want to see the pictures or get some of the older articles.

c. Inclusions: If the samples match at enough highly variable spots, then there is a very high probability the samples came from the same person, because the degree of variation is so high that only a few different people in the world should have the same pattern.

For a more recent example of an identification based on DNA evidence, see *NY Times* articles of 10/13 about 'Baby Hope.' In this case, an unidentified 4 year old victim who died in 1991 was identified by matching her DNA to that of her relatives, and the person responsible for her death was found. How a tip led to the DNA tests

is explained at http://www.nytimes.com/2013/10/11/nyregion/investigators-learn-baby-hopes-name-angelica.html . How her killer was found after so many years is explained in follow up articles.

d. Exclusions: If the two samples do not match, then it is clear that the two samples came from different individuals and the suspect could not have committed the crime (since the DNA at the scene came from someone else).

e. STR's: The variable sections that are tested are often ones that have different numbers of short tandem repeats (STR's). The primers hybridize to regions outside the section with the repeats. The number of repeats in each DNA can be figured out from the length of the sections amplified by PCR. The new FBI data base contains the information from checking 13 sections with variable numbers of STR's.

For a great site from the Dolan Learning Center with examples of how DNA is used for identification and forensics click here.

4. Bar Coding (See the article on DNA testing by students on handout side B from lecture 10. For more details on Fish bar coding, see the FishBol site.)

The tests of the DNA from different organism used a similar principle to the one used in forensics. A particular gene that varies from species to species was amplified and then sequenced. The procedure is called 'Bar coding' because the sequencing procedure produces a pattern that looks like a supermarket bar code. There is enough variation in the sequence (or Bar code) of that particular gene to identify the species of animal or fish from which it came. In this case, the amplified DNA from the different samples is compared to the DNA from reference samples. The actual base sequences of the various DNAs are compared. In forensics, the amplified DNA from the crime scene is compared to the amplified DNA from the suspect, and the comparisons are based on the lengths of the amplified fragments (not on their actual sequences).

5. Why you can't do this with proteins

There are very sensitive tests for presence of proteins (usually using the catalytic activities of enzymes and/or binding abilities of antibodies), but no way to amplify (make copies of) what you detect. You can't make more protein from a protein template. PCR takes advantage of fact that DNA replicates for a living to make more copies. You *can* make more DNA from a DNA template.

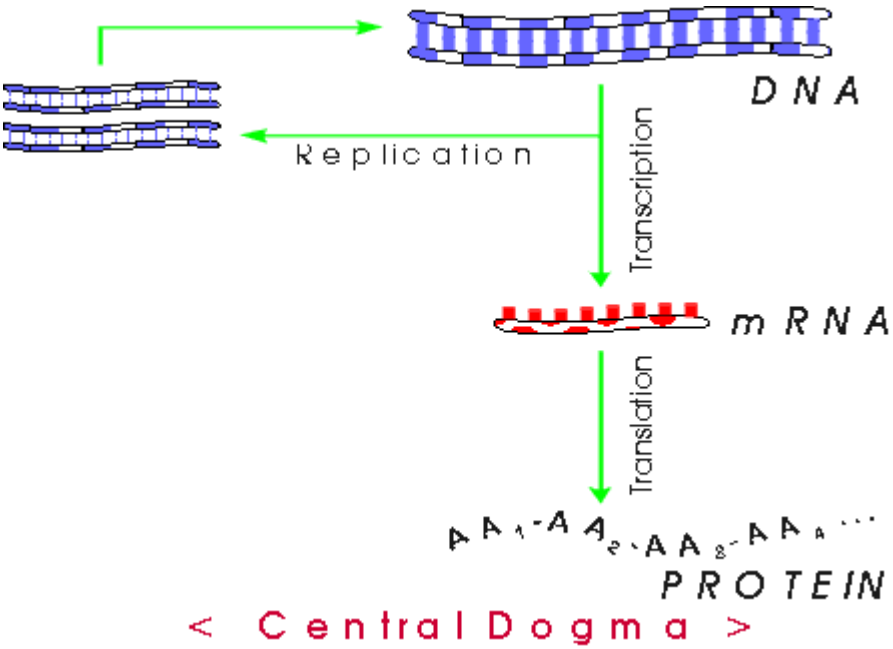
Note: So-called DNA fingerprints are characteristic of the ***person/DNA*** from which they came. So-called protein fingerprints are characteristic of the ***protein*** from which they came. That's why both are called 'fingerprints.' However the two types of 'fingerprints' are made differently and used for different purposes.

VII . Central Dogma -- How does DNA do job # 1? *Details in lecture #14.*

A. Big Picture. So we have a big DNA that includes a particular gene = stretch of DNA coding for a single peptide; how will we make the corresponding peptide?

Note: gene usually means a stretch of DNA encoding 1 polypeptide, but there are complications as we'll see later.

1. Basic idea -- See picture below; or see Becker fig. 18-1 (21-1) or Sadava fig. 14.2:



2. Terminology:

a. Replication = DNA synthesis using a DNA template.

b. Transcription = RNA synthesis using a DNA template.

c. Translation = Protein Synthesis. This term has two possible meanings (we will stick to the first):

(1) Usual meaning = protein synthesis using an RNA template (RNA → protein). Used in contrast to transcription (DNA → RNA).

(2) In some contexts, translation can mean the entire process (DNA → RNA → protein).

VIII. Why RNA, especially mRNA?

A. Basic idea**:** mRNA = Working, disposable copy vs DNA = archival, permanent master copy. DNA = big fat comprehensive reference book or complex web site. mRNA = Xerox of one (book) page or print out of one web page with information you need for a particular assignment. Book stays safe in library; web site remains unchanged. Xerox goes to your room, is actually used, gets covered with coffee stains, smudged, and thrown away.

B.Why mRNA?

1. Convenience. Small size (1 or a few peptides' worth) is much more convenient than many genes' worth. Xerox of one page much more convenient to work with than big fat book.

2. Preserve Master. Using mRNA to make protein saves wear and tear on master -- no coffee stains on the archival copy (DNA).

3. Flexibility. Different amounts of mRNA can be made when cell needs to make different amounts of different proteins. More on this when we get to regulation (operons).

C. Summary: How does RNA make protein?

1. "RNA makes protein" means two things:

a. Need mRNA (info goes DNA → RNA → protein)

b. Need several kinds of RNA to make protein -- See Sadava Fig. 14.2

- mRNA to act as template -- determines order of amino acids
- tRNA to carry the amino acids to the template, and line them up
- rRNA (in ribosomes) to align the tRNA's carrying the amino acids and hook the amino acids together
- Of course you need additional proteins (enzymes and other factors) to make protein

2. Hardware vs. Software. rRNA and tRNA are the hardware or tools or machines; mRNA is the software or working instructions or MP3/ tapes/CDs/punchcards. Cells use same old hardware and constantly changing, up to the minute, supply of new software.

D. How does RNA compare to DNA in structure, synthesis & function?

1. *Structure:* See Sadava table 4.1 & fig. 4.3 and *or* Becker table 3-4 & fig. 3-17 for comparison of DNA and RNA.
   - RNA is single stranded (although sections may double back on themselves → double stranded regions)
   - RNA has U not T, ribose not deoxyribose and is generally shorter, but otherwise like DNA.
2. *Consequences of Structural Differences*
   - RNA is less stable than DNA -- more easily damaged (because of reactive OH on ribose and because a single strand is more exposed)
   - RNA is less easily repaired (because there is no 2nd strand to use to correct mistakes on first strand).
   - DNA is also more easily repaired because it has T not U, so damaged C's (which are oxidized to U) can be recognized and removed.

These differences are summarized in the table on the next page.

Summary of DNA vs RNA

Property	DNA	RNA	Significance/Effect of Difference
ss or ds?	Double Stranded	Single Stranded*	For RNA: Ease of repair down; likelihood of damage up.
Bases	T not U	U not T	For DNA: Ease of repair of damaged (oxidized) C up. (Damage that coverts C to U can be detected & repaired.)
Sugar	Deoxyribose	Ribose	For RNA: Reactivity up, stability down
Length	Very long	Relatively Short	For RNA: Less Information carried per molecule but molecule is much more convenient size
Half Life	Very long (not turned over)	depends	mRNA – short ½ life, in prokaryotes tRNA & rRNA -- relatively long ½ life (much longer >mRNA but shorter < DNA)
* RNA is basically single stranded, but can fold back on itself to form hairpins -- short regions that are double stranded. See Sadava fig. 4.3			

3. Synthesis.

a. RNA grows just like DNA by adding nucleoside triphosphates (XTP's) to the 3' end of a growing chain. For RNA, enzyme for elongation is called RNA polymerase, XTP's are ribo (not deoxy) and U replaces T. Details next time.

b. Synthesis of RNA requires a DNA template.

4. Types. There are 3 major types of RNA involved in translation: messenger RNA (mRNA), transfer RNA (tRNA) and ribosomal RNA (rRNA). The roles of the different types of RNA are outlined above and will be explained in detail next time.

Reminder: Any kind of RNA -- tRNA, mRNA, rRNA, or any other type of RNA -- is made in the same way from a DNA template. The product of transcription does not have to be an mRNA -- it can be a tRNA, rRNA, etc. RNA is NOT used as template to make more RNA.

Next time: Anything above we don't finish, and then -- More on the role and structure of RNA, how the RNA is made, and how the RNA is used to make protein.