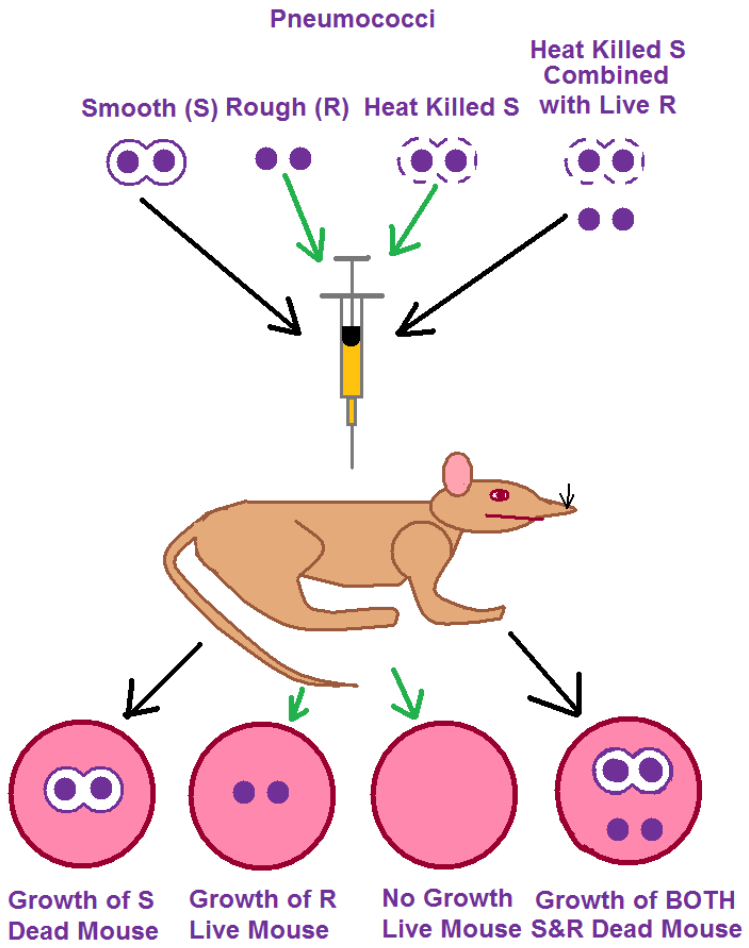
Introduction to Nucleic Acids and Nuclear Transgressions

How DNA, RNA, Enzymes and Ribosomes Work as An Intracellular Team

Griffith’s Transformation

- The first evidence that DNA was responsible for transmitting genetic information was shown by Griffith in 1928, LONG before Watson and Crick showed the secondary structure of DNA to be a double strand of DNA in an α-helix.
- Griffith's transformation, as his experiment has become known, demonstrated conclusively that DNA was "the stuff of heredity".

- Griffith began his experiment by astutely observing that when some strains of *Streptococcus pneumoniae* were injected into mice, they didn't die, while other strains of the same bacterium caused the mice to die.
- As Griffith delved further into this mystery, he noticed that there was a big difference between the two bacterial strains: one had a capsule around itself (he called this the smooth or "S" strain) and the other did not (he called this the rough or "R" strain).
- He then took some of the smooth bacterium (this was the one that had previously killed the mouse) and heat-killed it.
- This dead bacterium was injected into a mouse and the mouse lived.
- Up to this point, whenever Griffith had injected bacteria into the mouse, he had always been able to culture it.
- After injecting the heat-killed bacteria, he was unable to re-isolate any bacteria.
- This was a positive thing, for it conclusively demonstrated that the bacteria was, indeed, dead.
- It was the next step in Griffith's experiments that turned the heredity world on its head: Griffith took BOTH heat-killed smooth bacteria and live rough bacteria and injected them simultaneously into another mouse.
- The mouse died and when Griffith isolated bacteria from this animal, he observed the growth of both S and R strains of the bacteria in culture.
- Since the S strains were previously heat-killed, the only other answer to explain this phenomenon was that the R strains of the bacterium had taken up [some of] the genetic material and begun synthesizing and releasing a capsule based upon that genetic information.

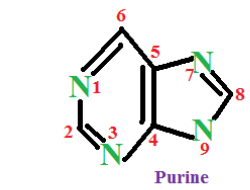


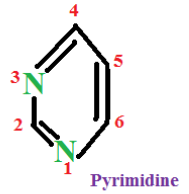
Definitions

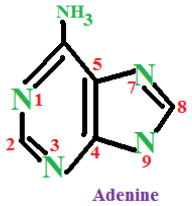
- By definition, nucleic acids are biomolecules that store genetic information in cells or that transfer this information from old cells to new cells.
- There are two groups of nucleic acids:
- DeoxyRiboNucleic Acid (DNA)
  - DNA codes for the functioning of the cell
  - DNA is located mainly in the nucleus of the cell (with a small amount in the mitochondrion of eukaryotic cells -- to be discussed at a later date);
  - DNA is double stranded
- RiboNucleic Acid (RNA)
  - RNA is the "worker" that helps get the DNA message out to the rest of the cell.
  - RNA is primarily in the cytosol of the cell.
  - RNA is single stranded – with one “exception.
- BOTH are "codes" for the cell and, hence, the body's activities at the cellular level.

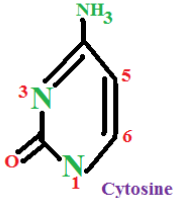
Nucleic Acids

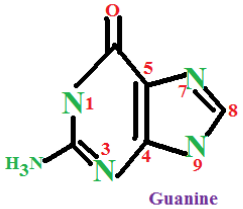
- Nucleic acids consist of nitrogenous compounds called purines or pyrimidines, a carbohydrate and phosphate.
- The figure shows what the structures of purine and pyrimidine look like in the lower right-hand corner.
- Purine is a 2-fused ring system that consists of one 6-membered ring fused to a 5-membered ring.
- In each ring, there are 2 nitrogens.
- Pyrimidine has only the 6-membered ring with the 2 nitrogens.

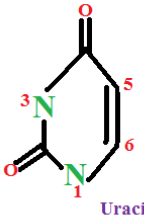


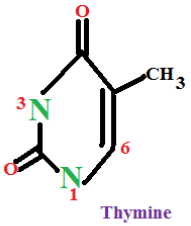






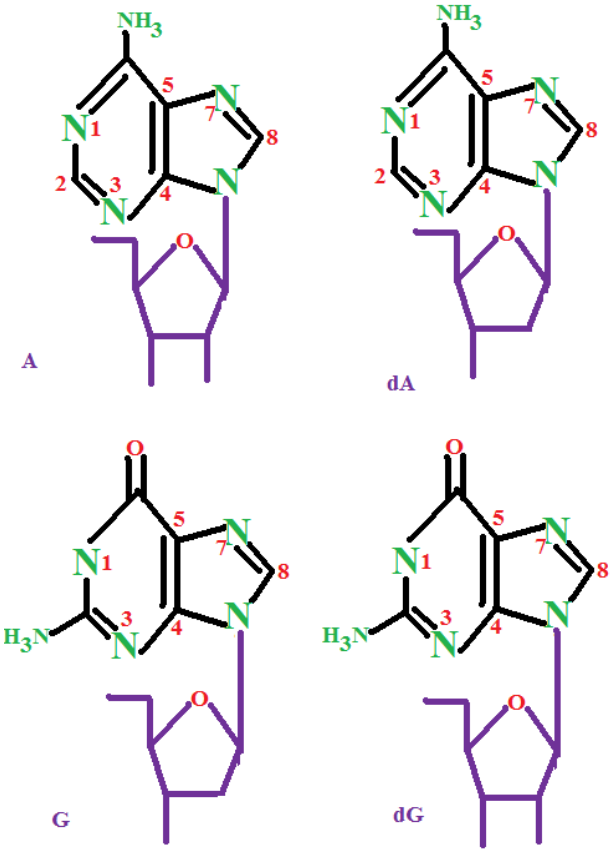






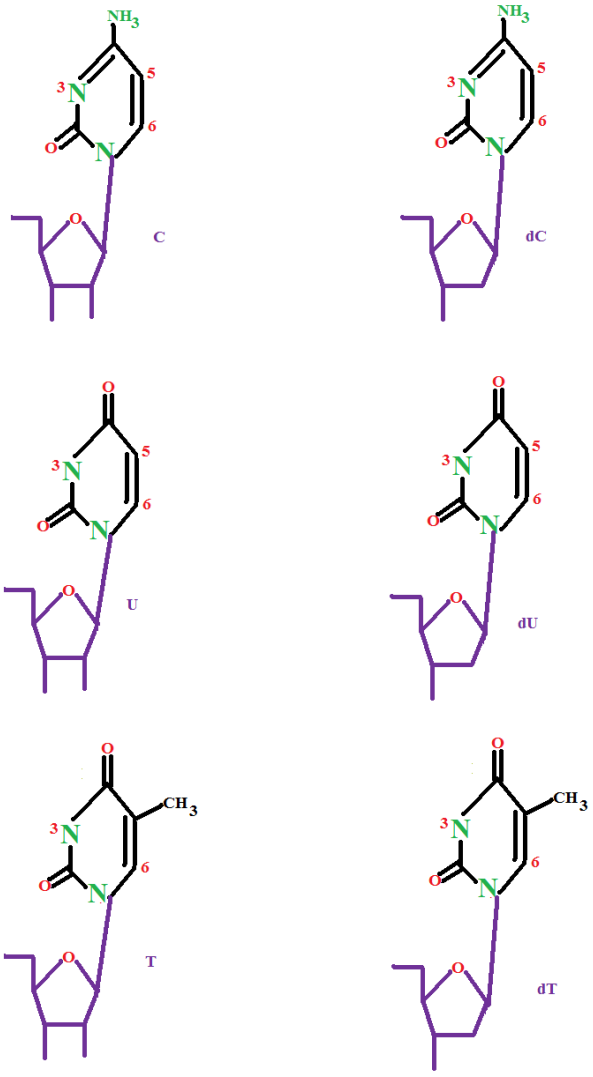
Purines

- The two purines of significance are adenine and guanine.
- Note that they differ very simply:
  - adenine has an amino group on the top of the 6-membered ring,
  - while guanine has an amino group between the 2 nitrogens on the 6-membered ring, a double bonded oxygen in place of adenine's amino group (called a ketone) and has lost a double bond to accommodate the ketone.



Pyrimidines

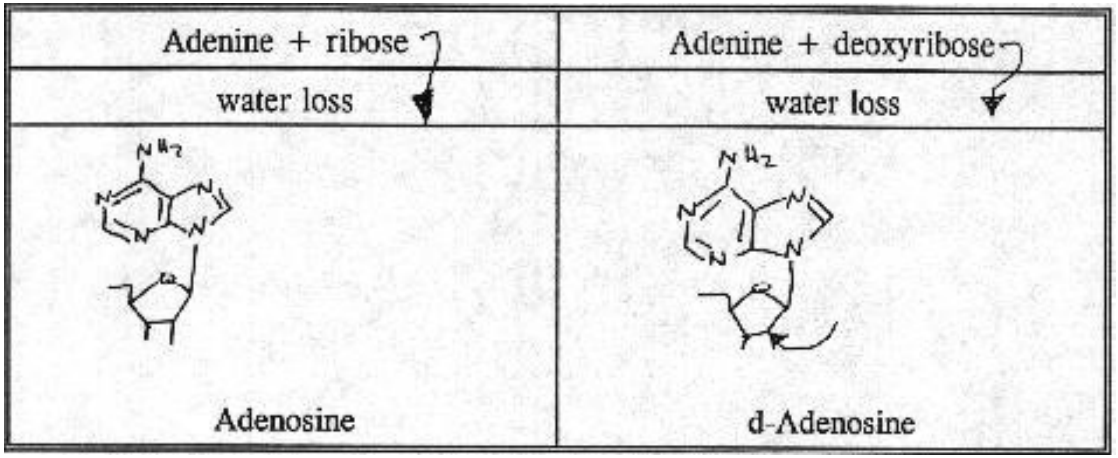
- There are three pyrimidines of interest in Biology:
- Cytosine
  - Cytosine has a ketone between its two nitrogens, an amino group atop its ring and has lost a double bond to accommodate the ketone.
- Uracil
  - Uracil is a modified form of cytosine, where the amino group is replaced by a ketone and a double bond in the ring is lost to accomodate the new ketone.
- Thymine
  - Thymine is uracil with a CH_3 (methyl) group attached adjacent to the ketone at the top of the ring.



- Generally, the 5 nitrogenous compounds with their carbohydrate moiety are abbreviated as one letter abbreviations: A, G, C, U, T.
- It is important to keep in mind that when referring to RNA, these shortcuts are o.k.
- When referring to them in DNA, it is important to remember that we're discussing dA, dG, dC, dU, dT.
- The reason for this is shown in the d-R.
- The carbohydrate that binds with the nitrogenous compounds in RNA is ribose.
- The carbohydrate that binds with the nitrogenous compounds in DNA is deoxy-ribose.
- Deoxyribose is ribose that has had the 2' -OH group removed.
- The last portion of nucleic acids is the phosphate group.
- This group is of immense importance, as it is through this group that DNA and RNA are "held together".

NucleoSides

- In the hierarchy of nucleic acid structure, there are two more levels of nomenclature: nucleoSides and nucleoTides.
- We'll address the nucleoSides first.
- Nucleosides consist of a purine or pyrimidine and a carbohydrate.
- When a purine or a pyrimidine reacts with ribose or deoxy-ribose, water is always one of the products.
- When the purine is adenine and it reacts with ribose, the other product is adenosine (A).
- When adenine reacts with deoxy-ribose, the other nucleoside is deoxy-adenosine (d-adenosine or dA).
- Likewise with the remainder purines and pyrimidines.
- One point to keep in mind is that d-uridine and thymidine are produced only in the lab, not in DNA or RNA, respectively.



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- When a purine or a pyrimidine reacts with ribose or deoxy-ribose, water is always one of the products.
- When the purine is guanine and it reacts with ribose, the other product is guanosine (G).
- When guanine reacts with deoxy-ribose, the other nucleoside is deoxy-guanosine (d-guanosine or dG).
- Likewise with the remainder purines and pyrimidines.
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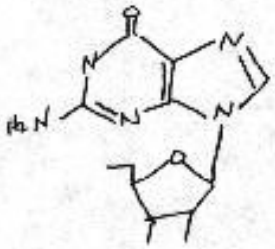
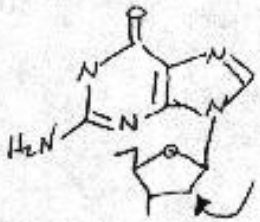
Guanine + ribose	Guanine + deoxyribose
water loss	water loss
	
Guanosine	d-Guanosine

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- When the pyrimidine is cytosine and it reacts with ribose, the other product is cytidine (C).
- When cytosine reacts with deoxy-ribose, the other nucleoside is deoxy-cytidine (d-cytidine or dC).
- Likewise with the remainder purines and pyrimidines.
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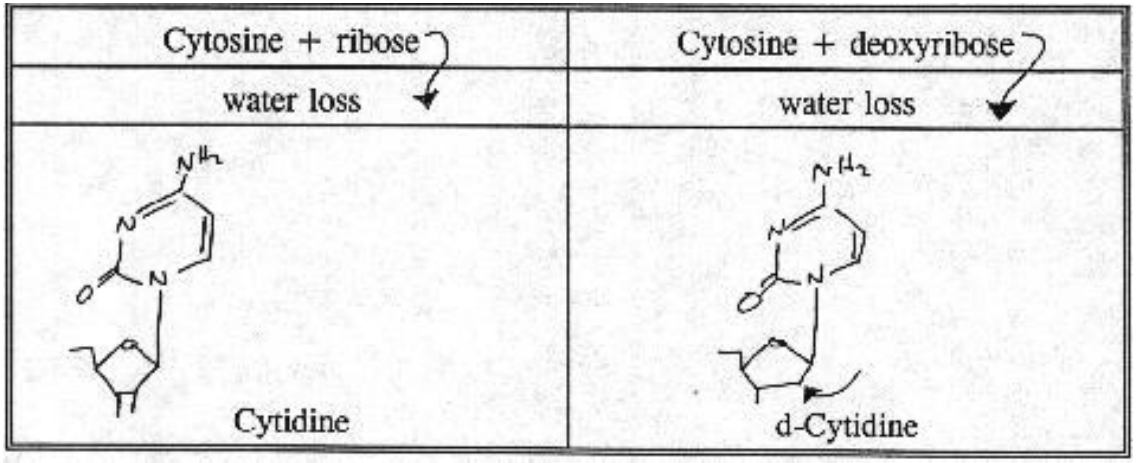

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- When a purine or a pyrimidine reacts with ribose or deoxy-ribose, water is always one of the products.
- When the purine is uracil and it reacts with ribose, the other product is uridine (U).
- When uracil reacts with deoxy-ribose, the other nucleoside is deoxy-uridine (d-uridine or dU).
- Likewise with the remainder purines and pyrimidines.
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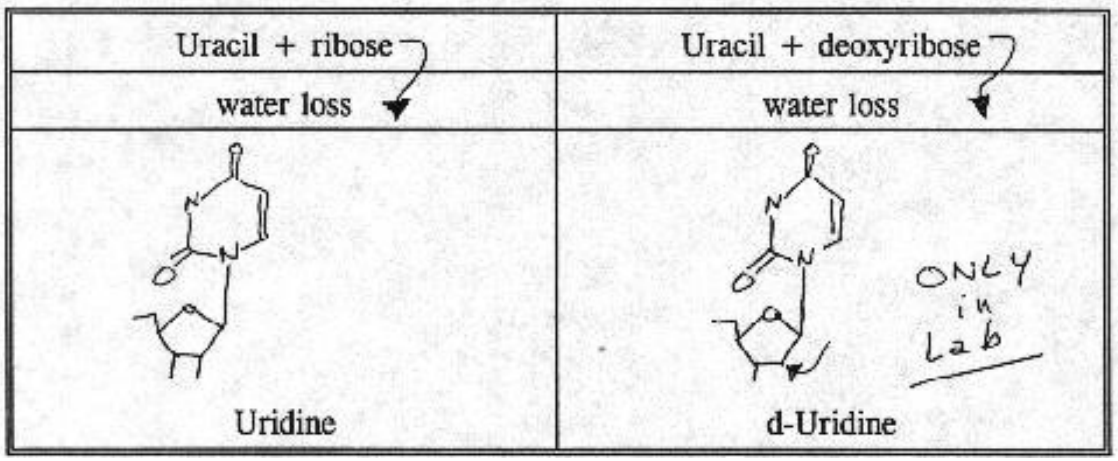

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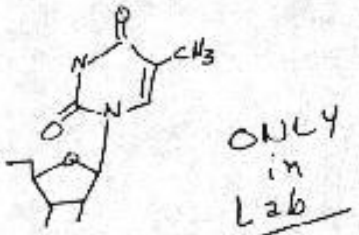
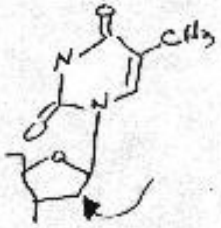
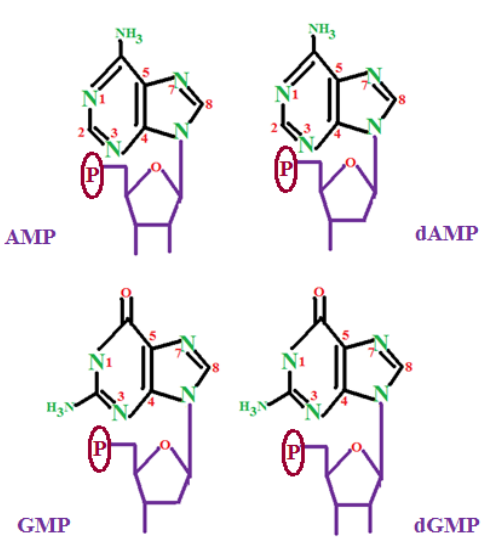
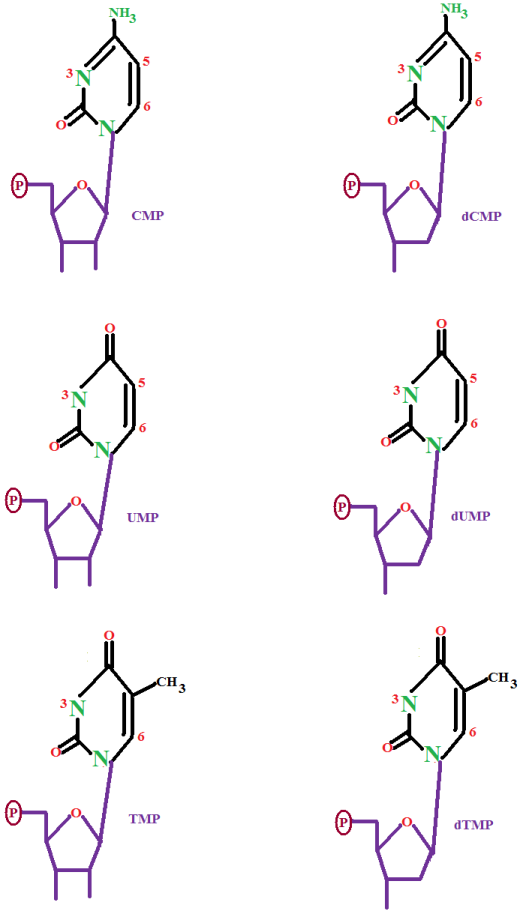
- When a purine or a pyrimidine reacts with ribose or deoxy-ribose, water is always one of the products.
- When the purine is thymine and it reacts with ribose, the other product is thymidine (T).
- When thymine reacts with deoxy-ribose, the other nucleoside is deoxy-thymidine (d-thymidine or dT).
- Likewise with the remainder purines and pyrimidines.
- One point to keep in mind is that d-uridine and thymidine are produced only in the lab, not in DNA or RNA, respectively.

Thymine + ribose	Thymine + deoxyribose
water loss	water loss
	
Thymidine	d-Thymidine

NucleoTides

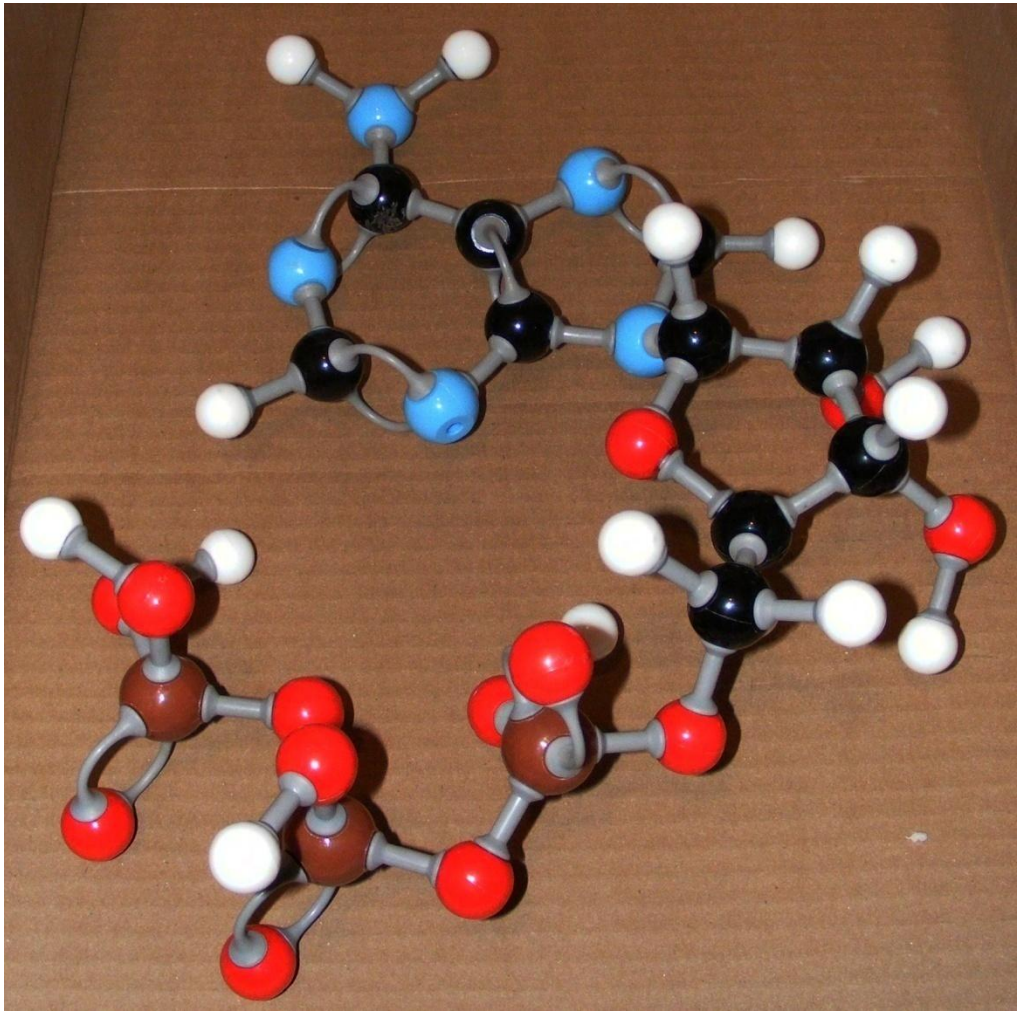
- The last simple level of nucleic acid nomenclature hierarchy is the nucleoTide.
- Nucleotides are nucleosides that have added a phosphate group to the 5' carbon of ribose or deoxy-ribose.
- Biochemist's short-hand the PO_4^{-3} as a "P" with a circle around it (Ⓟ).
- Note that if there were three (3) phosphates instead of one, the names would end as "triphosphate", hence, ATP is adenosine triphosphate and dATP is deoxy-adenosine triphosphate.





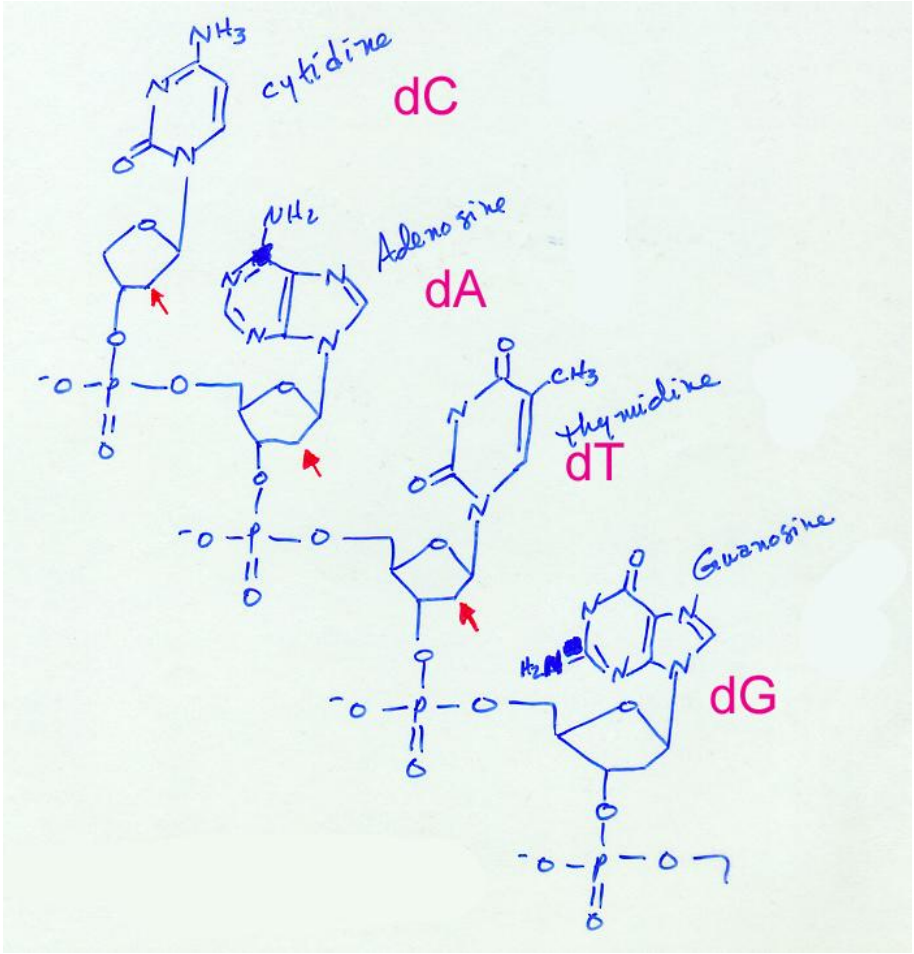
NucleoTides

- With three (3) phosphates instead of one, the names of the previous nucleotides would end as "triphosphate", hence, ATP is adenosine triphosphate and dATP is deoxy-adenosine triphosphate.
- ATP to right.



Primary Structure of Nucleic Acids

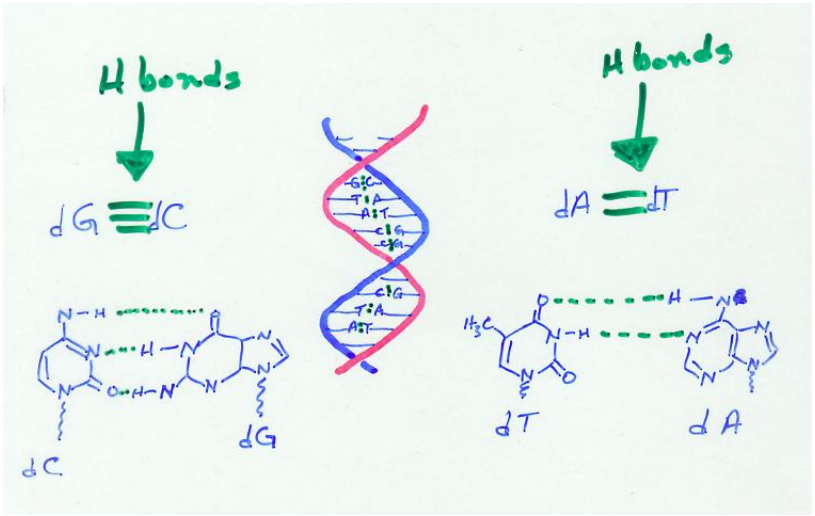
- Linked by phosphodiester bonds between [d] ribose.
- GC AT DNA
- (dGdC dAdT DNA)

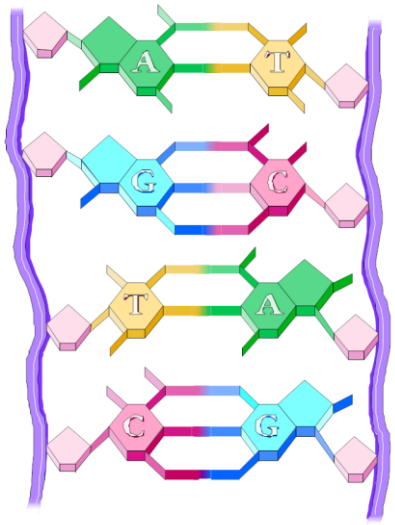


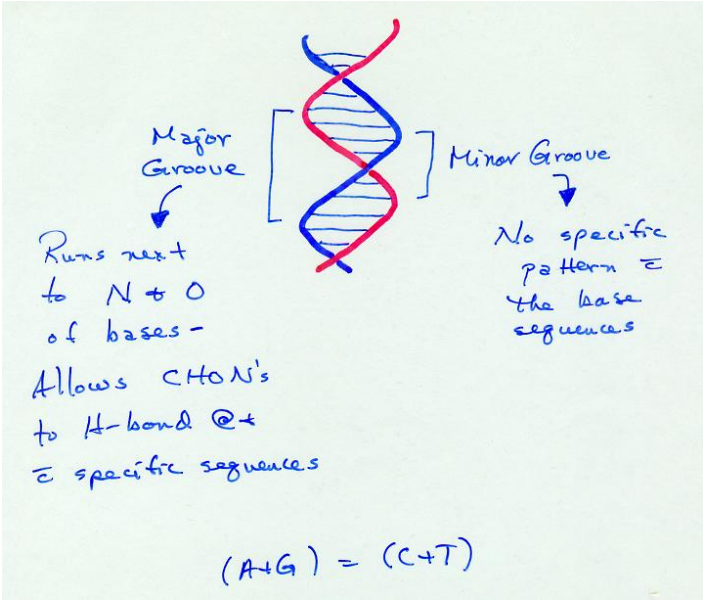
RNA – Primary Structure

- Also linked by phosphodiester bonds in the same manner as DNA
- Difference is that U is substituted for T in RNA
- GC AU RNA

Secondary Structure of DNA







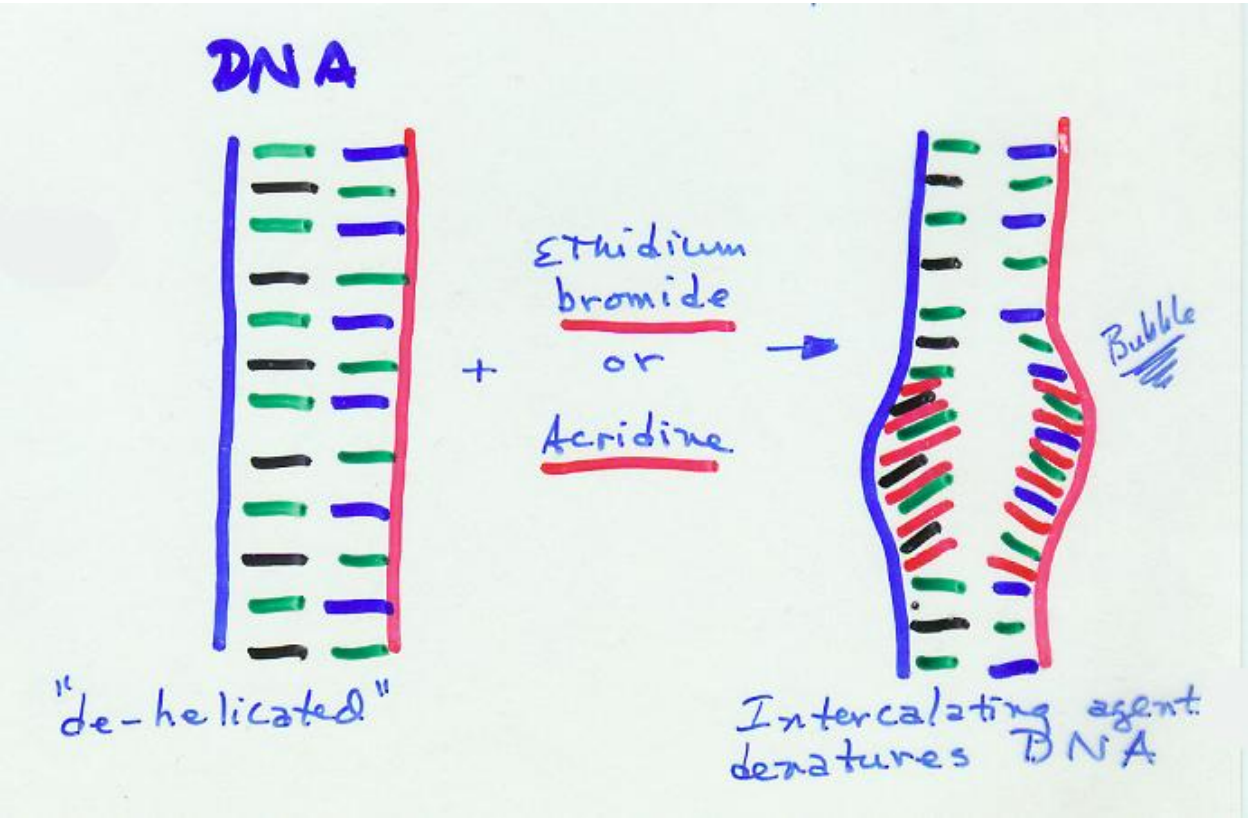
- 2 Linear strands of DNA in right handed double helix
- These strands run in ANTI-parallel directions:
- 3’----------5’
- 5’----------3’

DNA – Secondary Structure Comments

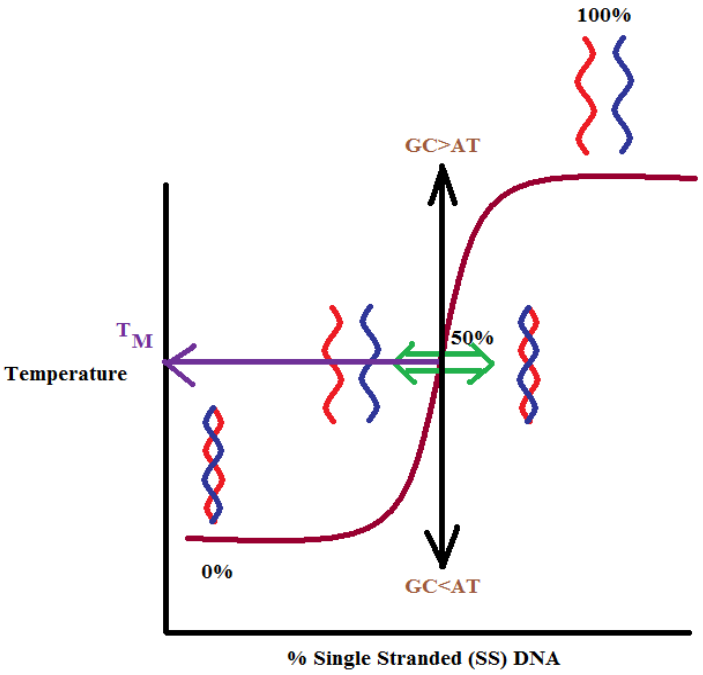
1. CHO-P backbone is on OUTSIDE of helix
2. Nitrogenous bases are on INSIDE of helix
3. OUTSIDE of helix is HYDROPHILIC
4. Inside of helix is HYDROPHOBIC

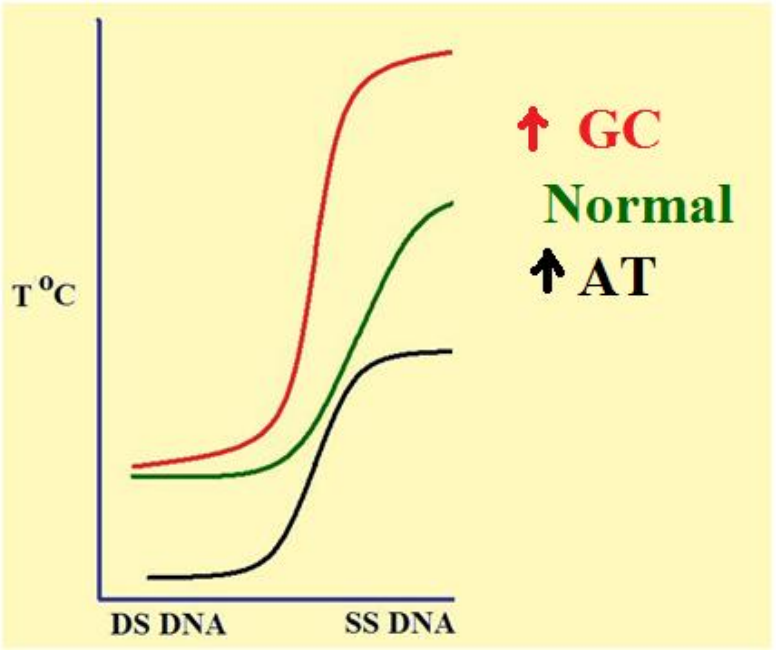
Cont'd

Bases are almost perpendicular to helical direction:

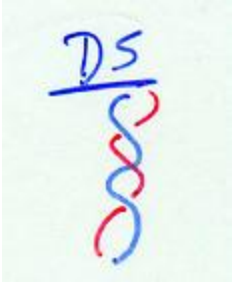
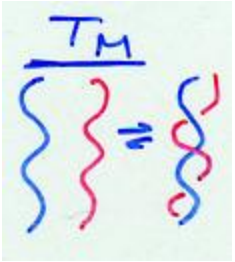
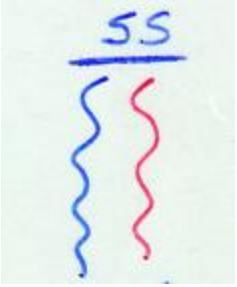


Physical Properties of DNA – Still on Secondary Structure





All dependent upon H bonds!

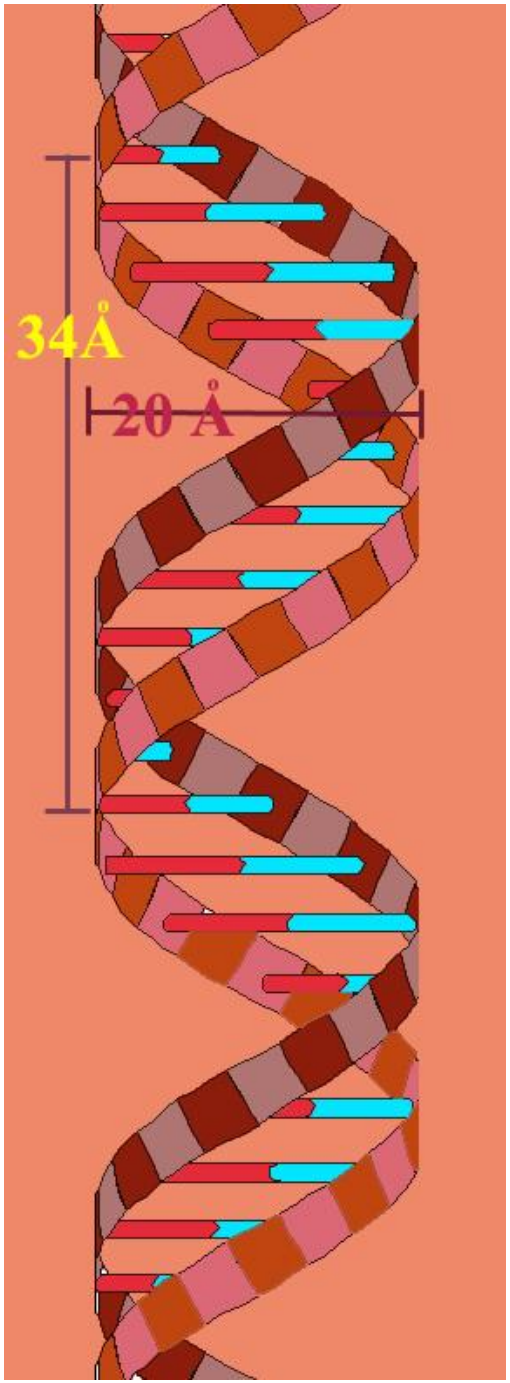
Other Physical Property Changes with Denaturation			
			
A_{260}	↓	↑	↑↑↑
Purines unstacking and opening up for greater absorption.			
Viscosity	↑	↓	↓↓↓
The less organized the DNA, the faster it flows.			
Buoyant Density	↑	↓	↓↓↓
The less organized the DNA, the less dense it is.			

- As DS DNA is denatured, three physical properties of DNA are altered (previous slide).
- These are
  - the absorbance of light at 260 nm,
  - the viscosity and
  - the buoyant density.
- DNA easily absorbs light at 260 nm. When the DNA is DS, not as much of this ultra-violet light is absorbed.
- This is because the nitrogenous bases are stacked up tightly and do not permit much of this light to be absorbed.
- As the DS DNA denatures, the ability to absorb light at 260 nm increases as the DS DNA denatures.
- This is because the purines unstack and open up to absorb much more light and is called the **hyperchromic shift**.
- The reverse process is called the **hypochromic shift**.

- The viscosity of DS DNA is very high, i.e., it doesn't flow well: it's rather sticky.
- You can liken this to when you have a cold and blow copious amounts of mucous from your nose.
- The mucous contains some cellular debris and lots of DNA.
- Consider how sticky (viscous) the mucous is and you have a good idea about how viscous DNA in the DS state is.
- As the temperature heats up, the viscosity of the DNA is greatly reduced.
- This is because the less organized the DNA, the faster it will flow (i.e., the less sticky it is).

- When DNA is in the DS state, it has a very high buoyant density, the density at which it will float.
- As the DNA becomes more disorganized, the buoyant density drops.
- This is because as the DNA becomes less organized, it becomes less dense.

- A segment of DNA that illustrates the width of the helix (2 nm) and the distance per turn of the helix (3.4 nm).
- Ångstroms, as seen, were the old way of measuring distances: 10 Å = 1 nm.
- As a general rule, one turn of the helix consists of 10 base pairs (bp).
- Remember this is DS DNA, so we refer to distance in terms of bp's.
- Each bp is roughly 0.34 nm in height.
- The form of DNA in the graphic is called the "B-form" of DNA.

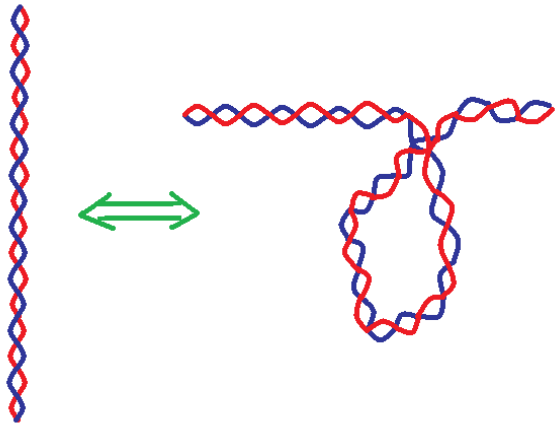


There are 3 forms of DNA known to man, Table below:

DNA Types			
	A-form	**B-form**	Z-form
Turn of helix	Right	**Right**	Left
Nucleotides/rotation	11 bp	**10 bp**	6 bp
Length of 1 rotation	18 Å	**34 Å**	45 Å
Thickness of one residue	2.55 Å	**3.4 Å**	3.7 Å
Turn (°)/nucleotide	33 (per bp)	**36 (per bp)**	-60 (per bp)
Major/minor grooves	No	**Yes**	Sort of
Phosphate backbone	Smooth	**Smooth**	"Z"-shaped; jagged
Physiological	No	**Yes**	No

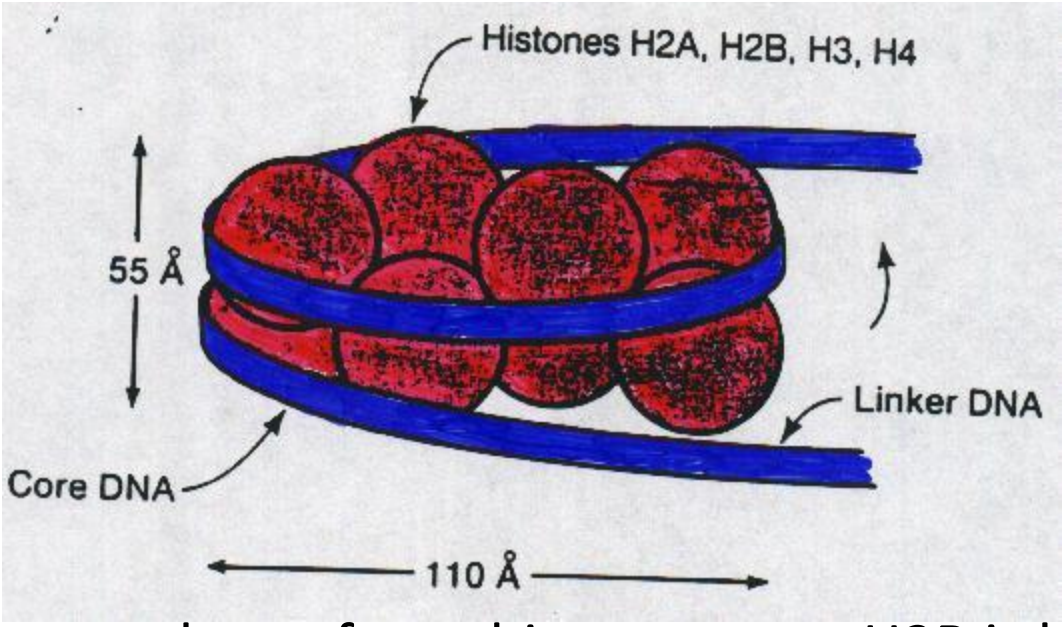
1° & 2° Structures do NOT Account for All the Physical Properties in DNA

- What else is there????
- 3° and 4° structures!



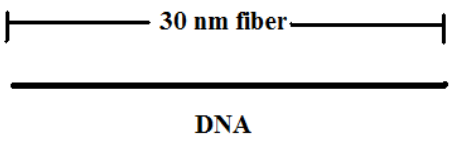
4° Structure

Depends on 5 classes of positively charged proteins called histones.		
Name	Quantity	Type
H1	1	Linker
H2A	2	Core
H2B	2	Core
H3	2	Core
H4	2	Core

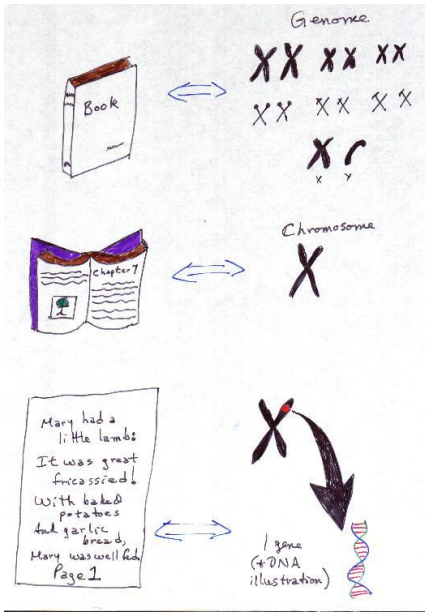


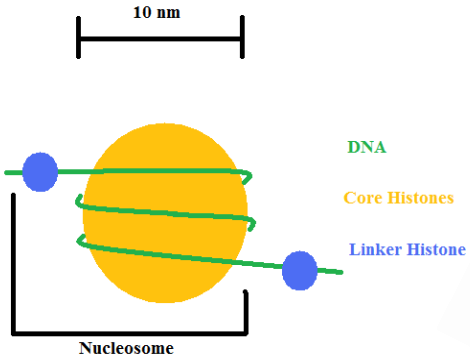
- "H" and the number refer to histones, e.g., H2B is histone 2B. H1 is a linker histone that holds DNA together from a region outside the "core".
- The core consists of the other 4 types of histones around which DNA has wrapped itself, above. There are a total of 8 histones inside this core. The reason these histones are so tightly bound to the DNA is because these histones are positively charged and the phosphate backbone of the DNA is so negatively charged.
- Depending on the literature source, the DNA is wrapped around the core histones anywhere from 1.6 to 1.9 turns.

DNA “Compression” – Rapid Summary

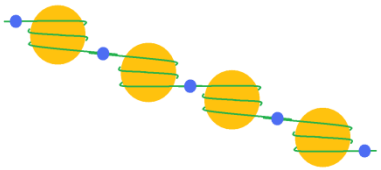


Step 1: 30 nm fiber of DNA

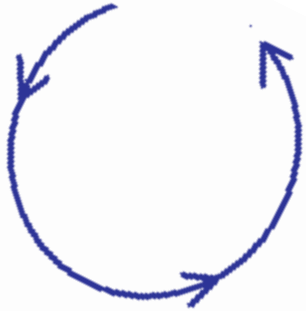




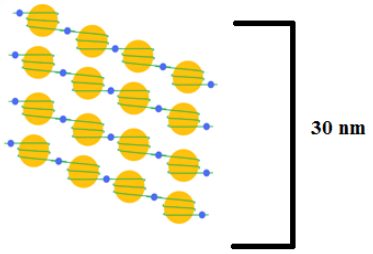
Step 2: “Bead on a String”



Step 2a: “Bead on a String”



Last step, many steps later: quaternary and super-secondary structure gives a chromosome, ca 1400 nm wide.



Step 3: “30 nm fiber” of DNA more densely packed

4 Mechanisms to Take DNA Apart

- 1) fracturing the strands with enzymes,
- 2) during replication,
- 3) during transcription and
- 4) in DNA repair

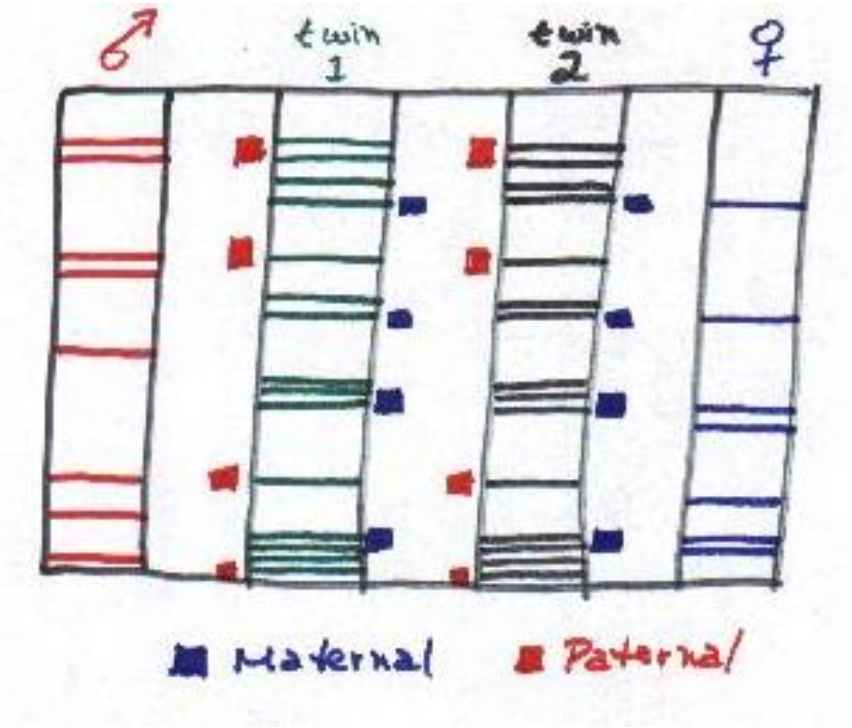
First: Fracturing the Strands of DNA

There are four classes of enzymes necessary to accomplish this task:

Exonucleases: remove a single nucleotide from the end of the nucleotide chain	Endonucleases: "cuts" strands from within leaving 3'-OH and 5' phosphate ends; very specific	Restriction enzymes: A special class of enzymes -- recognizes a target sequence in the DNA; used to detect paternity, criminal presence,	Topoisomerases: remove supercoiling as replication progresses (think overwound rubber band), e.g., DNA gyrase

Two Examples in The Use of Restriction Enzymes: Paternity and Criminal Presence

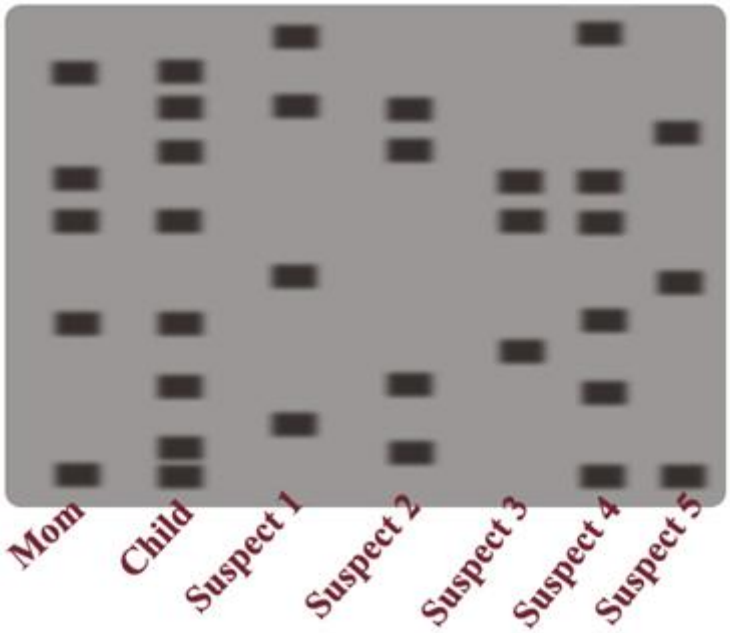
- A representation of the electrophoretic patterns of the DNA for
- 4 people:
  - a male,
  - a female and
  - 2 identical twin children.
- While this is an over-simplification, it nevertheless illustrates how paternity may be determined using restriction enzymes.
- The first "column" in red represents the lane in which the putative father's DNA was run out.
- Lanes 2 and 3 (twin 1 and 2, respectively) represent the DNA of each twin.
- The last lane shows the DNA from the mother.



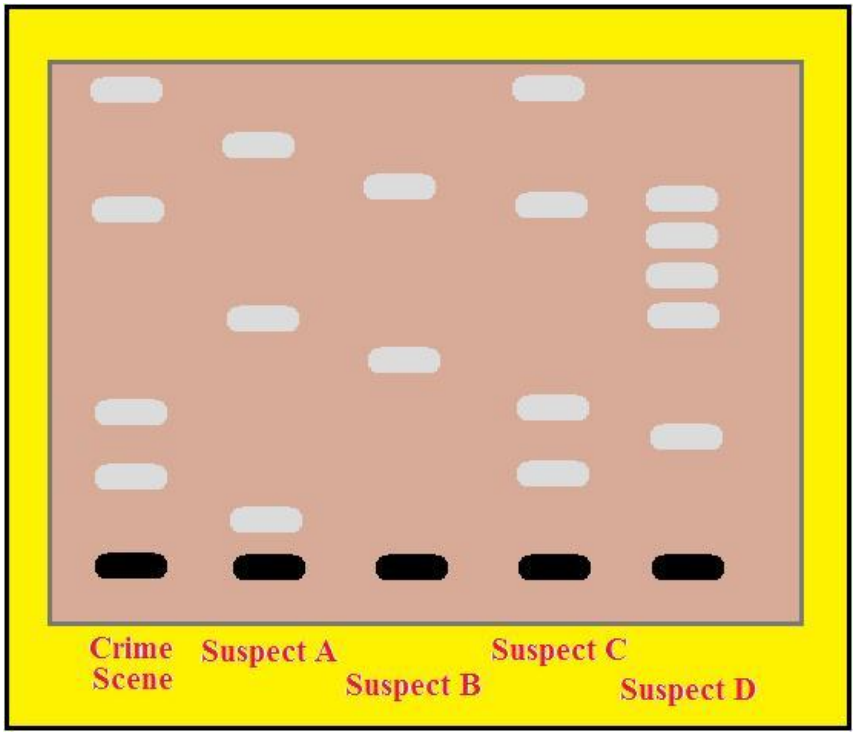
- Identical twins are not entirely identical: they have unique fingerprints and their retinal maps are unique, as well.
- Their DNA, however, is close enough that without special techniques it's difficult to tell them apart.
- Each twin inherited half of his or her genetic information from each parent.
- While each twin will have his or her own unique DNA, each will also have sequences identical to each parent.
- Note that there are red boxes and blue boxes next to the representative DNA bands.
- These boxes show which bands (lines) came from which parent, demonstrating paternity.

Paternity

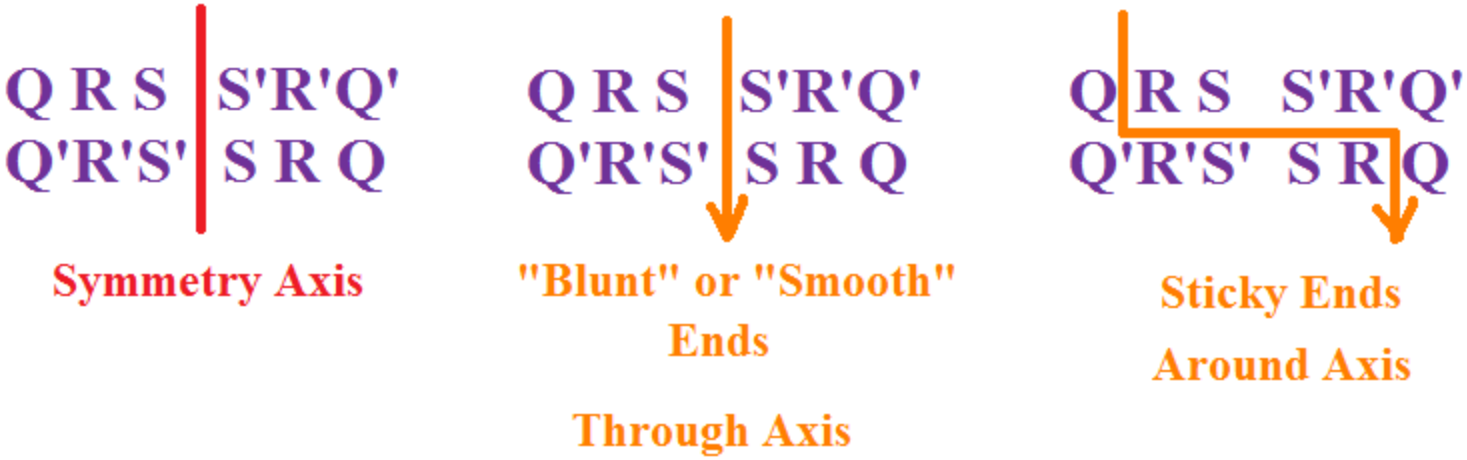
- A representation of the electrophoretic patterns of the DNA for 7 people is shown at right: a female, one child and 5 possible fathers.
- While this is an over-simplification, it nevertheless illustrates how paternity may be determined using restriction enzymes. When one matches up the bands from mom to child, it's pretty easy to see which bands came from "dad" – and when you match those remaining bands, it's easy to see that "dad" is Suspect #2.



- Restriction enzymes to include or exclude three individuals from having been present at a crime.
- The far-left lane is the crime scene DNA and the last four lanes are DNA from each of four suspects.
- Note that the patterns of the evidence and the DNA from Suspect C match.
- Suspects A, B and D may go home and Suspect C will be convicted.

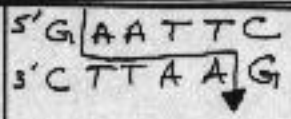
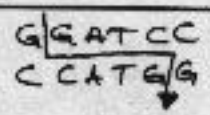
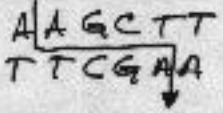
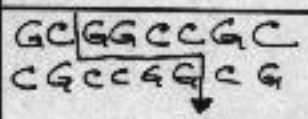


- The number of DNA fragments depend upon at least three features:
  - 1) the DNA itself (how was the sample cared for; how old is the sample; is there a mixture of DNA's),
  - 2) the restriction enzymes used for the separation (ya hafta use the same restriction enzymes for every technique in the "batch" you are running) and
  - 3) the technique used by the individuals running the gels (good technique gives good results; bad technique gives bad results; ya gotta run quality control with your samples).



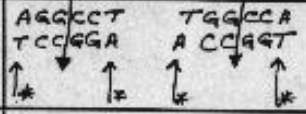
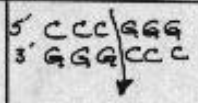
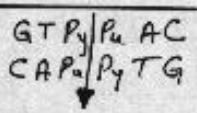
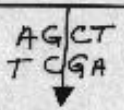
- Restriction enzymes are endonucleases that recognize target sequences in the DS DNA.
- Most sequences of recognition are palindromes (read the same way front to back and *vice versa*, e.g., **Madam, I'm Adam**).
- Above illustrates a palindromic sequence along with its axis of symmetry.
- It is about this axis of symmetry that the restriction enzyme will "clip".
- At this time, there are no known sequences less than 4 bp in length, hence, there are generally 4 or more bp's per recognition site for each specific restriction enzyme.

- Restriction enzymes may cleave 2 different ways:
- They may cleave right through the axis of symmetry leaving blunt or smooth ends or they may cleave around the axis leaving sticky or overlapping ends.
- As a general rule, the biological function of restriction enzymes is to cause the destruction of foreign DNA, e.g., viral DNA.
- Since it's possible that the cells' own DNA may contain sequences identical to binding sites for restriction enzymes, these sites are methylated (capped) to render them resistant to the restriction enzymes.

Source of Enzyme	Name of Enzyme	Recognition/Binding Site
E. coli	EcoR1	
B. amyloliquefaciens H	BamH1	
H. influenzae R_d	HindIII	
	NotI	

4 examples of restrictions enzymes and their bacterial sources.

Note that all of the examples in this figure are for leaving sticky or over-lapping ends and that each site is very specific for cleavage.

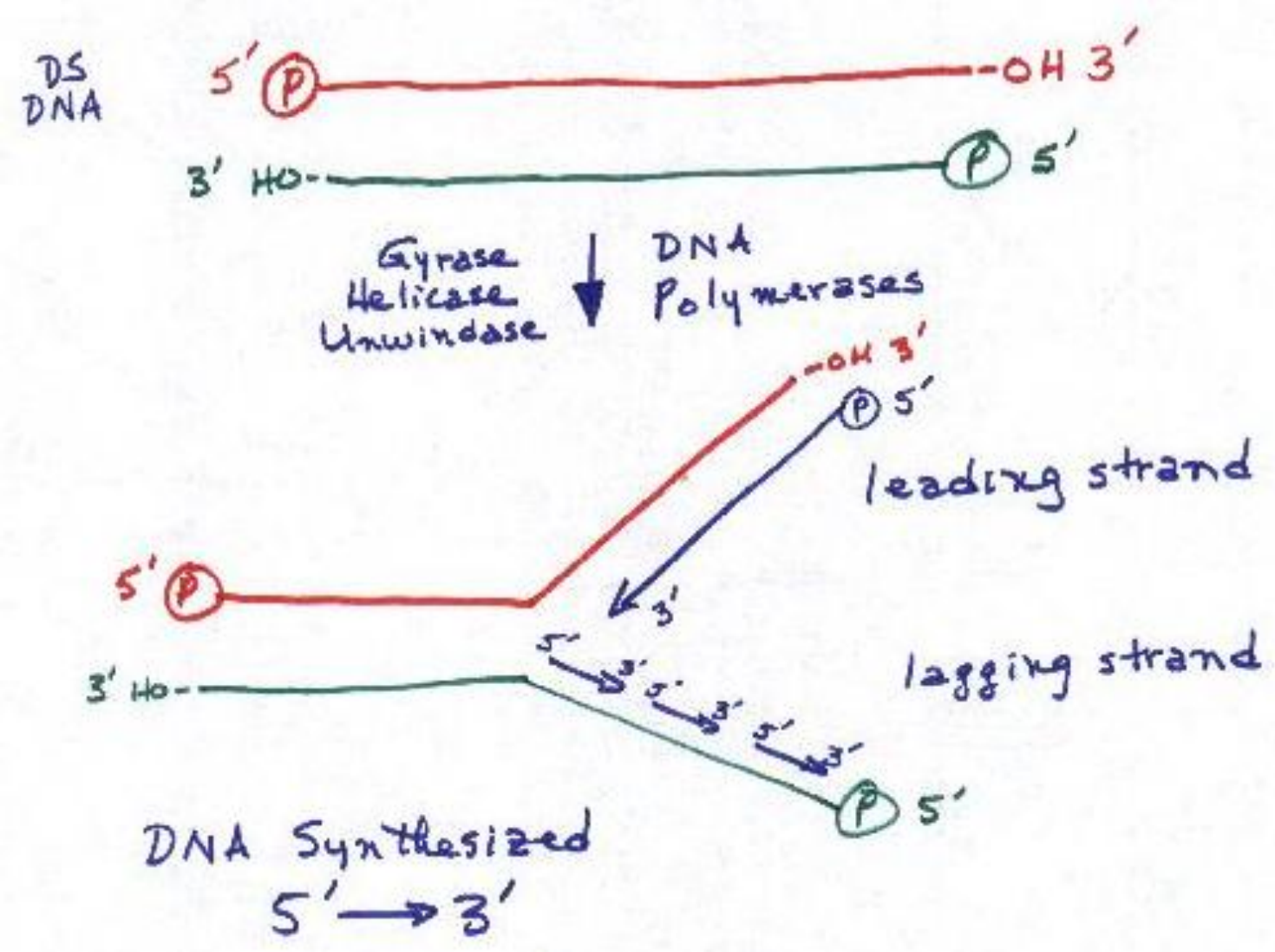
Source of Enzyme	Name of Enzyme	Recognition/Binding Site
H. aegyptius	HaeI*	
S. marcescens	SmaI	
	HindII	
	AluI	

* A and T orientation appears to not make much difference to this enzyme, AS LONG AS the organization of the two bases is symmetrical.

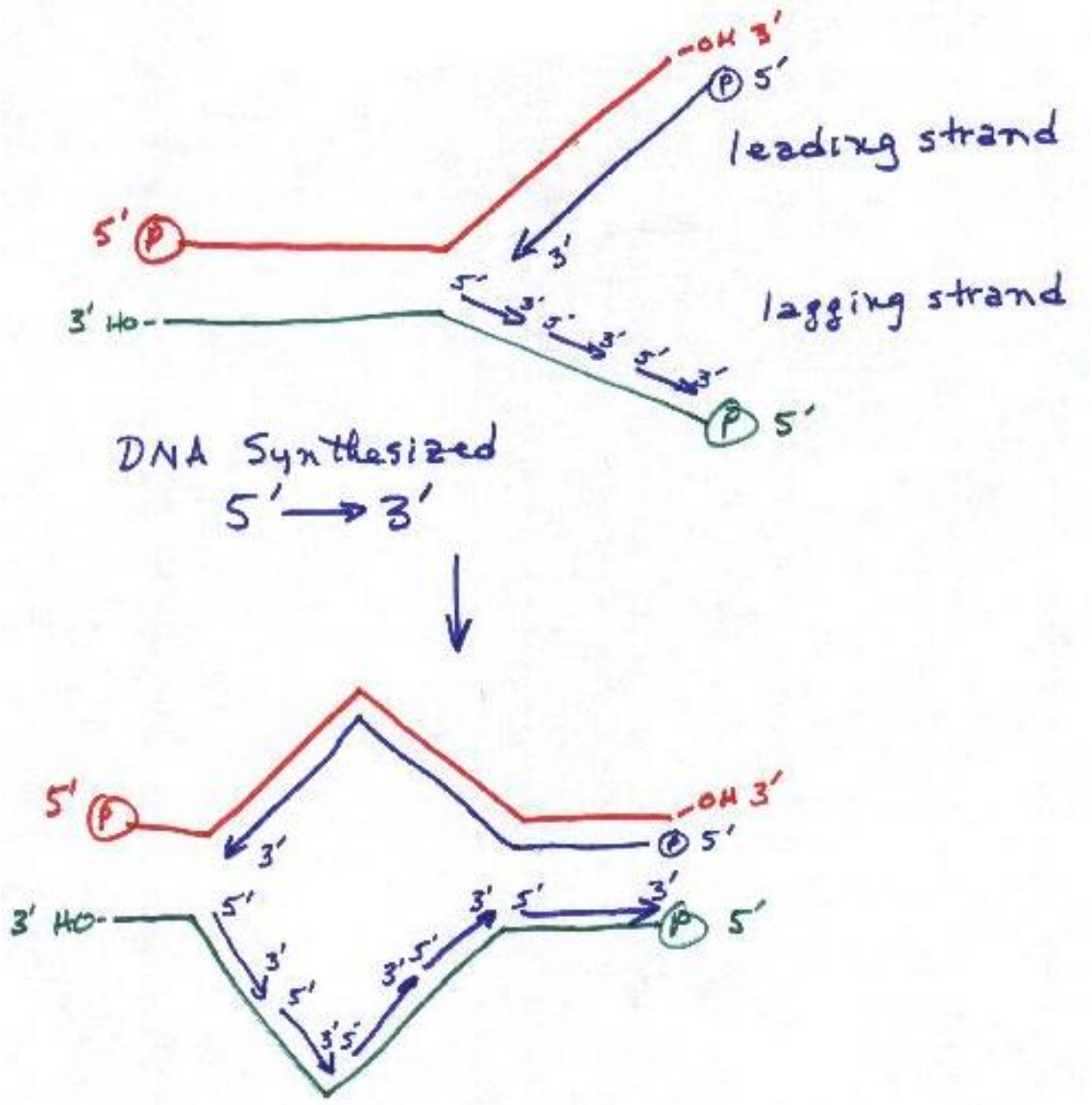
- 4 examples of restriction enzymes and their bacterial sources.
- Note that all of the examples in this figure are for leaving blunt, smooth or flush ends and each site is not as specific for cleavage as were those in the previous slide.
- The recognition site for HaeI relies upon the location of dA and dT as long as they are symmetrically arranged about the axis of symmetry -- note the "*'s" in the figure.
- Note, also, HindII's site: the axis of symmetry is through a pyrimidine-purine center that is complimentarily repeated.

Second: During Replication

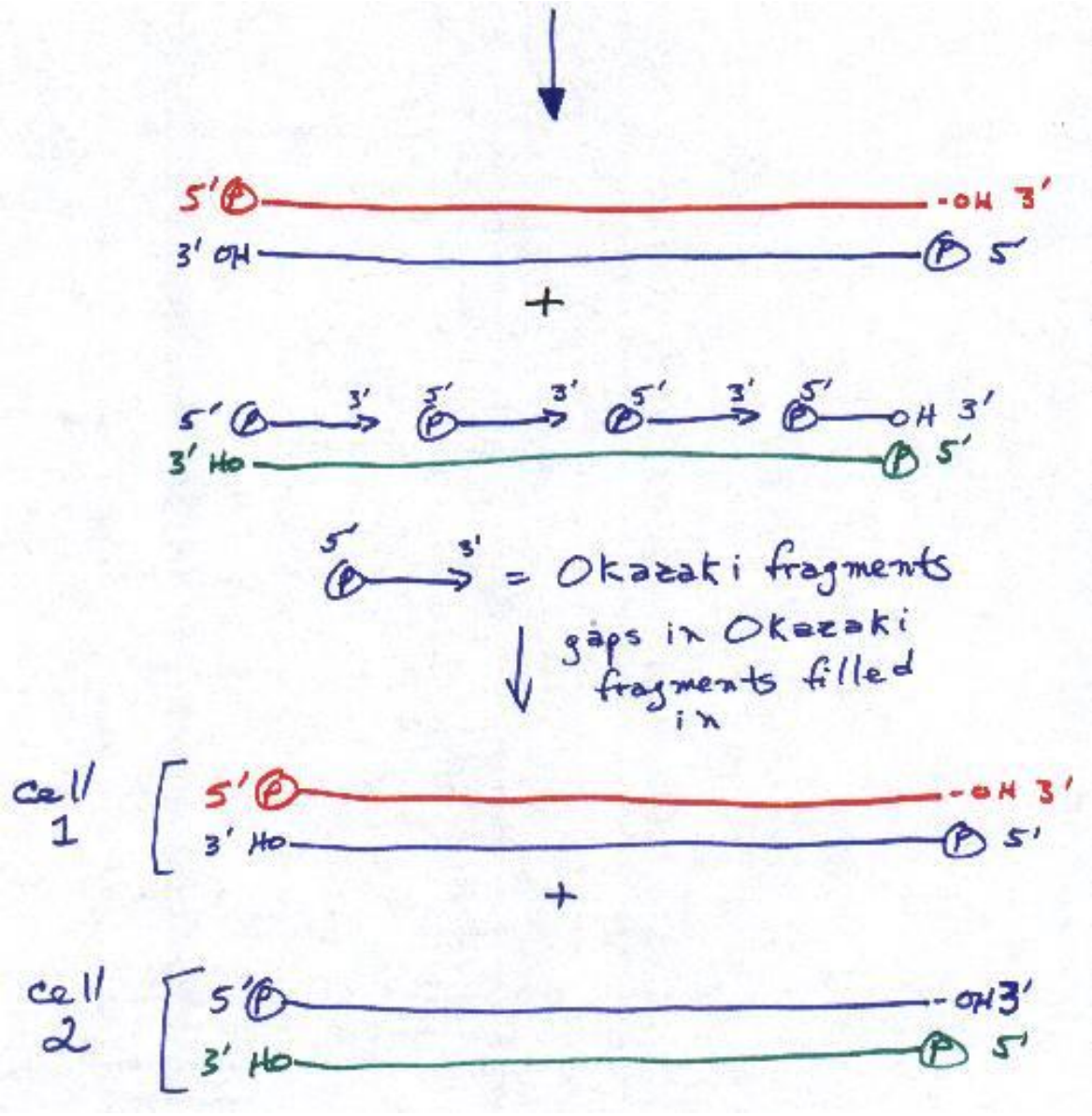
- The process of replication occurs during cell division and consists of the DNA making a copy if itself.
- The first thing DS, anti-parallel DNA must do is open a portion of its structure so that the necessary enzymes my "read" the DNA a strand at a time and in the opposite directions.
- Note that the 5' end has a phosphate and the 3' end has the -OH.
- A series of enzymes, gyrase, helicase, unwindase and DNA polymerases, must unwind the DNA, then read the DNA, then transport in the complimentary nitrogenous bases to accommodate
- DNA replication process. As the DS DNA opens up, it forms a replication fork.
- This replication fork allows both strands of the DNA to be replicated simultaneously, albeit in two different manners.


DS DNA
5′
P
-OH 3′
3′ HO-
P
5′
Gyrase
Helicase
Unwindase
DNA
Polymerases
-OH 3′
P
5′
leading strand
5′
P
3′
5′
3′
3′ HO-
lagging strand
P
5′
DNA Synthesized
5′ → 3′

- Note that in the previous slide that there is a strand of DNA called the leading strand.
- This strand is so-called as it is continuously replicated along its complimentary strand and is always ahead of the other strand being replicated.
- The latter strand is called the lagging strand because its synthesis is always lagging behind the lead strands.
- It is also incompletely replicated.
- The reason for this is that the synthesis of the lagging strand depends on the opening of the replication fork and the space that the opening permits to accommodate the enzymes, hence, its synthesis starts with a spit and a sputter, leaving small fragments as replication continues.
- These fragments are called Okazaki fragments after their discoverer.
- Note, also, that regardless of the direction, DNA is synthesized from the 5' to the 3' direction, i.e., in a complimentary fashion.
- As replication progresses, the replication fork forms a replication bubble in the DNA until, at the very end of the strand of DNA, it forms the last replication fork.


-OH 3'
5'
leading strand
5'
3'
3' HO-
lagging strand
5'
3'
5'
DNA Synthesized
5' → 3'
5'
-OH 3'
3'
5'
3' HO-
5'
3'
5'
3'
5'
3'
5'
3'
5'
5'
5'

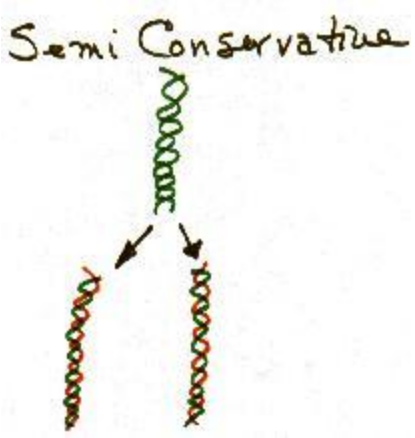
- Before the cell may continue on towards division and before G_2 phase is complete, the Okazaki fragments must be annealed.
- This requires energy (ATP).
- Once the gaps are filled in, the cell may leave G_2 and move into mitosis to make two identical cells to the parent cell.
- Also note that 50% of the old DNA goes to the daughter cells and 50% of the new goes to the daughter cells, i.e., an old strand and a new strand go to the new cells.

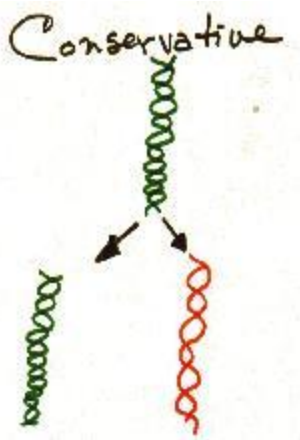

5' P
-OH 3'
3' OH
P 5'
+
5' P 3' 5' P 3' 5' P 3' 5' P OH 3'
3' HO P 5'
5' P 3' = Okazaki fragments
gaps in Okazaki fragments filled in
cell 1
5' P
-OH 3'
3' HO
P 5'
+
cell 2
5' P
-OH 3'
3' HO
P 5'

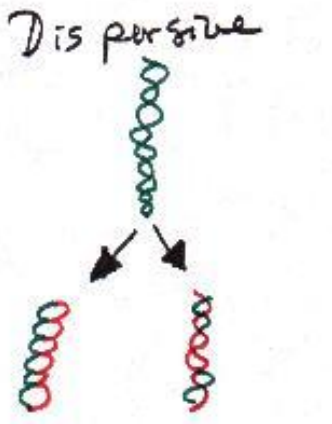
Semi-Conservative Replication

- This whole process is called semi-conservative replication.
- How, though, was it determined that DNA replicates in a semi-conservative manner?
- This came about from the elegant Meselson-Stahl experiments.
- They felt that there were three options available to explain how DNA was replicated.

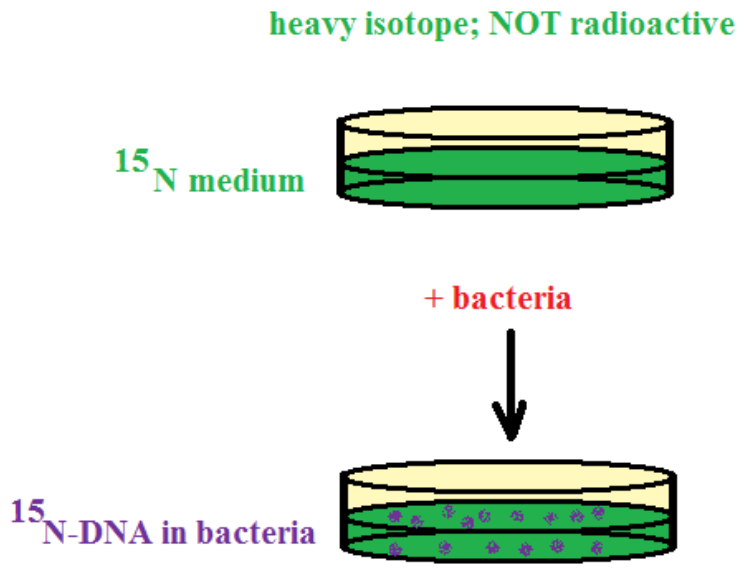
Semi-conservatively	Conservatively	Dispersively
Each strand of the original was used as a template for the synthesis of a complimentary strand, with which it is combined in the daughter cell.	Each strand of the DNA is used as a template strand for the synthesis of new strands that form identical DNA, but via old-old and new-new strand combinations.	The DNA is replicated willy-nilly and aligns however it wishes.





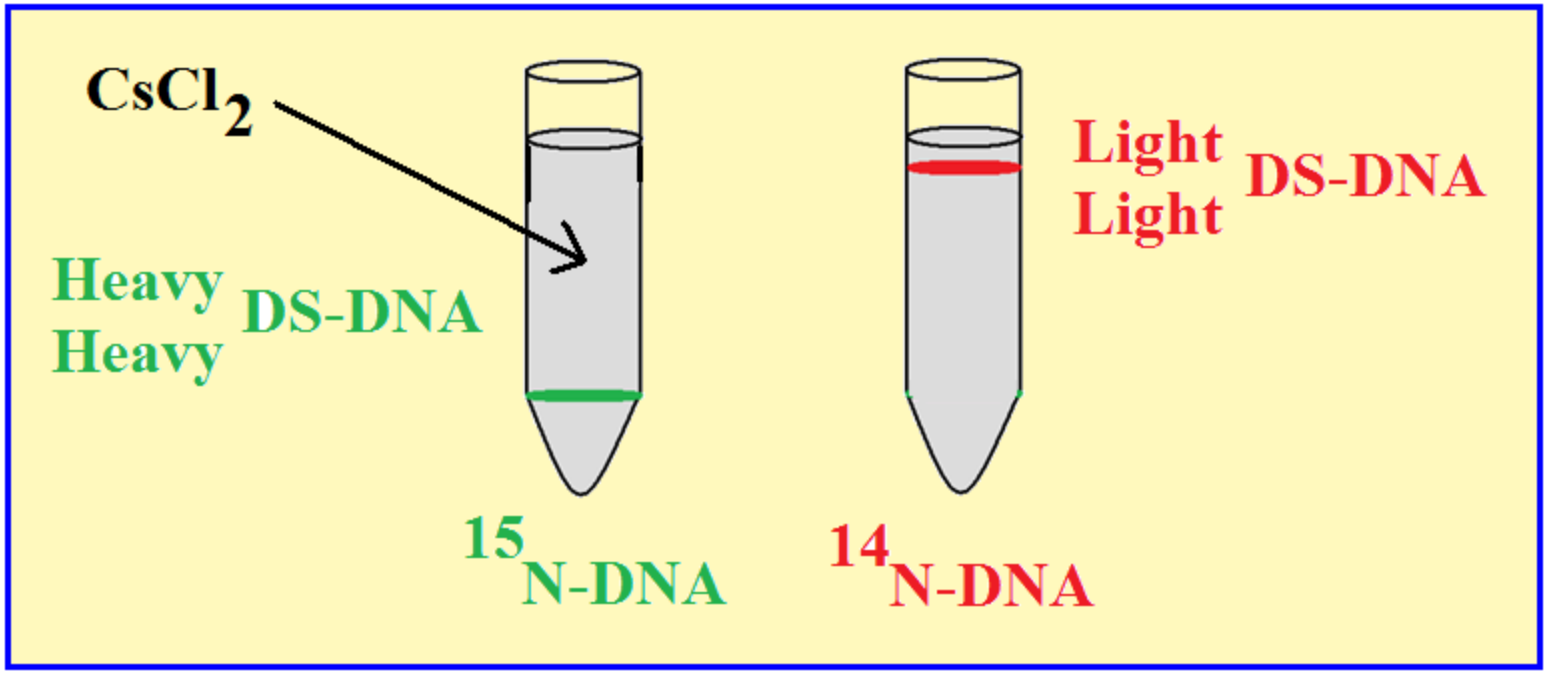


- The question was "Which one is correct?"
- Meselson and Stahl came up with a slick way to determine the answer to this.
- They made a medium for bacterial growth that contained ^{15}N (a heavy isotope of nitrogen, NOT radioactive) and grew their bacteria on it.
- The idea was that the bacteria would incorporate the ^{15}N into their DNA, making their DNA heavier than other DNA.
- Then the bacteria were removed from this heavy medium and re-grown on medium that contained ^{14}N (the "regular" (lighter) isotope of nitrogen) for one or more generations.

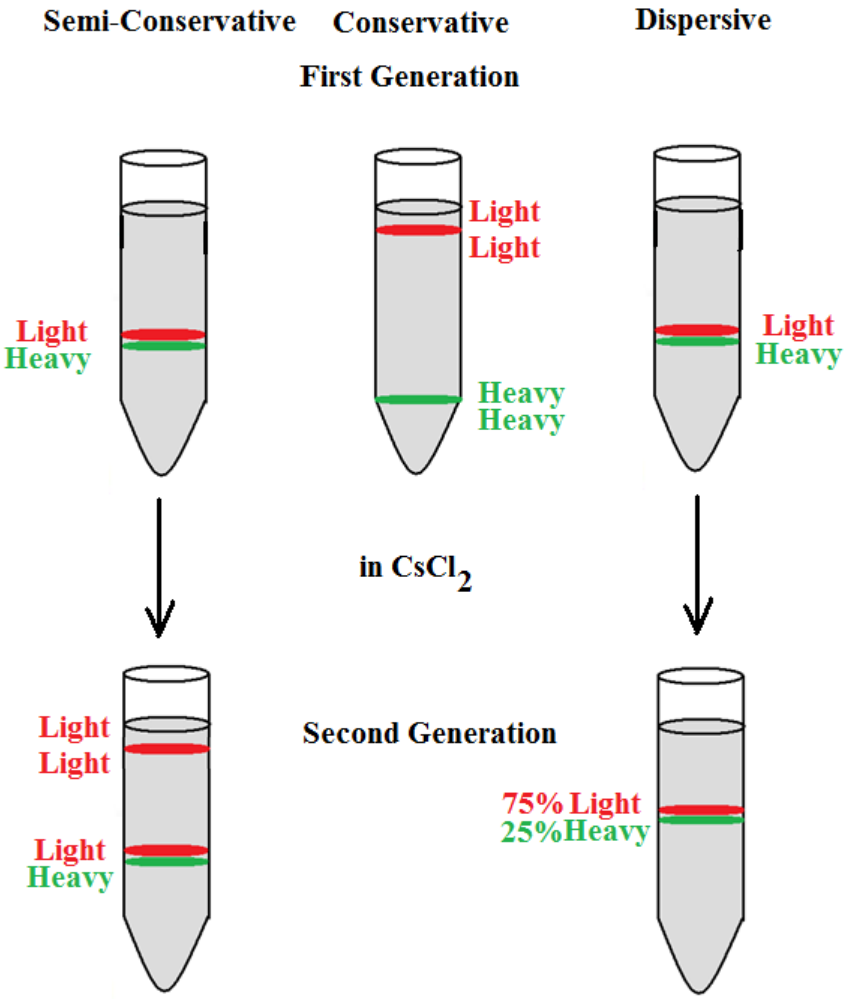


- They then harvested the bacterial DNA and studied it in $CsCl_2$ via ultra-centrifugation.
- In a nutshell, ultra-centrifugation is a process whereby tubes are spun at pre-determined speeds that permit forces on the samples in the tubes to go far beyond gravitational pull.
- By using $CsCl_2$, Meselson and Stahl were pretty clever. Cesium chloride solutions have a density about the same as DNA.
- When the cesium chloride solutions are spun, though, in a centrifuge, a density gradient (think about concentration gradients you learned about in A&P I) is set up in this salt solution.
- The DNA then floats, as it were, to the region of density that most closely approximates its own density in the cesium chloride as it's spun down.
- Typically, this is near the -- more or less -- middle of the tube.

- For the purposes of illustration, though, we'll use artistic license as shown below to visualize the differences between the heavy and light DNA in the cesium chloride solutions.

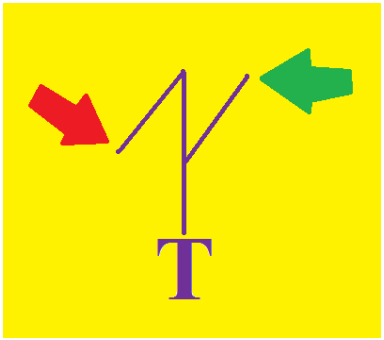


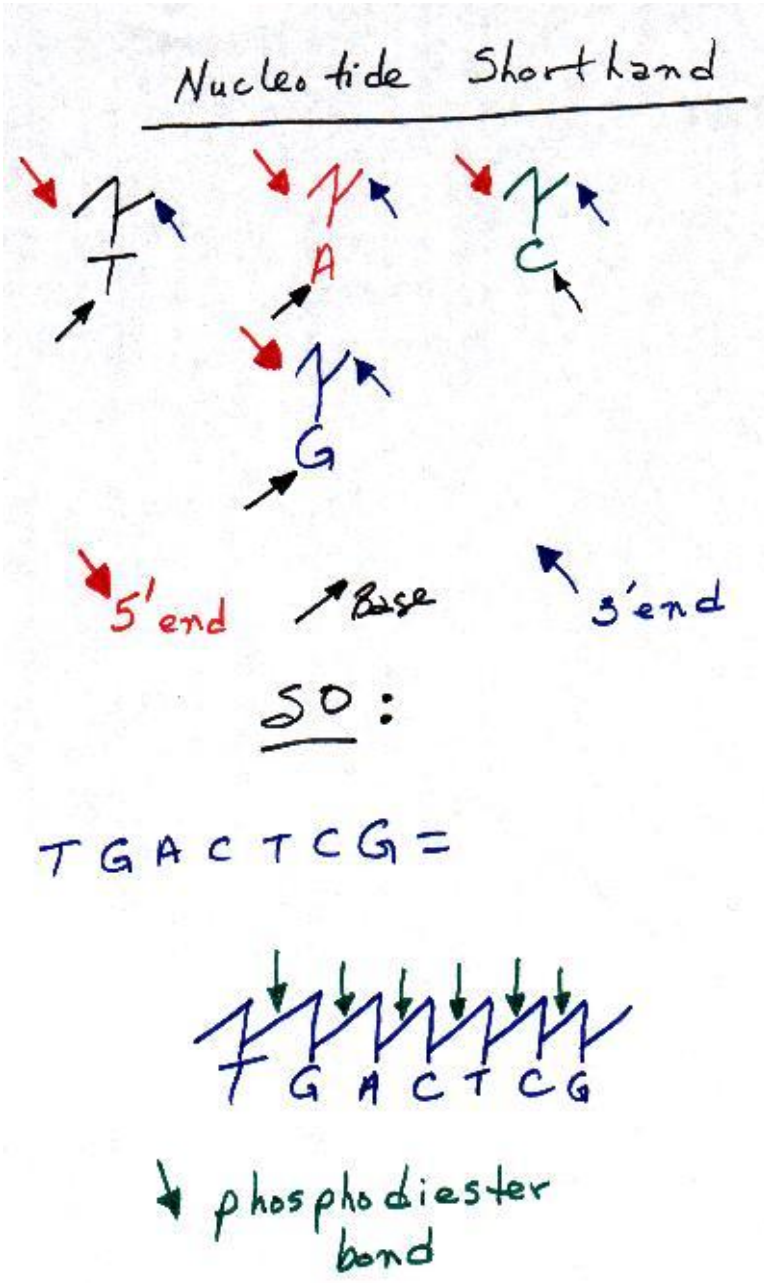
- Meselson and Stahl predicted DNA patterns for each of the three models of DNA replication, graphic right, in cesium chloride.
- For semi-conservative replication, they predicted that the first generation's band in the density gradient would consist of a strand of heavy and a strand of light DNA.
- For conservative replication, they predicted that the first generation would have two bands: one with two strands of light DNA and one with two strands of heavy DNA.
- Since this didn't happen, they moved on from there to the dispersive model.
- They predicted that the dispersive replication would give one band in cesium chloride that was part heavy DNA and part light DNA.
- Meselson and Stahl predicted that the second generation's DNA following semi-conservative replication would produce two bands: one that consisted of 2 strands of light DNA and the other that consisted of one light and one heavy strand of DNA.
- They, likewise, predicted that if the DNA was replicated in a dispersive manner that the second generation's DNA would be in one band: 25% heavy DNA and 75% light DNA.
- DNA continues to be studied on cesium chloride gradients, as well as on sucrose.



Basic Science and Clinical Techniques for Isolating, Purifying and Identifying Nucleic Acids

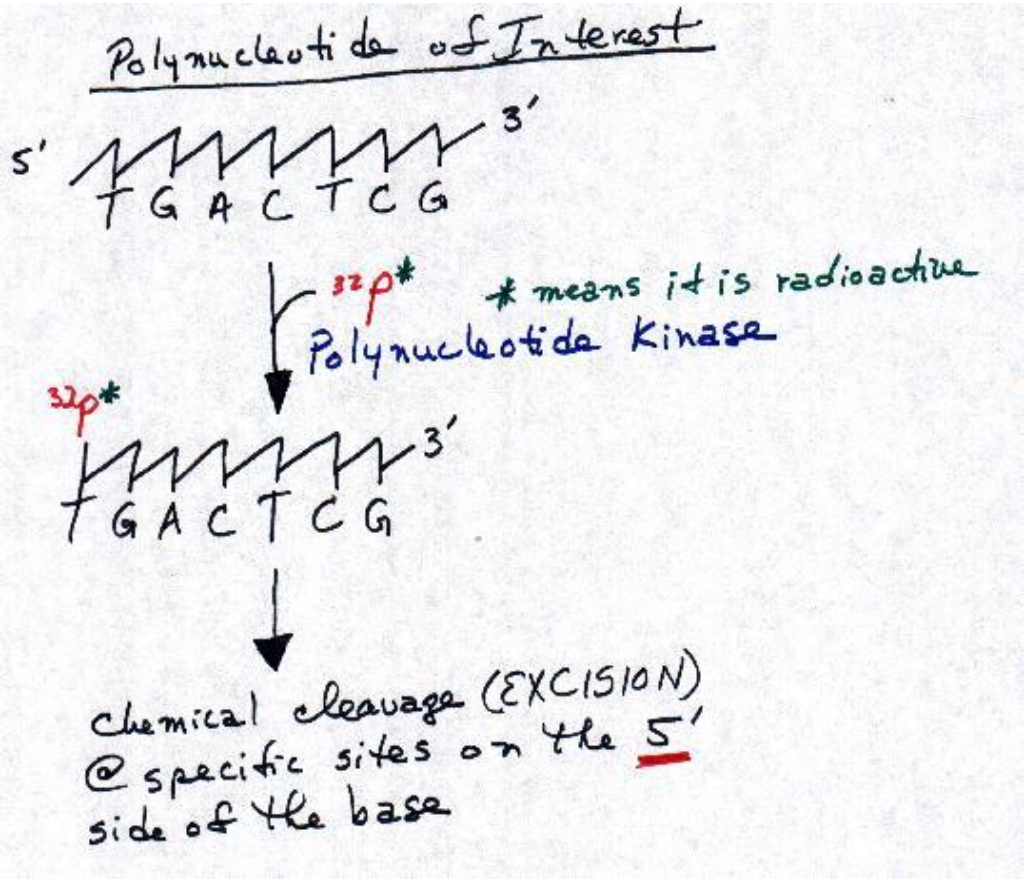
- Several techniques have been developed to determine the sequence of DNA: the Maxam-Gilbert and Sanger methods. In order to follow how the techniques work, it is necessary to understand a little nucleotide shorthand:



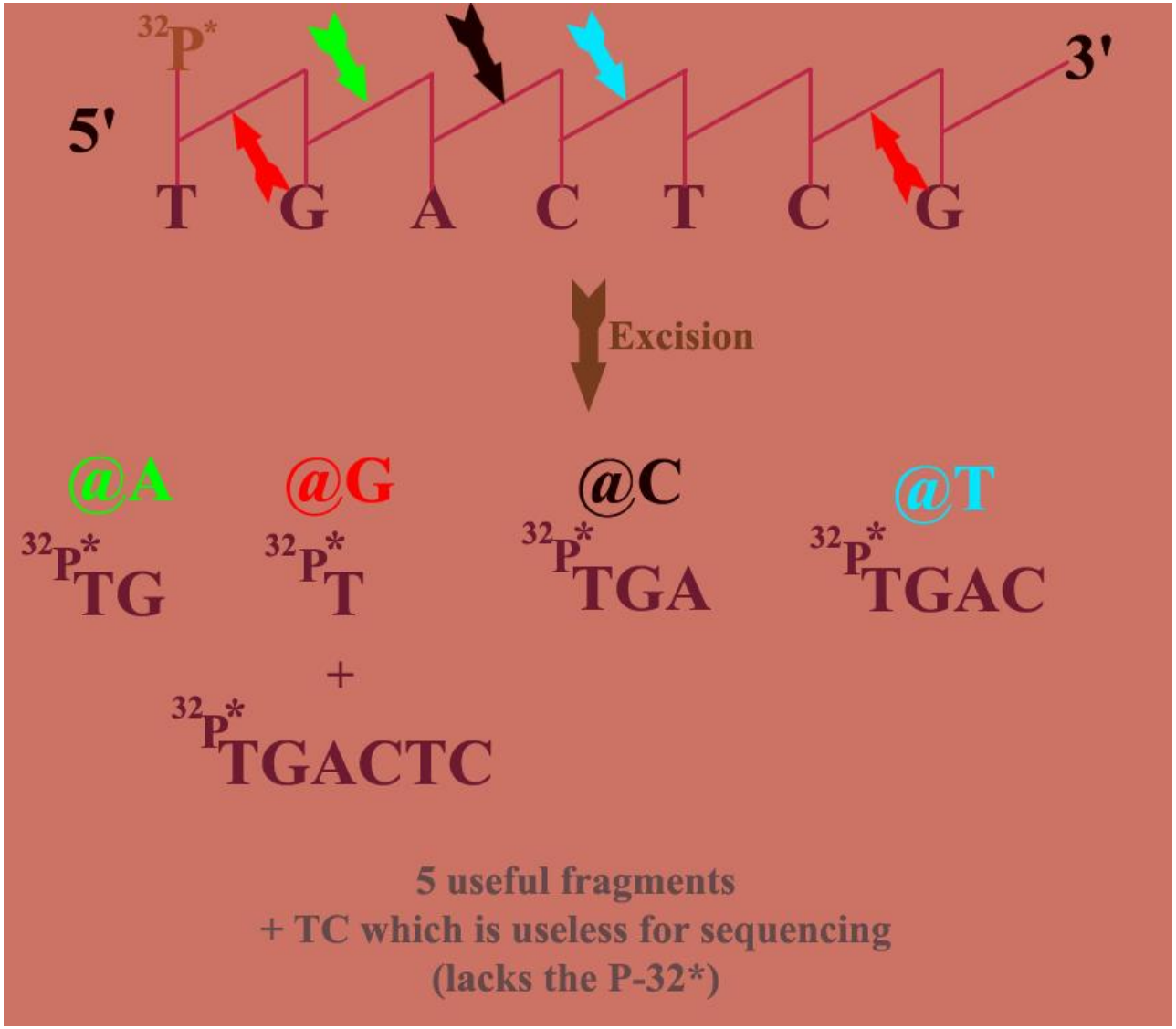


The Maxam-Gilbert Method of DNA Sequencing

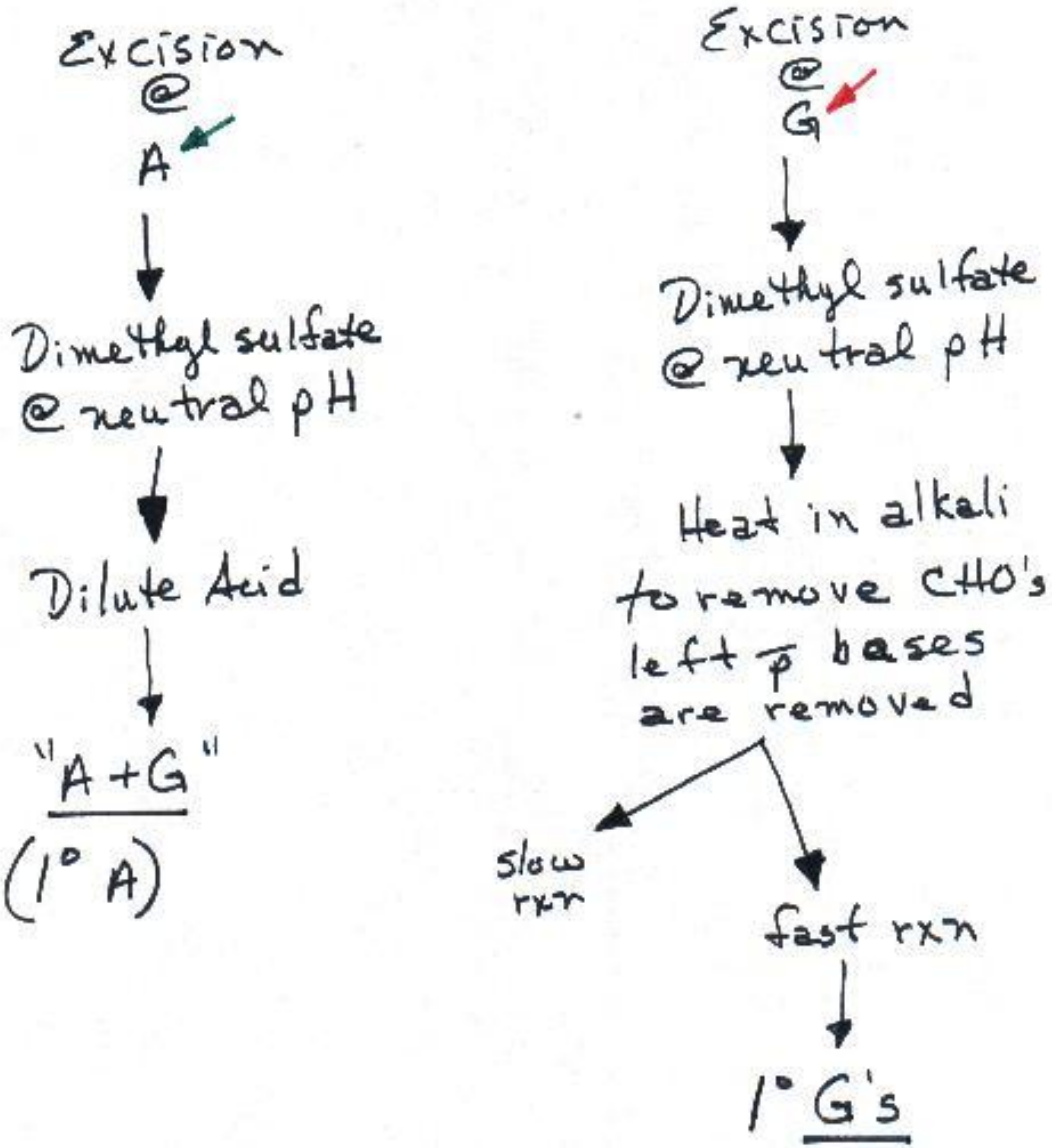
- To the polynucleotide, with polynucleotide kinase, radioactive ^{32}P (phosphorus with an atomic mass of 32; often labeled with an asterisk [*] to denote its radioactivity) is added.
- This allows for detection of nucleotide fragments (this will become clearer shortly.)



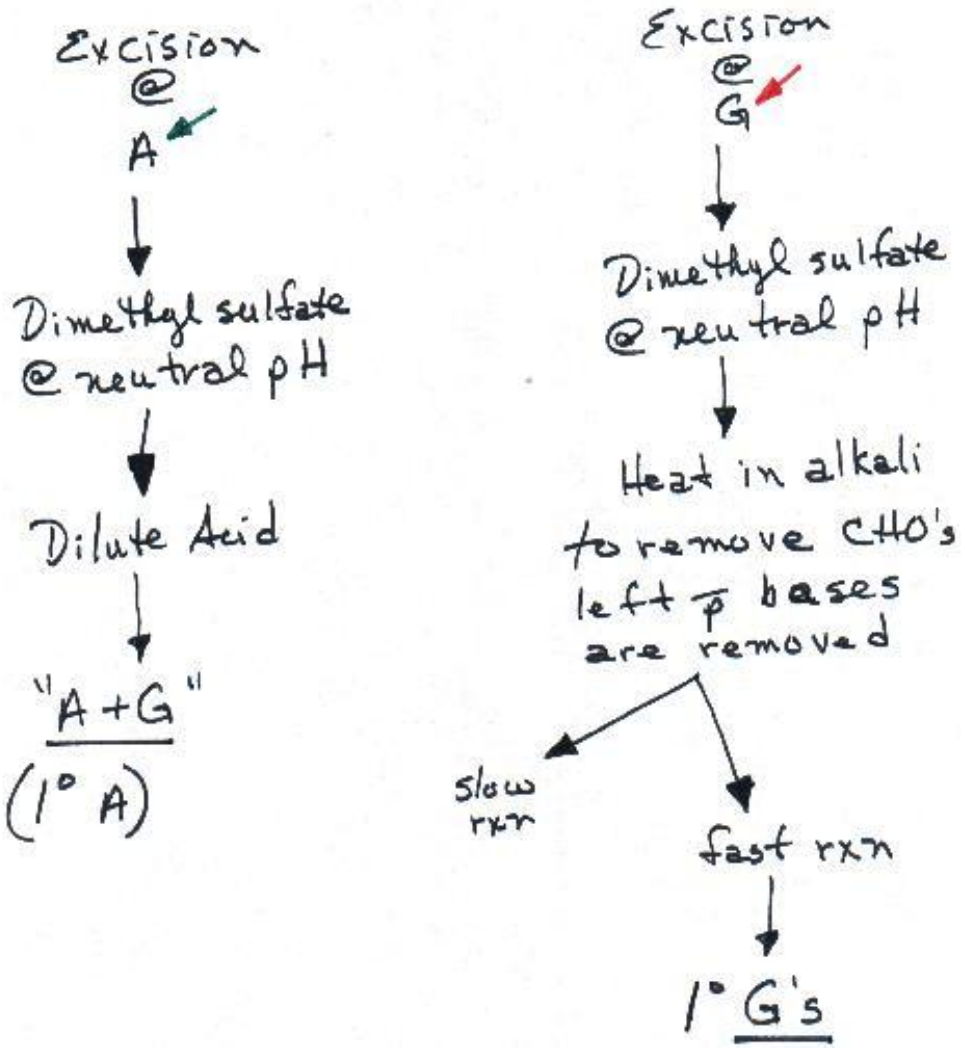
- Following ^{32}P addition, the polynucleotide sequence is subjected to chemical cleavage (excision) at specific sites on the 5' side of the base:



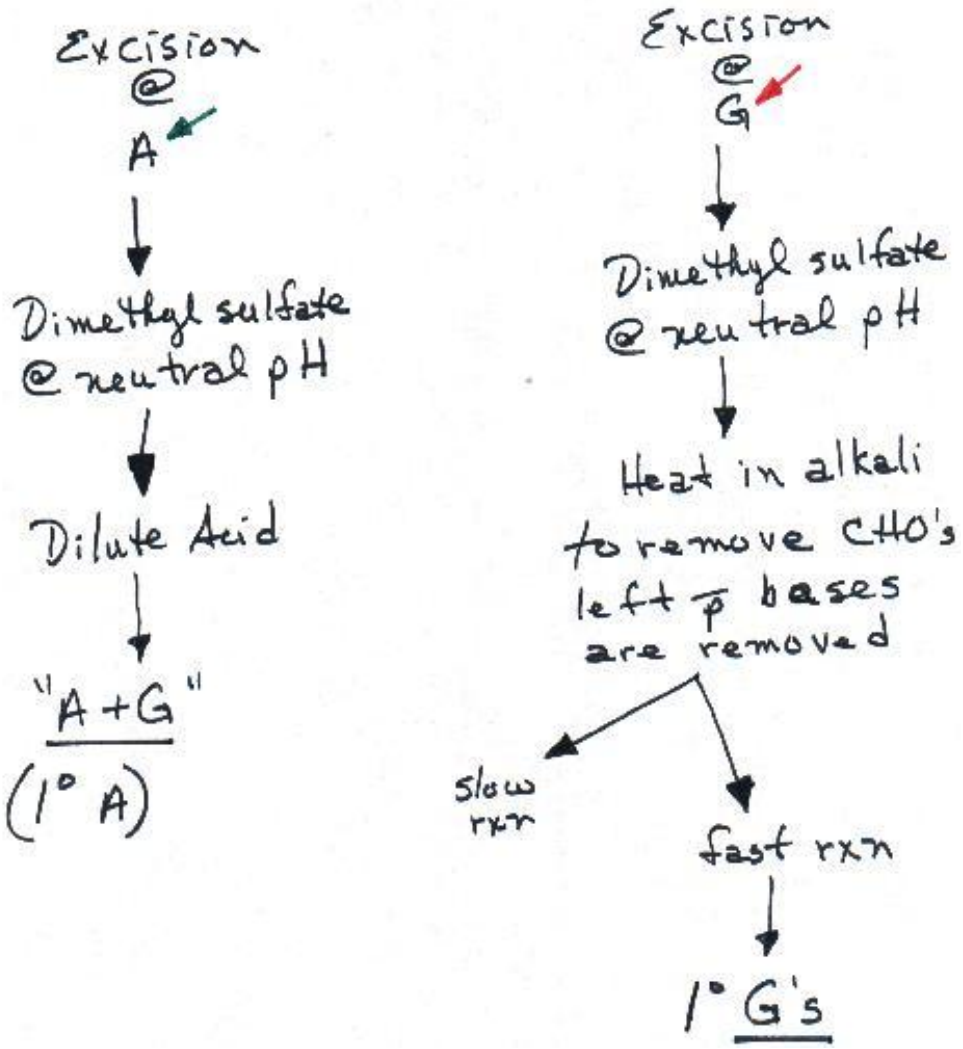
- The actual chemistry behind the cleavage at the 5' end of A- and G-sited segments from these sequences is illustrated, right:



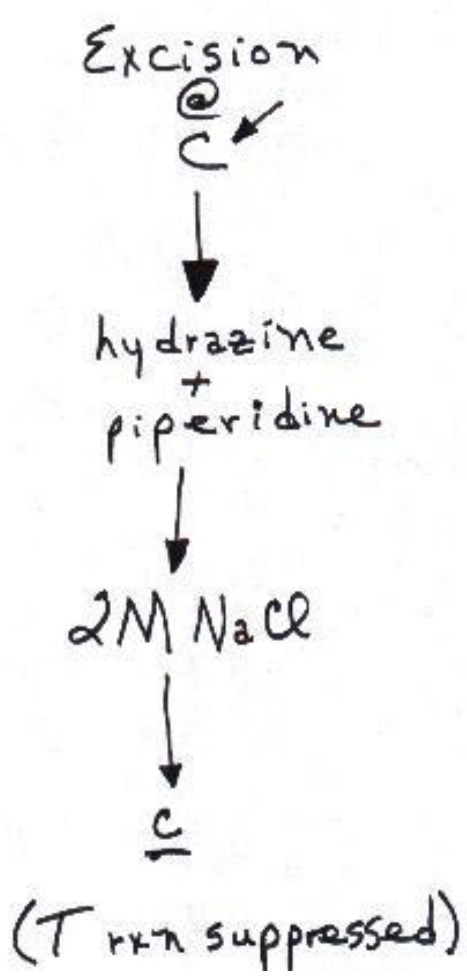
- Briefly, in order to excise a fragment at the 5' end of A, the sequence is reacted with dimethyl sulfate at a neutral pH, then with dilute acid.
- The products of this chemical surgery are a mixture of A and G.
- These products -- for reasons explained shortly -- are labeled "A+G" although the product contains primarily A.

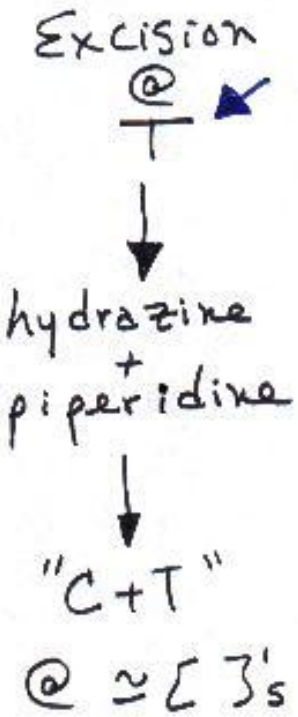


- To excise a fragment at the 5' end of G, the sequence is treated with dimethyl sulfate as with the A, then heated in alkali to remove carbohydrates left after the bases are removed.
- Two reactions results:
  - a slow one with which we have no interest and
  - a fast one that yields primarily G's.
- This latter product is labeled "G".

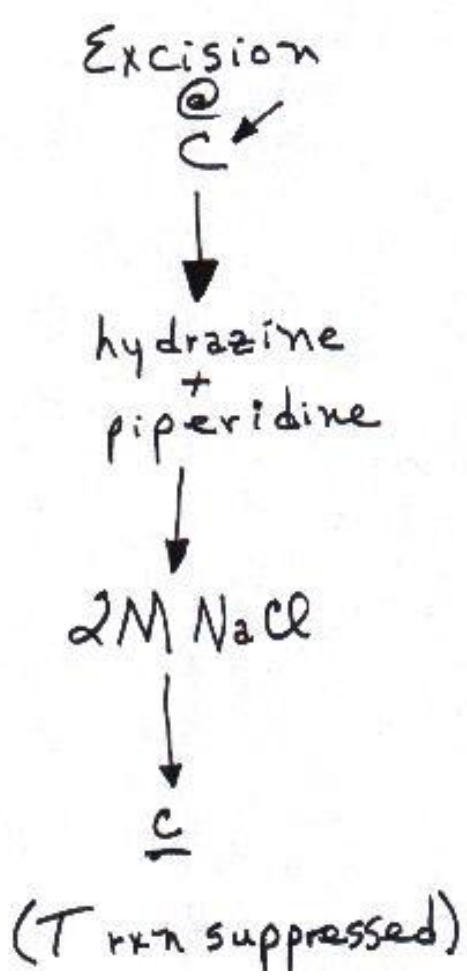


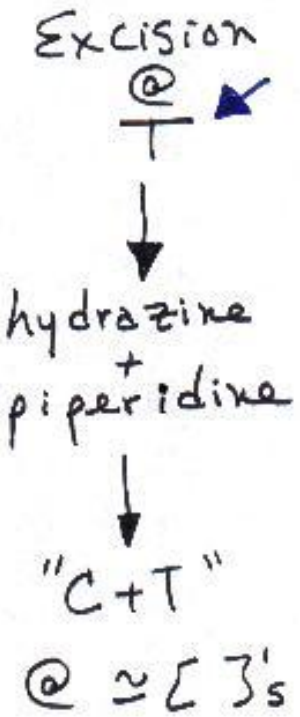
- To excise a fragment at the 5' end of C the sequence is treated with hydrazine and piperidine, followed by 2M NaCl. The product of this reaction is C. The salt solution suppresses the reaction of the reagents with T. The former product is labeled "C".





- To excise a fragment at the 5' end of T, the sequence is treated with hydrazine and piperidine.
- The product is a mixture of C and T, hence it's labeled "C+T" -- both are present at approximately equal concentrations.

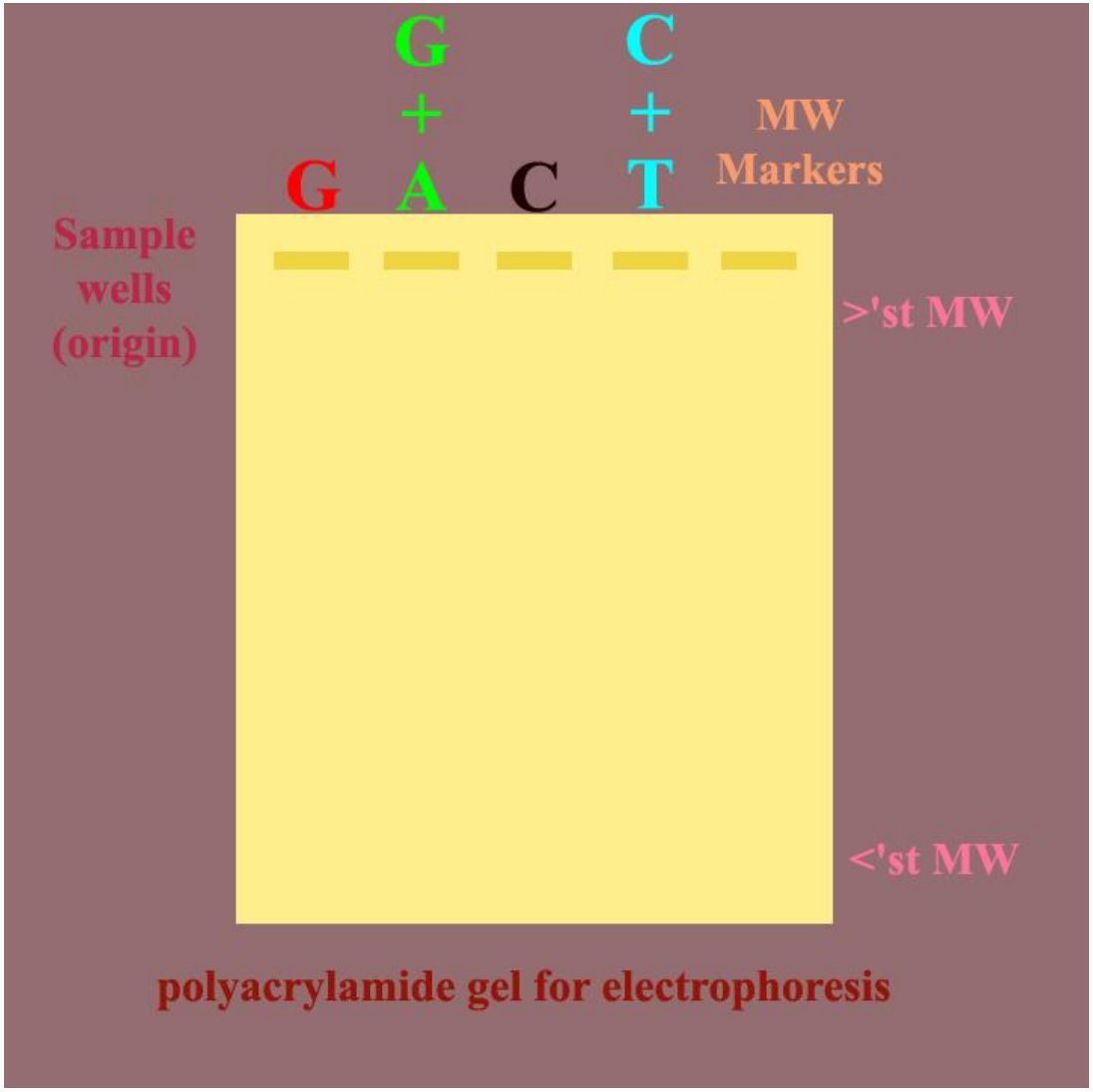




- We now have four products that are radioactively labeled.
- How are they analyzed?
- They are first separated on a polyacrylamide gel during electrophoresis.
- Remember that electrophoresis is the separation of molecules in a solid/formed matrix/support by molecular size driven by an electrical charge/through an electrical field

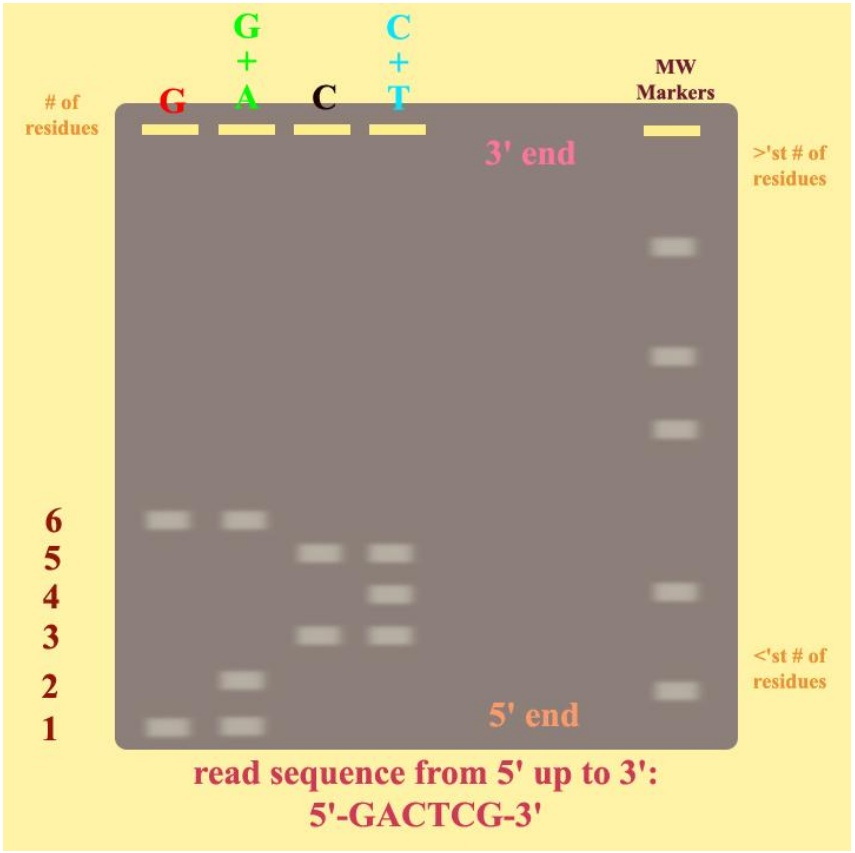
PAGE

- The samples labeled "G" are placed in a well (small depression specially designed to accept the sample so it may get "driven" into the gel) labeled G; G+A, C and C+T are likewise placed in their identified wells.
- Note that on the right of the gel that there is a well labeled "MW markers".
- This well contains sample with known molecular weights.
- In general, those fragments with the greatest (largest) MW are closest to the wells while those with the smallest MW are farthest from the wells (origin), i.e., travel the farthest from the origin.

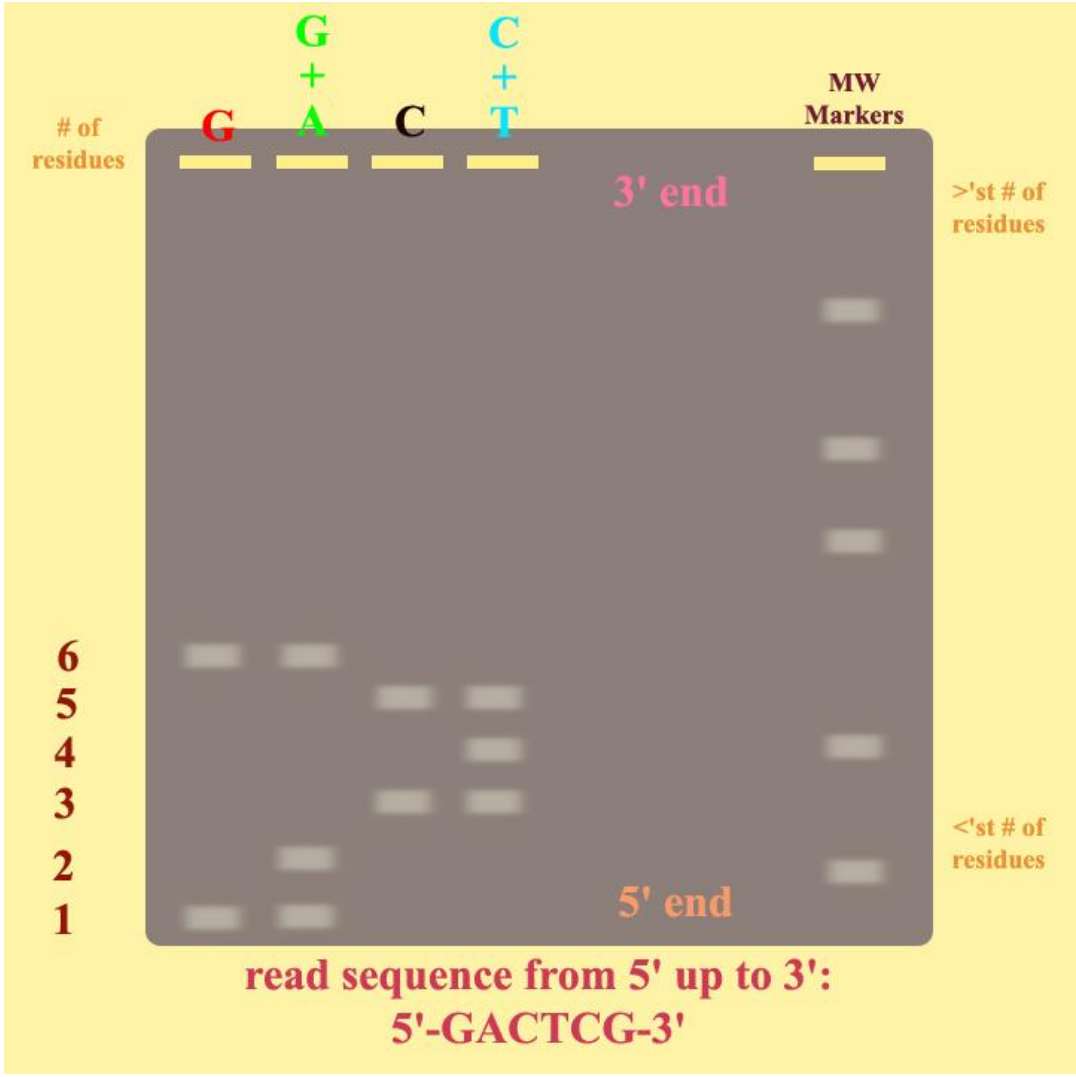


- The gels are placed in a gel holder or tank and a buffer solution is poured into the tanks and slightly over the gels. The samples are carefully placed in their wells with micro-pipets. Electrodes are attached to each end of the gel and the current is turned on. Depending on the gel, its thickness and length, and the buffer, the gels are run for a pre-determined period of time.

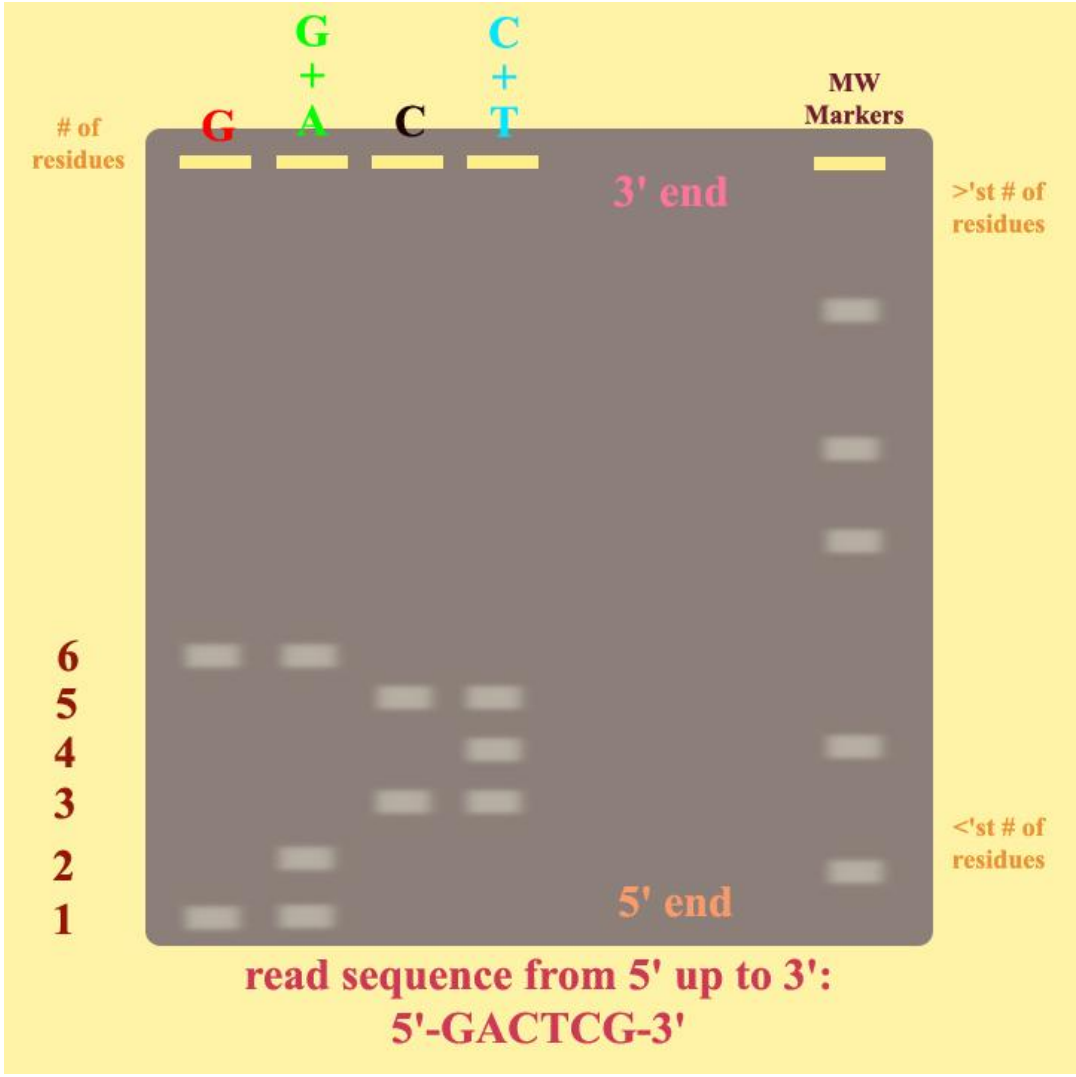
- Once the gels have been completed, they are incubated with a piece of film. Since the fragments are labeled with radioactive phosphorus (^{32}P), the film will be exposed wherever there are radioactively labeled bands.
- The film is exposed and viewed over a light box.
- The portion of the film where the least number of fragments are present is the 5' end of the sequence and the region closest to the origin is the 3' end.



- The DNA sequence is read directly from the autoradiograph (the picture it made of itself on film).
- It is important to remember that the sequence read from the autoradiograph is 1 nucleotide short of the original sequence.

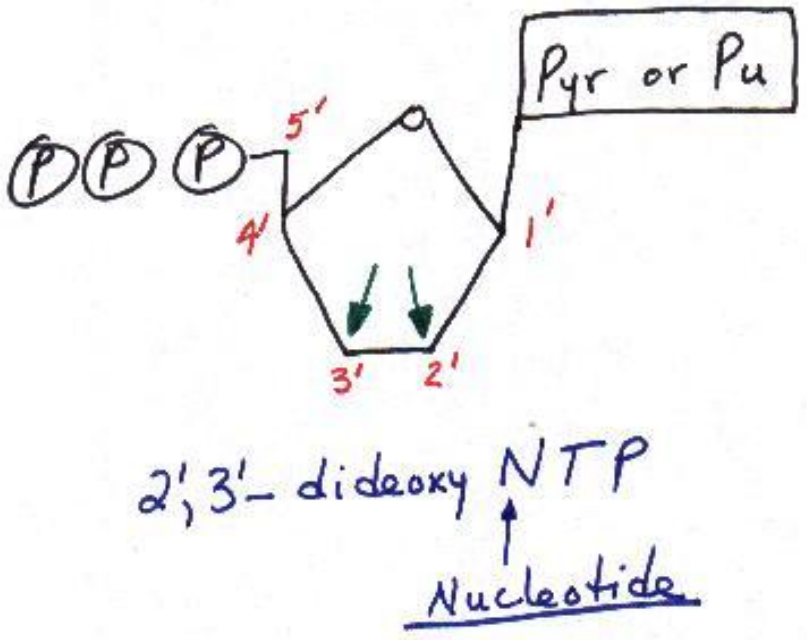


- It's also important to understand how to read the autoradiograph.
- Beginning at the 5' end, the first bands are G and G+A. Since both are present, the first nucleotide in this sequence is "G".
- The second nucleotide is in the G+A lane ONLY. This means that there is only an "A", here.
- The third nucleotide has bands at "C" and "C+T". Since both bands are present, the nucleotide is "C".
- The fourth nucleotide has a band in the "C+T" lane. Since there is not a band in the "C" lane, this nucleotide is a "T".
- The rest of the gel is read in the same manner. The sequence of this polynucleotide is (5' to 3') GACTCG.

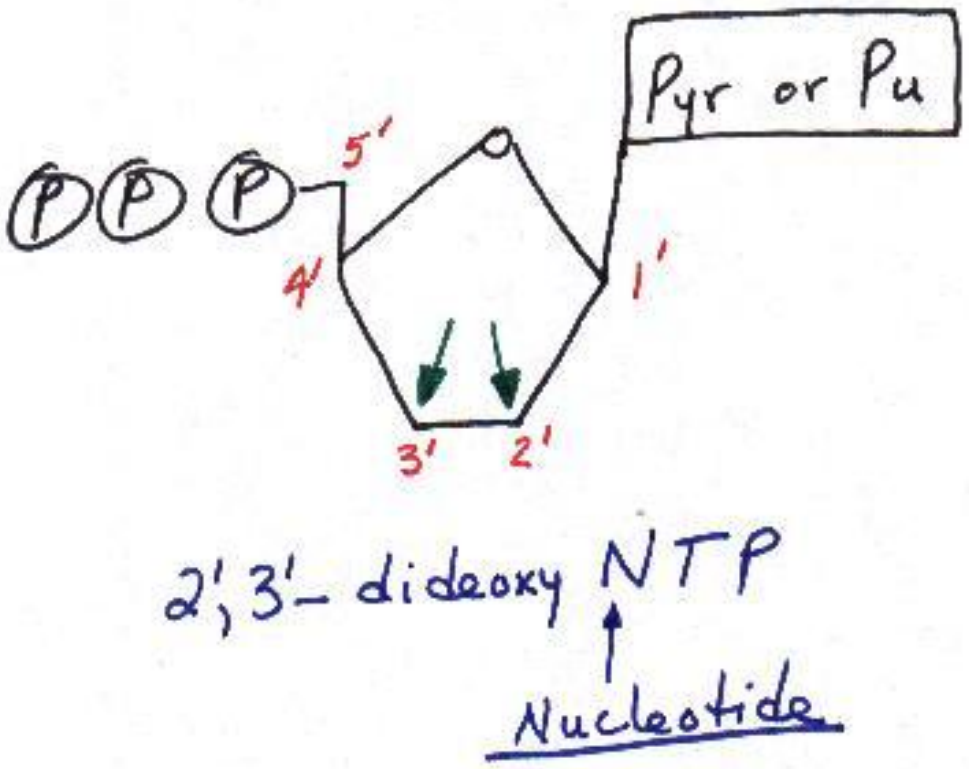


Sanger Method

- It is also known as the chain termination, controlled interruption of enzymatic replication and dideoxy methods of DNA sequencing.
- The key to this method is the utilization of a 2',3'-dideoxy compound, Figure, right, green arrows.



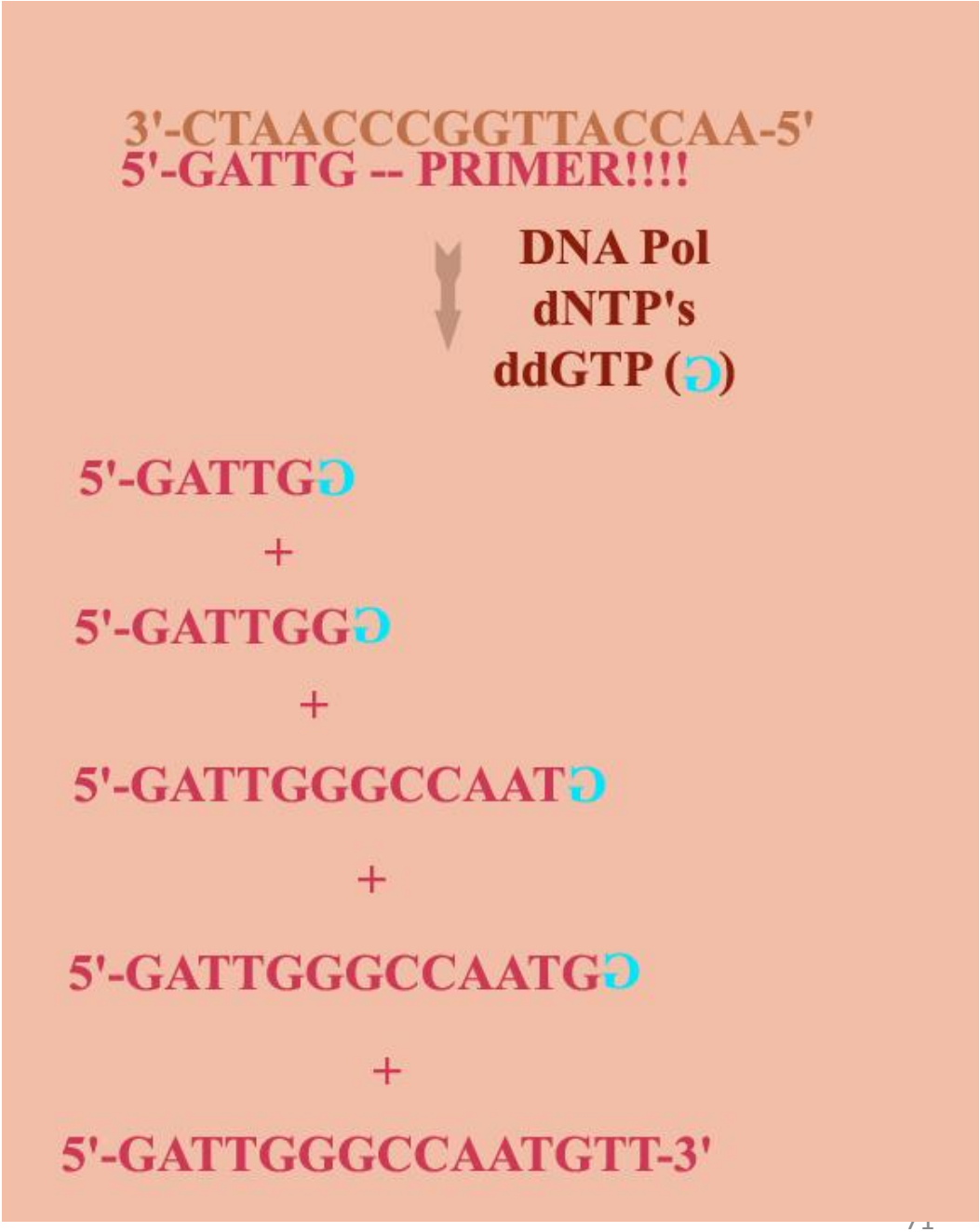
- In this compound, besides having no 2' -OH, there is also no 3' -OH.
- Without this -OH, it is impossible to make 3',5'-phosphodiester bonds and the DNA chain will not continue to elongate.



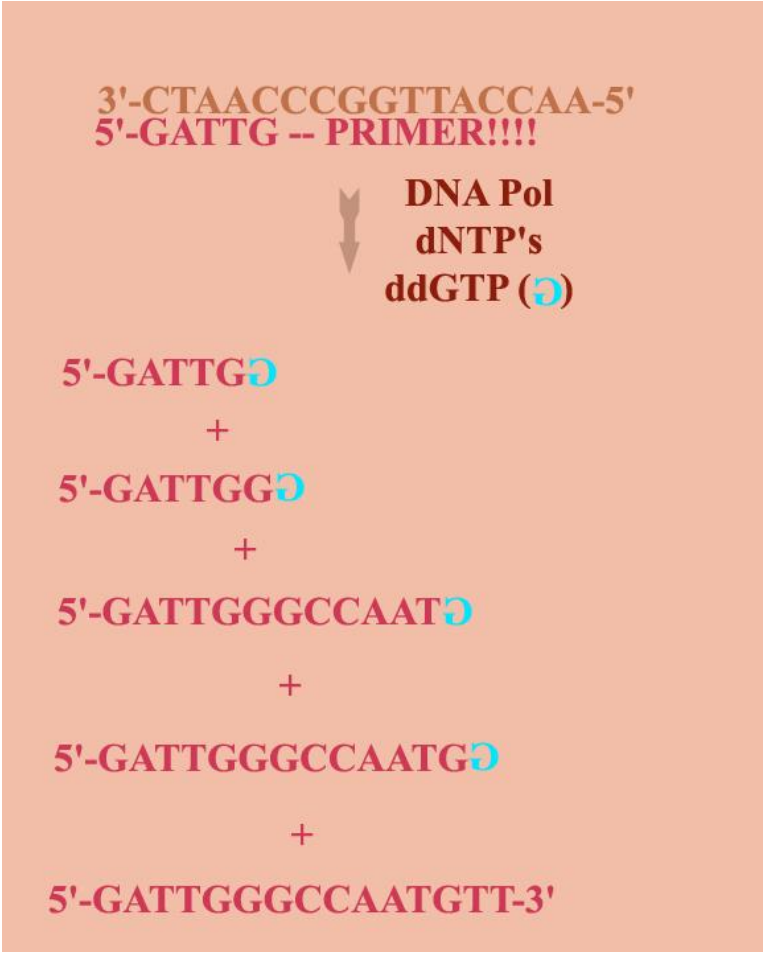
- In order to follow this method, let's consider the sequence:

- In addition, this method requires a primer that typically comes from a restriction digest. The primer for our sequence is **GATTG**, 5' to 3'.
- Note that the primer is complimentary to the first 5 nucleotides in the sequence we want to study

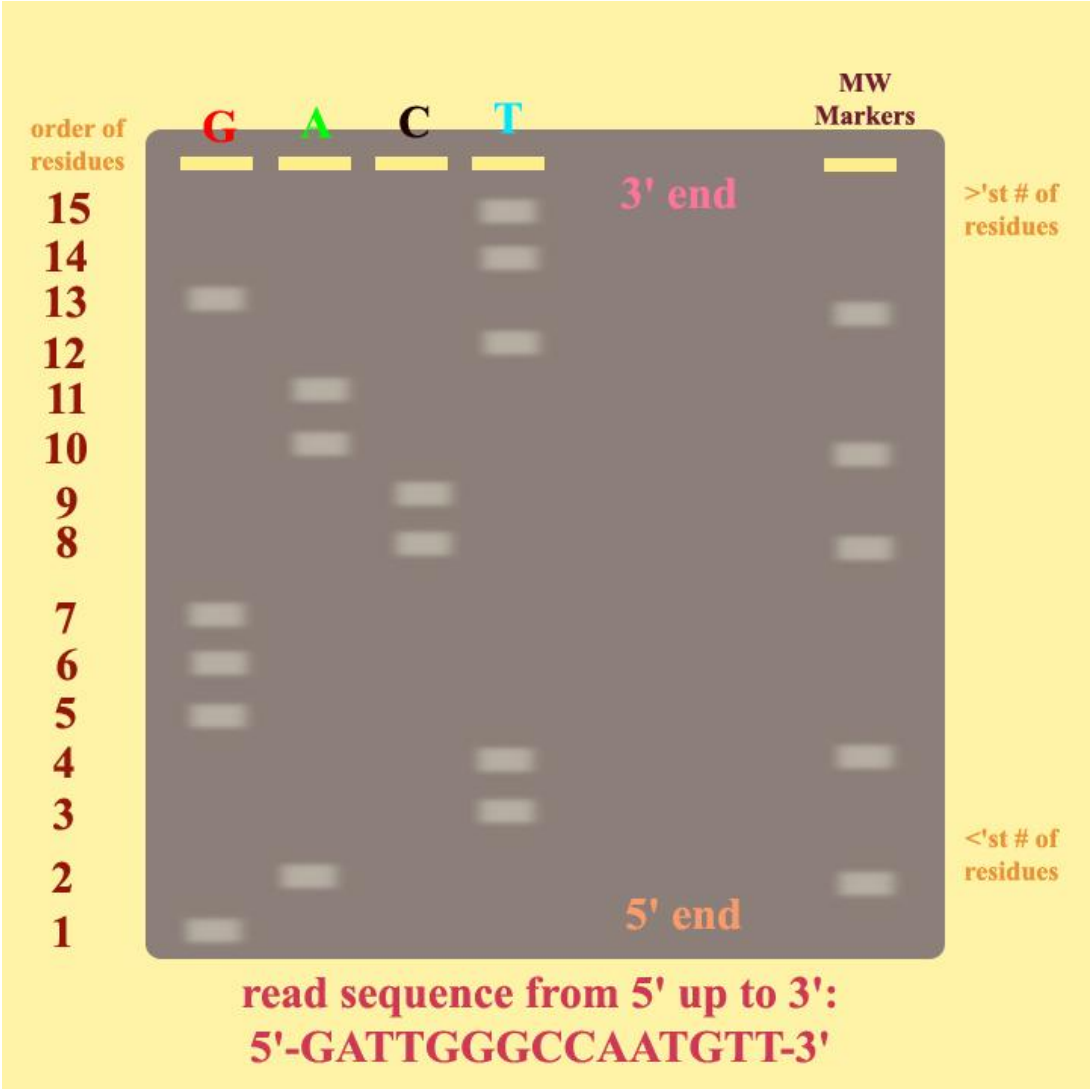
- The DNA fragment for sequencing is reacted with the complimentary primer, DNA Pol, radioactively labeled dNTP's (dNTP*) and ddGTP (dideoxy-GTP*; represented by the backwards G in the figure)



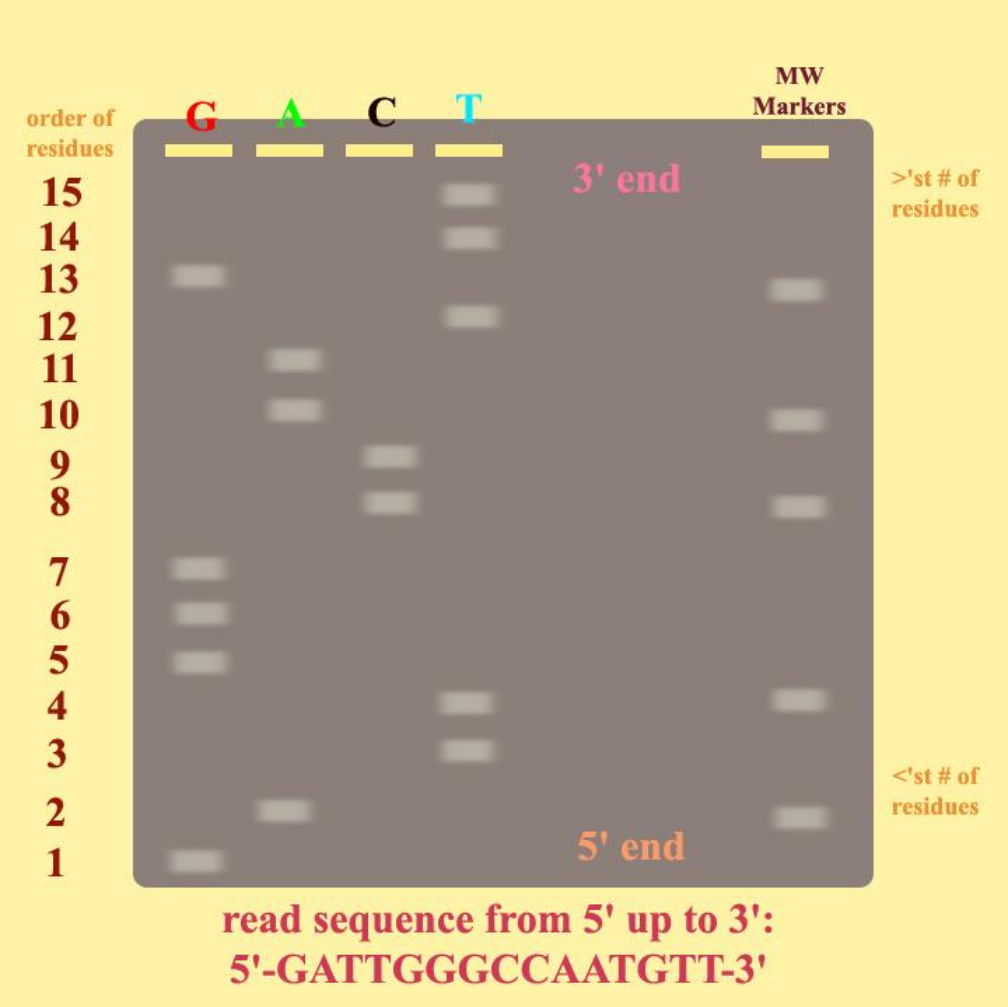
- Note that the sequence being synthesized is complimentary to the original sample.
- Whenever the newly synthesizing sequence is determined to need a "G" added to its sequence, either dGTP* or ddGTP will add on.
- If ddGTP adds on, that fragment will no longer elongate, i.e., the chain terminates -- the last residue of that fragment is a ddGTP.
- This cycle continues as desired, *ad nauseum*.



- Each ddNTP will be utilized in the Sanger method so that, essentially, 4 samples will be obtained, all of which are dideoxy-derivatives of G, A, C, T.
- These samples will be electrophoresed in the much the same manner as with the Maxam-Gilbert method



- In the Sanger method, the first residue is the first nucleotide from the original nucleotide's newly synthesized complimentary DNA after the primer.

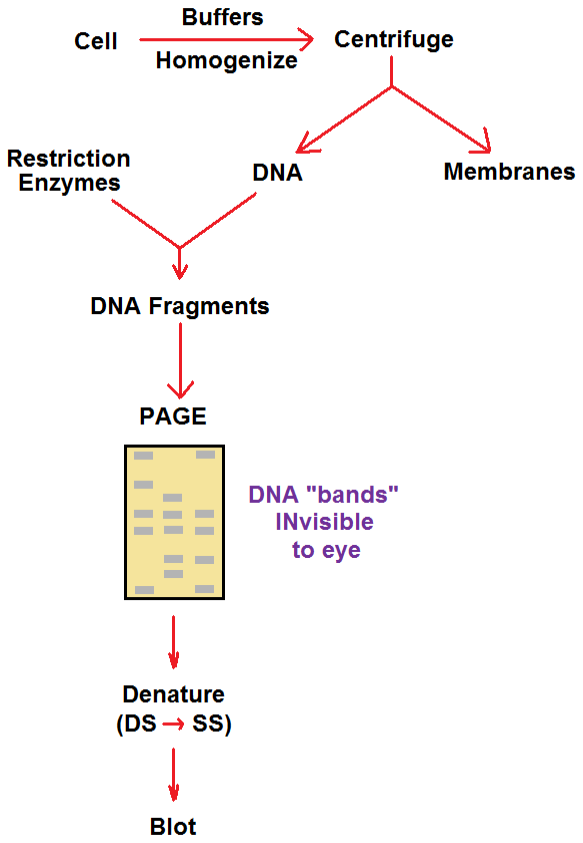


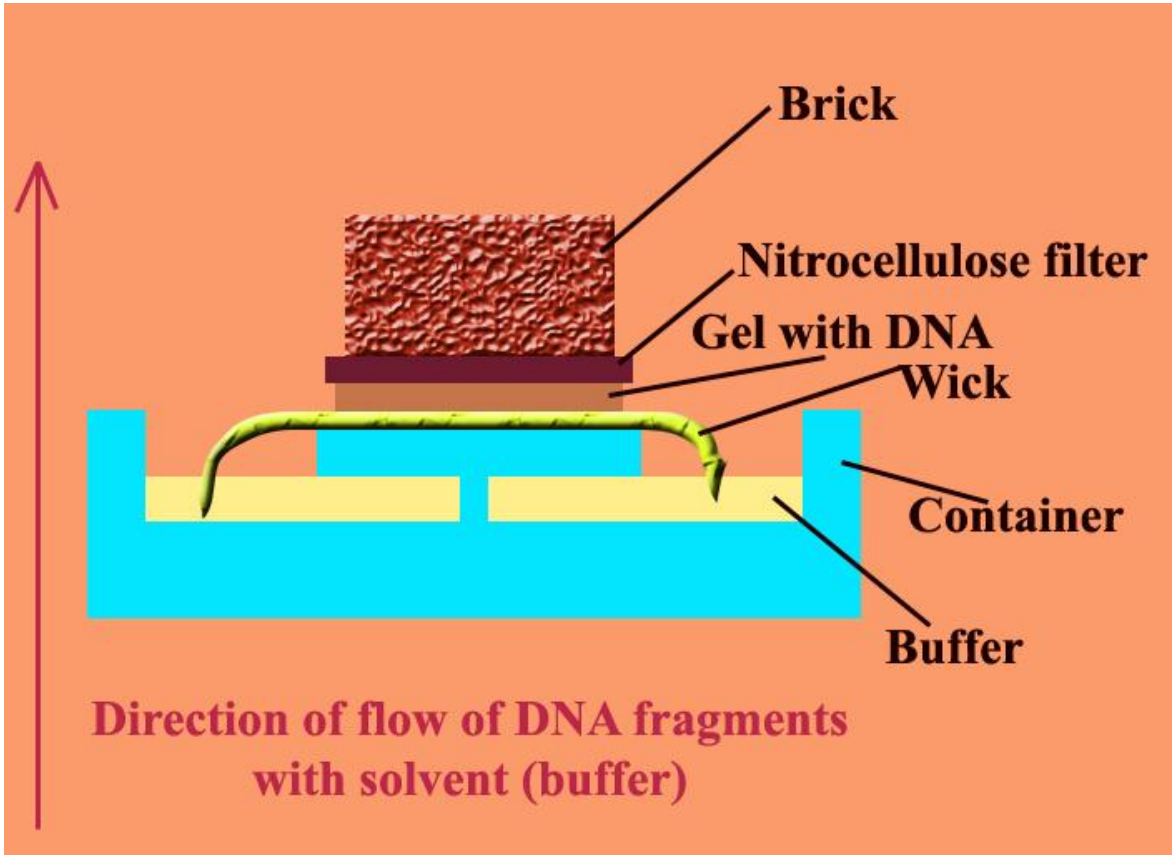
- The sequence is read directly from the autoradiograph and the **original sequence is then deduced from its complimentary strand sequence**.
- Although both methods are used, the Sanger method has been automated and is much faster and useful in DNA sequencing in the lab.

- With several questions now answered about DNA and its structure, there is still a burning question regarding techniques of identifying either specific DNA regions or individuals by their DNA sequences.
- About 25% of the human genome is in many different alleles (identical genes with different sequences). These are called polymorphisms.
- Each is an inherited pattern and segregate according to Mendel's rules (coming up in the future). These **restriction fragment length polymorphisms (RFLP's)** may be used to identify diseases, criminal acts or the release of suspects for lack of correct evidence, *ad nauseum*. Only identical twins have identical RFLP's, but NOT identical fingerprints or retinal scans.
- **VNTR's (variable numbers of tandemly repeated units)** comprise very unique RFLP's. They serve as "molecular finger prints" of an individual. Both of these fragments may be studied by Southern blot and PCR.

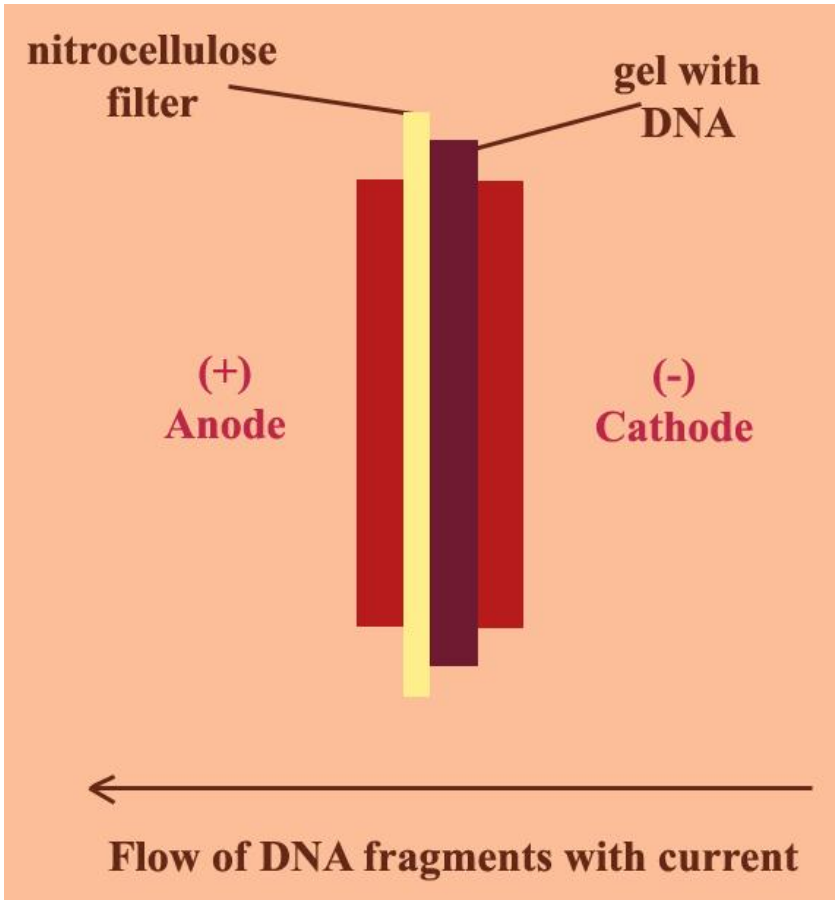
Southern Blot

- Cells are mixed with appropriate buffers and homogenized. The gimish is centrifuged to give various cell fractions, which include DNA.
- The DNA is digested by restriction enzymes to fragments and submitted to PAGE.
- There are NO fragments visible to the human eye in this gel -- yet. The gel is treated in such a manner that the DS DNA is denatured to SS DNA and then the gel is blotted onto a nitrocellulose filter.



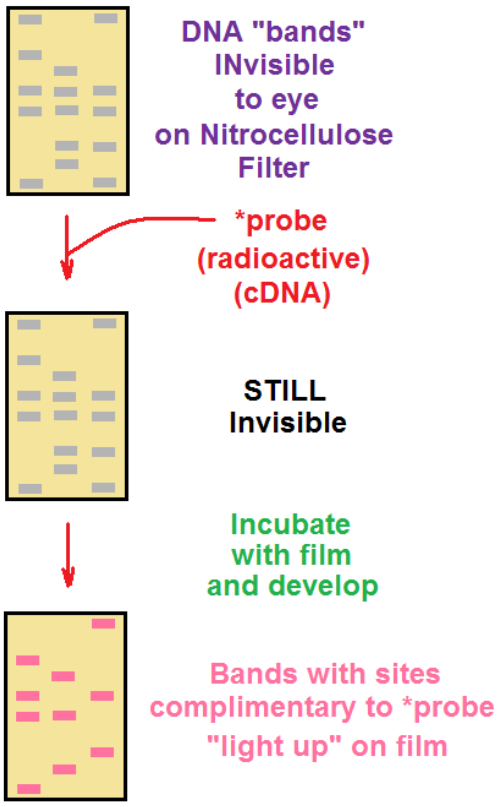


- Buffer is poured into a "pan" with a stand in its center. A wick (some just use paper towels) is laid over the stand, upon which the gel is placed. A nitrocellulose filter is placed on top of the gel and a brick (or some heavy weight) is placed on top of the filter. The solvent (buffer) flows up the wick and under the pressure of the brick, causes the fragments to be transferred to the nitrocellulose filter.



- The second way in which material is transferred from the PAGE gel to a nitrocellulose filter: by electrophoresis. The gel with the SS DNA is placed between two electrodes with a nitrocellulose filter placed on the positively charged electrode (anode) side. Electrophoresis is run as described earlier. The DNA (negatively charged) is attracted to the anode and the SS DNA is transferred to the nitrocellulose filter.

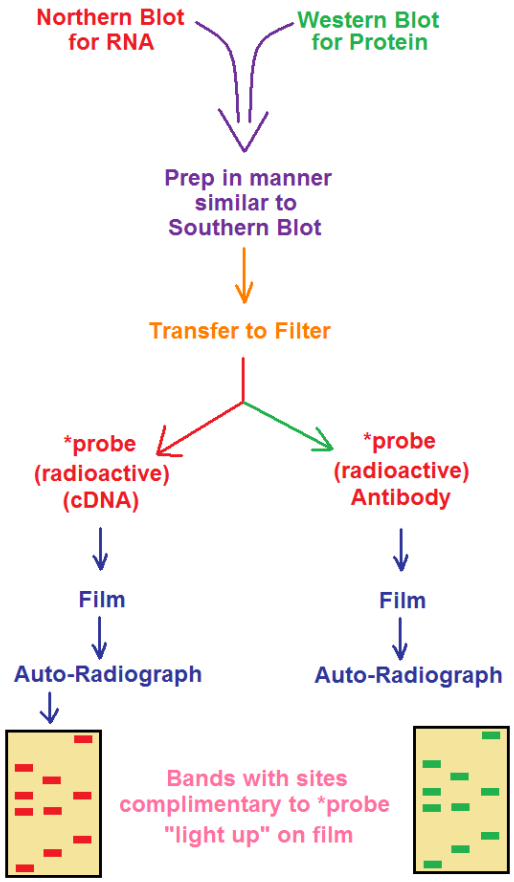
- There are STILL no bands visible on the nitrocellulose filter, yet, Figure, right.
- A radioactively (^{32}P) labeled "probe", a specific sequence of DNA complimentary to what you are looking for (cDNA -- complimentary DNA) is added to the nitrocellulose filter and incubated. Once incubation is complete, the irradiated filter is incubated with a piece of film and, then, developed. The only bands, which show up on the autoradiograph, are those bands that are complimentary to the probe and light up on the film. Those fragments may be identified on the nitrocellulose filters by matching it up with the film; the fragments may then be studied as desired.



Northern and Western Blots

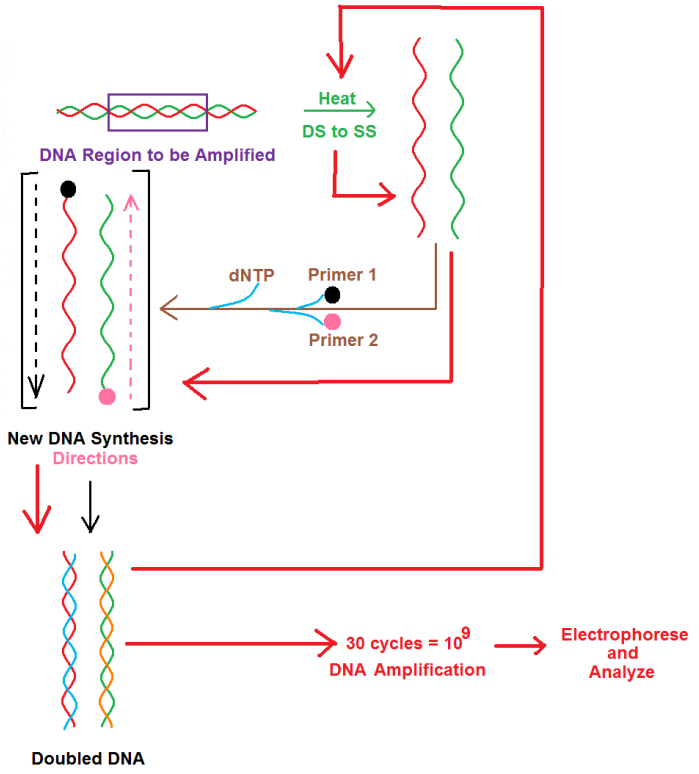
- Two other blots are of significance, as well:
  - the Northern and
  - Western blots.
- While the techniques to do these blots are similar, they examine DIFFERENT macromolecules.

- The Northern blot (named for geography rather than someone's name) examines RNA fragments.
- The probe for the Northern blot is radioactively labeled cDNA.
- The Western blot (more warped geneticist's humor) looks for proteins, Figure, right.
- The probe for the Western blot is radioactively labeled antibodies (more on this in the future).
- The Western blot has gained notoriety as it is the confirming test to positively diagnose people who are infected with HIV.



Polymerase Chain Reaction

- What happens, though, if there isn't enough DNA in a sample to be studied adequately? A technique called the PCR (polymerase chain reaction) was developed just for this purpose.
- In brief, a fragment of DS DNA is heated to denature it.
- To the two strands of SS DNA, two primers are added, as well as DNA Pol and dNTP's.
- As this chain reaction gets going, after 20 cycles, this small amount of, previously unstudyable, DNA is amplified one million fold! After 30 cycles, this DNA is amplified a billion-fold!



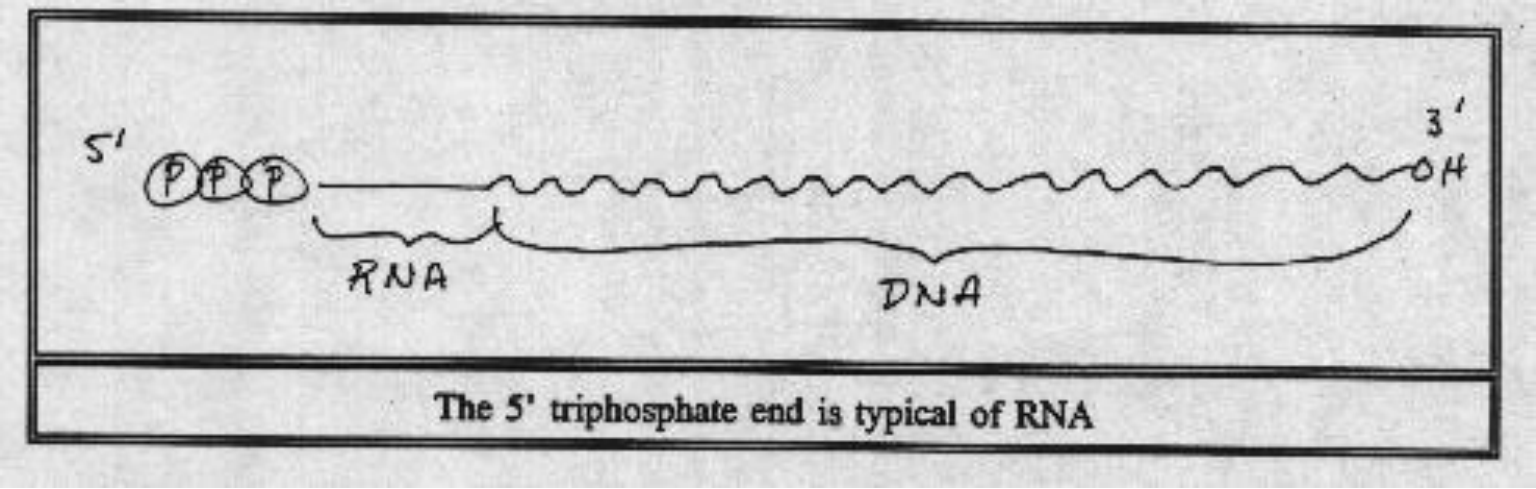
- This technique has proven useful in numerous trials.
- The PCR is also used
  - 1) to detect viruses of a "sneaky" nature (e.g., HIV),
  - 2) prenatally, e.g., to identify genetic defects,
  - 3) to detect polymorphisms,
  - 4) in tissue typing to reduce tissue rejections,
  - 5) in old DNA samples, e.g., bacteria from pyramids in amber to track evolutionary changes and
  - 6) in forensics, e.g., to identify perpetrators and separate them from innocent suspects.

There are some fine points to keep in mind when considering DNA replication

ONLY 5' triphosphates are appropriate substrates for DNA polymerase (DNA Pol)

1.

- A primer is required for DNA replication.
- This primer is an RNA oligonucleotide, 4-60 bp in length.
- The primer requires RNA Pol and Primase that copies the DNA sequence from 1 DNA strand.
- This primer is different from classical RNA as this RNA remains H-bonded to the DNA template.
- Illustrates the appearance of the RNA primer/DNA strand.
- Note that on the 5' end there is a triphosphate. This is typical of RNA.



The 5' triphosphate end is typical of RNA

2. Nick translation is very important in DNA ligature.

- It occurs in the lagging strand to force the Okazaki fragments to join together at the expense of ATP (the enzyme, here is a ligase).
- When DNA Pol makes enough DNA to run into the next chain of RNA primer (each Okazaki fragment has its own RNA primer), its exonuclease activity removes the RNA and replaces it with DNA (DNA Pol is a multi-functional enzyme).
- The 2 Okazaki fragments are then joined together with ligase.

3. The helix is unwound with helicases.

- The exact mechanism is not understood.
- It is known that helicases require ATP.

4. After the DNA is unwound to for two SS DNA, it must be stabilized or it will "re-fuse" or form H-bonds with itself.

- The DNA remains stabile with SS DNA binding proteins (SSDBP).
- Although the mechanism for the removal of the SSDBP's is unknown, they are replaced by the advancing polymerases.

5. The rate of polymerization (formation of many nucleotides into a strand) in eukaryotes in DNA synthesis is about 0.5-5 kbp (kilo base pairs) per minute!

6. When DNA is replicated, the histones are dissociated from the replicating fork in the immediate area, then by unknown mechanisms, octamers and DNA reassociate.

- Makes one wonder about the relationship between the histones and the SSDBP's, doesn't it?

7. Once DNA has been replicated there is still one little problem: there will be an RNA sequence at the end.

- It's easy to remove, but it seems to be replaced, instead.
- The replacement mechanism is unknown although we DO know that the synthesis of DNA necessarily depends on the presence of this RNA primer.

We also know the following:

1. The RNA can not remain, because its properties would alter the properties of the DNA.
2. It doesn't seem reasonable to expect that the ends would remain SS after the RNA was removed, as this would alter the properties of the DNA, as well. It would also effect the function of telomerase.

All the above suggests that the following may play a role in the RNA replacement:

1. Perhaps a "terminase", a DNA Pol that remains to be identified, is involved in altering the RNA end into a DNA end.
2. Perhaps a 2'-hydroxylase, heretofore unknown, simply strips the 2' -OH group from the ribonucleotides to change them into deoxyribonucleotides -- challenge: how would this take care of the T vs. U characteristics between DNA and RNA? (DNA Pols are not capable of distinguishing between dUTP and dTTP since H-bonding occurs between the two and A in DNA: hence dUTP becomes incorporated into the DNA -- therefore, no major problem, since the dUMP will be removed from the newly synthesized strand of DNA and replaced under the influence of ligase.)
3. Although unlikely, perhaps the enzyme ligase is a multifunctional enzyme, i.e., has ligase activity and hydrolase activity or has ligase activity and transferase activity, and simply has not been elucidated with current technology.

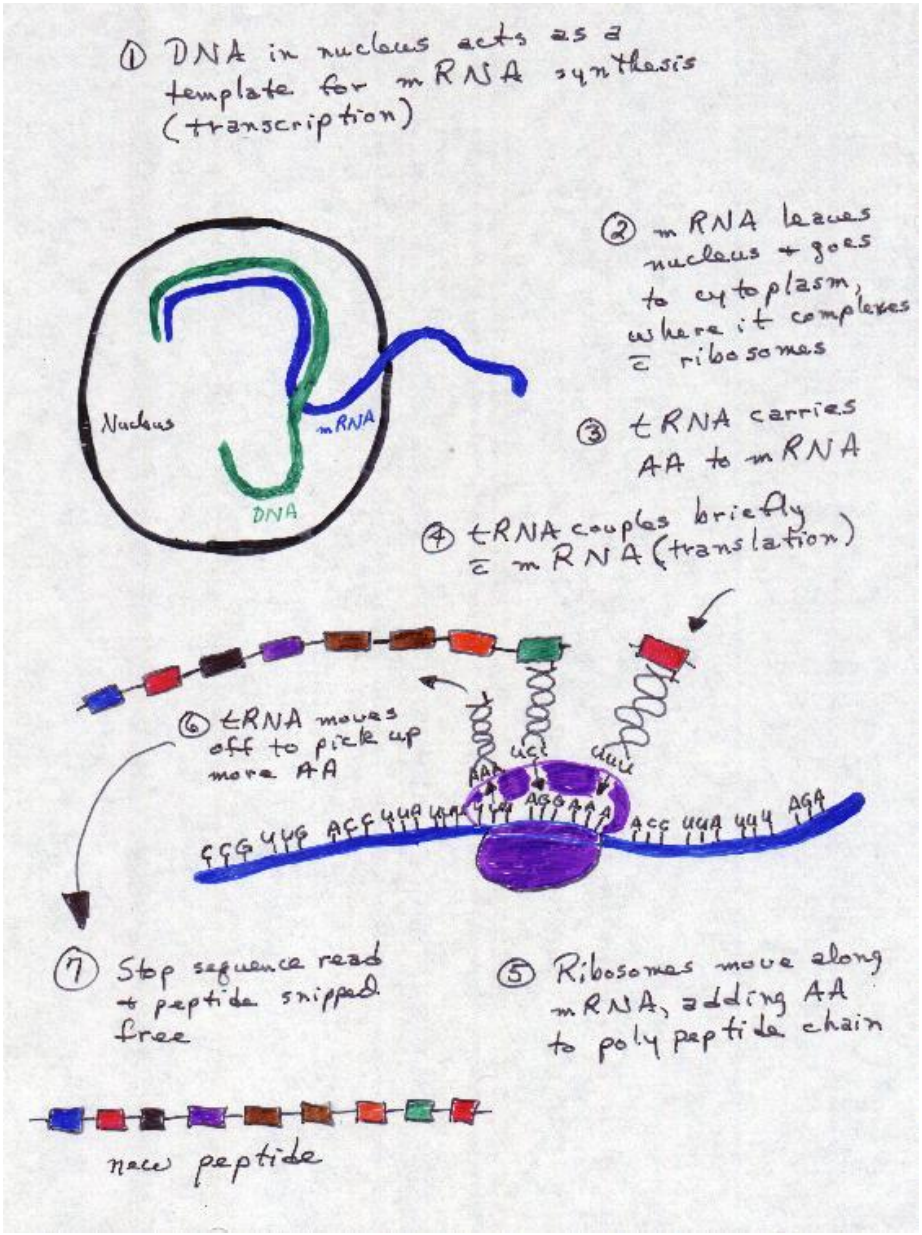
- DNA Synthesis may be inhibited by numerous chemicals and/or drugs.
- The following table summarizes 3 classes of compounds used to inhibit DNA synthesis:

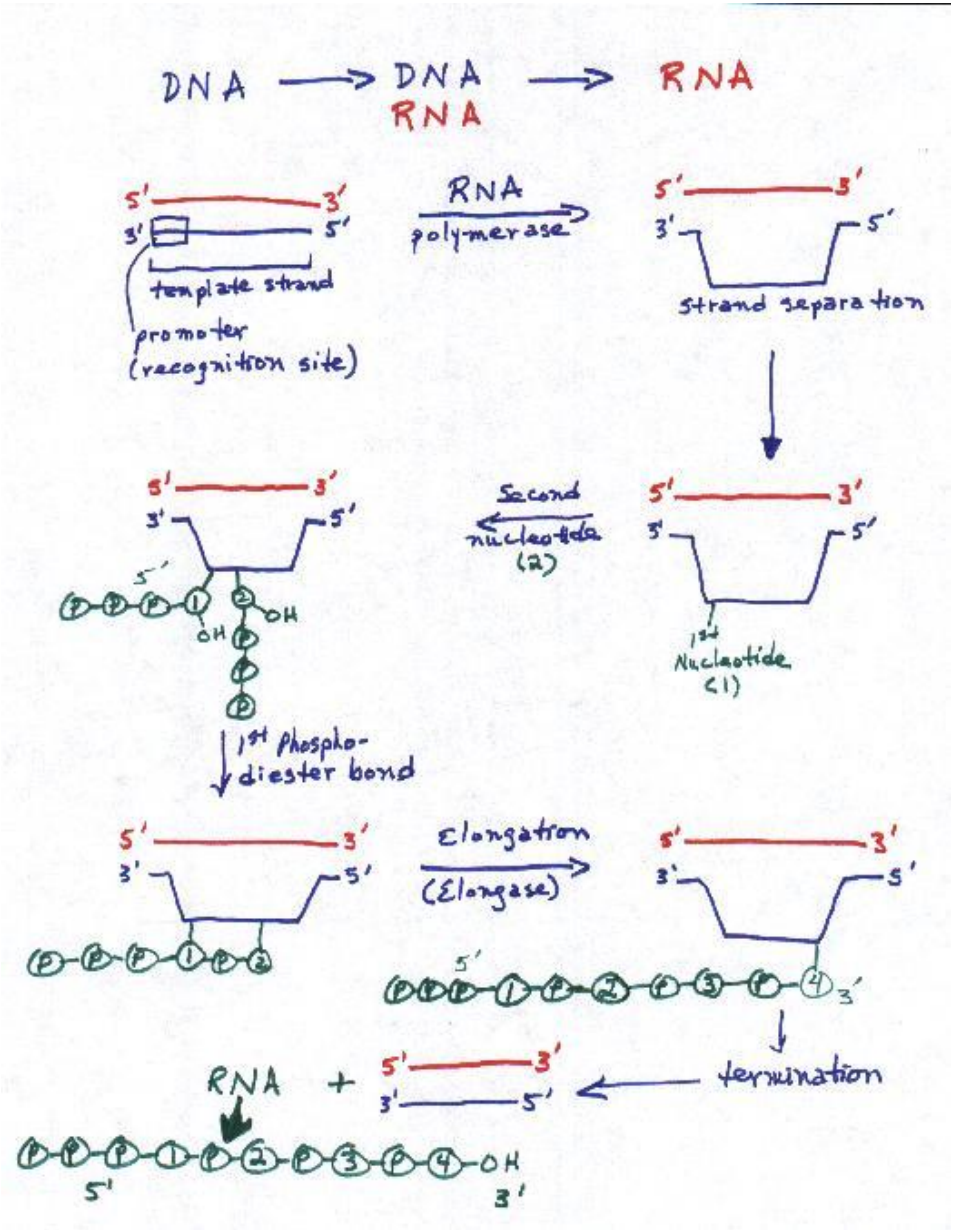
Precursor Synthesis Inhibitors	Template Altering Drugs/Inhibitors of Strand Priming	Polymerase/Other Enzyme Inhibitors
Covered in Future Courses; examples listed below are NOT inclusive	**Intercalating agents (review ethidium bromide or acridine); Daunorubicin, doxorubicin and plicamycin**	**Chain terminators: 2',3'-dideoxyribonucleotides, cordycepin Acyclovir in Herpes Simplex; Aphidicolon on SOME DNA Pol in some viruses**
DHFR* inhibitors: Methotrexate in bacteria and protozoans; Pyrimethamine in protozoans; Trimethoprim in bacteria and protozoans	**Chain breakers: Bleomycin, Zinostatin**	
Inhibitors of purine biosynthesis: sulfonamides in bacteria; Azaserine in humans (inhibits inosinic acid synthesis); 6-diazo-5-oxo-L-norleucine (DON), also	**Chain cross linkers: Alkyl sulfonates, anthramycin, nitrogen mustards, mitomycin C**	**Bacterial DNA Gyrase inhibitors: novobiocin, oxolinic acid, nalidixic acid**
Inhibitors of AMP and GMP formation: Hadacidin (from fungi) in bacteria, plants and tumors -- AMP inhibition; Ribavirin monophosphate -- GMP inhibition	**Template activity inhibitors: platinum coordination compounds (review Chem 122)**	

*DHFR inhibitors are those inhibitors that block dihydrofolate reductase from reducing dihydrofolate to tetrahydrofolate so that our bodies (or bacterial cells or protozoans) won't be able to adequately utilize folic acid to its utmost.

The Third manner in which DNA may be taken apart is through the process of transcription, i.e., RNA synthesis

- During transcription, only ONE strand of the DNA is actively read and transcribed. The strand to be transcribed contains a promoter (recognition site) that is recognized by RNA Pol. This enzyme causes the DS DNA to separate and it adds on the first of a sequence of nucleotide triphosphates (NTP's) to initiate transcription.
-
- Note that the DNA is the template for the new strand of RNA. In this instance, the bases in RNA will H-bond with the DNA in a complimentary manner, i.e. AU and/or AT (depending on the nucleic acid and orientation) and GC.
-
- As the polynucleotide progresses, the enzyme elongase extends the polynucleotide chain until termination occurs with the release of RNA (much more on this in the RNA chapter).


① DNA in nucleus acts as a template for mRNA synthesis (transcription)
Nucleus
mRNA
DNA
② mRNA leaves nucleus + goes to cytoplasm, where it complexes ∈ ribosomes
③ tRNA carries AA to mRNA
④ tRNA couples briefly ∈ mRNA (translation)
⑥ tRNA moves off to pick up more AA
UCU
UUU
AAA
AGG
AAA
CCG GUG ACC GUA GUA GUA ACC UUA GUA AGA
⑦ Stop sequence read + peptide snipped free
⑤ Ribosomes move along mRNA, adding AA to polypeptide chain
new peptide


DNA → DNA RNA → RNA
5′ 3′
3′ 5′
template strand
promoter (recognition site)
RNA polymerase
5′ 3′
3′ 5′
Strand separation
5′ 3′
3′ 5′
Second nucleotide (2)
5′ 3′
5′ 5′
1st Nucleotide (1)
OH
1st Phospho-diester bond
5′ 3′
3′ 5′
Elongation (Elongase)
5′ 3′
3′ 5′
termination
RNA + 5′ 3′ 3′ 5′
OH
5′
3′

Regulatory Proteins: DNA Binding for Transcriptional Control

- There are three motifs of DNA binding proteins:
- All three of these bind to DNA
  - Helix-turn-helix,
  - zinc (metallo) fingers and
  - leucine zippers.

ALL three motifs are capable of positive/negative regulation of transcription.

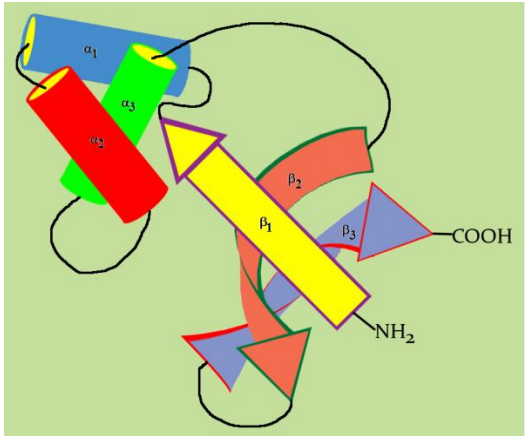
There are some general rules that are applicable to these DNA binding proteins:

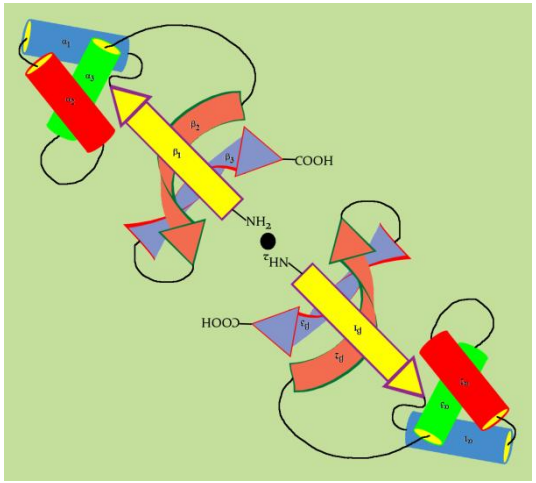
1. They have a high affinity binding (binds tightly and favorably) to a specific site on the DNA.
2. They have a low affinity for the rest of the DNA.
3. Only a "small bit" of these proteins may directly contact the DNA.
4. The styles of binding sites for all 3 motifs increase cooperative binding and increase the affinity of the protein of/for/with DNA -- analogy: cooperative binding of oxygen onto hemoglobin as you learned in A&P II.

Helix-turn-helix Motif

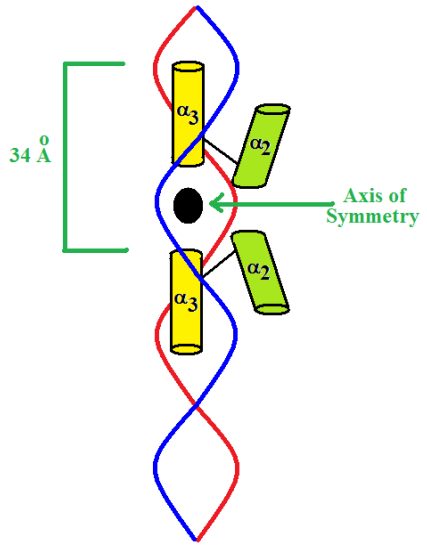
- Typically this motif has 3 antiparallel β-pleated sheets and 3 α-helices .
- Each sub-unit plays a different role in the binding of this protein with DNA:
-
- The α_3 subunit interacts with the major groove over about 5 bp's.
- β_3 interacts with another helix-turn-helix protein as it dimerizes.
- Therefore, 2 α_2 subunits then interact with the major groove to regulate this section of DNA.
- α_3 and α_2 sub-units are perpendicular to each other to maintain the position of α_3.

- The structure of the dimeric form of this motif. Note the point of symmetry between the two β_3 sub-units.



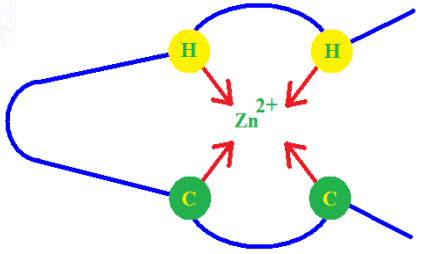


- The binding of two dimers in two regions of DNA.
- Note that the rest of the protein is not shown to simplify this illustration.



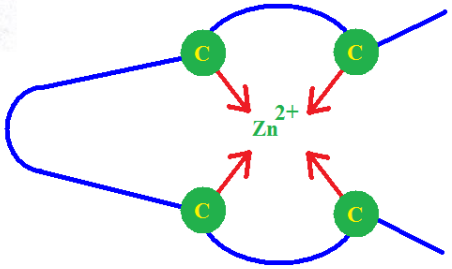
Zinc Finger Motifs

- These were the second DNA binding proteins to be studied.
- Studies revealed in a protein called TFIIIA (Transcription Factor IIIA) that it has 9 Zn^{2+} ions, each of which are complexed by 2 Cys residues close to each other and by 2 His residues 12-13 residues later.



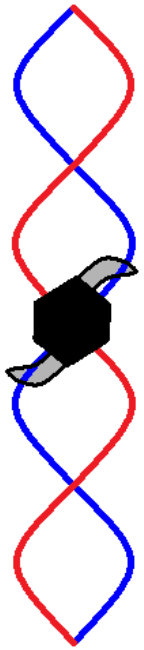
- This protein is known as a Cys-His Zn Finger or a C_2H_2 Zn Finger.
- The "C"'s stand for cysteine residues and the "H"'s stand for the histidine residues.

- Of the forms of Zn fingers known, a change occurs in steroid receptors and thyroid hormone receptor families: the 2 his residues are replaced with 2 cys residues.
- This is a Cys-Cys Zn Finger or C_4 Zn Finger.
- It is important to note that in all known Zn fingers, the Zn^{2+} is in tetrahedral geometry (review Chem 121 or 122).



- A variation of the C4C4 finger is the C3HC4 RING finger, so called due to its appearance of two fingers turned back on each other forming a ring.
- RING fingers are found in BRCA-1, the gene identified as being the cause in familial breast cancer -- this is under controversial discussion, at this time.
- It is possible that the same RING finger style may be in BRCA-2, a gene implicated more in familial ovarian cancer than breast cancer -- again, this is under discussion at this time.

- Proteins with Zn fingers lie on a face of the helix with its fingers "stuck" into the major groove.
- Each finger spans 5 bp's (some sources suggest 2-5 bp's) as does the helix in the helix-turn-helix binding motif.

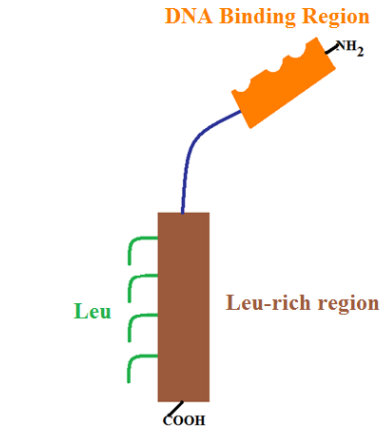


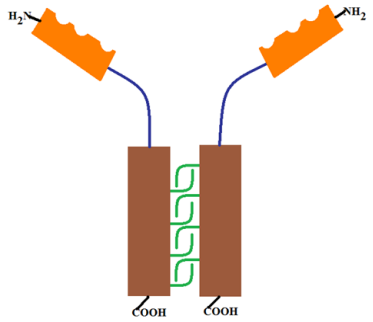
How Important Are Zn Finger Proteins?

- **Vitamin D receptor proteins have 2 Zn fingers.**
  - **If ONE mutation occurs in either finger, this receptor will NOT bind vitamin D and Vitamin D resistant rickets manifests.**
- **Zn fingers seem to regulate CNS development.**
- **Zn fingers may function between DNA-RNA hybrids.**
- **Zn fingers may be oncoproteins in leukemogenesis (development of leukemias).**
  - **Under normal conditions/structures, hematopoietic cells develop into blood cells.**
  - **When abnormal, lead to cancers of the blood (leukemias).**
- **Zn (RING) fingers may be required for viral "growth", indeed, it now appears that Zn fingers are involved with HIV.**
- **Zn fingers may prevent inappropriate major histocompatability class (MHC) II expression (more on MHC in BIOL 251), immunoregulatory genes which are responsible for causing multiple sclerosis as well as insulin dependent diabetes mellitus (IDDM).**

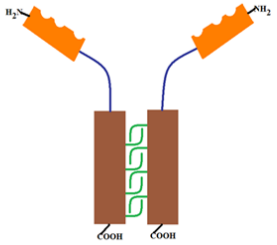
Leu Zipper Motif

- This motif is found in the C-terminus of enhancer binding proteins.
- The leu zipper has α-helical conformation and every 7 residues there is a leu.
- This occurs for 8 turns with 4 repeats, i.e., leu is found at numbers 1, 8, 15 and 22.
- This motif is monomeric until it's needed to bind with/to DNA, next slide.
- The "zipper teeth" in the image represent the leu residues.
- When two leu zipper monomers dimerize, these two leu-rich regions zip together into a coiled-coil which appears to increase the association of the DNA binding sites with their DNA target sites, next slide.
- Note that the "zipper" is located on the C-terminus of the proteins and the binding region is in the N-terminus of the proteins.
- The binding region of the leu zipper seems to consist of basic amino acids (positively charged amino acids).
- This works out reasonably well, since the phosphate backbone is negatively charged and affords good binding to basic amino acids.





Seems to be basic (alkaline -- positively charged) amino acids.
Slips into major groove of DNA

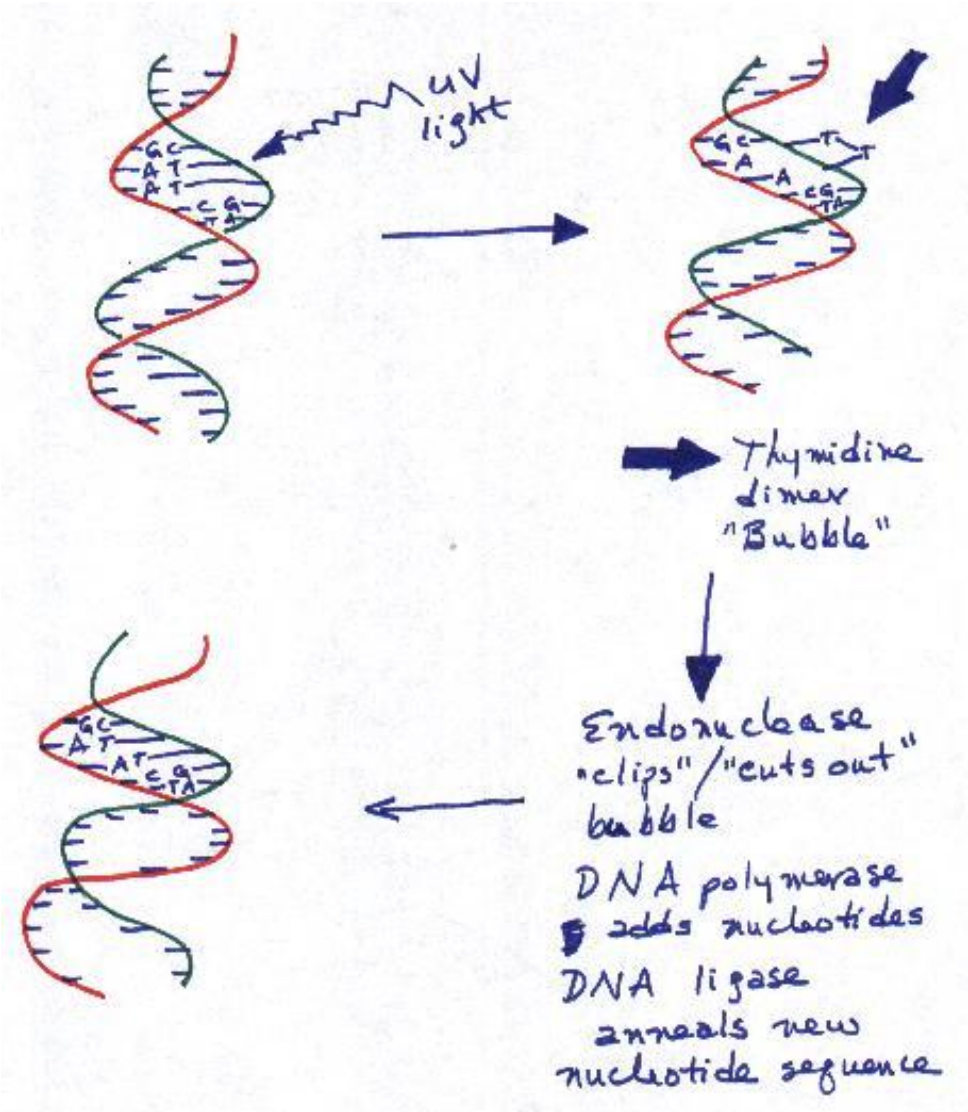


Leu "zipped up"

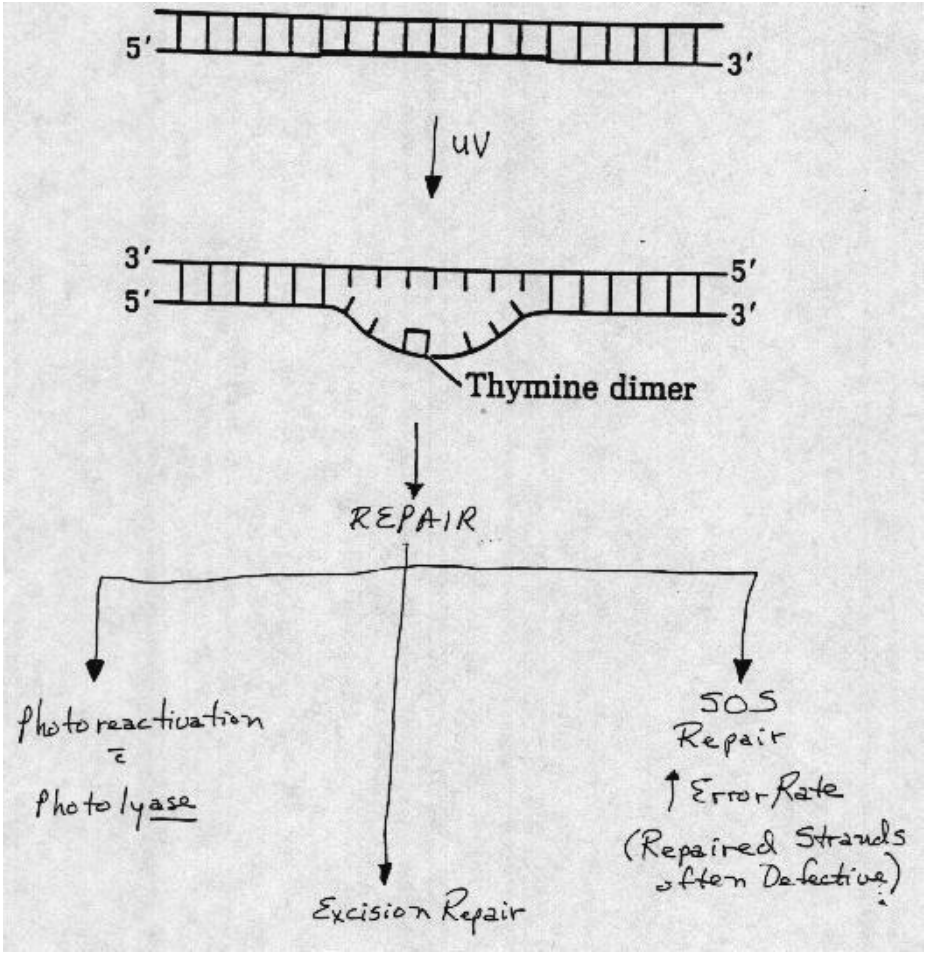
The Fourth and last manner in which we can take DNA apart has to do with DNA repair.

- **The first we want to examine is the effect of UV light and the last is the effect of ionizing radiation.**
- **UV light is absolutely miserable when it comes to DNA repair.**
- **This is the same UV light that causes malignant melanoma.**
  - **The UVA range is wavelengths from 320 to 400 nanometers (used for suntanning; shorter UVA wavelengths are considered possibly cancer-causing; responsible for cutaneous aging changes).**
  - **UVB refers to wavelengths from 290 to 320 nanometers (These wavelengths are more hazardous than UVA wavelengths, and are largely responsible for sunburn. The ozone layer partially blocks these wavelengths. UVB is largely blamed for malignant melanomas. Important in cutaneous vitamin D metabolism).**
  - **UVC refers to shorter UV wavelengths, usually 200 to 290 nm (Wavelengths in the UVC range, especially from the low 200's to about 275 nM, are especially damaging to exposed cells. Relatively little reaches our skin; well absorbed by atmospheric ozone).**
- **It is because of the UVB light-DNA interactions that we eventually develop malignant melanoma following unprotected sun exposure.**
- **These interactions trigger four (4) types of repair mechanisms: 1) photoreactivation with photolyase, 2) excision repair, 3) endonuclease repair and 4) SOS repair.**
- **The latter has an incredible error rate, as the repaired strands are often defective.**

- The following slide illustrates what happens when UV (ultra-violet) light interacts with DNA and how endonuclease activity repairs it.
- UV light "looks" for dTdT pairs on the same strand of DNA.
- When these pairs are hit by UV light, the "naturally" react to form thymine dimers, which are held together by a cyclobutyl bridge.
- These thymine dimers make bubbles in the DNA, much like a speed bump through J.C. Penney's parking lot.
- Just like the speed bump bouncing cars when they drive too fast over the bumps, the dimers knock the enzymes off the DNA, thereby making inappropriate DNA.
- Endonuclease "clips" or "cuts out" the bubble.
- DNA Pol adds nucleotides to the "cut area" and DNA ligase anneals the new nucleotide sequence.

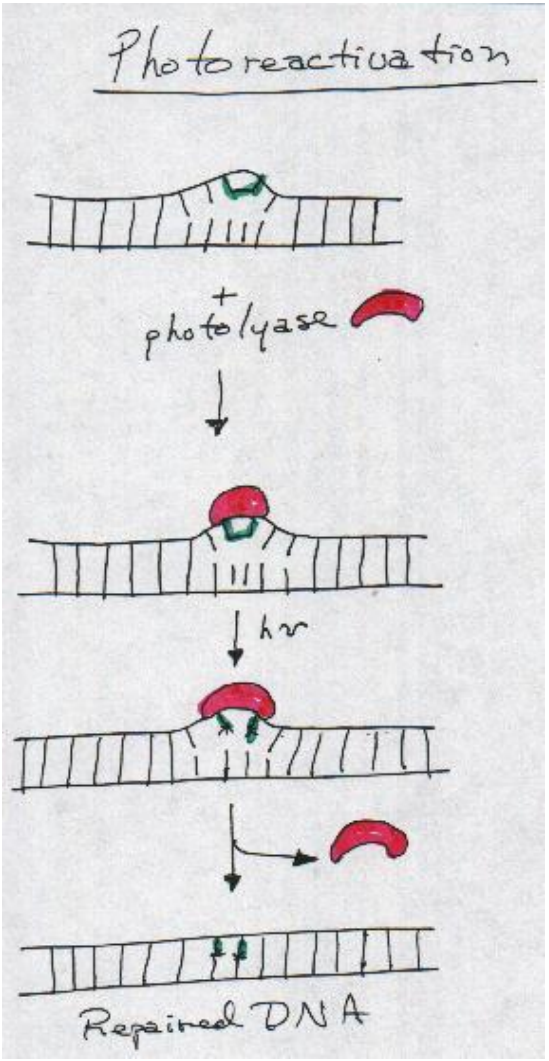

UV light
Thymidine dimer "Bubble"
Endonuclease "clips"/"cuts out" bubble
DNA polymerase adds nucleotides
DNA ligase anneals new nucleotide sequence

3 More Significant Repair Mechanisms Following UV-induced DNA-damage



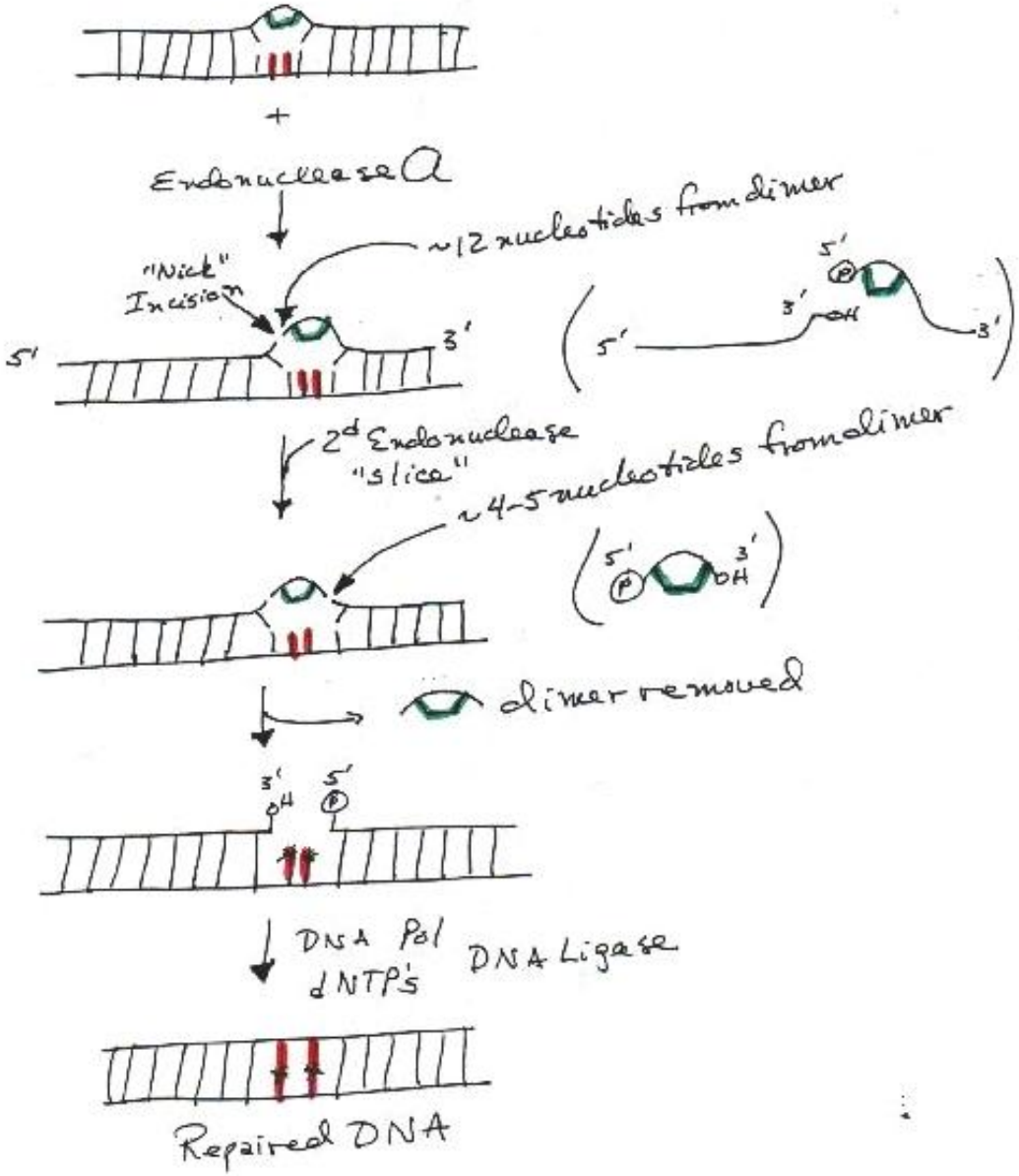
The first mechanism under discussion is photoreactivation.

- This mechanism works by having an enzyme (photolyase) bind to the site of the thymine dimer formation, once dimer formation has occurred.
- Light then activates photolyase to "clip" the bonds holding the cyclobutyl bridge together, permitting the dT's to reanneal with their complimentary base[s] (dA).



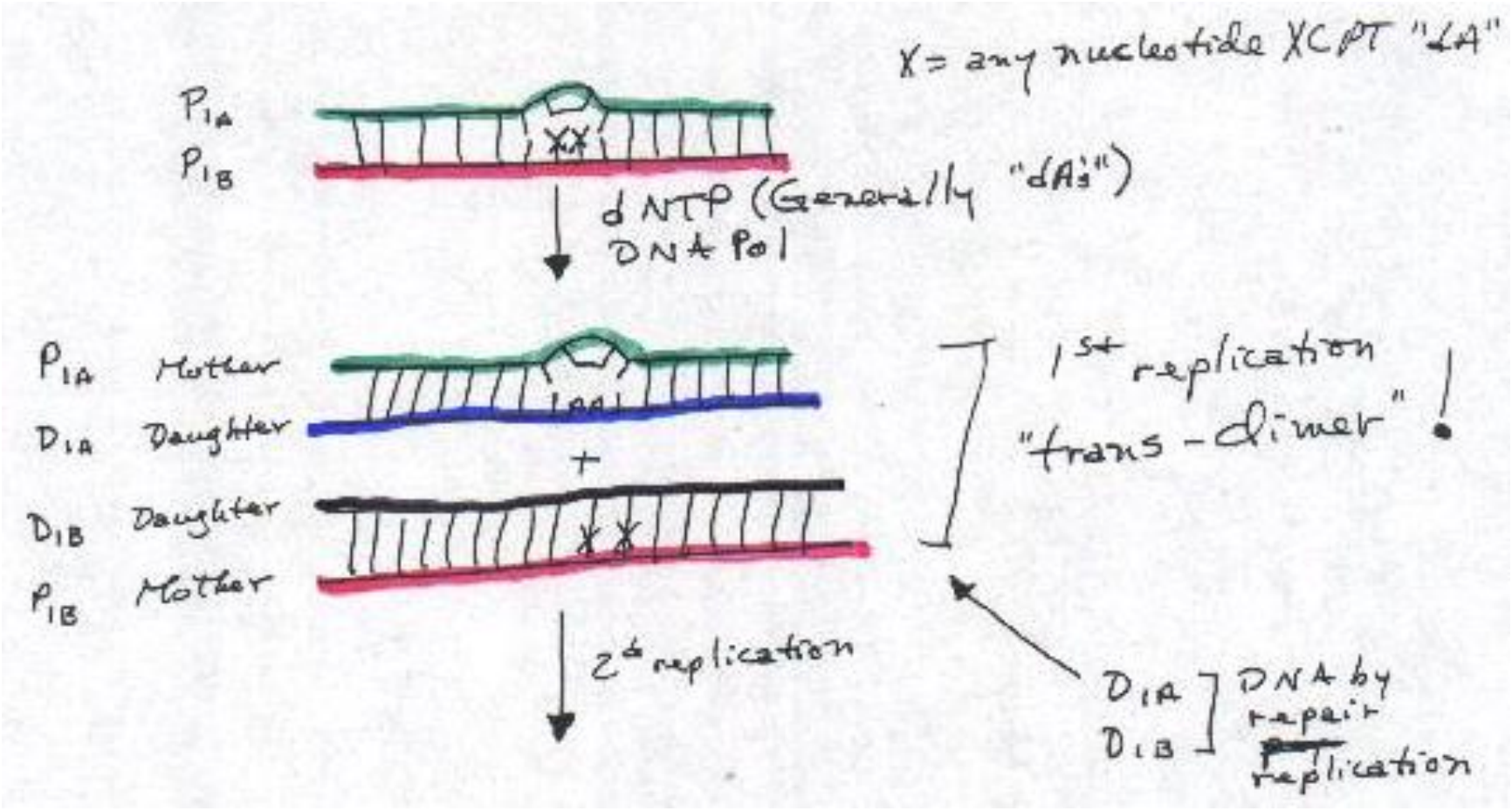
The Second Mechanism is Excision Repair.

- The second mechanism is excision repair.
- This is similar to the endonuclease activity repair we examined earlier, but is of sufficient enough difference that it has another name.
- Two endonuclease activities are required: 1 to clip on the 5' end of the dimer about 12 nucleotides from the dimer ("nick") and the 2d to clip on the 3' end of the dimer 4-5 nucleotides from the dimer ("slice"), following slide.
- This "nick" and "slice" leaves 3' -OH's and 5' phosphates on both the excised dimer and the intact strand.
- Once the dimer is removed, DNA Pol and ligase with dNTP's will "fill in" the excision and leave repaired DNA.

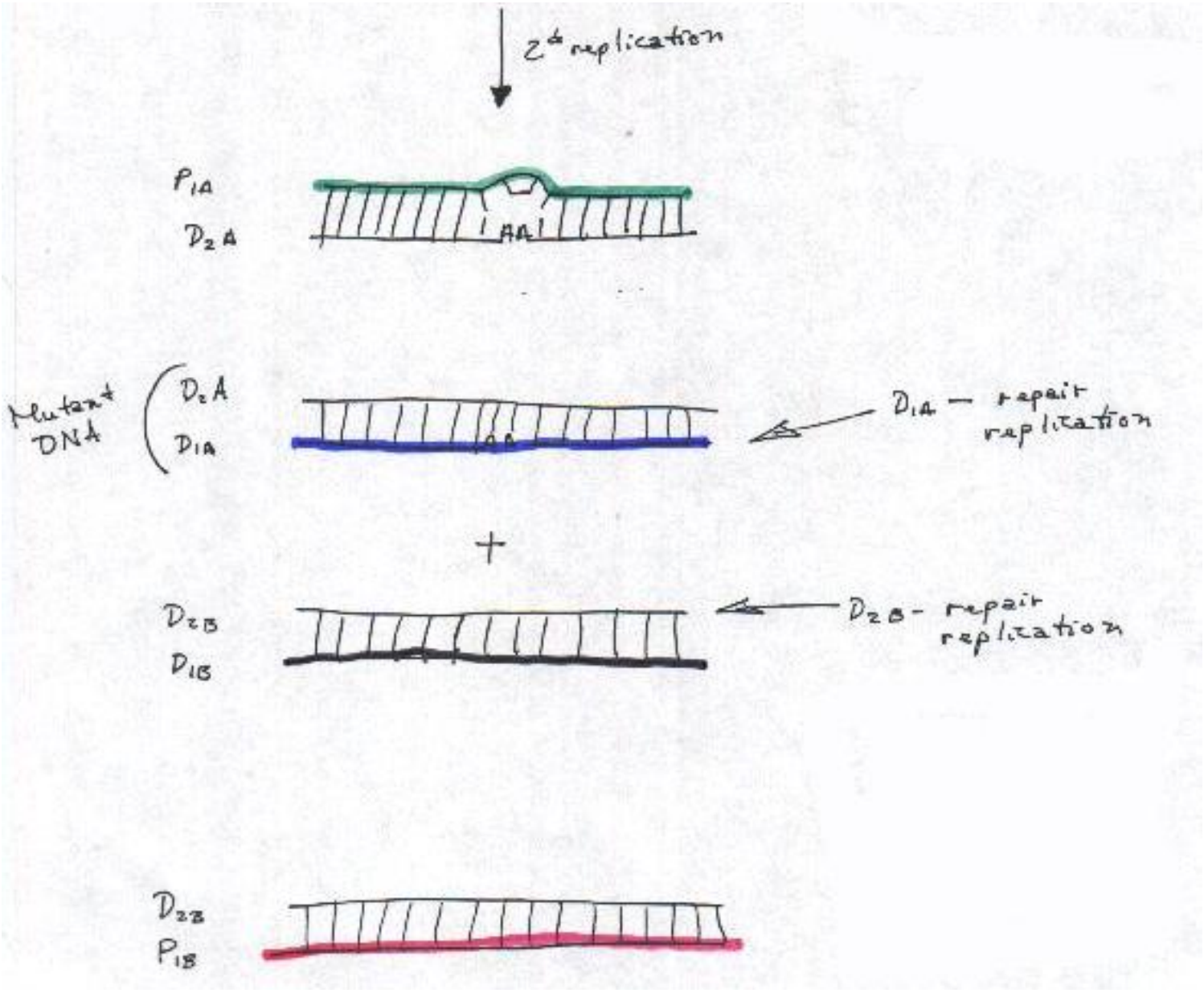

+
Endonuclease a
~12 nucleotides from dimer
"Nick" Incision
5'
3'
5'
P
3'
OH
3'
2d Endonuclease "slice"
~4-5 nucleotides from dimer
5'
P
3'
OH
dimer removed
3'
OH
5'
P
DNA Pol
dNTP's
DNA Ligase
Repaired DNA

The Third Mechanism is Called SOS Repair

- This is the system that, when induced, wreaks havoc with DNA.
- The way in which this mechanism works is as follows: a dimer is induced in one strand of the DNA.
- DNA Pol and dNTP's (generally dATP's) induce DNA replication.
- The product of this first replication is two double strands of DNA: 1 with the dimer still there and 1 that is, more or less, repaired.
- This replication is said to be "trans-dimer" as the process read right over (across) the dimer and permitted its semiconservative replication and repair replication.
- Note also that the first generation of daughter cells is half parent (P) and half daughter (D) DNA.

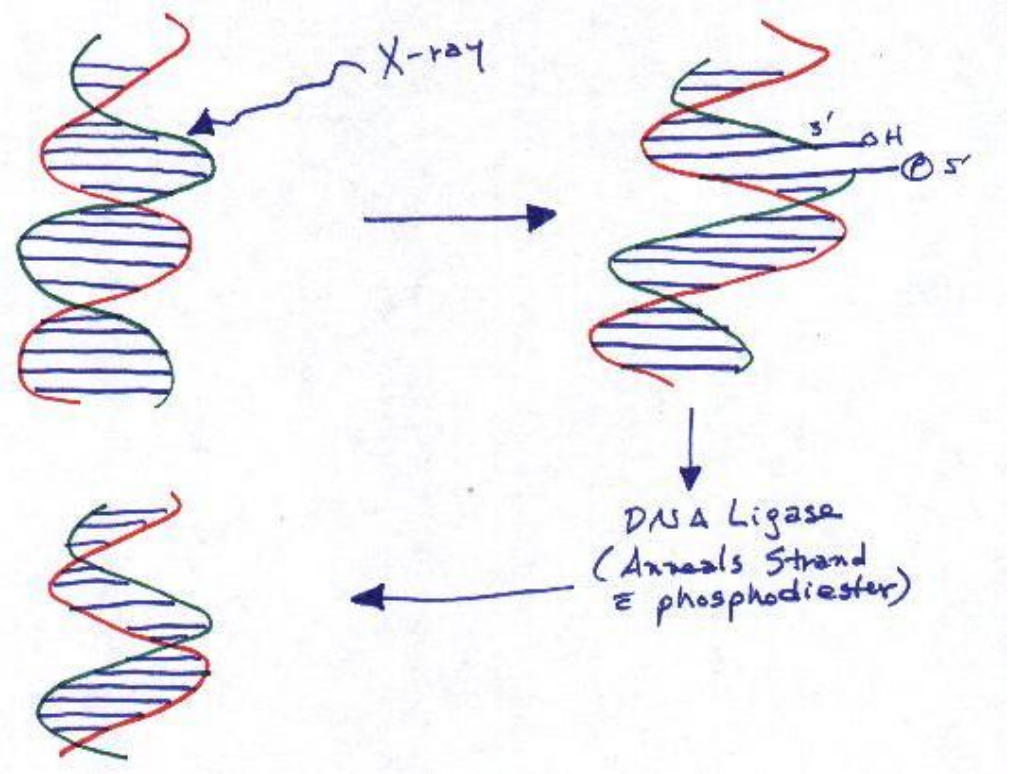

X= any nucleotide XCPT "A"
P_{1A}
P_{1B}
XX
dNTP (Generally "dA's")
DNA Pol
P_{1A} Mother
D_{1A} Daughter
+
D_{1B} Daughter
P_{1B} Mother
1st replication
"trans-dimer"!
2d replication
D_{1A}
D_{1B}
DNA by repair replication

- The second replication/generation is when and where the problems really fire up: now these four strands of DNA from the first replication will ALSO be semi-conservatively replicated.
- Of the four new sets of DS DNA, 1 has the dimer still intact and one has no error -- both of these contain half-original parent DNA and half brand-spanking, new DNA.
- The cell will eventually discard these.
- The other two contain half of their DNA from the first replication and the other half from the second replication.
- These two DS DNA are the product of repair replication.
- One has no errors; the other (designated D2A,D1A in the figure) is the mutant DNA.
- The SOS system has to crank up its protein synthetic machinery to effect its "repair".
- It is also the MAJOR cause of UV-induced mutagenesis.


2d replication
P1A
D2A
AA
Mutant DNA
D2A
D1A
D1A — repair replication
+
D2B
D1B
D2B - repair replication
D2B
P1B

The Last Repair Mechanism to be Examined is The Effect of Ionizing Radiation (e.g., x-ray, gamma ray) on DNA

- When ionizing radiation interacts with DNA, it causes a "nick" in the DNA.
- DNA ligase anneals the "nick" with a phosphodiester bond at the expense of ATP to repair the DNA.



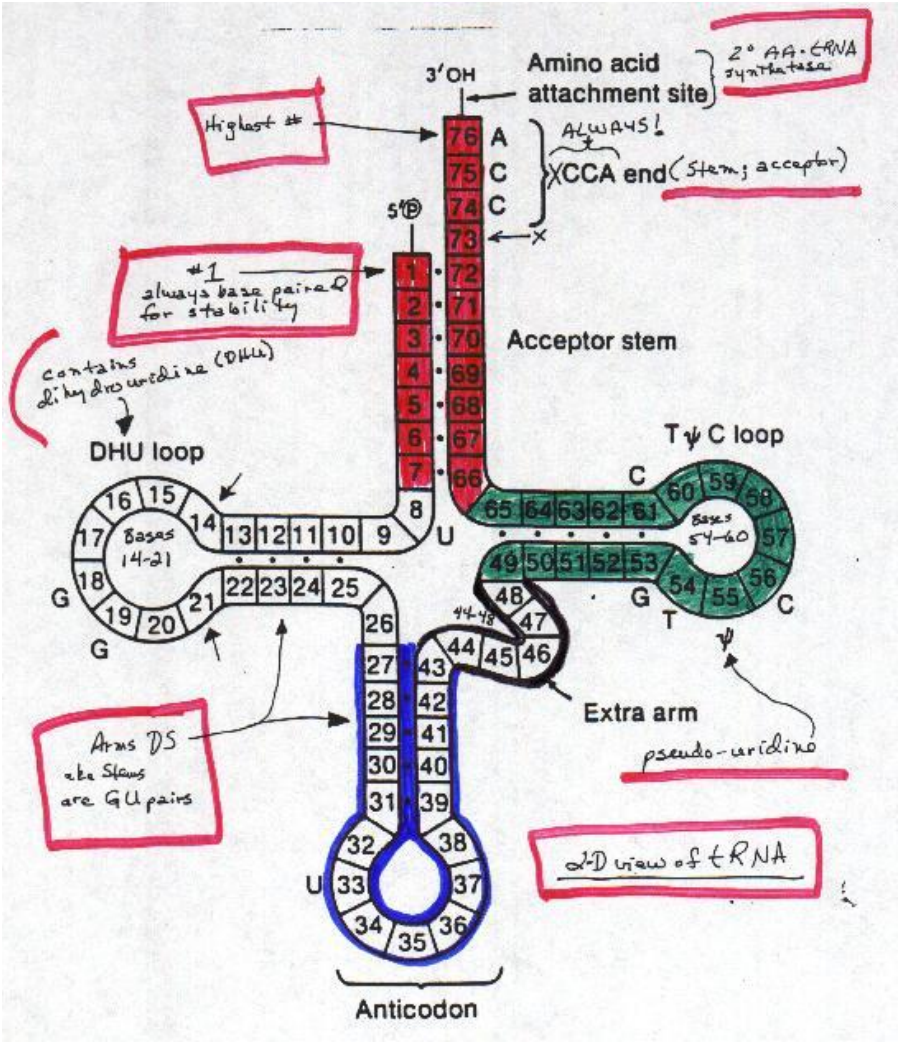
RNA Characteristics with Introductory Transcription and Translation

How DNA, RNA, Enzymes and Ribosomes Work as An Intracellular Team

There are Five Kinds of RNA, All of Which are Templated from DNA.

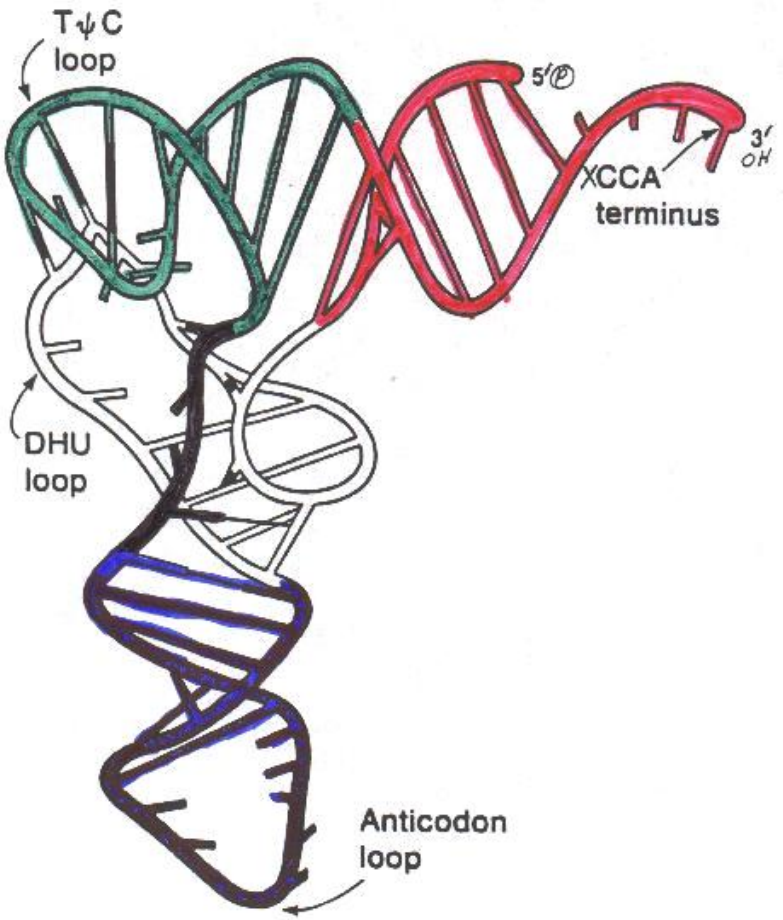
- The first type of RNA is tRNA.
- The "t" stands for "transfer".
- This RNA is the RNA that transfers amino acids to the growing peptide as it is elongating on the ribosome.
- It is single-stranded (SS) and found in the cytosol of the cell.
- tRNA makes up about 15% of all RNA's.

There are at least 20 different kinds of tRNA's as there are at least 20 different amino acids. tRNA has a cloverleaf shape:



1. Bases 1-7 are paired with bases 66-72 to form a double stranded (DS) region in the tRNA that makes it stable/stronger. This region extends through bases 73-76. The whole "arm" is known as the acceptor stem. Note that the 3' -OH is the site of attachment of the amino acid under the direction/catalysis of aminoacyl-tRNA synthetase.
2. Bases 10-13 are paired with bases 22-25 in the DHU loop (on left in graphic). The "H" stands for dihydrouridine (DHU).
3. Bases 27-31 are paired with bases 39-43 to form the anticodon loop of the tRNA (bottom of graphic). Bases 34, 35 and 36 make up the triplet that is the anti-codon.
4. Although not DS, bases 44-48 make up the "extra arm" of tRNA.
5. Bases 49-53 pair up with bases 61-65 in the TψC loop. ψ is pronounced "sigh" and looks like a three-pronged pitchfork; it represents the presence of pseudo-uridine in this loop.
6. The arms or stems of the loops seem to be primarily GU pairs, a rather odd combination, as we typically think about G and C pairing and A and U pairing.
7. The amino acid acceptor end ALWAYS ends with XCCA, where X is any nucleotide, so that A is always attached to the binding/transferable amino acid.

That folds to a more compact "L" shape:



- The region of the tRNA that bonds with the mRNA is called the anti-codon.
- Typically, the tRNA's are identified by which amino acid they transfer, e.g., alanyl tRNA would be represented as "$tRNA^{Ala}$".
- As with ALL RNA's, the concentration of adenine is not equal to the concentration of uracil ([A] [U]), nor are the concentrations of guanine and cytosine equal ([G] [C]).
- This is due to the fact that RNA is single stranded (SS).

- The second type of RNA is "rRNA" or ribosomal RNA.
- It, too is SS.
- It is found in the ribosomes in the cell.
- rRNA makes up about 80% of the RNA's in the cell.
- It is the most stable of the RNA's and is synthesized only when the cell needs more ribosomes.
- The code for the rRNA is found in nuclear DNA.

Third Type of RNA

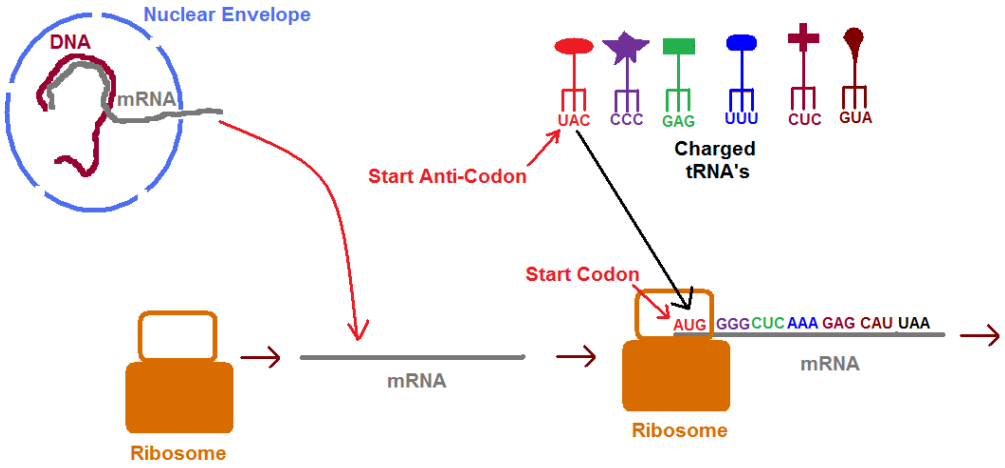
- Messenger RNA (mRNA) is also SS.
- It is synthesized in the nucleus, is sent to the cytosol of the cell and binds with ribosomes.
- It makes up less than 5% of the RNA's as it doesn't "survive" long enough to make up much of the RNA's.
- It has a half-life ($t_{½}$) of 4-24 hours.
- mRNA is around only long enough to drive the synthesis of its specific protein, then it is recycled.
- mRNA is synthesized from a single gene unlike t and rRNA.
- mRNA may range from 70 nucleotides in length up to 20000 nucleotides in length.
- The 3' terminus carries a poly-A "tail" that consists of 20-200 adenosine residues that are added after mRNA is synthesized.

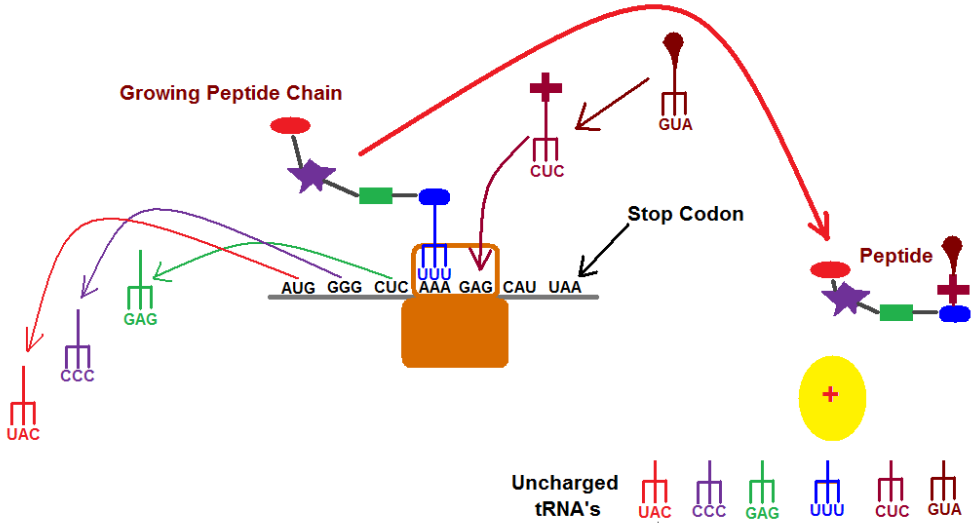
Fourth Kind of RNA

- The mitochondrion makes some of its own RNA, as well, called mitoRNA.
- It is SS and found in, believe it or not, the mitochondrion.
- mitoRNA may be t OR rRNA-type.
- It is utilized in the synthesis of mitochondrial protein.
- Remember, though, that the mitochondrion requires nuclear-coded proteins to function, as well.

- The fifth type of RNA is called small nuclear RNA or snRNA (called "snurps").
- It is found in the nucleus of the cell.
- Some snurps are involved in/with RNA processing.
- It consists of and interacts with ribonucleoprotein.
- They are typically named with a "U" followed by a number., e.g., "1", then completed with RNA: U1RNA, U2RNA, U3RNA, *ad nauseum*.

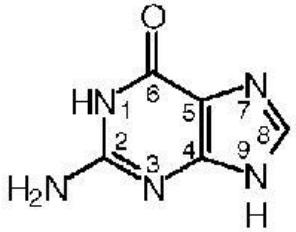
Transcription and Translation Rapid Overview



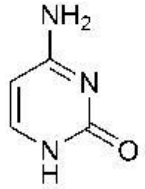


DNA, RNA, Transcription and Translation

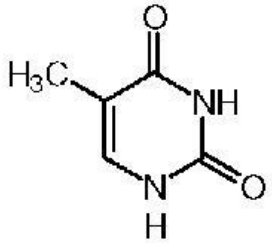
Adenine



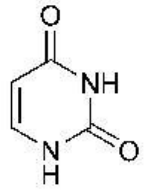
Guanine



Cytosine

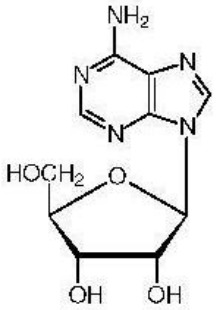


Thymine



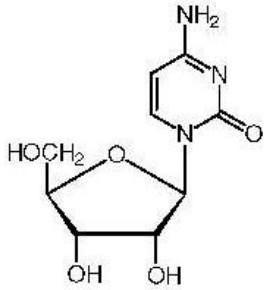
Uracil

DNA, RNA, Transcription and Translation

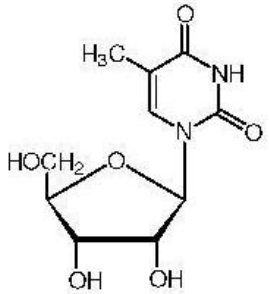


Adenosine
A

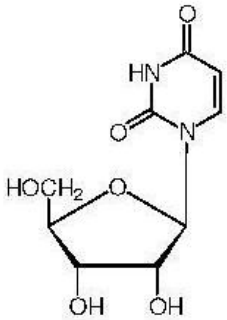
Guanosine
G



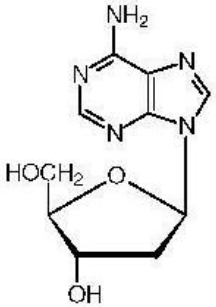
Cytidine
C



Thymidine
T

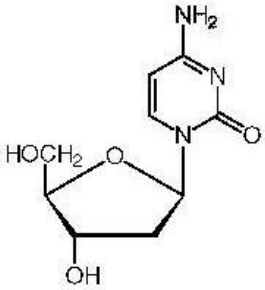


Uridine
U

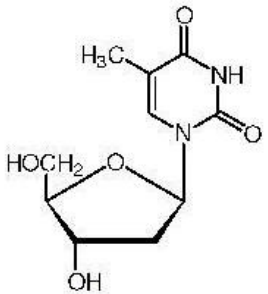


deoxy-Adenosine
dA

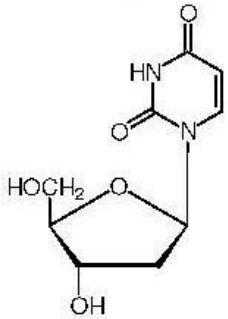
deoxy-Guanosine
dG



deoxy-Cytidine
dC

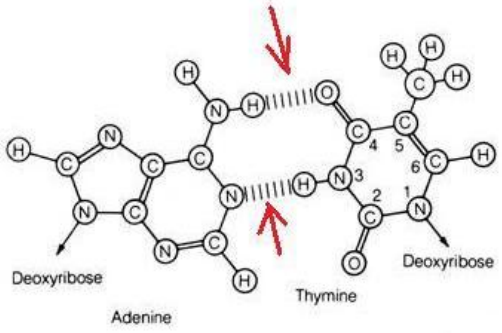


deoxy-Thymidine
dT

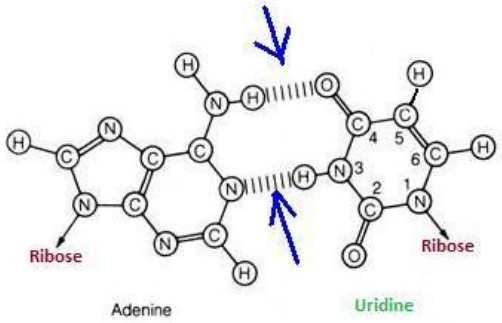


deoxy-Uridine
dU

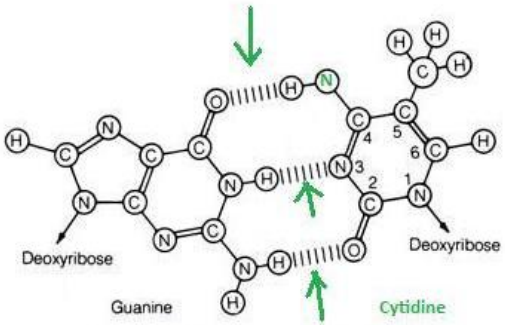
DNA, RNA, Transcription and Translation



In DNA, dA and dT pair up with 2 H bonds linking them; editors reduce the dA and dT to simply A and T to save $$$.

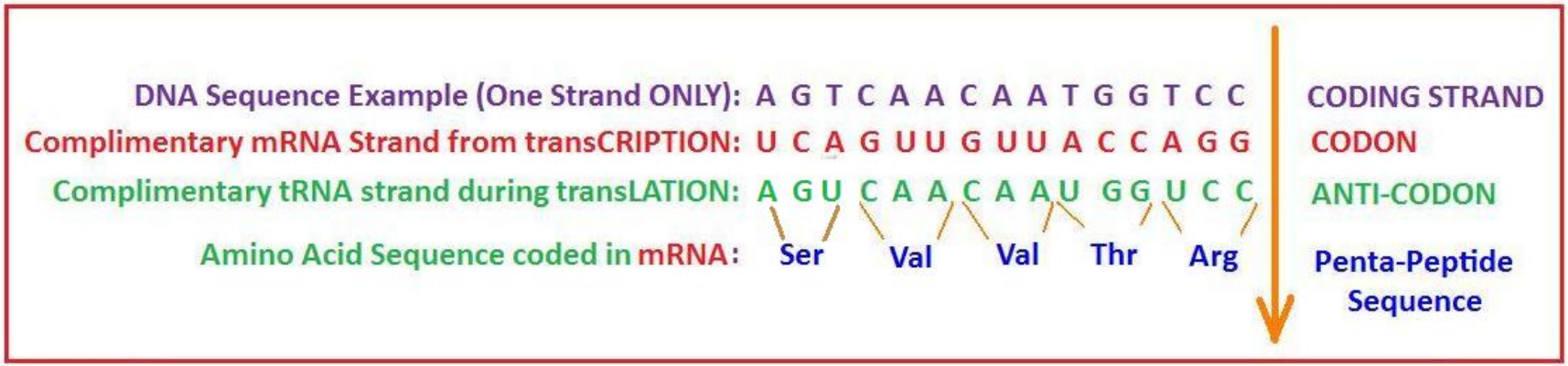


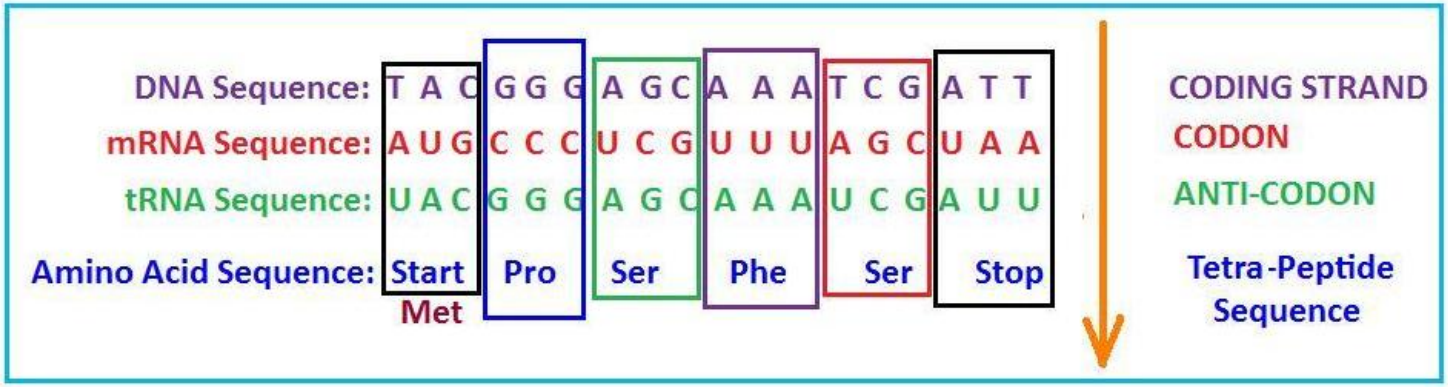
In RNA, A and U pair up with 2 H bonds linking them -- no editorial reduction needed (A,G,C,U are in RNA). G and C continue to pair up, as well, in RNA.



In DNA, dG and dC pair up with 3 H bonds linking them; editors reduce the dG and dC to simply G and C to save $$$.

DNA, RNA, Transcription and Translation





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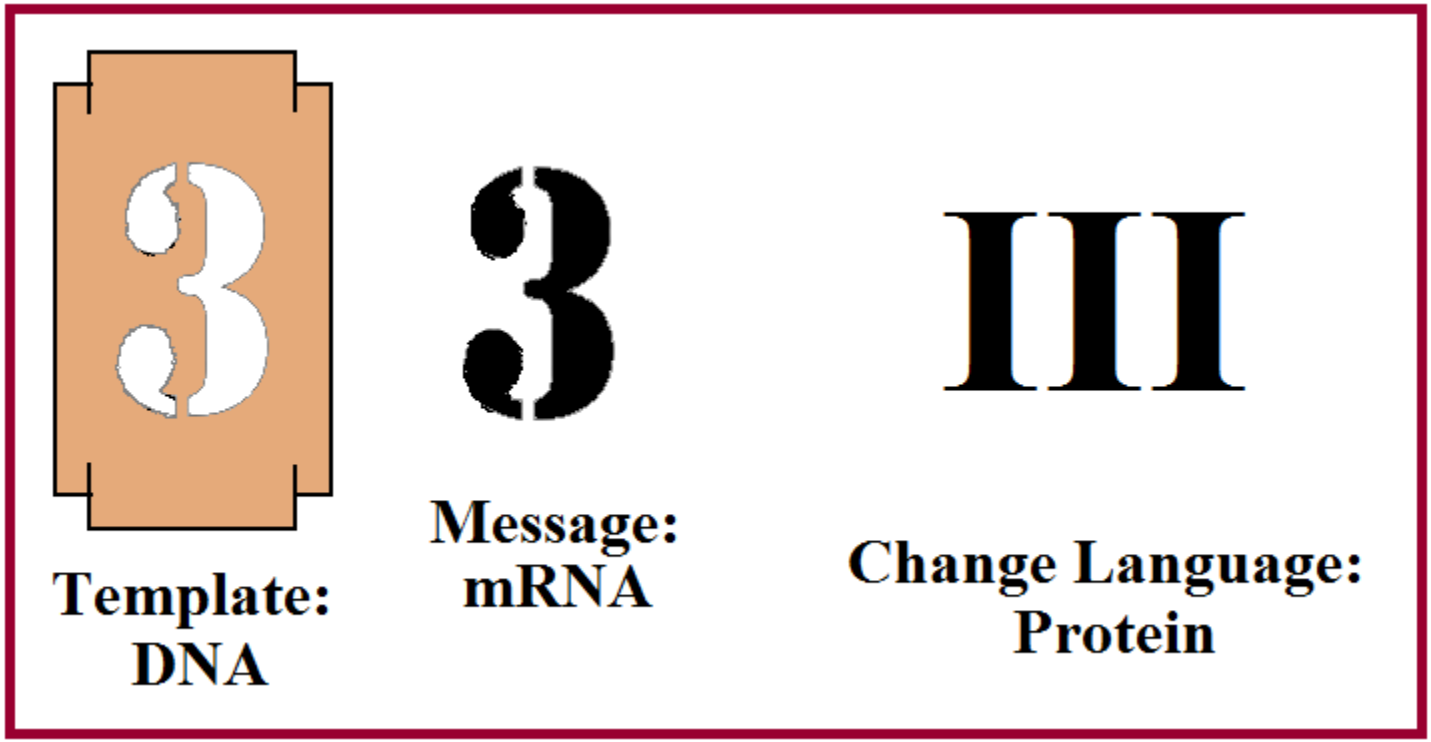
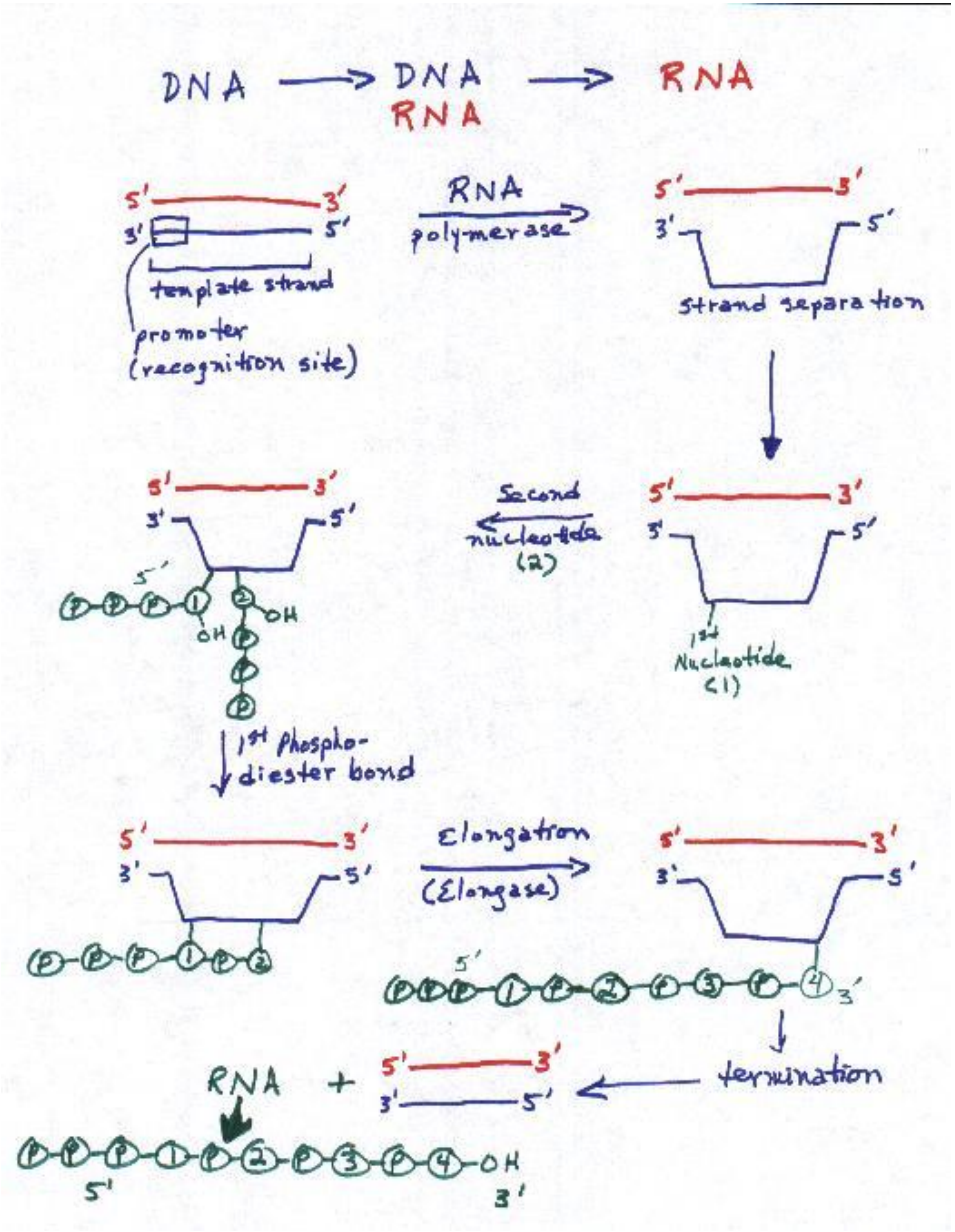


Transcription, i.e., RNA synthesis

- During transcription, only ONE strand of the DNA is actively read and transcribed. The strand to be transcribed contains a promoter (recognition site) that is recognized by RNA Pol. This enzyme causes the DS DNA to separate and it adds on the first of a sequence of nucleotide triphosphates (NTP's) to initiate transcription.
-
- Note that the DNA is the template for the new strand of RNA. In this instance, the bases in RNA will H-bond with the DNA in a complimentary manner, i.e. AU and/or AT (depending on the nucleic acid and orientation) and GC.
-
- As the polynucleotide progresses, the enzyme elongase extends the polynucleotide chain until termination occurs with the release of RNA (much more on this in the RNA chapter).


DNA → DNA → RNA
RNA
5′ 3′
3′ 5′
template strand
promoter
(recognition site)
RNA
polymerase
5′ 3′
3′ 5′
Strand separation
5′ 3′
3′ 5′
Second
nucleotide
(2)
5′ 3′
5′ 5′
1st
Nucleotide
(1)
1st Phospho-
diester bond
Elongation
(Elongase)
termination
RNA + 5′ 3′
3′ 5′
5′
3′
OH

- The genetic code is based upon triplets, i.e., a set of three nucleotides in sequence that code for a single amino acid.
- Each triplet in mRNA is read from 5' to 3'; this triplet in mRNA is called the codon.
- Each triplet in tRNA is called the anti-codon and is read complimentarily to the codon.

Listed below in the table is an incomplete list of codons for some of the amino acids:

Triplet Code (Codon with Amino Acid -- NOT Inclusive)		
AGA = Arg	CCC = Pro	CUA = Leu
AAG = Lys	CAC = His	CGA = Arg
AAC = Asn	UAC = Tyr	UGG = Trp
ACA = Thr	AGC = Ser	UGC = Cys
GAU = Asp	AUA = Ile	GUG = Val
GAA = Glu	GGG = Gly	UUU = Phe
CAA = Gln	AUG = Met (Start)	GCA = Ala
UAA = Stop "Ochre"	UAG = Stop "Amber"	UGA = Stop "Opal"

Note that, in some instances, the difference between amino acids is one (1) nucleotide in the triplet, e.g.,

- mRNA sequence: AUG-CAC-AGA-CCC-UGC-UAA
-
- amino acid sequence:

(Start) Met-His-Arg-Pro-Cys-Stop

-
- If you alter this sequence by placing an "**A**" after 9 bases, the new sequence is:
- mRNA sequence: AUG-CAC-AGA-**A**CC-CUG-CUA-A__
- New amino acid sequence:

(Start) Met-His-Arg-Thr-Leu-Leu-----

Mutations

- One shift, one base change alters the whole protein after the insertion of the "**A**".
- A *mutation* is any change that presents in the DNA of a cell.
- Mutations are chance activities and occur spontaneously in all nuclear material.
- Mutations are neither good nor bad (like emotions) and are simply a natural occurrence of cellular activities.
- Mutations can not be predicted, nor can the effects be predicted - caveat: some ARE predictable, i.e., the ones we recognize and have studied extensively.

Definitions

- *Wild-type DNA* is typical DNA found in nature.
- *Mutagenesis* is the production of a mutation.
- A *mutation* is any change that presents in the DNA of a cell. Mutations are chance activities and occur spontaneously in all nuclear material. Mutations are neither good nor bad (like emotions) and are simply a natural occurrence of cellular activities. Genetic changes are not as a result of a mystical punishment as thought around the turn of the century.
- (One of the biggest myths was that "God" was punishing a woman who had a child with Down Syndrome. Of course, men were the ones who pushing this idea. We now know that at least a third of all cases of children with Down Syndrome are paternal in origin. As research unfolds, I expect that we'll find that it will come out to 50/50 between parents.)

- Mutations can not be predicted, nor can the effects be predicted - caveat: some ARE predictable, i.e., the ones we recognize and have studied extensively.
- Those compounds that interact with DNA and increase the frequency with which bases are altered or which causes the likelihood of mutation are called *mutagens*.
- Most mutations impair cell function rather than increase useful cell functions, therefore, mutations are though of as being harmful. Even with incredible minimal exposure to mutagens, there is still a small likelihood that a gene may mutate.
- This is called a *spontaneous mutation*. The frequency of spontaneous mutations vary greatly between genes and organisms. When this frequency increases, it is assumed that some mutagen is causing it.

The table, below, summarizes different types of mutations.

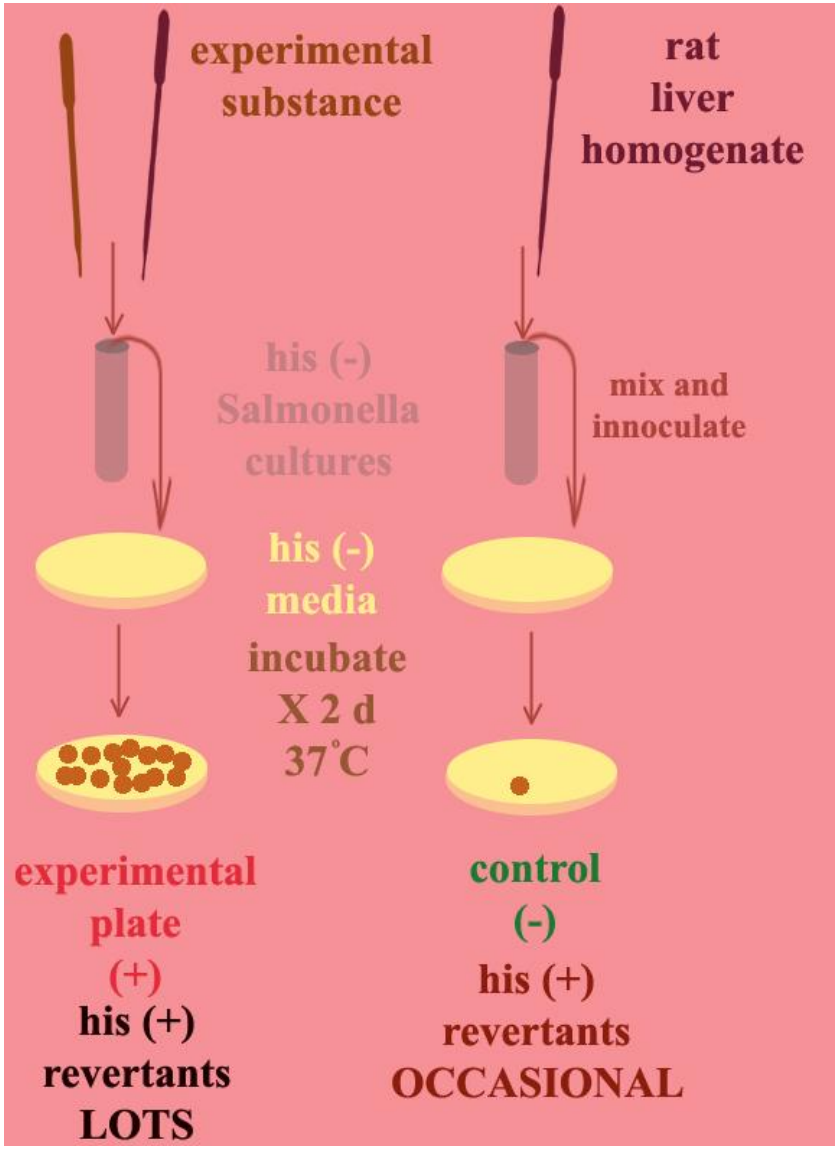
Type of Mutation	Example
Wild type ("normal")	**DNA is in cell nuclei.**
Point	**DNA is in cell nuclei (NO period)**
Insertion	**DNA is NOT in cell nuclei.**
Gene duplication	**DNA is in IN cell nuclei.**
Gene duplication with point mutation	**DNA is in AN cell nuclei.**
Chromosome duplication	**DNA is in cell nuclei.** **DNA is in cell nuclei.**
Translocation	**DNA is cell nuclei in.**
Inversion	**DNA is in cell ielcun.**
Frameshift*	**DN Ai sin cel lnu clei.**

*removal of or insertion of bp sequences that alter the reading of the DNA sequence.

- In terms of mutations of hereditary and somatic forms, when the mutation is passed onto the offspring it is said to be inherited, hereditary, genetic for lower organisms.
- For MAN there are two sort of mutations:
  - hereditary, which occurs ONLY in sex cells: sperm and ova (includes BRCA-1 and BRCA-2); and
  - somatic mutation, which consists of most mutations.
- These occur in all other cells.
- Somatic mutations are NOT hereditary and are NEVER passed onto progeny.
- Hence, there is a genetic and an environmental element involved in cancer.
- It is of significant interest that mutation, aging and cancer are tightly linked by somatic mutations.

- A *carcinogen* is an agent that causes cancer.
- A *forward mutation* causes DNA to change from wild type to a mutant form. This is to what mutations refer.
- A *reverse mutation* is a mutation that causes reversion from mutant to wild-type. This form of mutation is also known as *revertant*.
- A *pseudorevertant mutation* is a "double mutant". These grow under wild type conditions and are not easily distinguishable from wild types. They are generally due to one gene "out-regulating" another.

- A great example of applied mutational studies is the Ames Test. This test uses a strain of *Salmonella typhimurium* that lacks the ability to synthesize its own histidine (his-).
- The way it works is as follows: under normal, wild-type conditions, this micro-organism has the ability to synthesize his (his+).
- In the presence of dextrose (glucose), electrolytes and suitable medium, it grows well.
- The his- variant when grown in the presence of identical medium as the his+ wild-type variant.
- The "trick" to this test is to take the his- variant and grow it in the presence of glucose, electrolytes, rat liver homogenate and varying concentrations of suspected mutagen.
- If the suspected mutagen is a mutagen, it will cause the his- to mutate to either a true revertant or a pseudo-revertant and the bacterium will grow.
- The growth will be proportional to the concentration of mutagen/carcinogen in the medium.



- One example of a chemical that causes mutations is sodium nitrite ($NaNO_2$). Sodium nitrite is found in spinach, naturally, and is a food preservative that is added to keep the meat colored red and to reduce spoilage. The problem with sodium nitrite is that when it reacts with stomach acid (HCl) it forms nitrous acid (HNO_2)(remember this from the Diazotization experiment?)
- The nitrous acid kills cells and increases mutations. The way that this works is several-fold.
- **First** nitrous acid oxidizes the amine on Adenine to a ketone, causing the synthesis of hypoxanthine (HX). HX hydrogen bonds with C. The problem, here, is that A and T generally double hydrogen bond and G and C triple hydrogen bond. By altering the hydrogen binding "sites", as it were, there are only two hydrogen bonds between HX and C. The DNA is altered due to this altered bonding, i.e., is mutated.
- **Secondly**, nitrous acid oxidizes the amine on C to a ketone to form U. Remember that U is generally in RNA and binds with A during transcription/translation. Because of this mutation, i.e., forms U=A base pair rather than T=A base pair, the U HAS to be changed to T. The mechanism is one of those previously studied in DNA Replication and Repair.

- Other chemicals include nitrosamines (related to sodium nitrite), e.g., pesticides, herbicides, 2d hand smoke.
- Indeed it is WELL known that the more one smokes, the faster one dies. Current research is also showing that second-hand smoke is emerging as a killer, as well. Recently, third-hand smoke has been identified.
- Mutations are identical whether they are spontaneous (occur as a result of no known cause) or induced (due to something from the environment).

- Transition mutation: Those that occur with the highest frequency occurs where the point mutated is accomplished by changing 1 Pu (purine) for another Pu; or 1 Pyr (pyrimidine) for another Pyr. Those that occur with the least frequency are to change 1 Pu for a Pyr; or 1 Pyr for a Pu.
- Missense mutation: these are point mutations that lead to amino acid changes in the codon, e.g., HbA and HbS (hemoglobin A in healthy individuals and hemoglobin S in those with sickle cell disorder).
- A variant of this mutation is the temperature sensitive (Ts) mutation: This occurs if the new protein is active at one temperature (generally 30°C) and inactive at another (generally between 40°C and 41°C).
- Nonsense mutation: A point mutation that produces stop codons from a "normal" amino acid codon. These are also known as chain termination mutation and there are three sequences known: Amber (UAG), Ochre (UAA) and Opal (UGA) - you learned UAA as one of the stop codons back prior to translation. These are the ONLY stop codons produced as a point mutation. Amber is usually denoted Am, Ochre as Oc and Opal as Opal.
- Neutral Mutation: A point mutation in a codon that does not change the amino acid or the phenotype.

- More complicated mutations may add, delete or move pieces of, or complete, chromosomes. There are four of these that we are interested in studying:

- Deletions: occur with a loss of base pairs in the range of 100's to 1000's of bp's. These are not able to revert to wild-types as are point mutations.
- Duplications: occur when a piece of DNA is added to a chromosome that contains an identical sequence of DNA. This suggests having "spares" of chromosomes incase of some sort of catastrophe at the nuclear level. It also suggests that this "night" increase the survival of the organism.
- Translocations: Occurs when a piece of DNA is moved from 1 new site to a new site, e.g., 14/21 in Robertsonian translocation, previously discussed.
- Inversions: Occurs where a piece of DNA is snipped out, turned around and replaced in the same spot. This mutation alters the genotype and phenotype, as well. These sequences are valuable since they do NOT recombine during meiosis so they don't pair up since they are different. This mutation seems to permit gene preservation spanning multiple generations.

- In terms of mutations of dominant and recessive natures, they will occur either on the autosomes (22 pairs of chromosomes that code for the body) or the sex chromosomes (23d pair of chromosomes; X and Y for either XX (female) or XY (male)).
- Remember, too, that the genotype is the genetic information (the DNA sequence) while the phenotype is the expression of the genetic information.
- If alleles are identical, they are homozygous; if not identical, they are heterozygous. The allele expressed as phenotype is said to be dominant; the allele NOT expressed as phenotype is said to be recessive. (This last sentence is not altogether true as we'll see in later lectures.)
- A deviation from homozygous to heterozygous is a mutation, too.

- In terms of mutations of hereditary and somatic forms, when the mutation is passed onto the offspring it is said to be inherited, hereditary, genetic for lower organisms.
- For MAN there are two sort of mutations: hereditary, which occurs ONLY in sex cells: sperm and ova (includes BRCA-1 and BRCA-2); and somatic mutation, which consists of most mutations. These occur in all other cells. Somatic mutations are NOT hereditary and are NEVER passed onto progeny.
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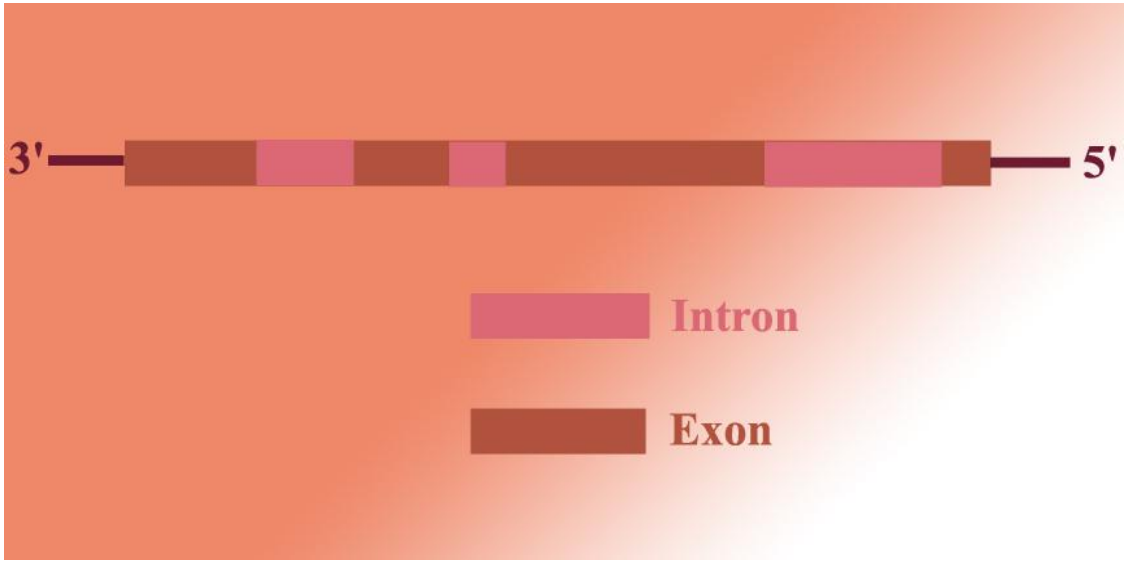
- Even with incredible minimal exposure to mutagens, there is still a small likelihood that a gene may mutate.
- This is called a *spontaneous mutation*.
- The frequency of spontaneous mutations vary greatly between genes and organisms.
- When this frequency increases, it is assumed that some mutagen is causing it.

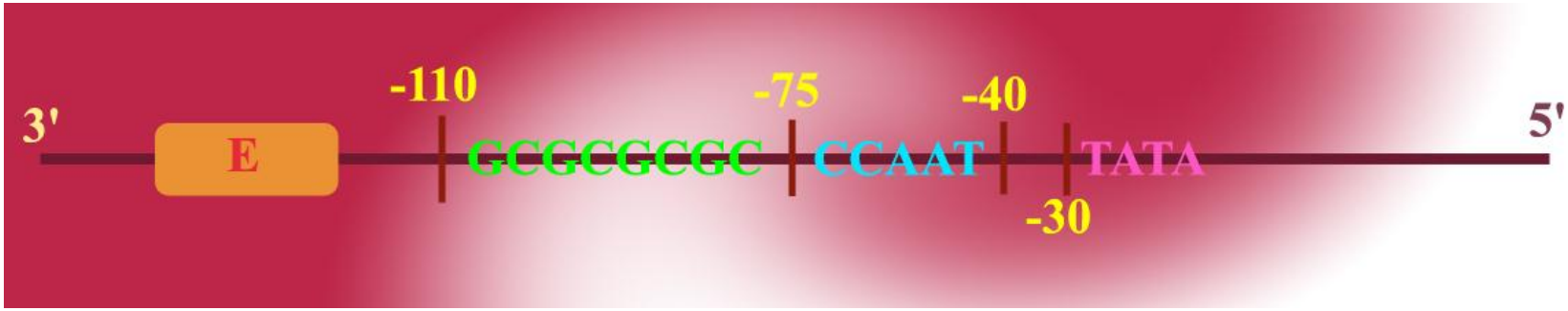
Transcription and Translation: From DNA to Protein[s]

A Generic Gene "Schematic" on An SS Piece of DNA



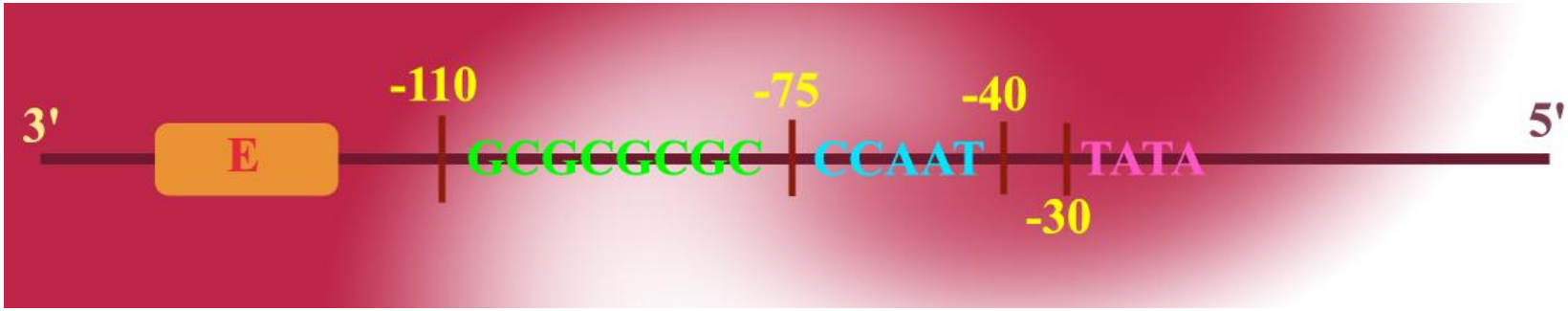
- Each gene contains units called exons (these contain coding information that takes up a very small space in the gene) and introns (contains non-coding information; they are not well understood and they take up a lot of space in the gene).
- Hence, not all of the DNA in a single gene is required for the synthesis of a protein.

Enhancers - 1



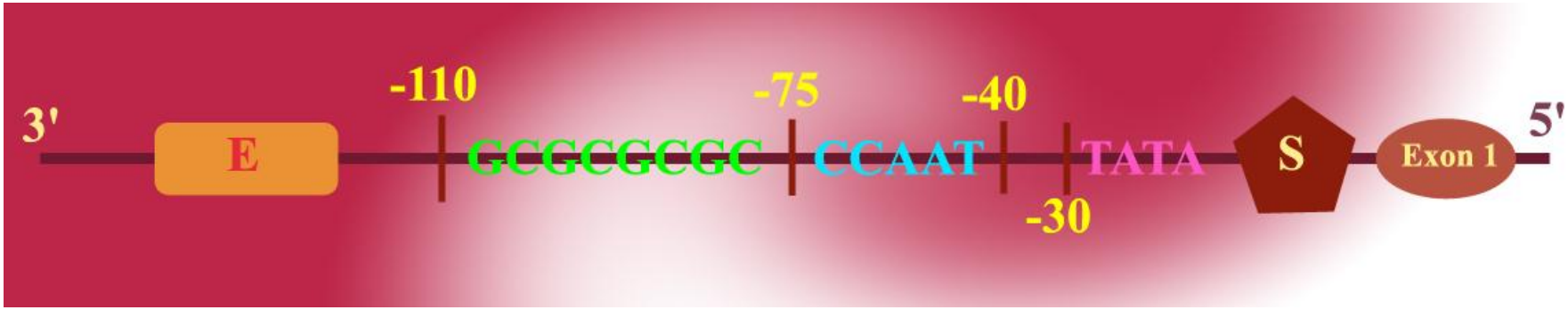
- This is a gene-specific sequence of DNA, 50-500 nucleotides long and may be repeated a number of times.
- Enhancers are located 100 base pairs (bp's) to thousands of bp's (kbp's) up OR downstream of the DNA sequence that will be read or transcribe.
- Enhancers are conditional, i.e., not every thing "turns them on".
- Enhancers are also known as recognition sites.
- Since enhancers are DNA-coded, they are called "cis" acting.
- Factors that bind to enhancers are called "trans" acting.

Enhancers - 2



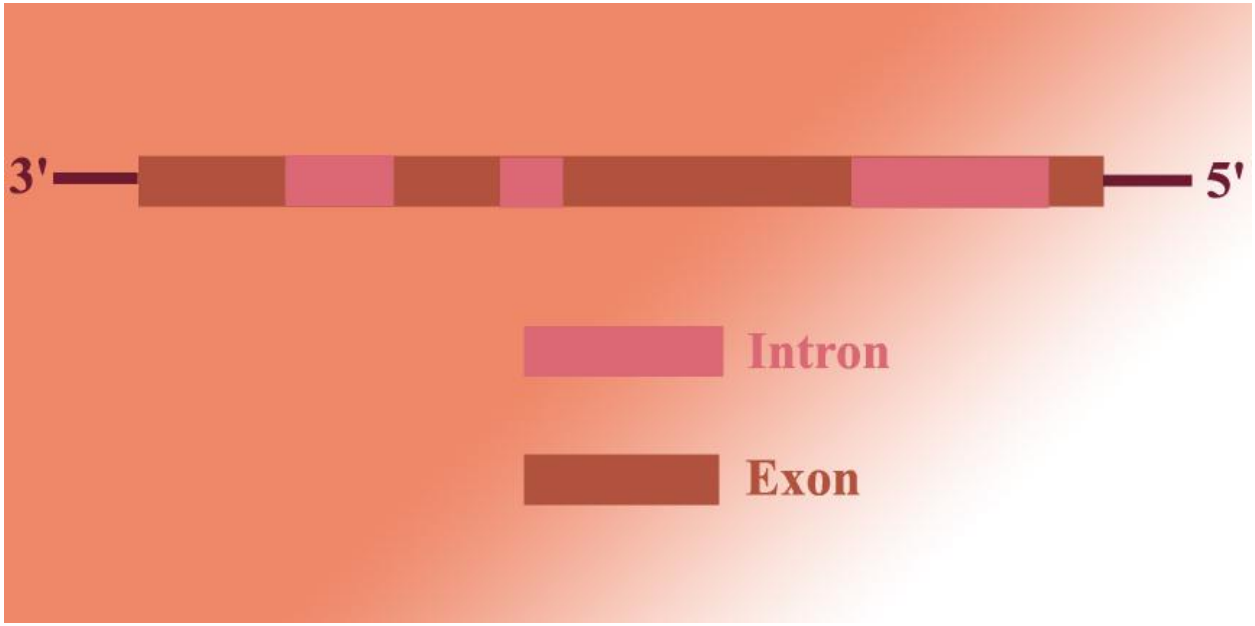
- Three other regions are observable:
  - a sequence of GC's,
  - a region of CAAT and
  - a TATA region.
- All three of these regions are called promoter sites. These are promoters for RNA Pol II (Slide coming). The region between 75-110 bases downstream of the transcription start site (more coming on this later; written as -75 - -110) is called the GC box.
- This is observed only every now and again, i.e., this is rare.
- Between -40 and -75 is a region of four nucleotides, CAAT, called the cat box. This is observed a bit more frequently than the GC box.

Enhancers & Start Box



- The promoter site almost always present is the TATA region between the start site (S) and -30.
- This latter region is called the Hogness box and is about 25 bp up from the transcription start site.
- Data indicates that the TATA box, as it's also called, is the "code" for the first base to be transcribed.

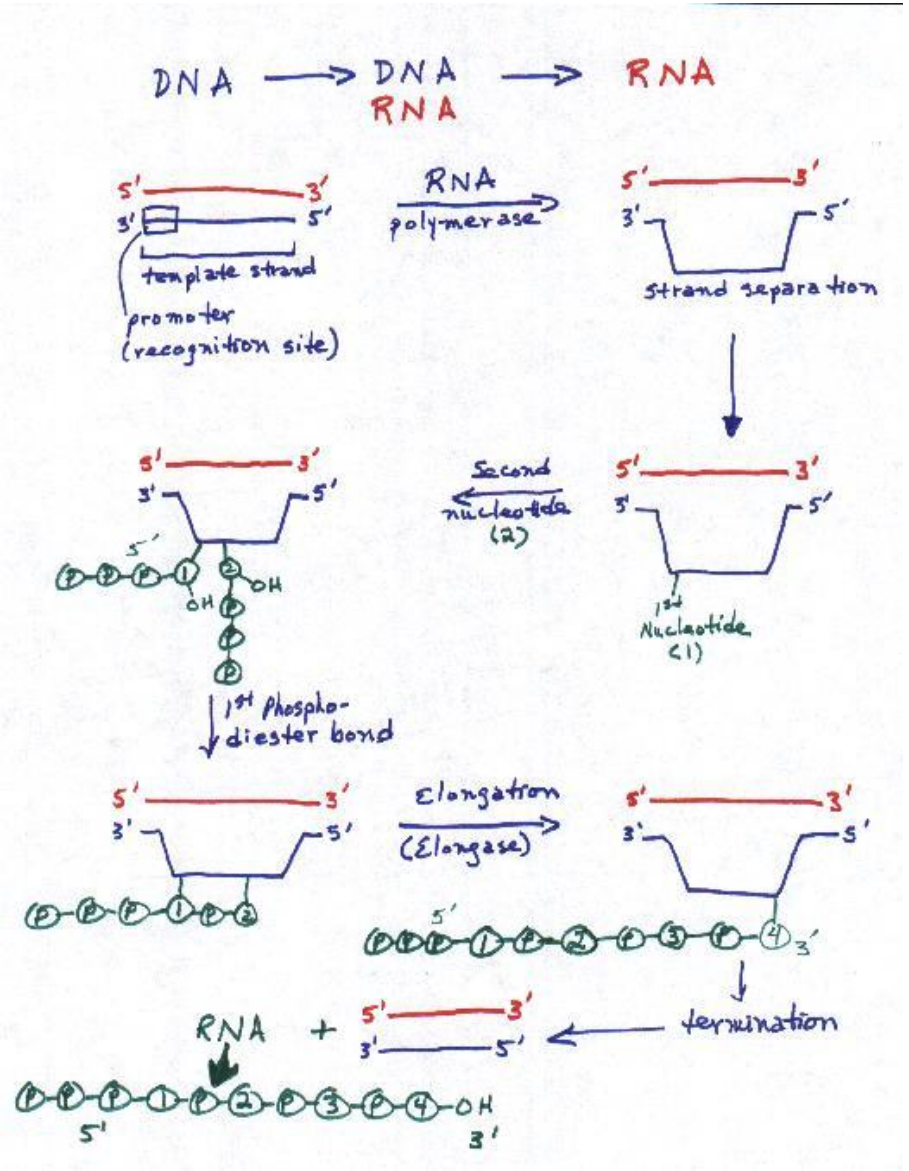
- Enhancers increase transcription more than 100-fold. The properties of a good enhancer are as follows:
  1. They must not possess promoting activity.
  2. They can be 1 kbp up OR down from the promoter.
  3. Some work only in 1 kind of cell, 1 kind of tissue and/or 1 kind of cell type.
  4. Some work where ever they "desire".
  5. They may be spliced into other regions of DNA.
  6. Their removal slows transcription to one-hundredth the activity that it was.
  7. They may be cut out and inserted backwards into the same spot without loss of genetic activity. This latter characteristic says that the enhancers are read front-to-back OR back-to-front for full activity.



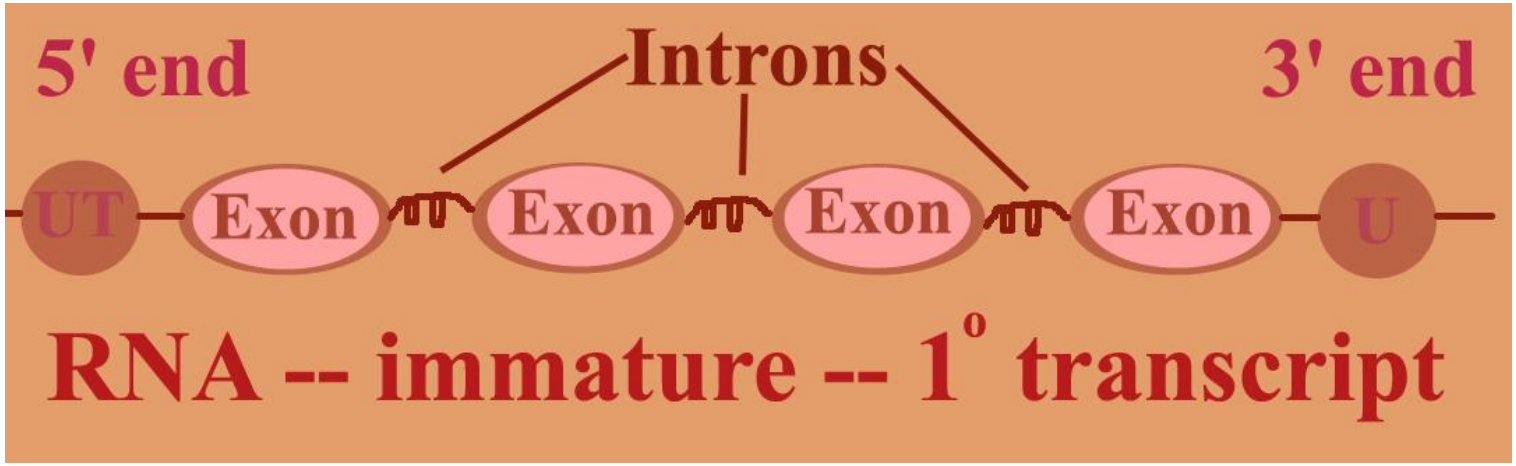
- In order for transcription to continue, the DNA must be unwound.
- Once the DNA is unwound and opened up, RNA Pol II may begin reading this SS strand of DNA

RNA Polymerases (RNA Pol) – An ASIDE		
RNA Pol I	RNA Pol II	RNA Pol III
--in nucleolus --for small rRNA synthesis	--requires transcription factors to bind to its promoters (*opens up chromatin to less organized DNA for transcription ; remember that histones reduce the availability of DNA to DNA'ses making the DNA more stable and less prone to breakage; RNA Pol knocks out this property to perpetuate transcription*) --synthesizes all mRNA does not bind to promoters, but around transcription start site --reaches ahead to locate the transcription start site --in nucleus	--in nucleolus and cytosol --for tiny rRNA and tRNA synthesis --promoters for III are downstream from the transcription start site and within the region being transcribed --reaches behind to locate the transcription start site

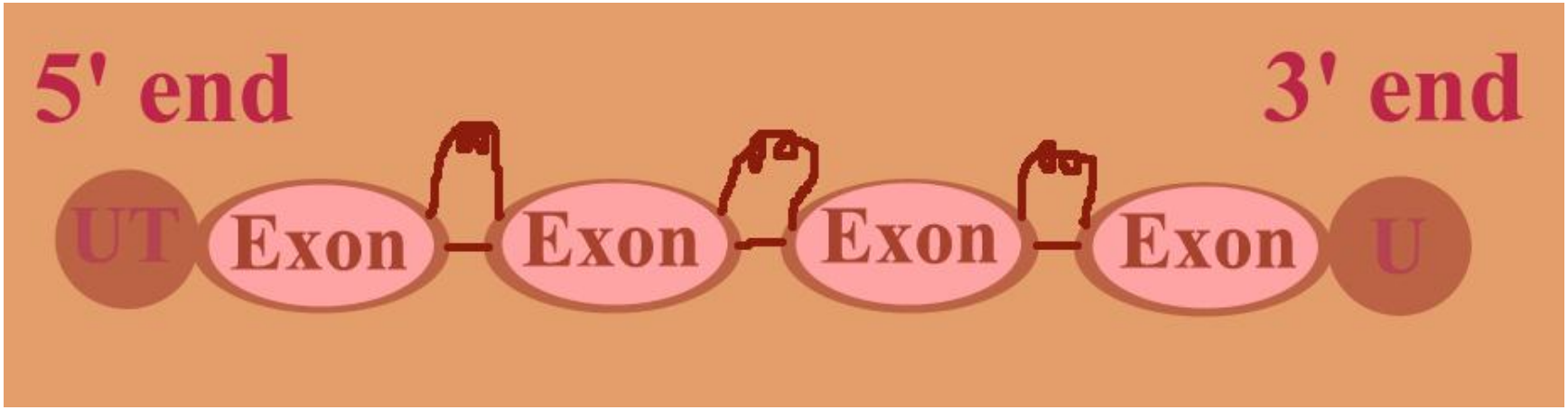
- This strand that is opened up for transcription is called the **template strand**, the **noncoding strand** or the **nonsense strand**. It is so called because the RNA that will be synthesized is complimentary to this DNA strand.
- The NONIDENTICAL strand of DNA is complimentary to the template strand and is identical to the transcript *non obstante* T to U variations and is called the **coding or SENSE strand**.
- As opposed to replication, RNA Pol's require no primer or primase to initiate transcription.



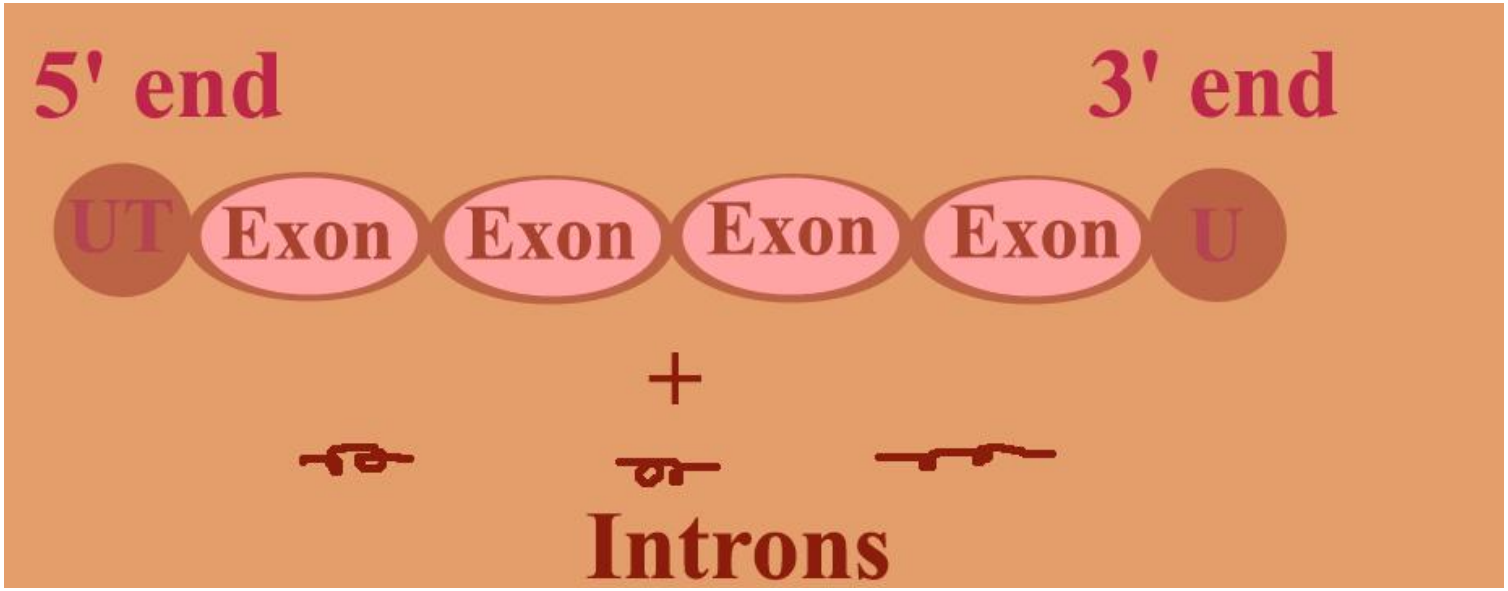
A Simplified Version of Transcription Followed by Post-Transcriptional Modification



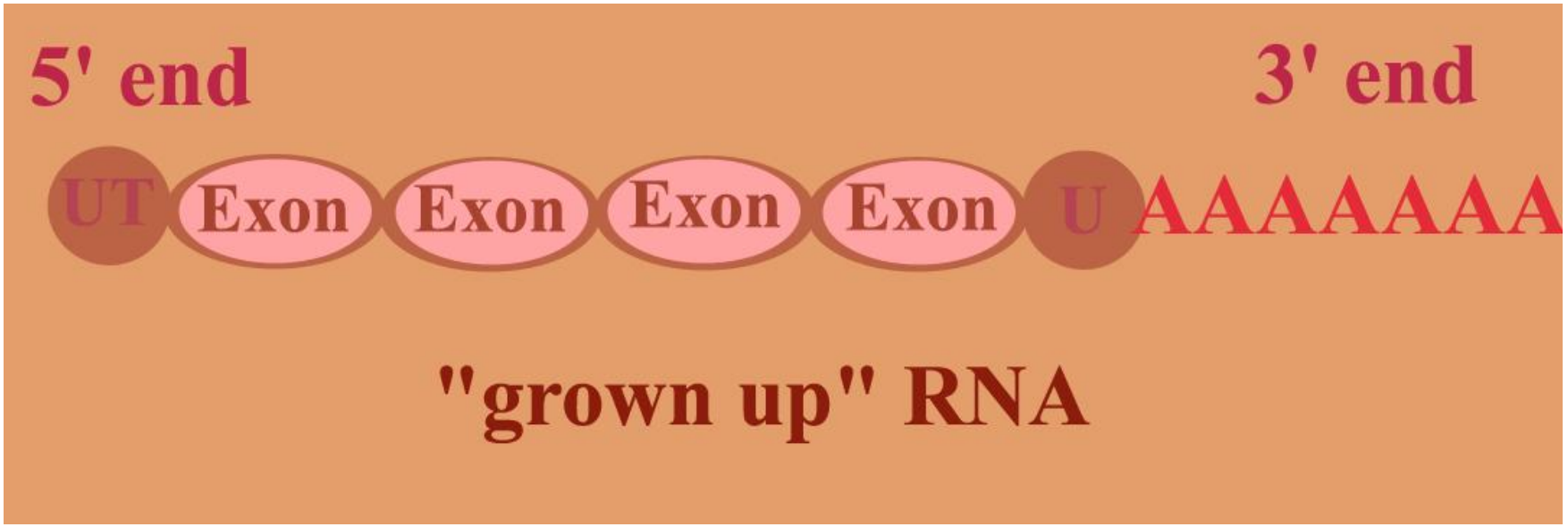
- The 5' region of the immature transcript contains the untranslated leader (UT) that contains NO amino acid information.
- The 3' region contains the untranslated region (U) that, likewise, codes for no amino acid information (the UT and U regions MAY provide the structure that the ribosome uses to bind the mRNA - do NOT confuse UT and U regions with the snurps: See Below).
- The bottom line in this process is to remove the non-coding introns and splice the coding exons together to bring them into closer proximity for ribosomal-reading.



- The first step in post-transcriptional modification is to isolate the introns. Once they are isolated, they are spliced out.



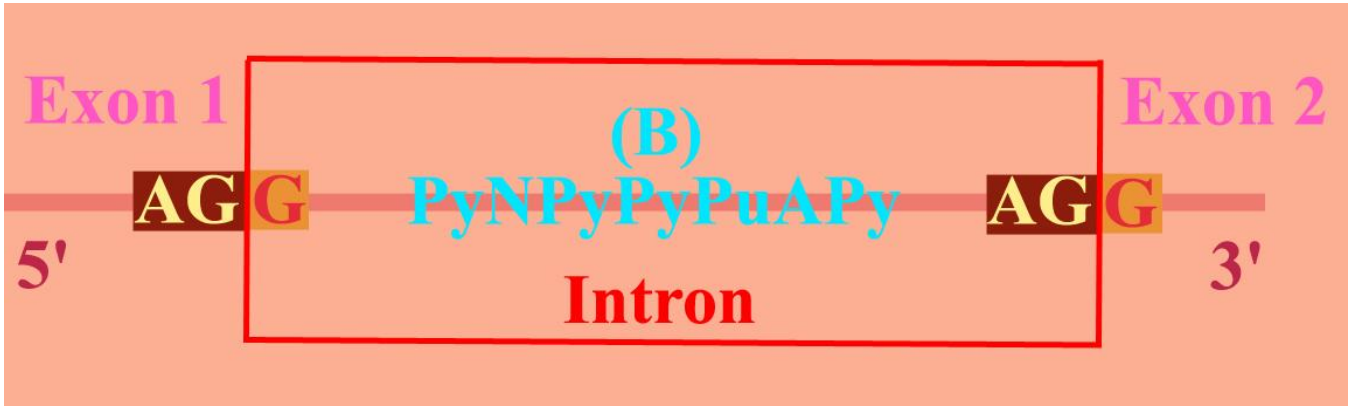
- This process is catalyzed by the RNA, itself. When RNA acts as an enzyme, it is called a ribozyme. This is one of several examples/exceptions of enzymes that do not fit the traditional definition of enzymes, i.e., they must be proteins; RNA breaks this "rule". Once the exons are spliced together, the introns are removed (30-50% of the primary transcript).



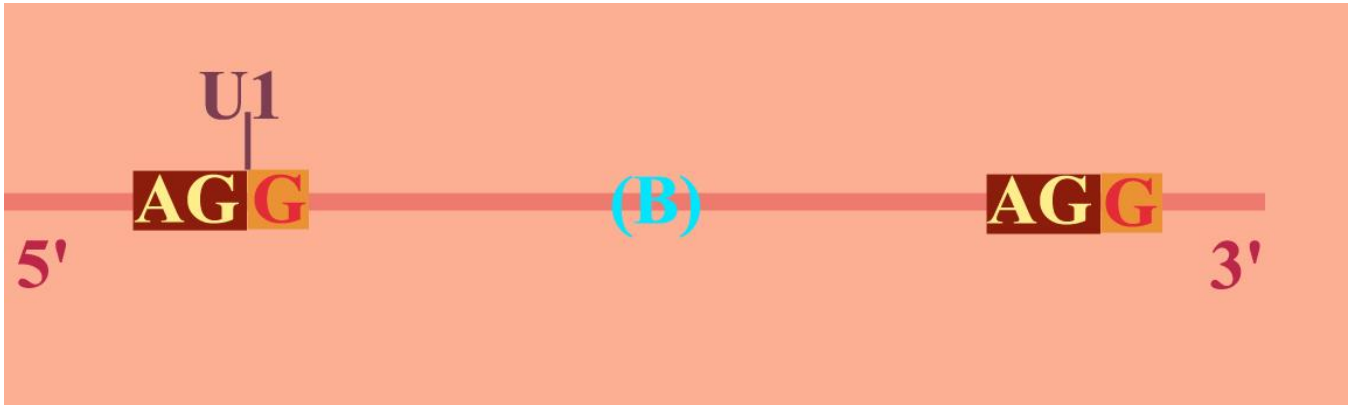
- The third processing, the addition of the poly-A tail, is directed by poly-A polymerase - no DNA template is required for this step.
- The mature RNA is now readable.

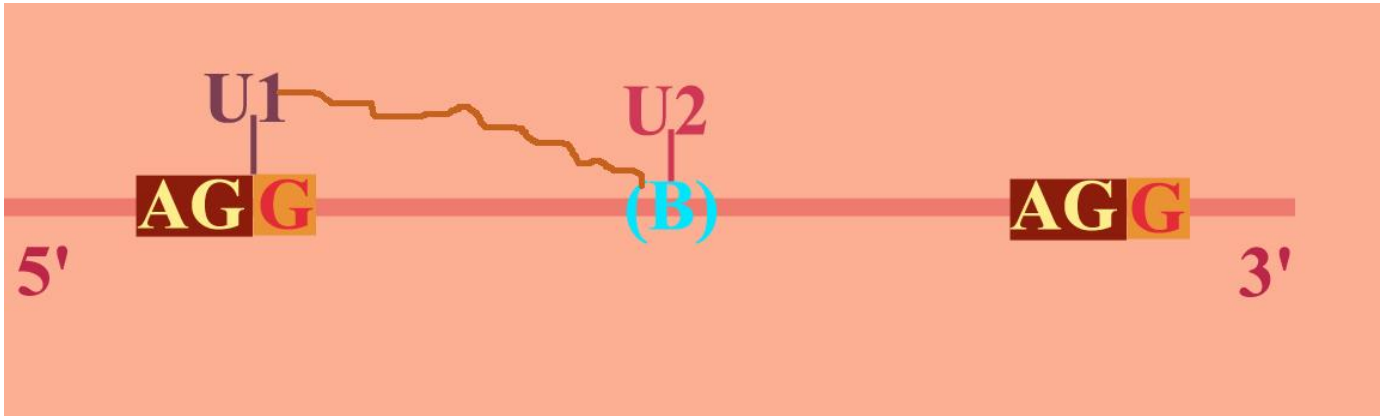
How, then, does all of this splicing occur?

- Splicing requires the primary transcript (also known as hnRNA: heterogeneous nuclear RNA), snurps (U1, U2, U4, U5, U6) and an unknown amount of proteins (perhaps they are used as scaffolding, for binding or for both).
- When all of these are present, they make up a **spliceosome**.

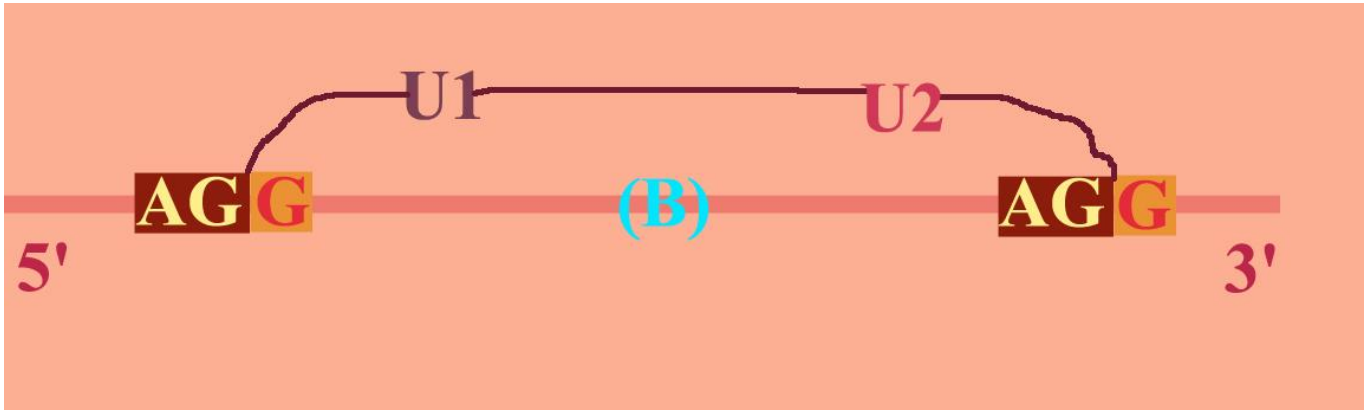


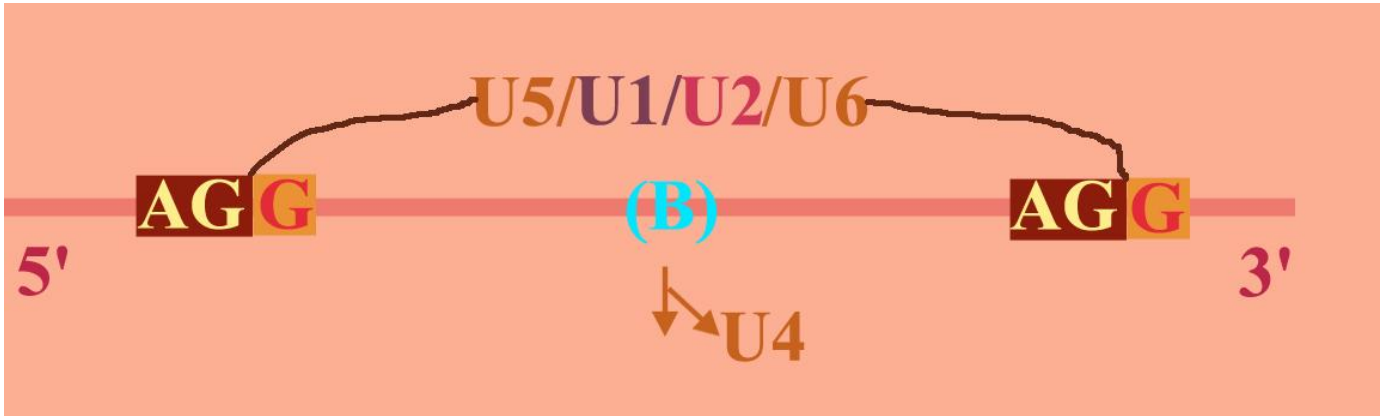
- The ends of exons seem to be AGG from 5' to 3'. Inside the intron is a sequence of pyrimidines (Py) and purines (Pu) with any nucleotide (N) and adenosine. The sequence is PyNPyPyPuAPy, again, from 5' to 3'. This sequence is called a consensus sequence, a conserved sequence that seems to act as the identifying feature of the splice site. **Sometimes this consensus sequence is called a branch site (B).**
- The primary transcript reacts with U1. U1 binds to the 5' end of the intron.



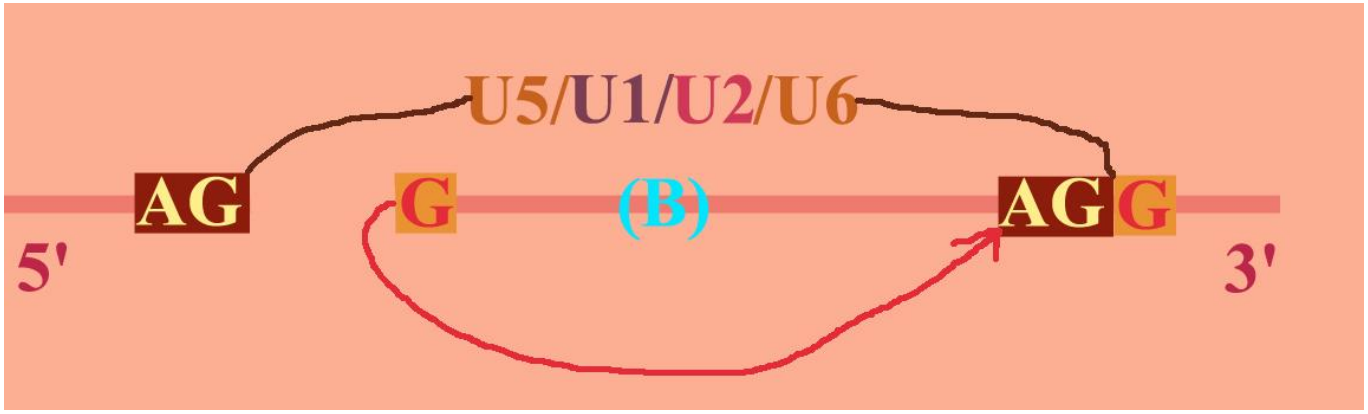


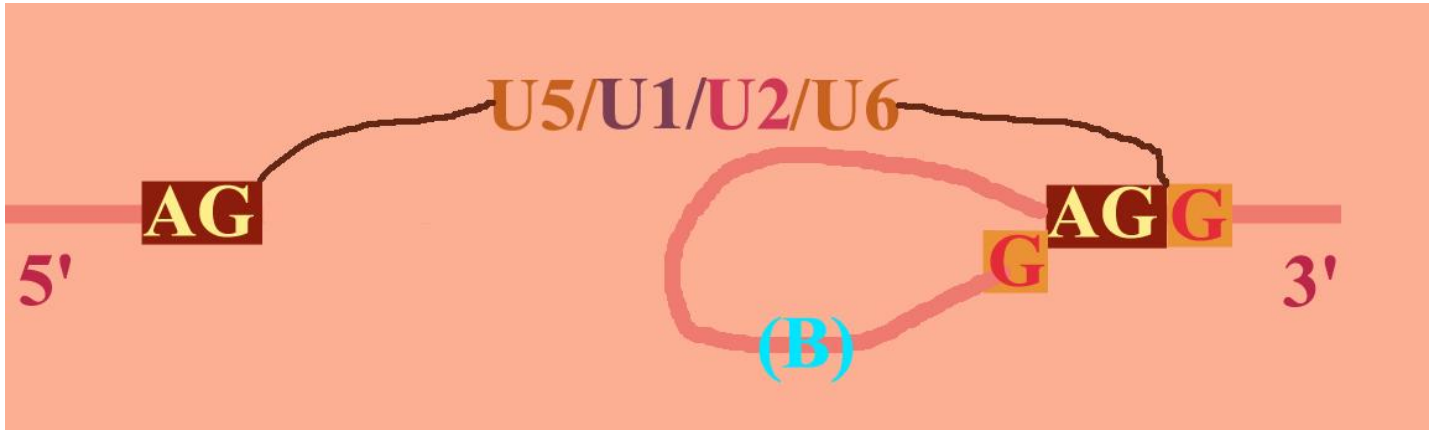
- U2 binds to the branch site (B) and also directs U1 to bind to B, as well as to the 5' end of the intron.
- U1 and U2 rearrange so that U1-U2 spans the intron, i.e., the U2 end binds to the 3' end of the intron.



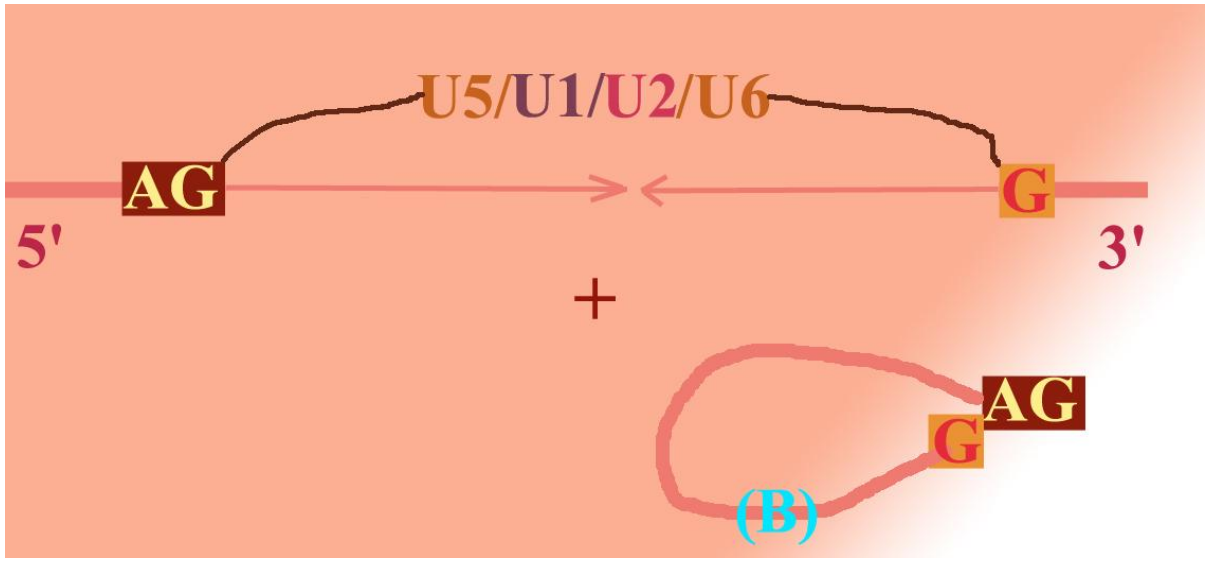


- U4, U5 and U6 then bind together (not unlike complement 1 proteins q, r, s, that initiate the classical complement cascade) to form a catalytically active complex that causes U4 to be released with the formation of a U1· U2· U5· U6 complex that causes the formation of a "lariat" between the 5' region of the partially "clipped" transcript and the 3' region of the transcript.

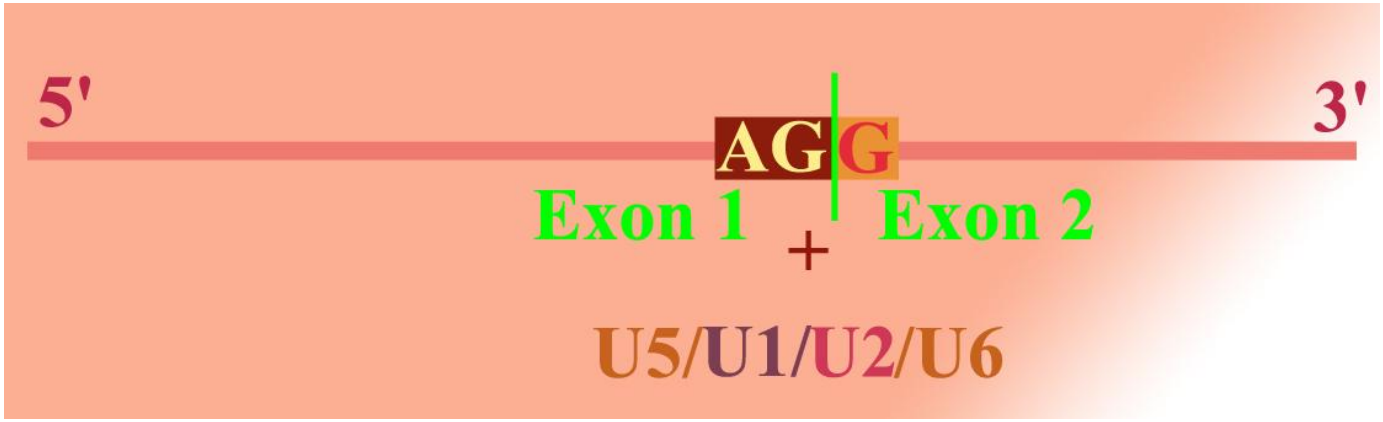




- Once the lariat (derived from the intron) is removed, the snurps catalyze the rearrangement of the exons, butting them up against one another.
- As the ends of the exons are annealed, the snurps are released, the lariat is metabolized and the nucleotides are recycled.
- This process is called the "lariat hypothesis".
- Another form of the "lariat splicing mechanism" has to do with a very unusual 5' to 2' lariat formation.



FINALLY!



- The termination of transcription is brought about by polyadenylation (poly-A). This is a sequence of A's about 200 bp's long.
- While there is no DNA template for poly A, there is a signal sequence in mRNA that "triggers" poly A addition. An RNA endonuclease cleaves the poly-A recognition site and poly-A is added.
- The AAUAAA sequence is a required base sequence for enzymatic activity.
- At this point, the hnRNA is matured and is ready to be "read" by ribosomes.
- mRNA processing also includes capping and methylating: these processes may protect RNA from RNA'ses OR may be recognition sites for ribosomes OR may be a transport sequence to get the mRNA out of the nucleus.

- Although gene regulation is complex and not fully understood, there are some initial regulations of transcription about which we do have some knowledge.
- Transcription may be positively regulated
  - 1) hormonally at the level of the DNA; with RNA Pol and with proteins necessary for Pol interaction. This probably does not occur in man;
  - 2) hormonally at the level of the DNA that causes conformational changes of DNA so RNA Pol may bind to it;
  - 3) hormonally where the hormone binds to the transcriptional factor that has to bind to DNA site before RNA Pol may bind;
  - 4) cAMP is even involved: it increases tyrosine aminotransferase, PEPCK and prolactin syntheses.

- Conversely, transcription may be negatively regulated
  - 1) hormonally where the hormone acts as an inducer that turns off repressors and/or
  - 2) hormonally where the hormone binds to the DNA to cause a conformational change of chromatin to make the DNA susceptible to RNA Pol.

- Once the mRNA has been synthesized and matured in the nucleus, it is ready for transport through the nuclear envelope into the cytosol to bind to ribosomes.
- tRNA, then, transports the necessary amino acids to the mRNA-ribosomal complex to continue the process of protein synthesis (translation).
- How is it that the two RNA's code for the amino acids?

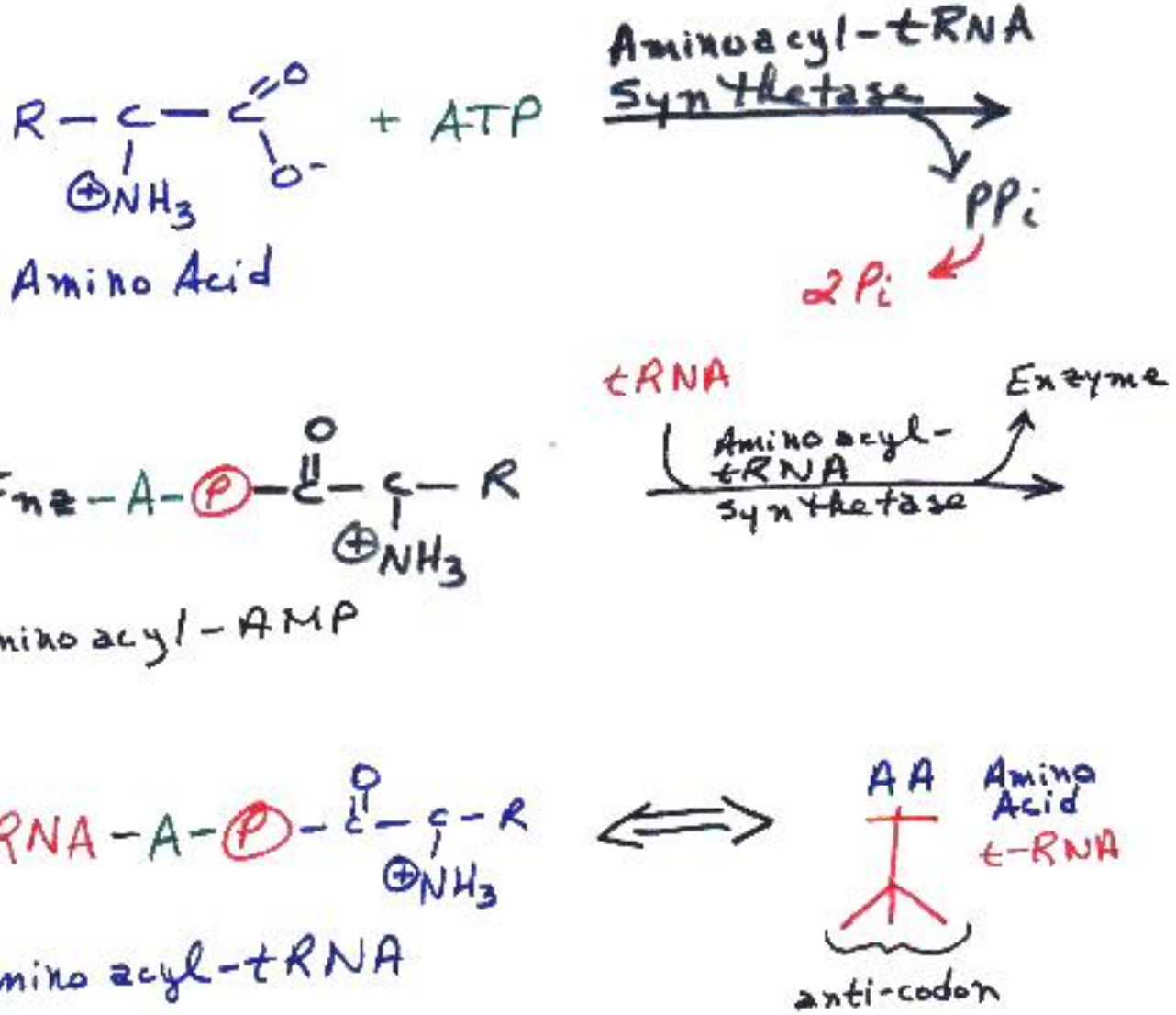
The process of using the mRNA to synthesize proteins is called translation and that's the next topic for study.

Translation: A Four-Step Process

Translation!

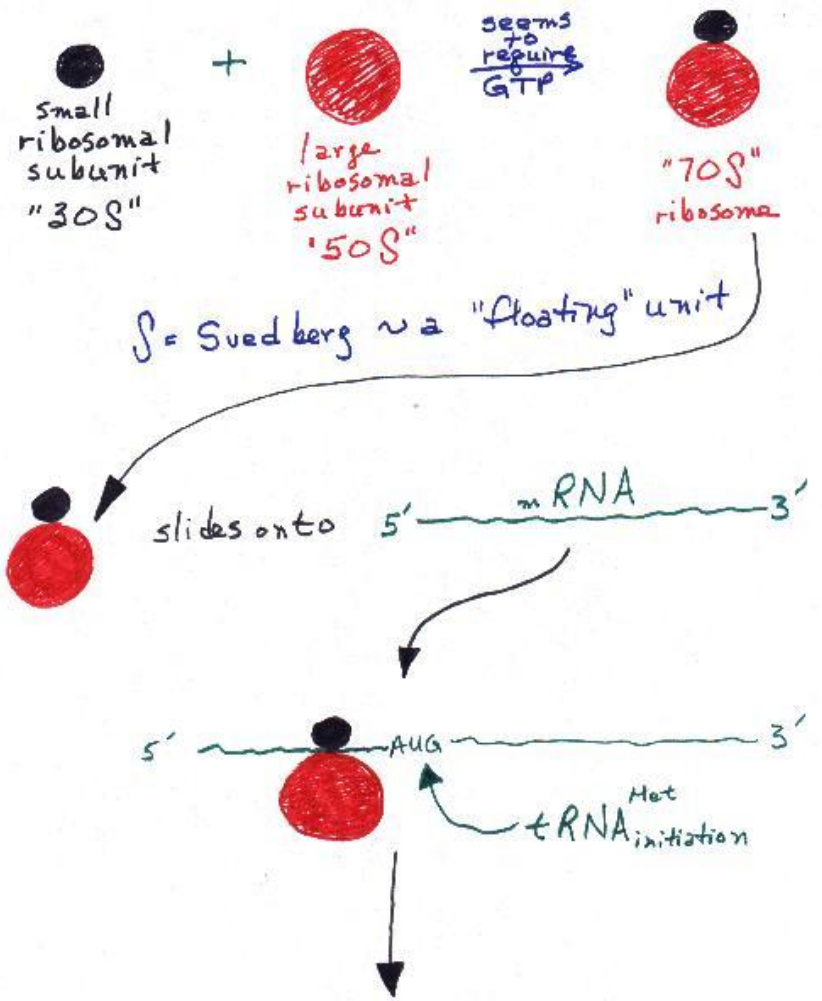
- The four steps, in order, in translation are
  - 1) Activation,
  - 2) Initiation,
  - 3) Elongation and
  - 4) Termination.

- Activation requires the activation of a tRNA such that it binds to its proper amino acid.
- The amino acid reacts with ATP in the presence of aminoacyl-tRNA synthetase to form the aminoacyl-AMP-aminoacyl-tRNA synthetase derivative/complex and inorganic pyrophosphate (PPi; the equivalent of 2 phosphates [Pi]).
- This reaction is driven by the hydrolysis of the ATP to the AMP derivative and the PPi.
- The release of the PPi is what provides the energy to drive this reaction forward.
- The aminoacyl-AMP derivative then reacts with the appropriate tRNA, releasing the aminoacyl tRNA synthetase and the aminoacyl-tRNA.

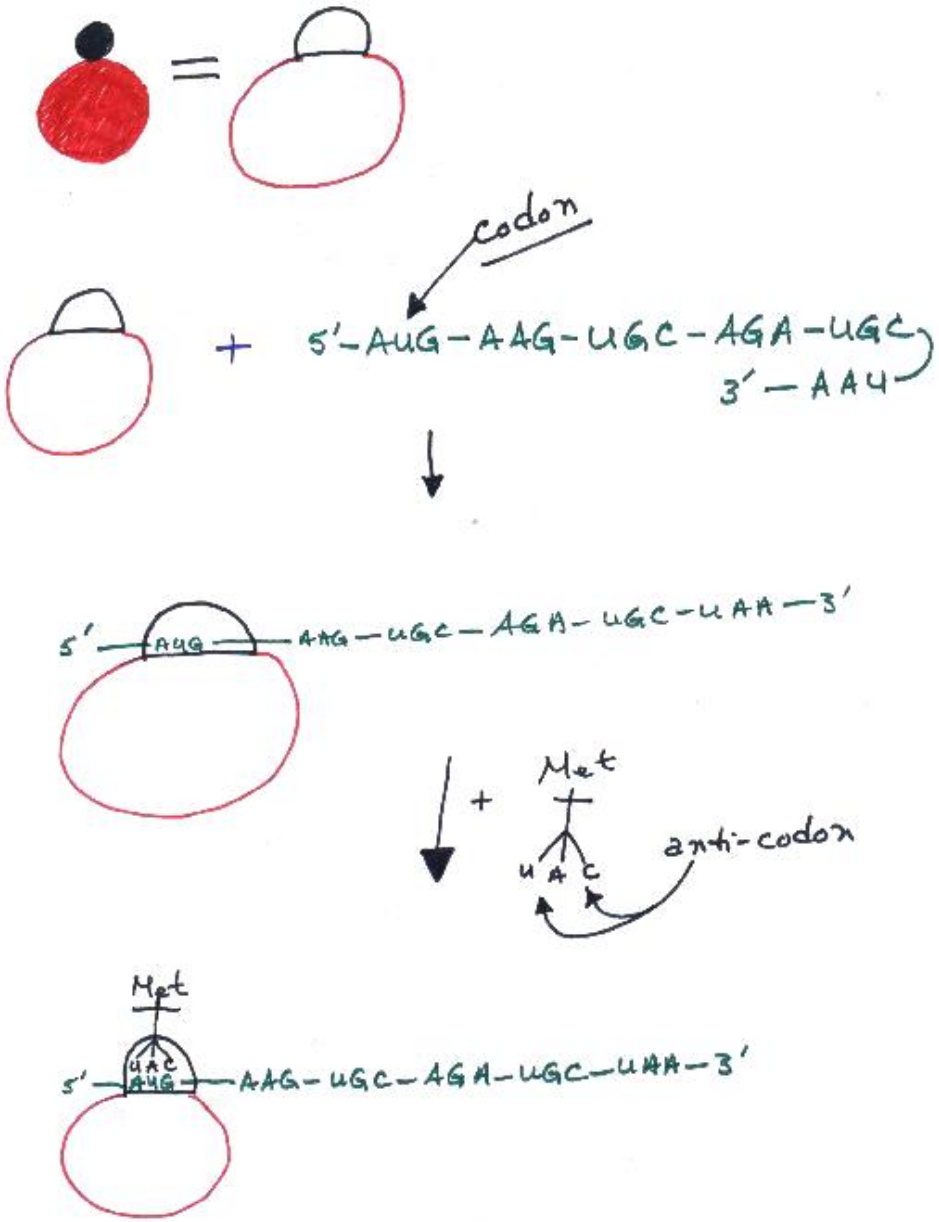

Amino Acid
+ ATP
Aminoacyl-tRNA Synthetase
PPi
2 Pi
Enz-A-P-C-C-R
NH3
Aminoacyl-AMP
tRNA
Enzyme
Aminoacyl-tRNA synthetase
tRNA-A-P-C-C-R
NH3
Aminoacyl-tRNA
AA
Amino Acid
t-RNA
anti-codon

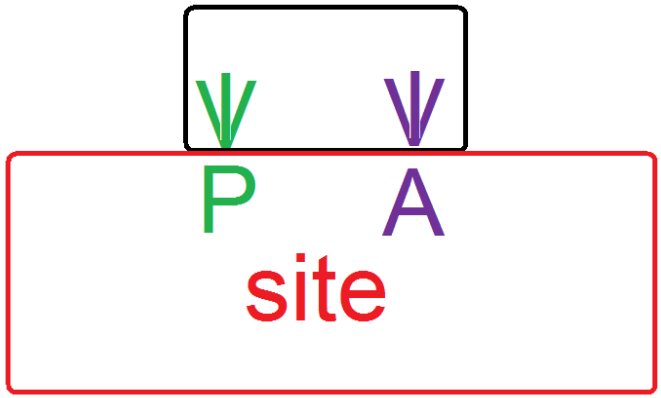
The second step in translation is Initiation

- A small ribosomal (30S) subunit binds with a large ribosomal (50S) subunit following the hydrolysis of GTP to form the 70S ribosome.
- The "S" is the Svedberg Unit which is a unit that measures the floating ability of the particle.



- At this point, translation "goes". The beauty of this mechanism is that only a unique protein is translated from this specific mRNA sequence.





70S Ribosome

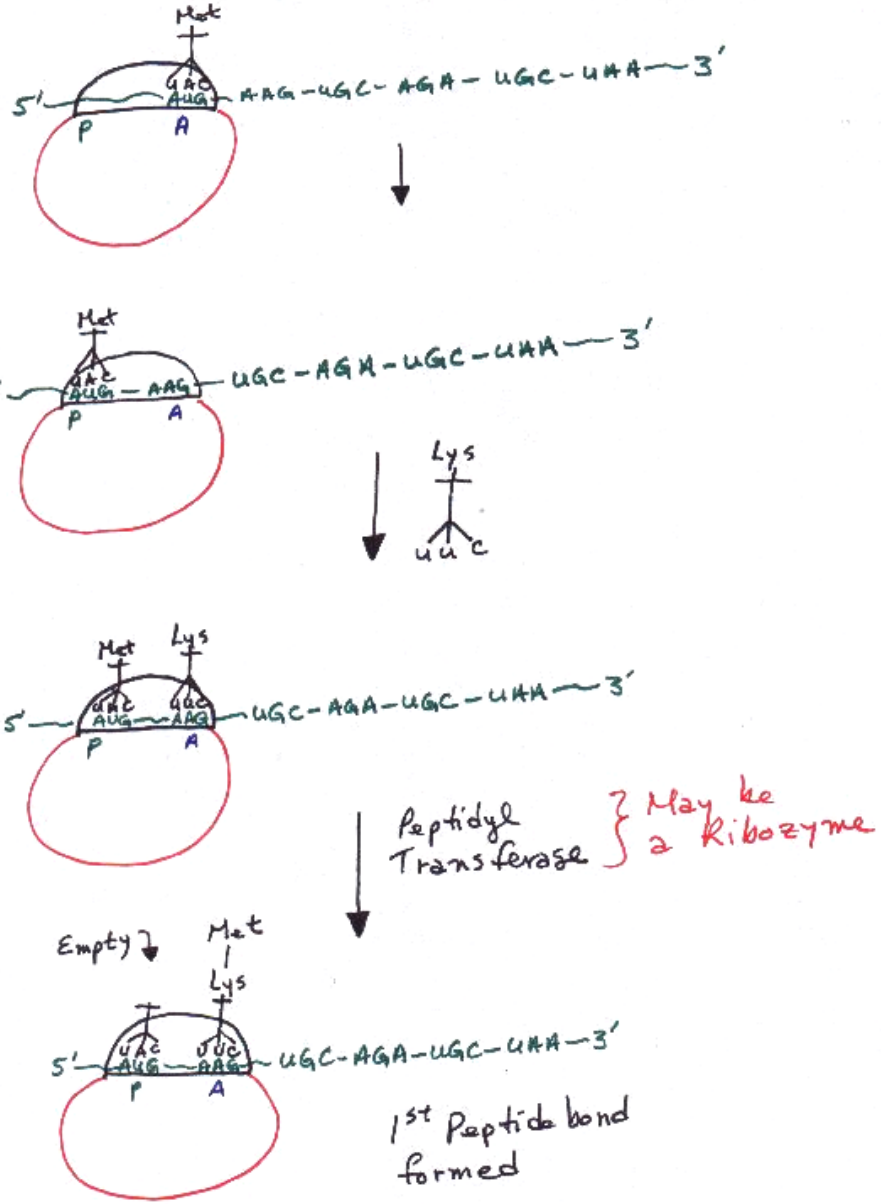
- There are two sites in the 70S ribosome: the "P" site and the "A" site.
- The "P" site is the peptidyl site and contains the growing peptide chain.
- By convention, this site is on the left-hand side of the 70S ribosome.
- The "A" site is the acyl site.
- This latter site contains the charged (activated) tRNA and is drawn on the right side of the 70S ribosome.

- The $tRNA^{Met}_{Initiation}$ is transported to the A site of the 70S ribosome.
- The ribosome slides down the mRNA in such a manner that the $tRNA^{Met}_{Initiation}$ is "shifted" -- presumably by the translocase regulated by $EF_{1\ and\ 2}$ -- into the "P" site, exposing the next codon (AAG; codon for Lys).
- Thus ends initiation and begins elongation.

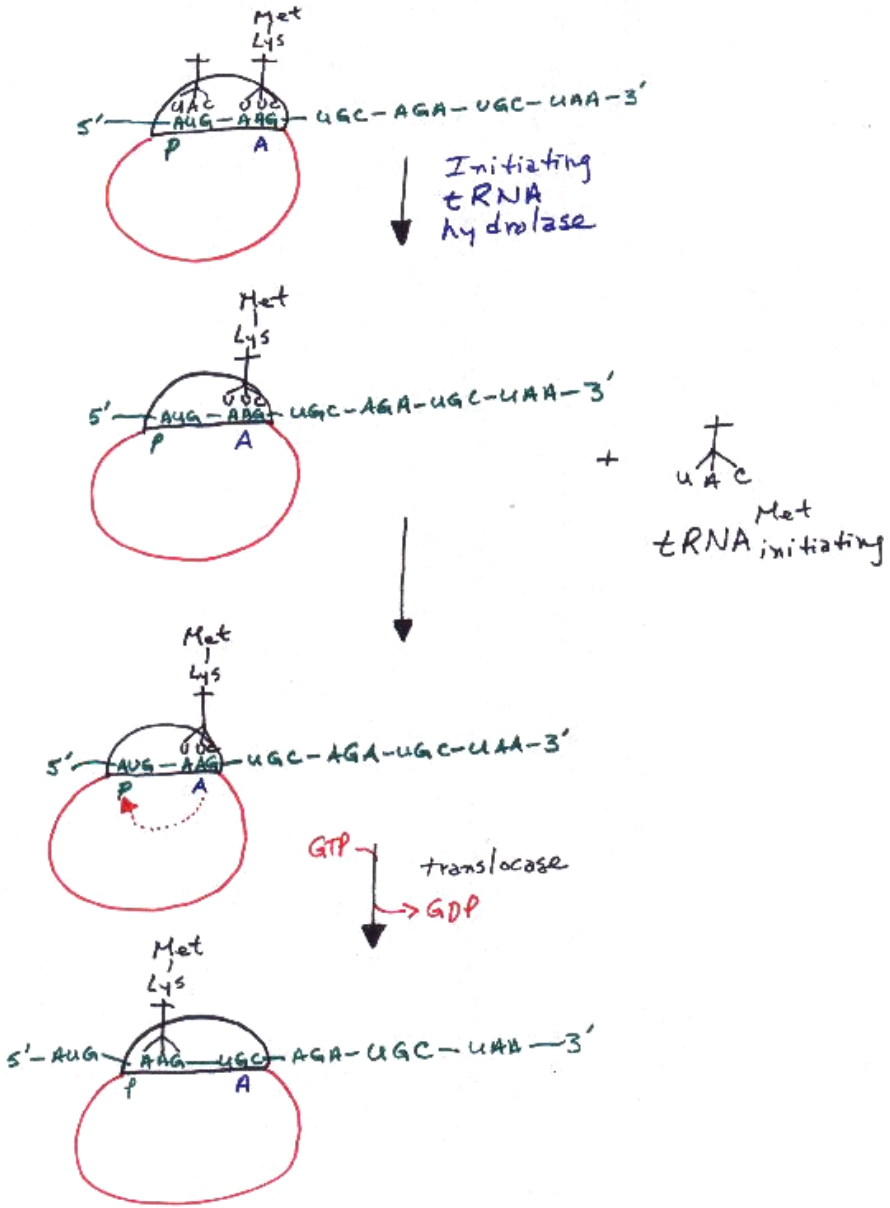
- Elongation depends upon elongation factors (EF's).
- EF_1 consists of $EF_{1\alpha}$ (a GTP binding protein) and $EF_{1\beta\gamma}$ (a GDP-GTP exchange protein).

- EF_2 regulates translocase activity (this enzyme is coming up shortly).
- Both EF's are highly conserved, i.e., they are found across nature having closely related structures/sequences.

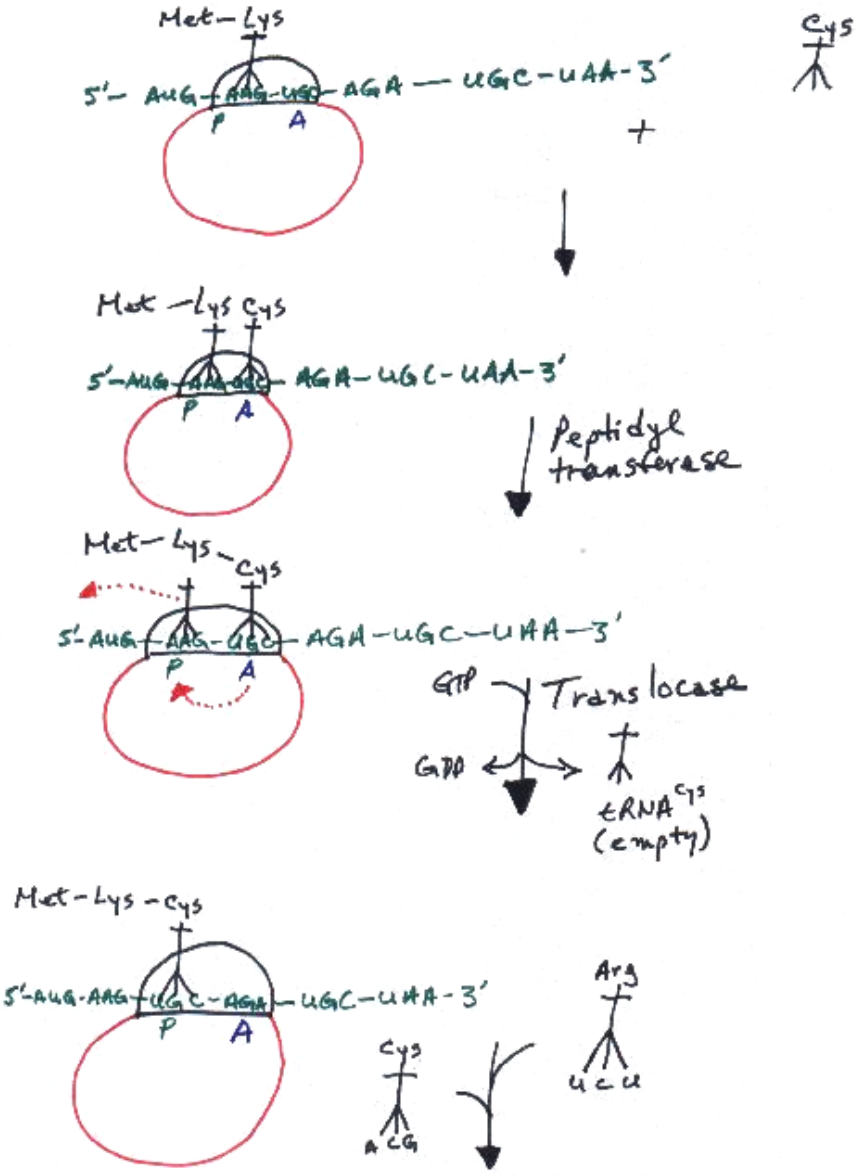
- Lysinyl-tRNA is then transported to the ribosome-mRNA complex.
- Once the latter tRNA is bound, peptidyl transferase (in all likelihood a ribozyme, i.e., RNA acting as an enzyme) tweaks the Met from its tRNA and forms the first peptide bond between the Met and the Lys.
- Lys remains bound to its tRNA in the "A" site of the 70S ribosome.

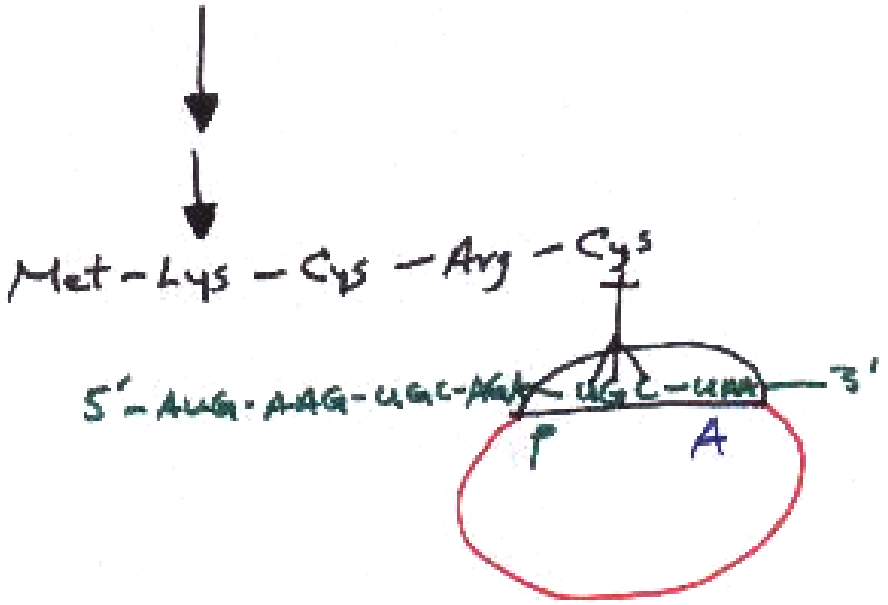

Met
5'
AAG-UGC-AGA-UGC-UAA—3'
P
A
Met
5'
UGC-AGA-UGC-UAA—3'
Lys
Met
Lys
5'
UGC-AGA-UGC-UAA—3'
Peptidyl Transferase
May be a Ribozyme
Empty
Met
Lys
5'
UGC-AGA-UGC-UAA—3'
1st Peptide bond formed

- Elongation continues and the following slide demonstrates how the initiating tRNA ($tRNA^{Met}_{Initiation}$) is removed from the tRNA-mRNA-ribosome complex: by an initiating tRNA hydrolase.
- Translocase then drives the 70S ribosome one triplet towards the 3' end of the mRNA, placing the dipeptidyl-tRNA in the P site and making the A site available for the next tRNA.
- Translocase requires GTP for this reaction, i.e., it is energy requiring.


Met
Lys
UAC
UUC
5'
AUG – AAG
UGC – AGA – UGC – UAA – 3'
P
A
Initiating
tRNA
hydrolase
+
UAC
Met
tRNA initiating
GTP
translocase
GDP
AUG – AAG – UGC – AGA – UGC – UAA – 3'

- This cycle continues, next two slides, until the stop codon (UAA) is in the A site.
- When UAA is in the A site, this signals for termination to begin.


Met–Lys
5'– AUG–AAG–UGC–AGA — UGC–UAA–3'
P
A
Cys
+
Met–Lys Cys
5'–AUG–AAG–UGC–AGA–UGC–UAA–3'
P
A
Peptidyl transferase
Met–Lys–Cys
5'–AUG–AAG–UGC–AGA–UGC–UAA–3'
P
A
GTP
Translocase
GDP
tRNA^Cys
(empty)
Met–Lys–Cys
5'–AUG–AAG–UGC–AGA–UGC–UAA–3'
P
A
Cys
A CG
Arg
U C U

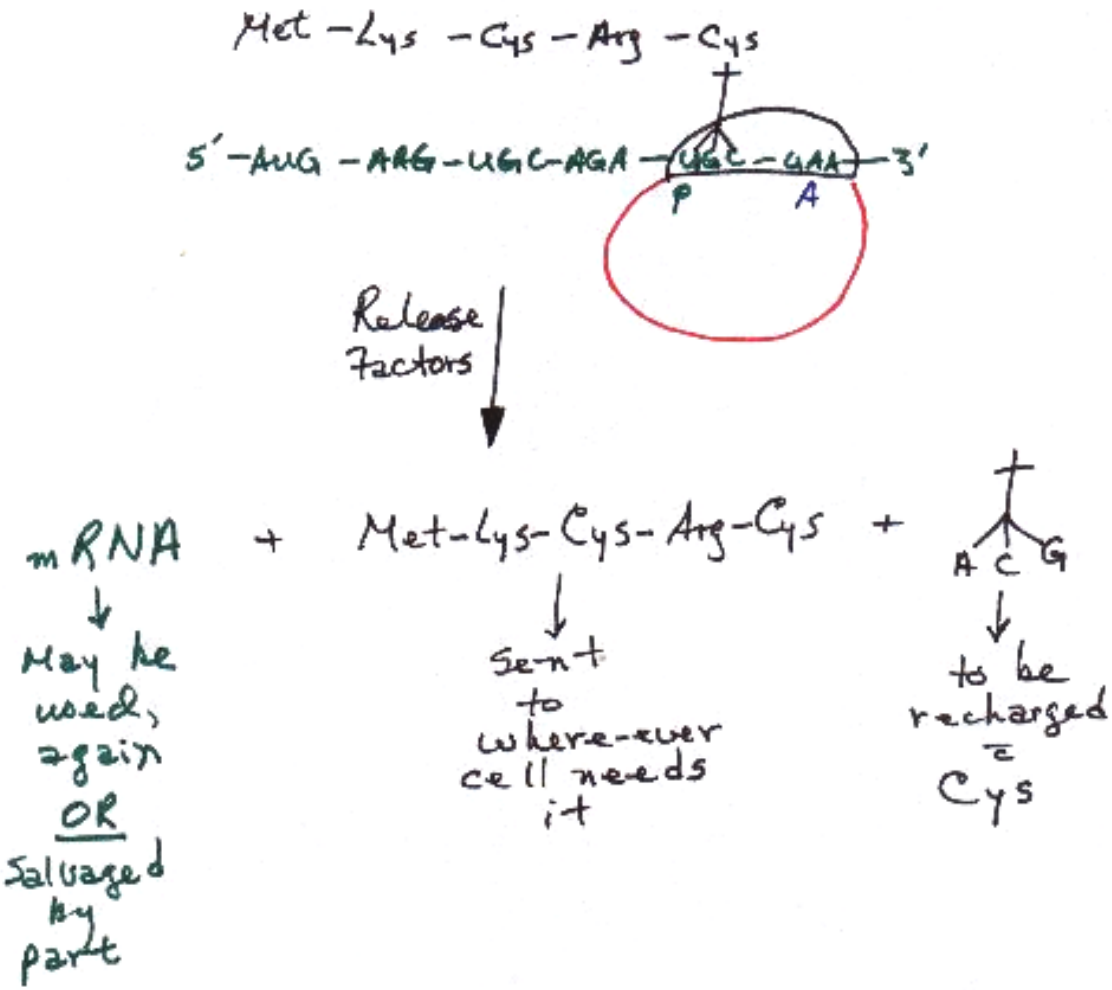


When UAA in A site

↓

TERMINATION

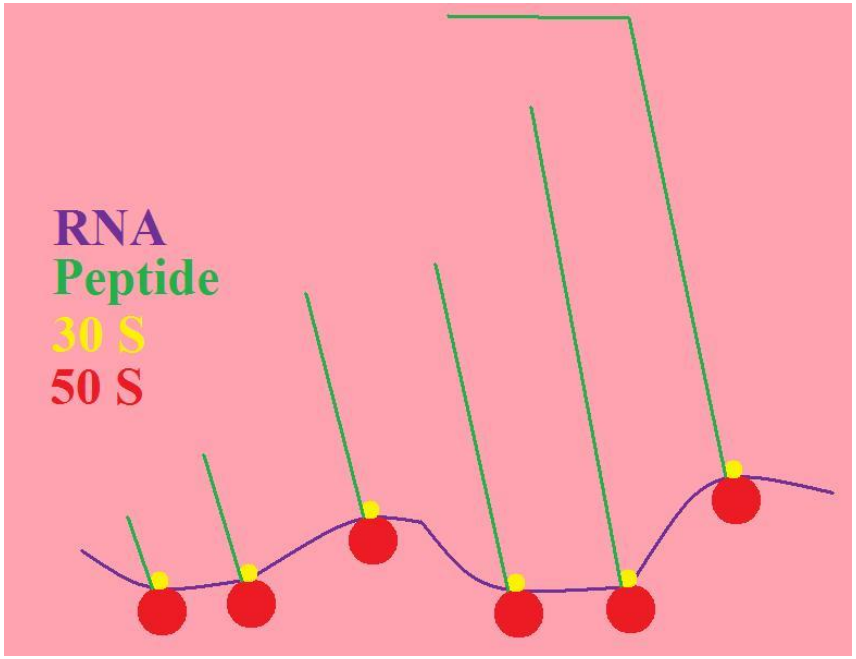
- Termination, next slide, is catalyzed by Release Factors that cause the mRNA to be used, again, or salvaged by part, the peptide to be released, modified and sent to where-ever the cell needs it and releases the last tRNA to be re-charged with the appropriate amino acid for future use (in the case of our example, to be re-charged with Cys).


Met – Lys – Cys – Arg – Cys
5′ – AUG – ARG – UGC – AGA – UGC – GAA – 3′
P
A
Release Factors
mRNA
+
Met-Lys-Cys-Arg-Cys
+
A C G
May be used, again
OR
Salvaged by part
Sent to where-ever cell needs it
to be recharged = Cys

Energy Requirements and Perspective of Translation:

-
- 2 ATP's are required to charge each amino acid
- 2 GTP's are required to elongate per elongation step
- 1 calorie = the energy necessary to raise 1 gram of water by 1° C
- 2 ATP's and 2 GTP's give approximately 28,000 calories of energy: this is equivalent to the energy necessary to raise 28 liters of water 1° C.
-
- In short, it takes LOTS of energy to synthesize proteins.
- A portion of that energy has to do with how the proteins are sequentially synthesized: once 25 amino acids (more or less) are linked by peptide bonds during translation, the AUG site is available/exposed for binding by ANOTHER 70S ribosome. This new ribosome initiates ANOTHER round of translation, *ad nauseum*.

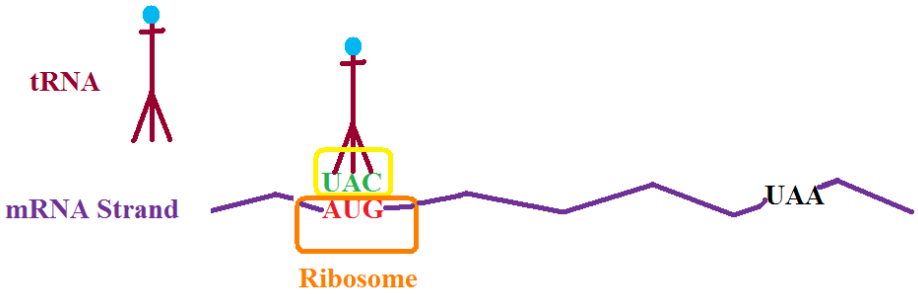
- Eventually, the mRNA is literally smothered by ribosomes every 25 or so amino acids, i.e., about every 75-80 nucleotides on the mRNA.
- This smothered mRNA by ribosomes is called a polysome or polyribosome.

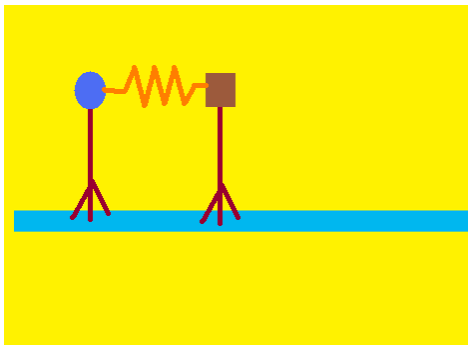


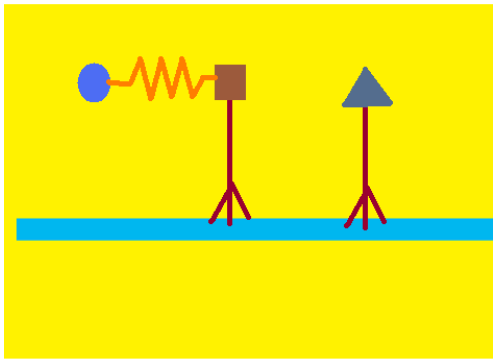
- This is the general form of the "translation unit in all cells".
- Polysomes increase the rate of translation per unit of time as compared to 1 ribosome on a mRNA strand – and translates 5’ to 3’ (left to right in graphic).

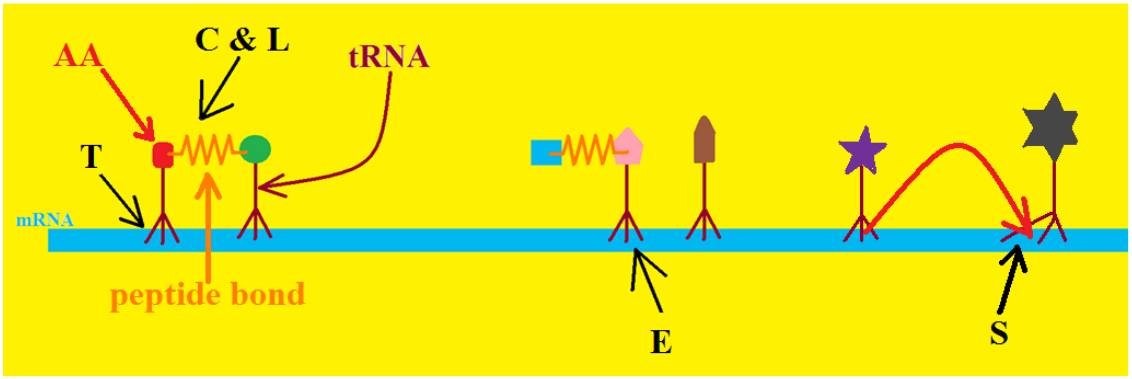
- Once translation is completed, one of at least 4 modifications will occur to the protein[s] (called post-translational modification):
  - 1) glycosylation -- addition of carbohydrate to the protein;
  - 2) phosphorylation -- add a phosphate;
  - 3) proteolytic cleavage -- proteins may be synthesized in an inactive form and require cleavage to become active, e.g., insulin and C-peptide. C-peptide is the portion from pre-insulin that is cleaved to leave active insulin;
  - 4) sub-unit binding -- quaternary structure formations, e.g., the 4 sub-units of hemoglobin binding together, myoglobin subunits binding together, the 3 subunits of arginase binding together.

- Translation is inhibitable (**normal translation = top and middle 2 graphics**).
- That very fact makes it of significance to any one going into health care as many micro-organisms are capable of being killed by translation inhibitors (**bottom graphic**) such as chloramphenicol (C), tetracycline (T), streptomycin (S), lincomycin (L) and erythromycin (E) to name five.
- C inhibits/blocks peptidyl transferase,
-
- T inhibits binding of charged tRNA to the A site of the ribosome,
- S blocks proper codon-anticodon binding to cause different peptides to be synthesized,
- E inhibits the translocase and
- L blocks peptidyl transferase and blocks tRNA from binding, although not at the same time.



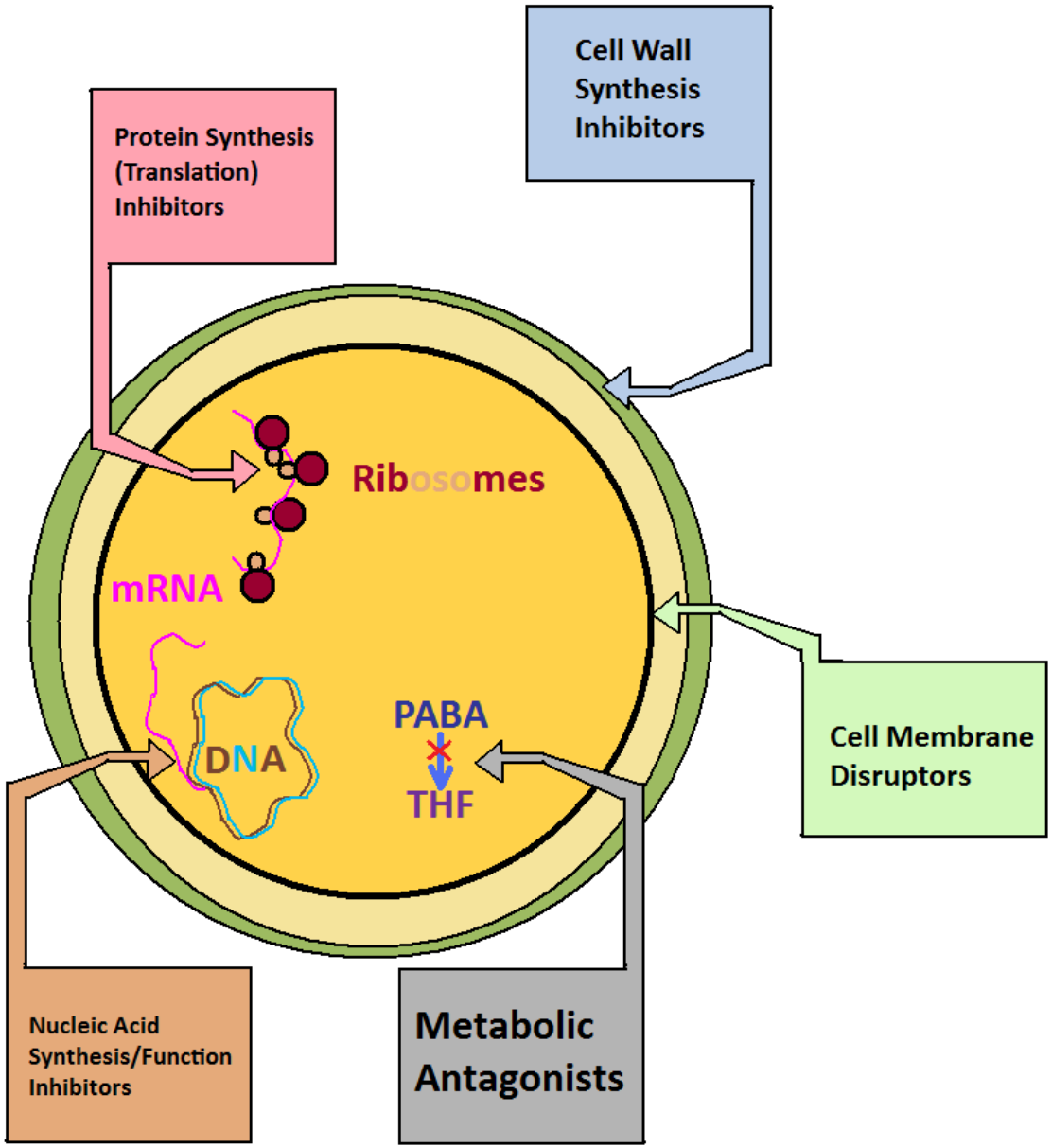




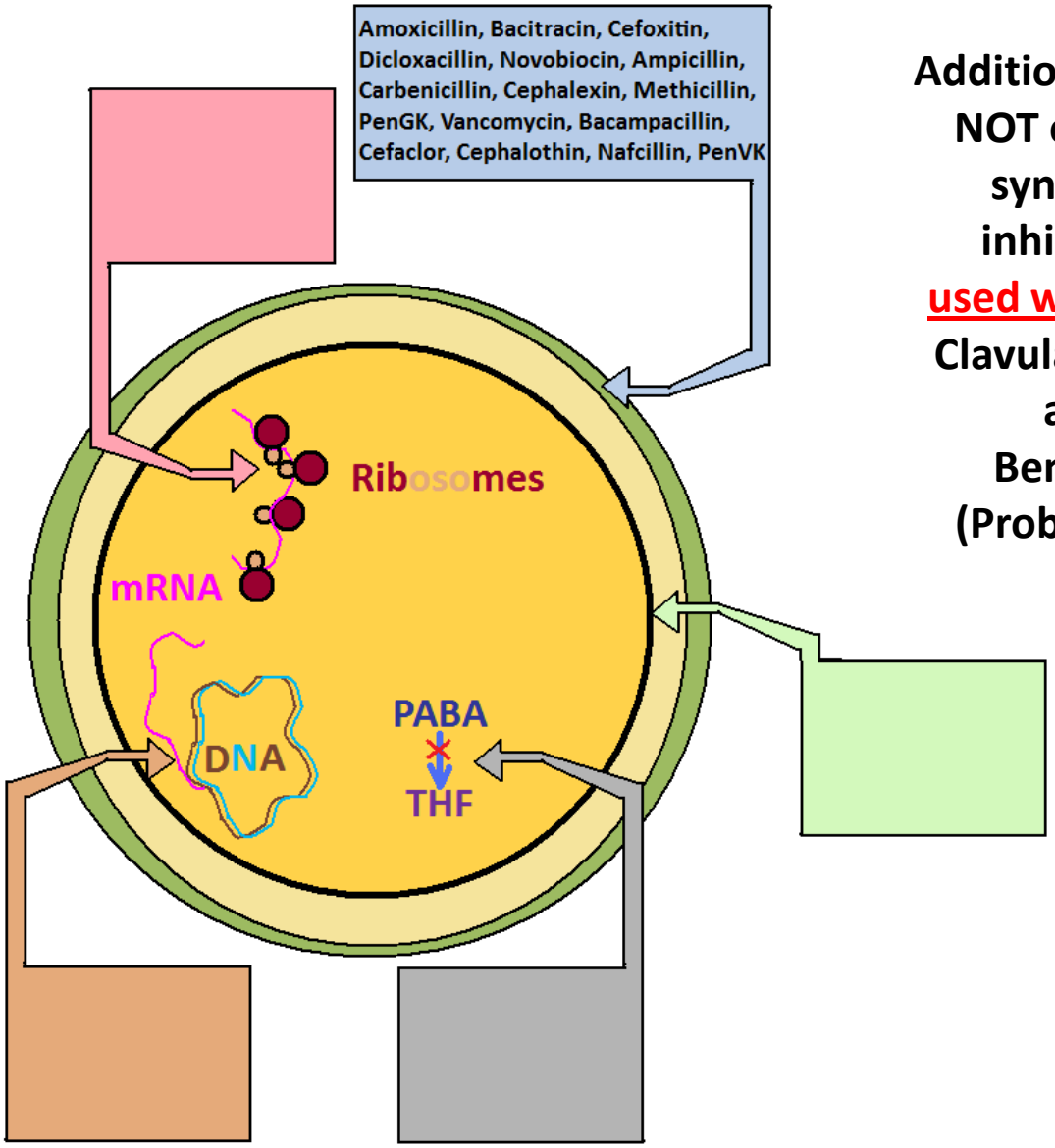


Mechanisms of Antibacterial Drug Action

Five Classes of Antibiotics

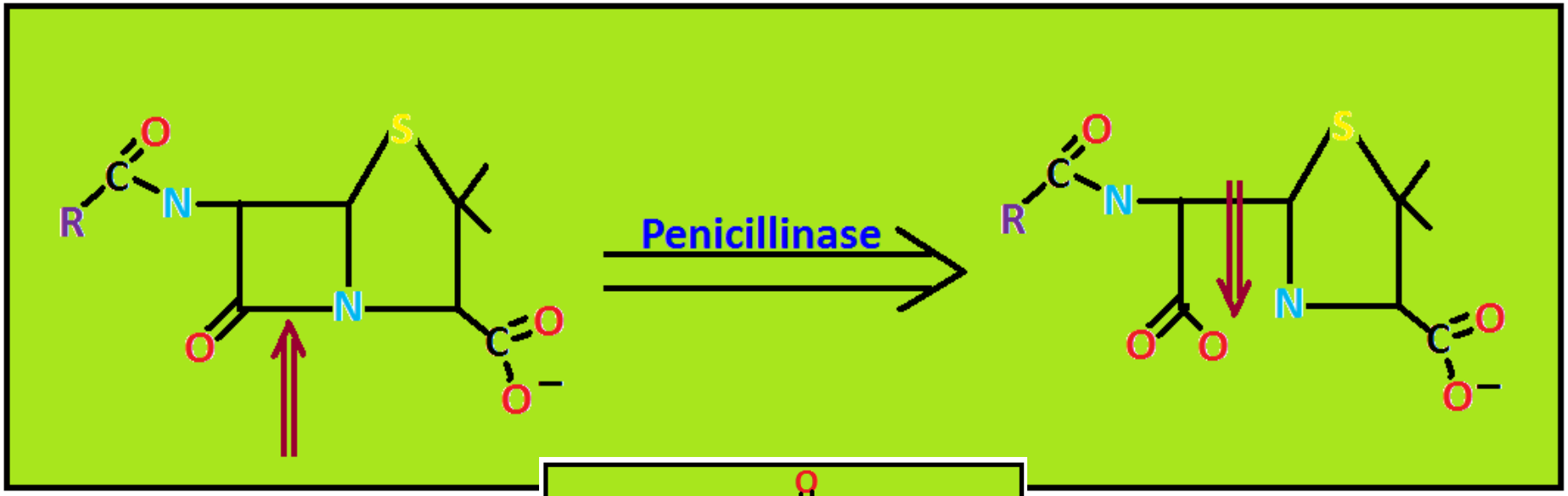


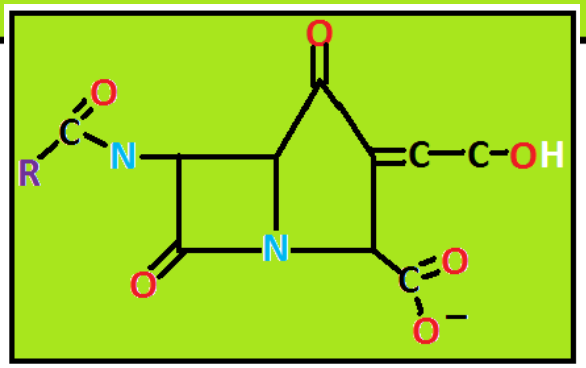
Cell Wall Synthesis Inhibitors Examples



Additional drugs, NOT cell wall synthesis inhibitors; used with them
Clavulanic Acid and Benemid (Probenecid)

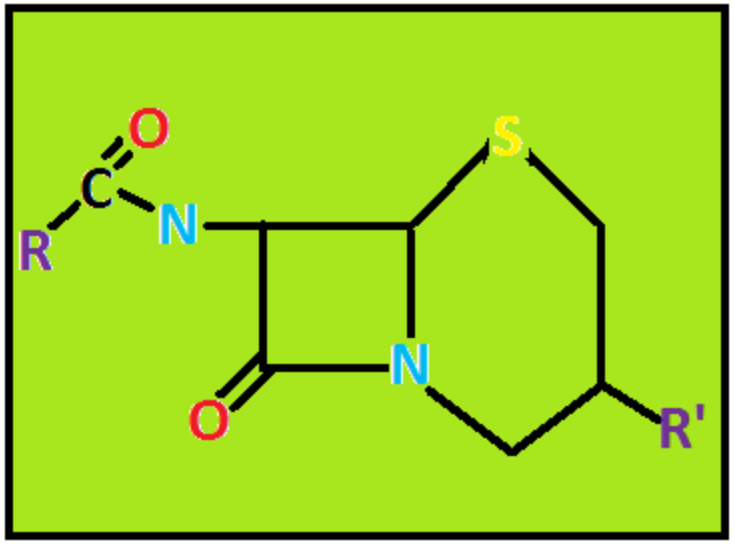
β-Lactamase Inhibition





Clavulanic acid; β-lactamase inhibitor; comes in Augmentin with Amoxicillin

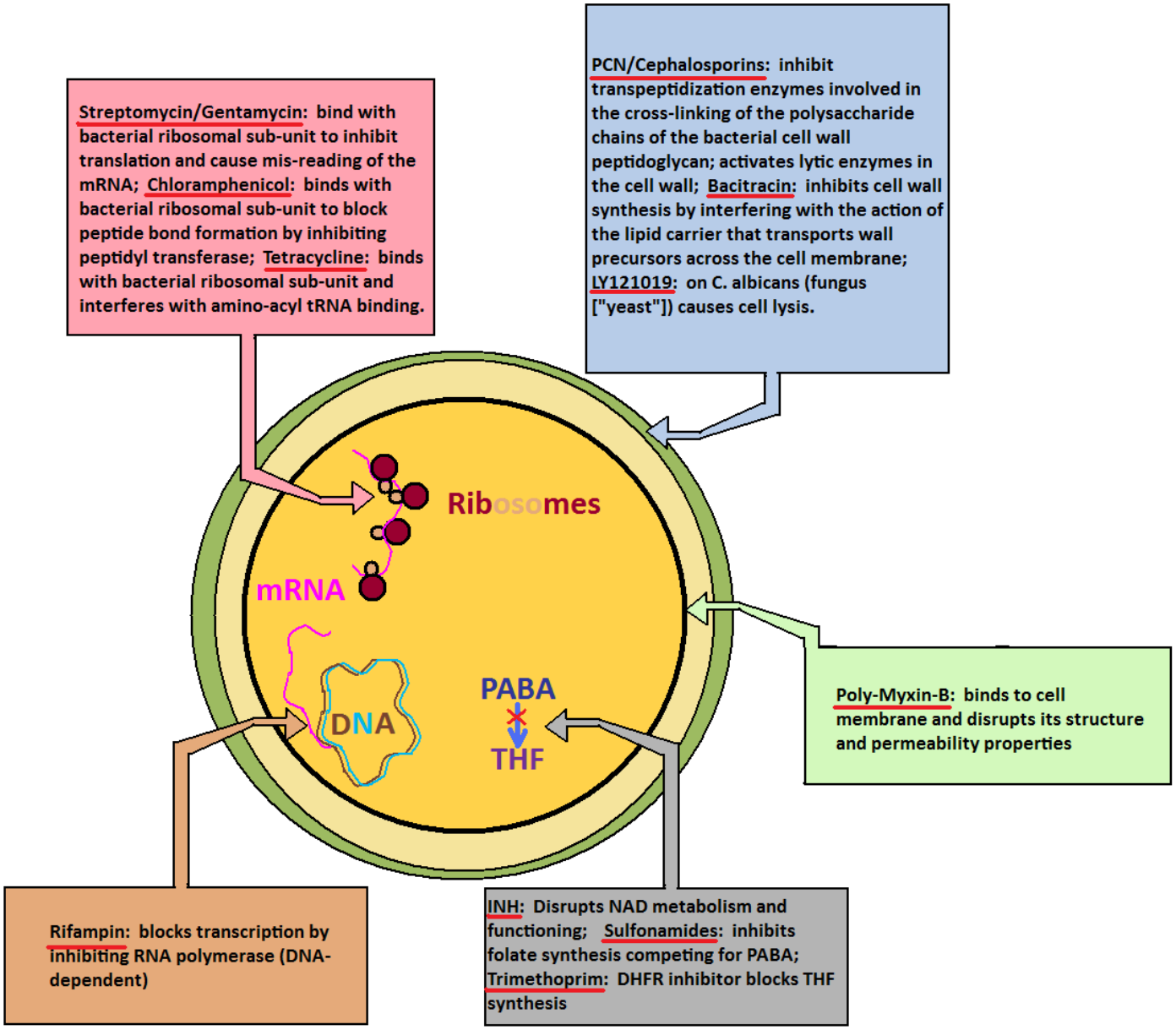
Cephalosporinase v Penicillinase



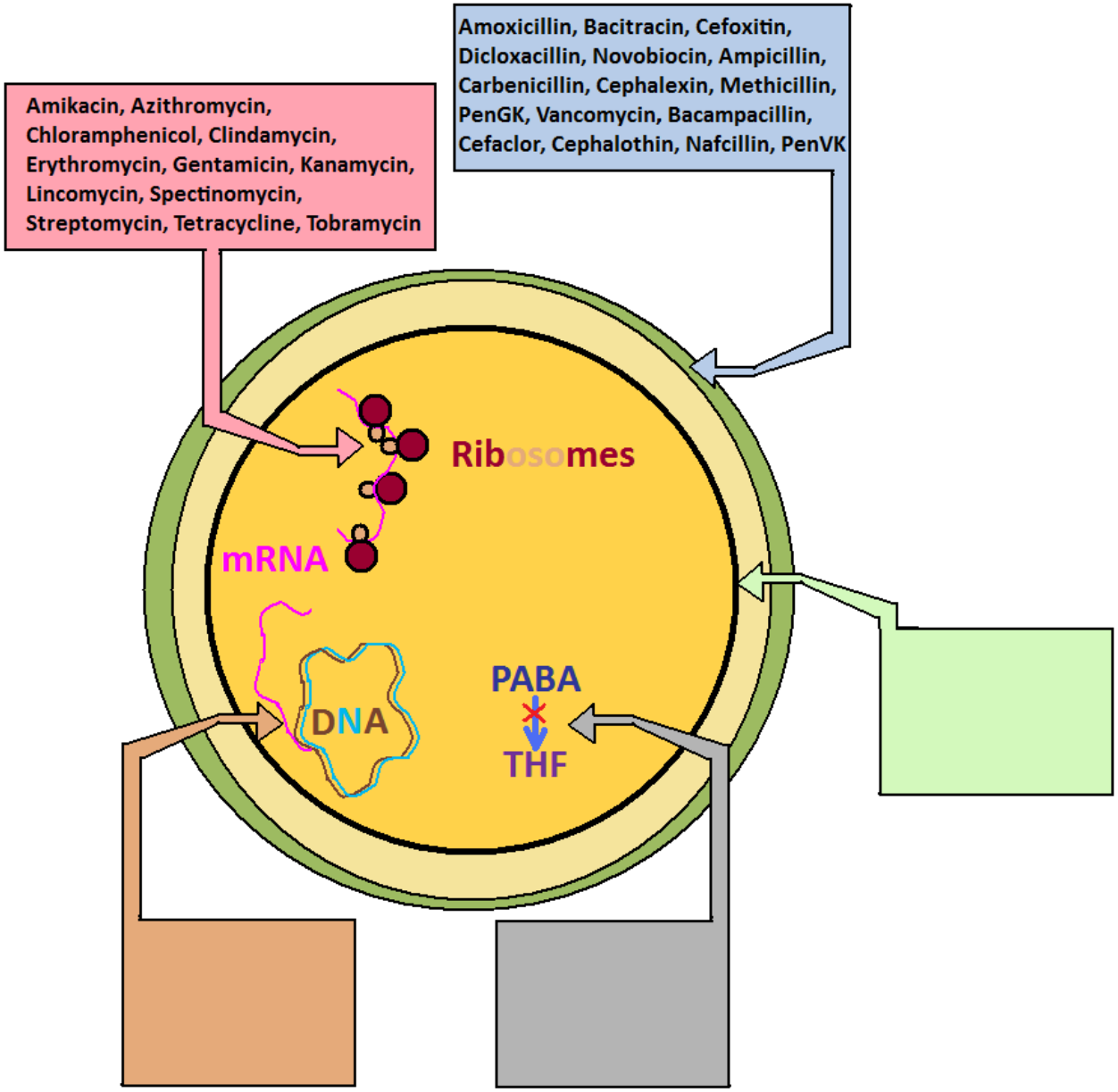
Penicillinase	Cephalosporinase
Inactivates “SOME”, but not with efficiency	Inactivates

Resistance of cephalosporins to cephalosporinase depends on the size of the R’ groups (steric hindrance), i.e., the greater the size, the greater the resistance.

Example Action Mechanisms

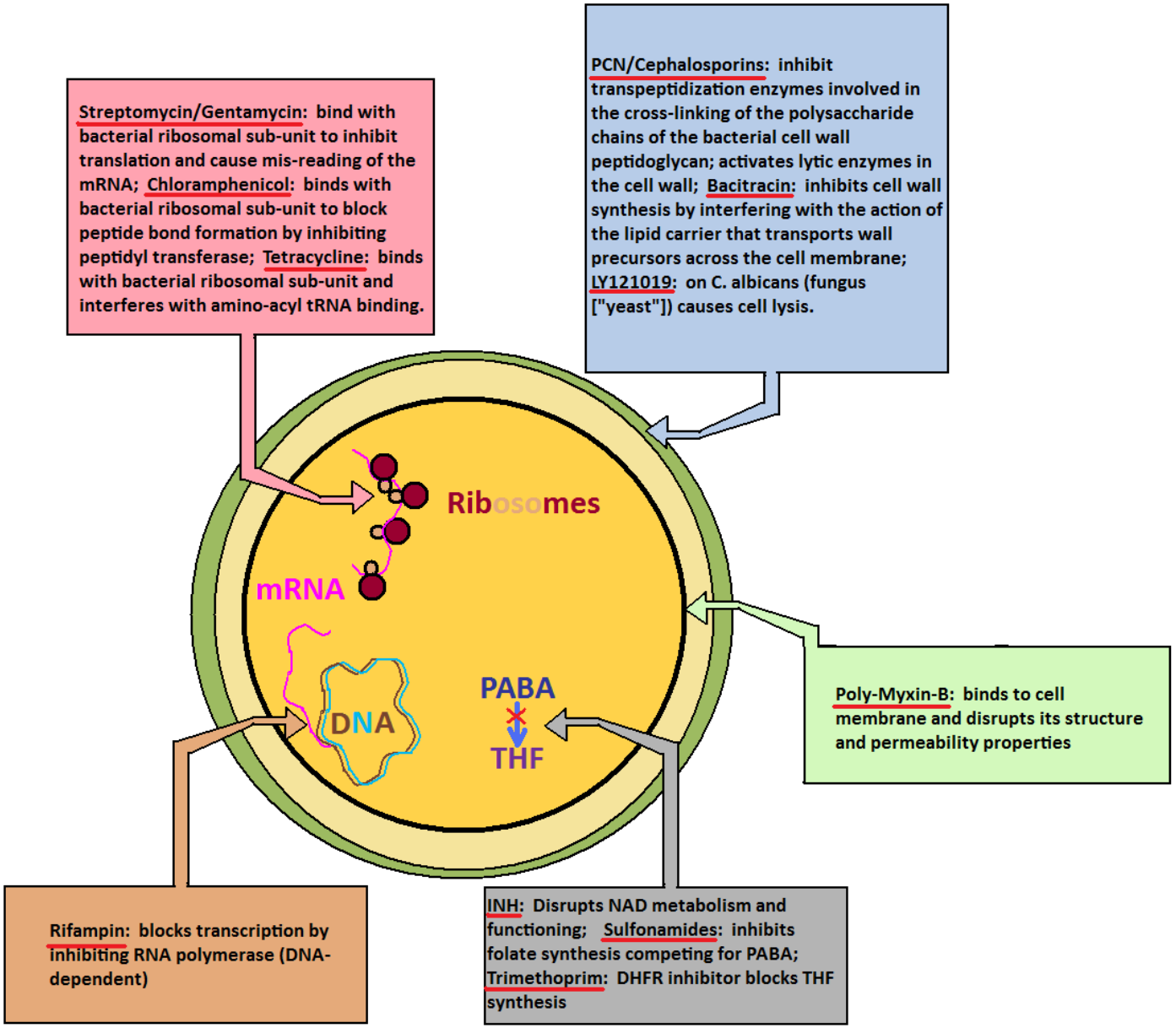


Translation Inhibitors Examples

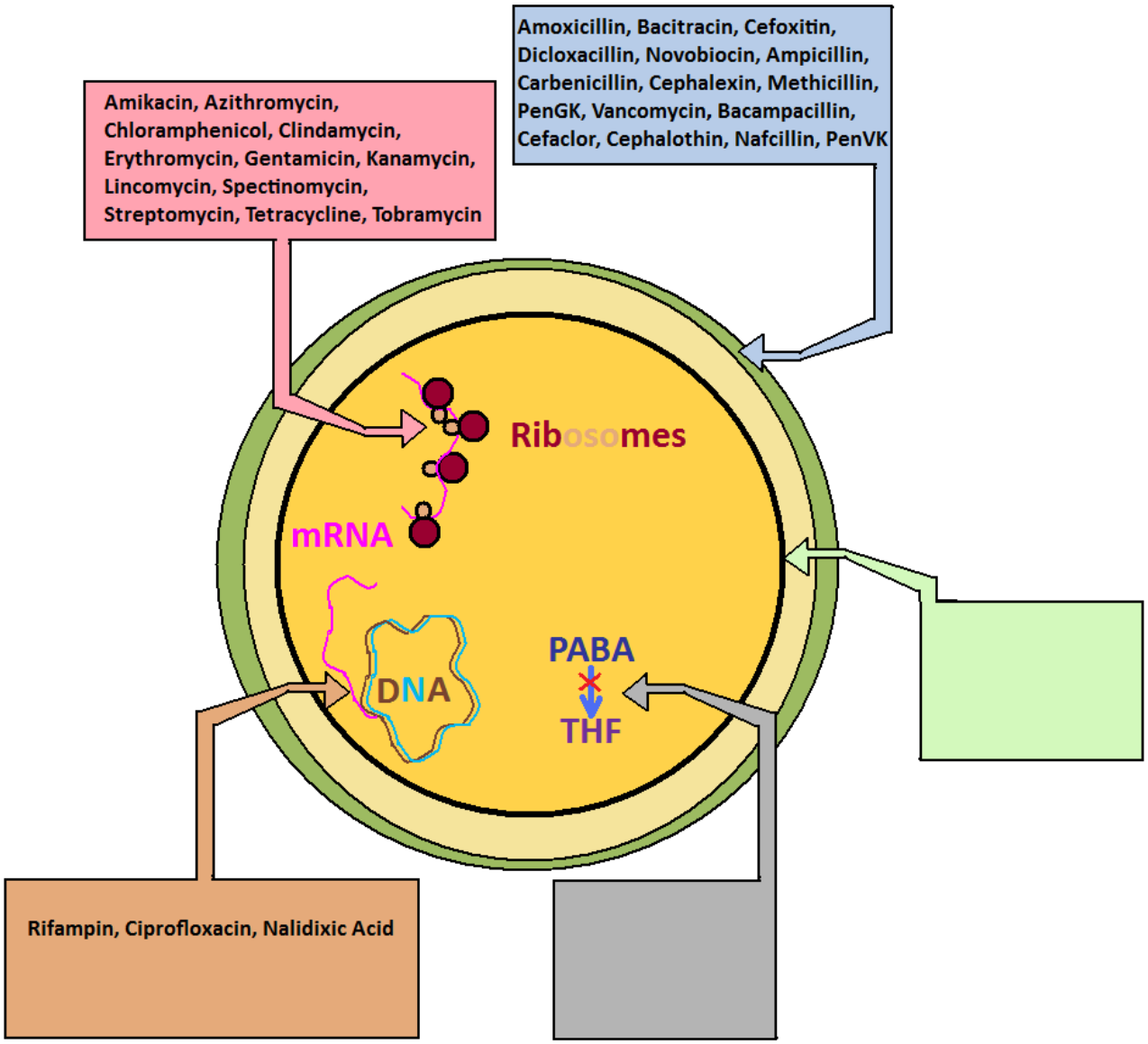


Example Action Mechanisms

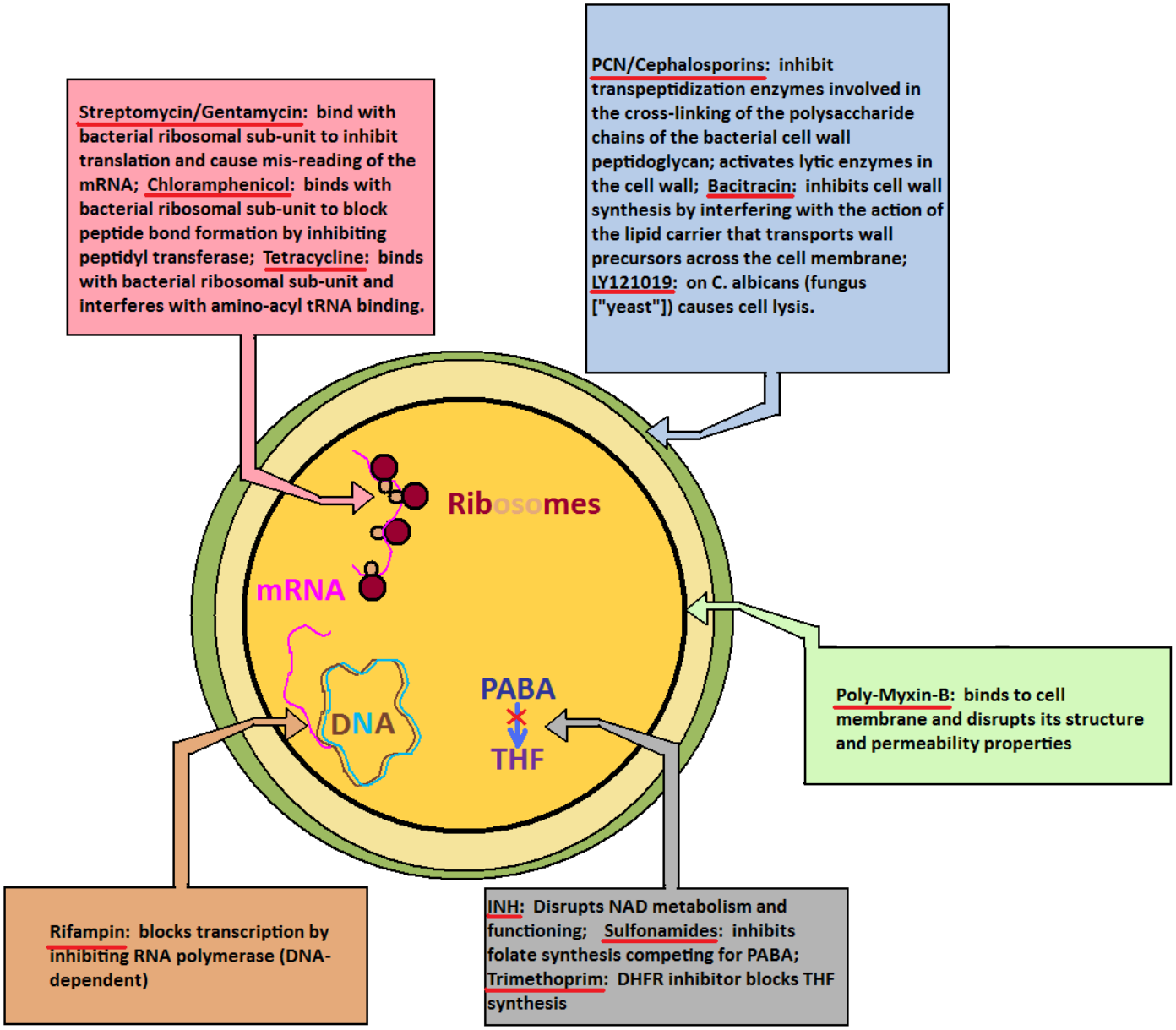
Tetracycline NOTE: Reduces plasma PT* activity and causes an increased risk of bleed out UNLESS anticoagulants are reduced.



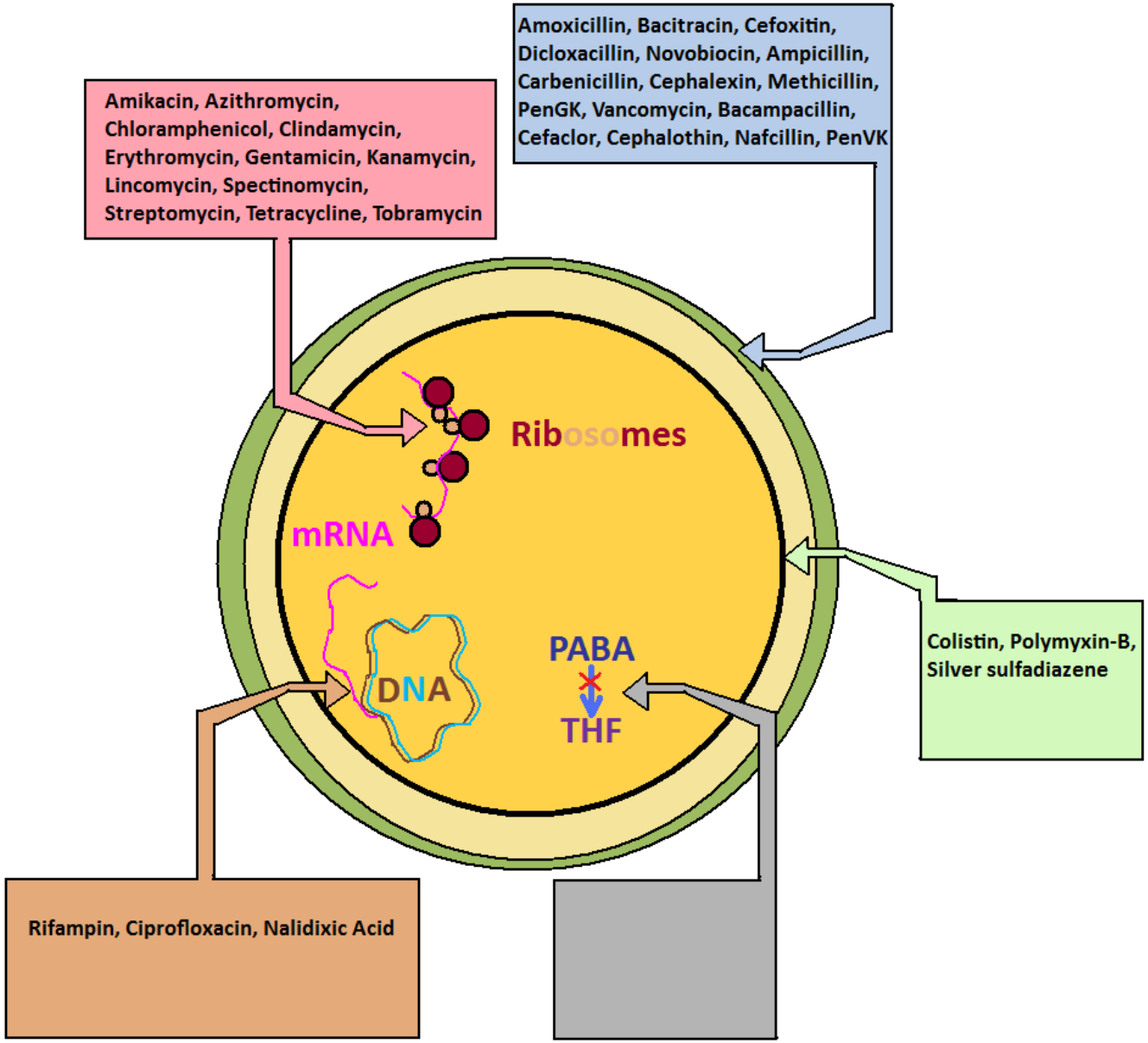
Nucleic Acid Synthesis/Function Inhibitors Examples



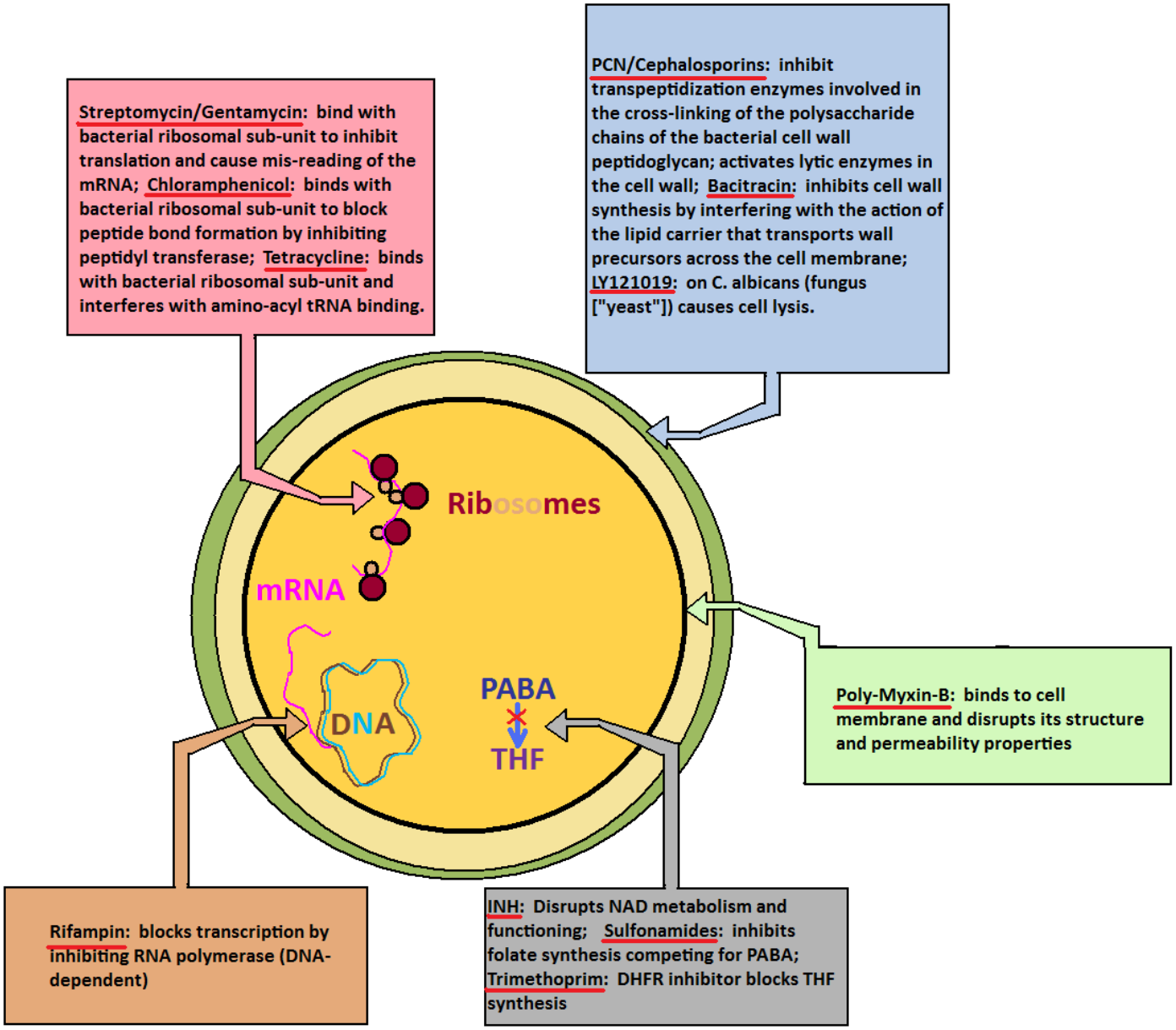
Example Action Mechanisms



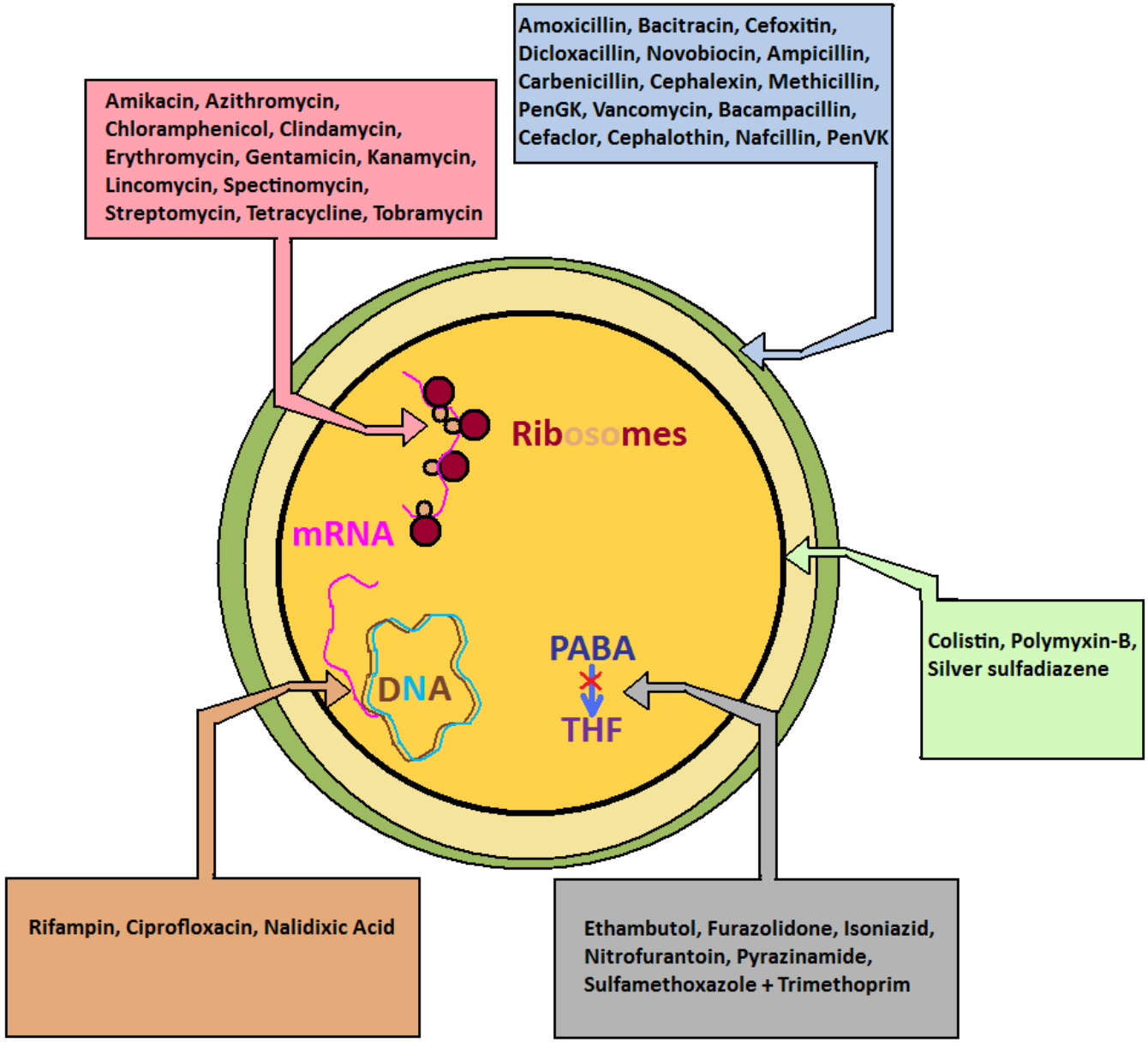
Cell Membrane Disruptors Examples



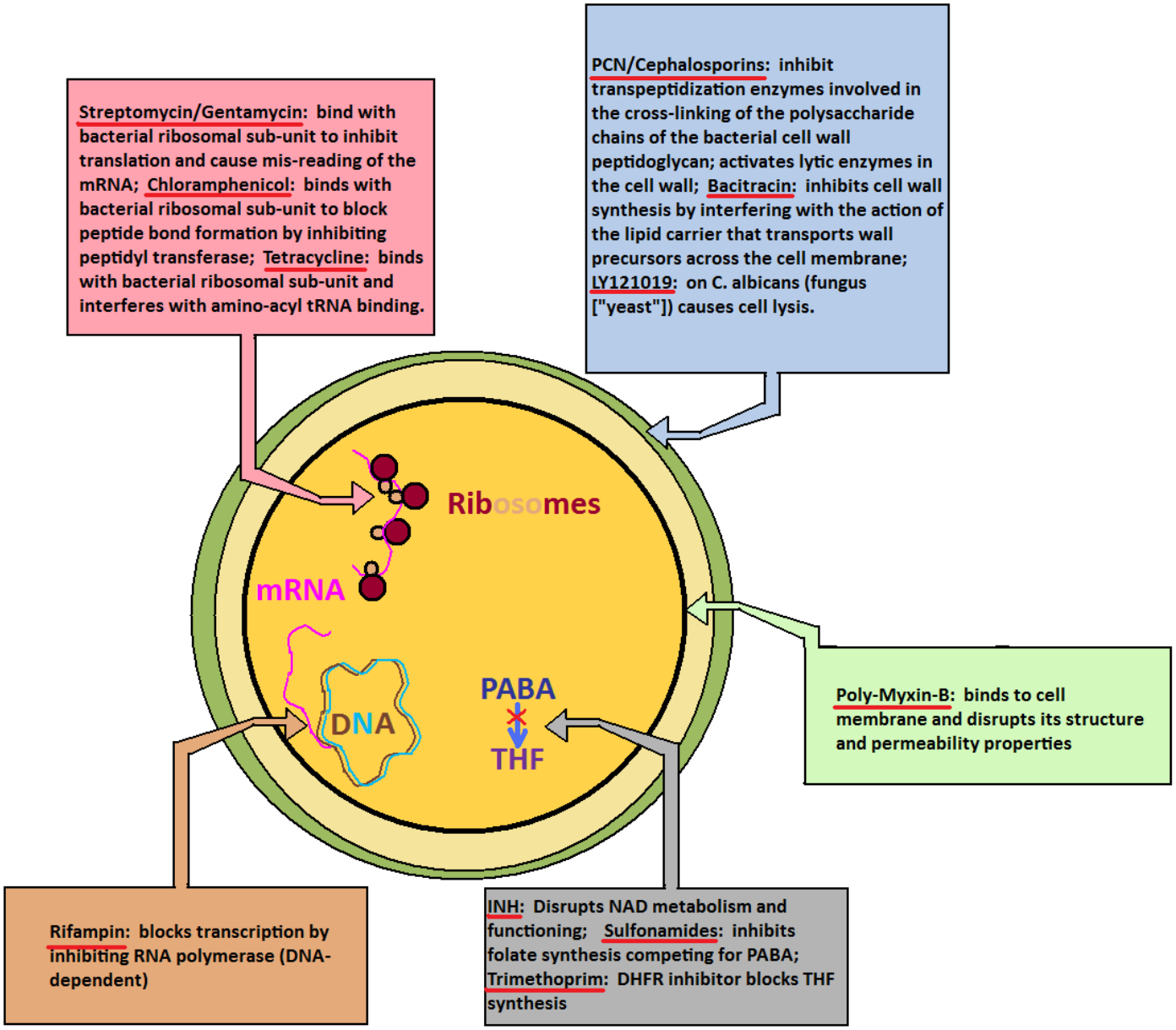
Example Action Mechanisms



Metabolic Antagonists Examples



Example Action Mechanisms

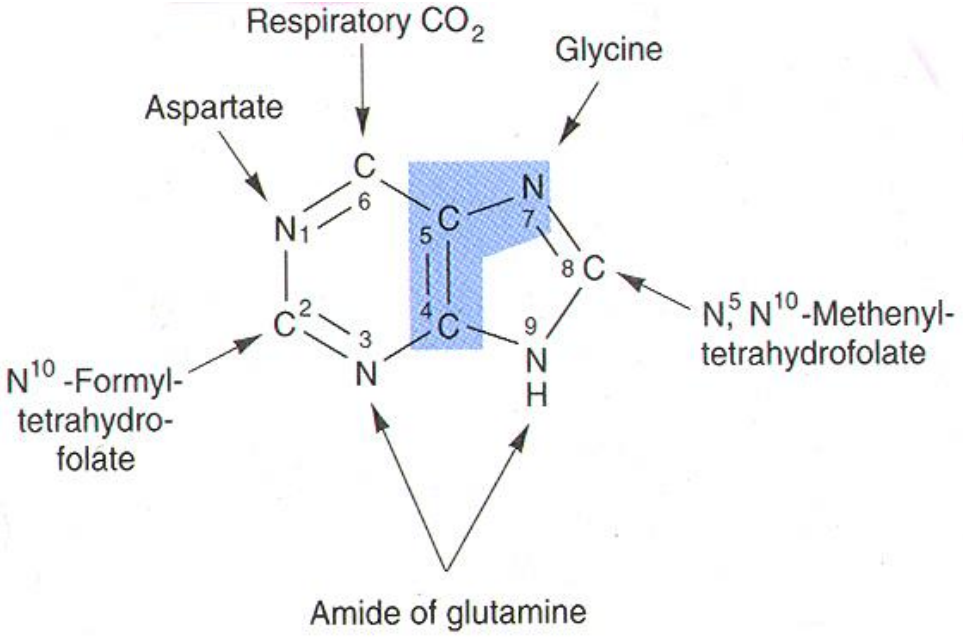


Nucleic Acid Metabolism: A Proper Introduction

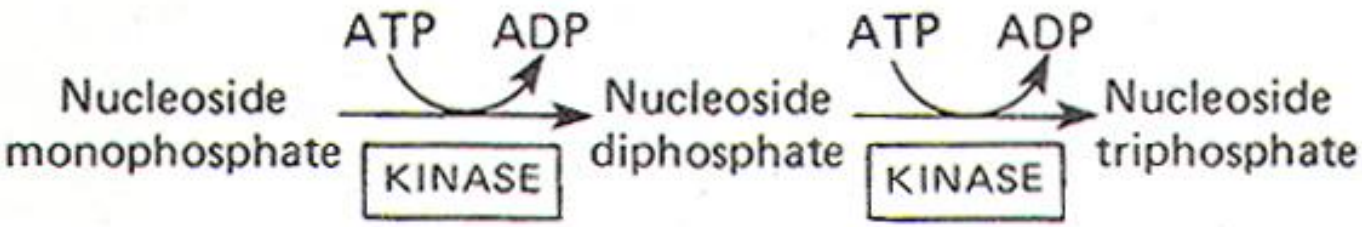
The Nickel Tour

Purine Nucleotide Biosynthesis/Anabolism

1. From Asp
2. Folic acid derivative
3. From Gln
4. From Gly
5. From Gly
6. From Cellular respiration
7. From Gly
8. Folic acid deriviative
9. From Gln

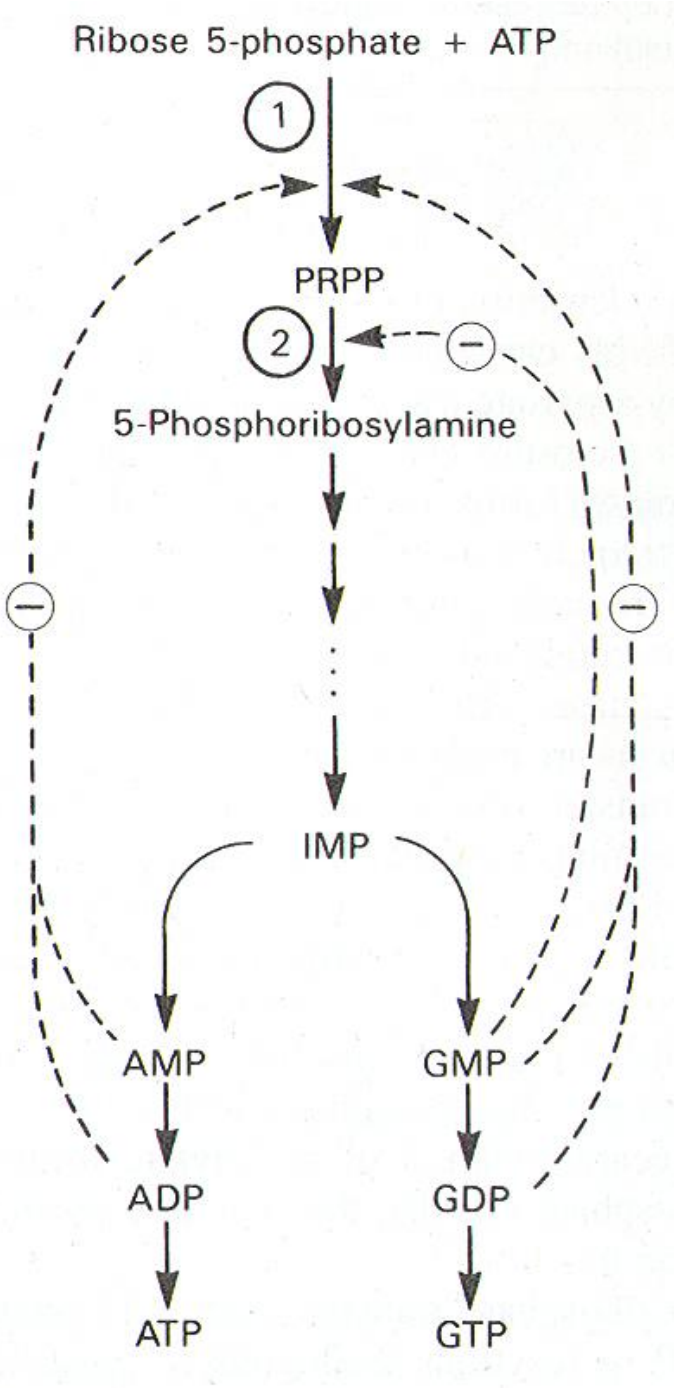


NMP → NDP → NTP Conversion



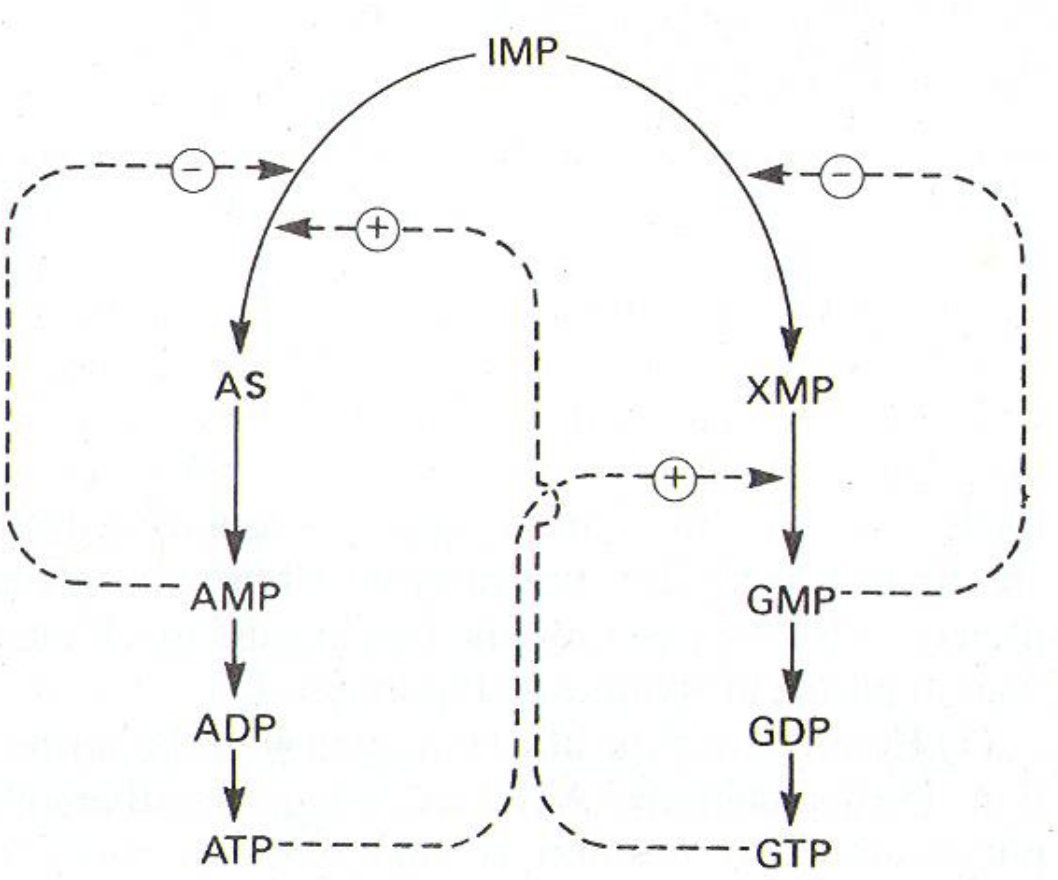
de novo synthetic rate control

- (-) = inhibition
- (+) = stimulation
- Both are feedback types of inhibition

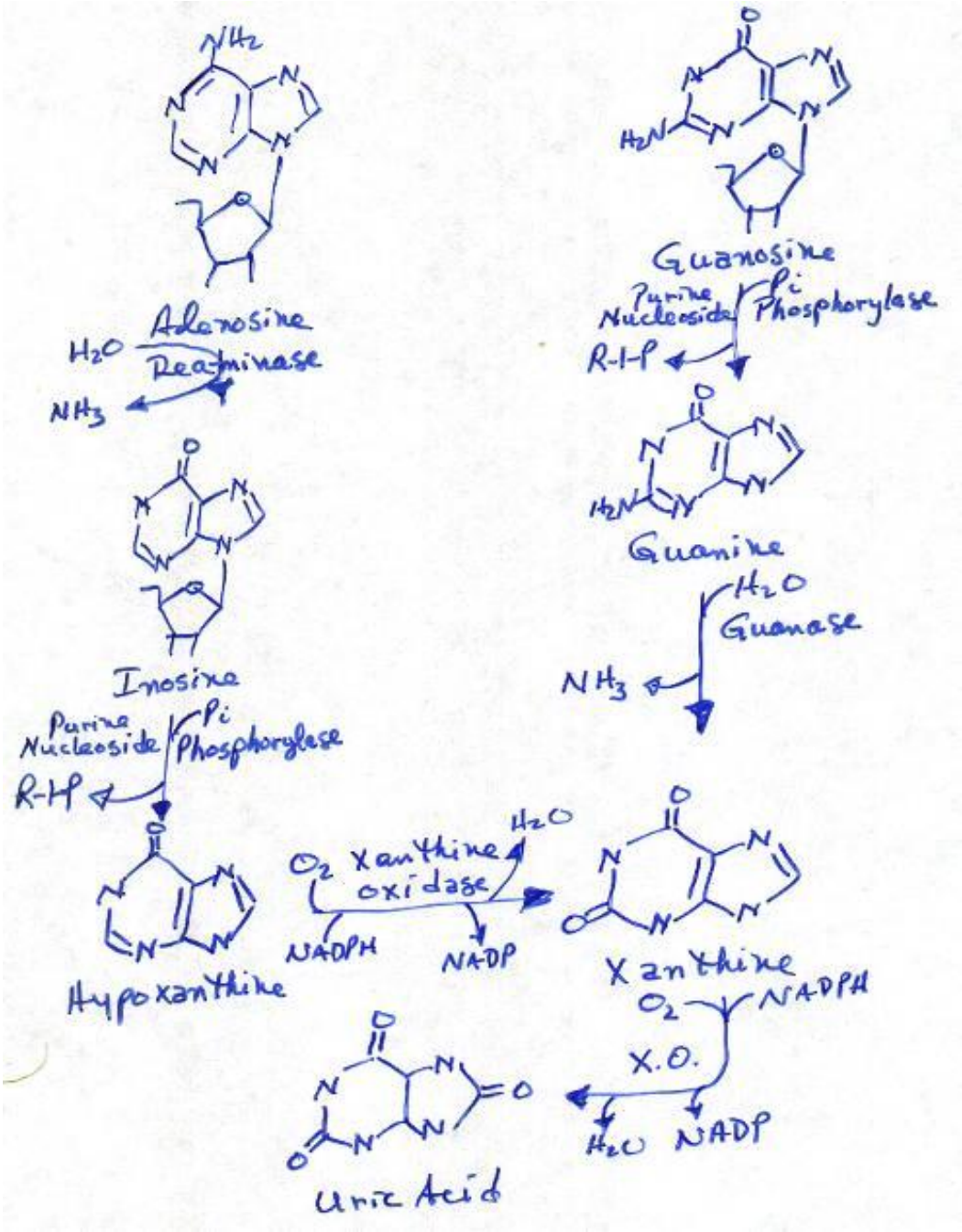


Ibid

X = xanthosine



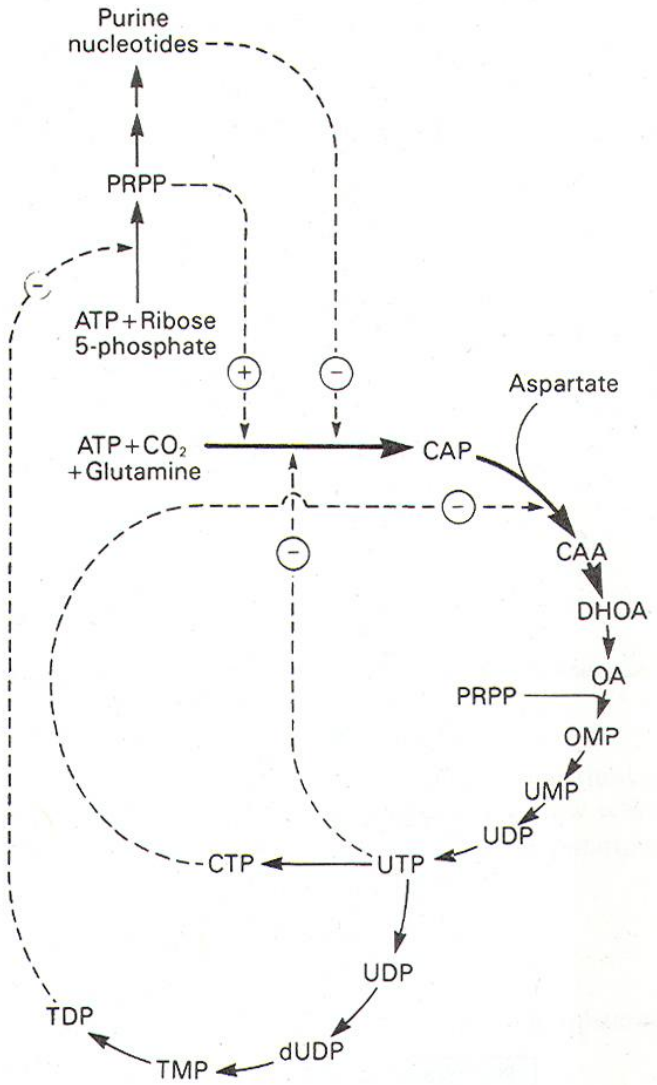
Purines: Catabolism



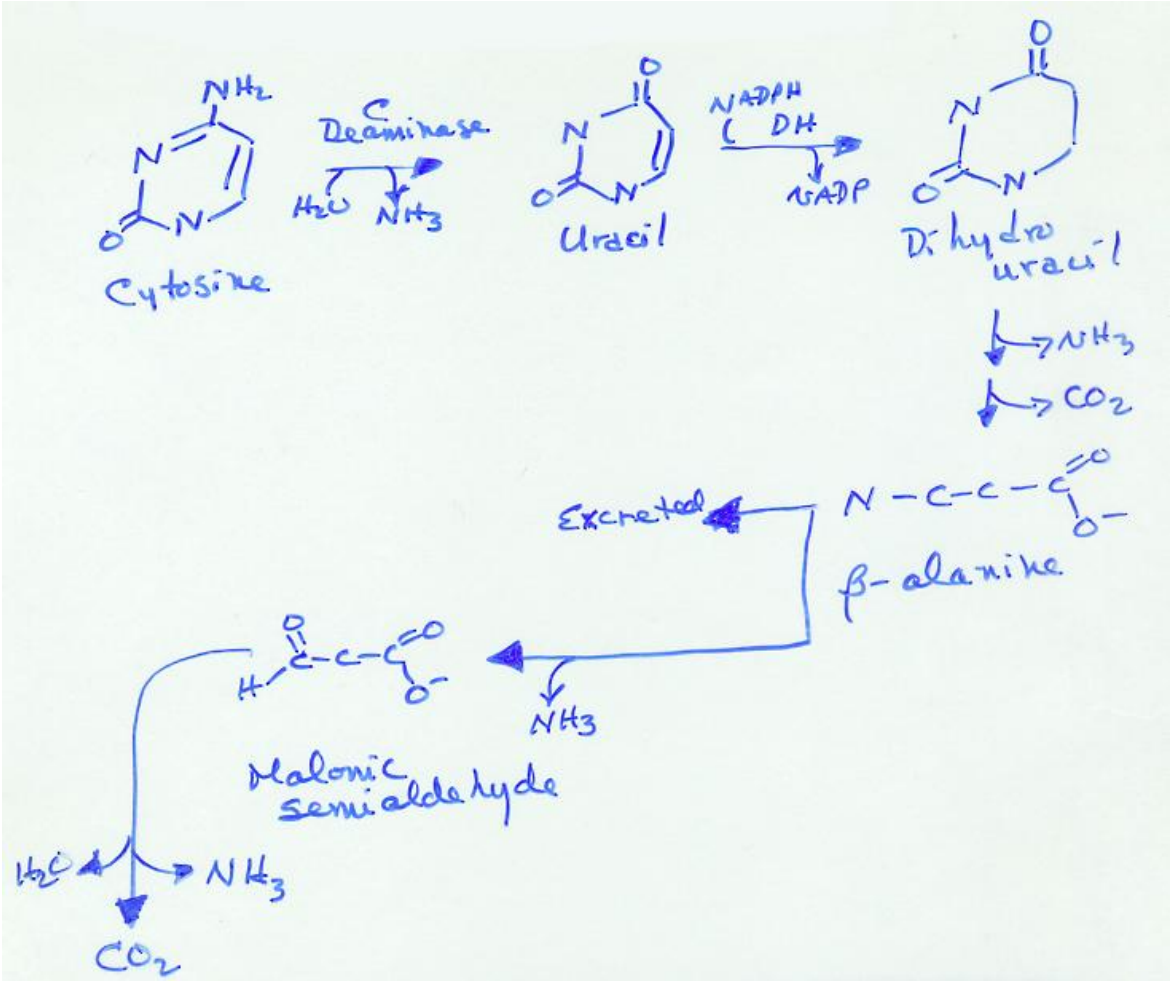
Purine-Pyrimidine Inter-Relationships

CAP = carbamoyl phosphate

CAA = carbamoyl aspartic acid



Pyrimidines



- Cytosine deaminase is highly elevated in some solid tumor cells
- Inhibition with tetrahydrouridine improves therapy due to reduced drug degradation

More Pyrimidines