What is DNA? Made of Nucleotides

5' end — Adenine, Thymine — 3' end

Note: when we add a nucleotide we add it to the 3' end.

Note: we don't label Carbons

Guanine

ie.

Phosphate Deoxyribose Backbone

① Nitrogenous Base (4 types) (lots of N)
- 2 types
- Purines
- Pyrimidines

Guanine (Purine)
Cytosine (Pyrimidine)
Thiamine (Pyrimidine)
Adenine (Purine)

ie Guanine

② Deoxyribose Sugar (In DNA)
In RNA it's ribose sugar

③ Phosphate group

Every 3 letters code for an amino acid

Purines (Adenine, Guanine)
Pyrimidines (Cytosine, Thymine, Uracil)

A ≡ T (2 H-bonds)
G ≡ C (3 H-bonds)
Hydrogen Bonds (weak bond)

Interphase
- G_1 - grows
- S - copy phase
- G_2 - grow

Mitosis

DNA replication

Eukaryotes — Cell cycle

Prokaryotes — Binary Fission

Genome: All genetic material in Body

B/C DNA is so long it is packaged up with a Protein called Histones

DNA is double stranded and made of Nucleotides

DNA — H1 (Ties nucleosome beads together) — H2A, H2B, H3, H4 — Histones

H1 (not in nucleosome core)

We can replicate DNA in Mitosis

Note: If wrapped too tight then Transcriptionally inactive. "Heterochromatin"

Euchromatin: less condensed, Transcriptionally active

Chromatin condenses to Chromosomes.

Genes can make proteins

Ie. Muscle, Skin, Liver (All cells in our Body have same DNA sequence)

Skin — DNA

The DNA and histones can Tag genes to either "Turn on, Turn off"

Methylation

makes DNA mute

CpG site — CpG site — CpG Island

Methylation of CpG sites in Promter regions is a/k/a "gene Silencing"

Epigenetics

Level of DNA methylation

sperm — egg — zygote — morula — Fetus — uterus

In Zygote phase male DNA goes thru rapid DeMethylation

By morula stage the methylation pattern has been erased

Moms gradual

Enzymes responsible for DeMethylation of Mom/Dads are called TET (Ten Eleven Translocation)

methylcytosine dioxygenase I (TET1)

DNMT1

responsible for maintaining methylation during cell division

DNMT3a, DNMT3b

responsible for DE NOVO methylation

Histone Modification

are added to Histone tails

Acetyl, methyl groups

relaxes the coil

Acetylation H3K9 (activates Chromatin)

Methylation H3k27 (Represses)

What can we do with DNA?
1) Replicate it (mitosis)

To make proteins and enzymes in our body the DNA must make RNA first.

DNA is wrapped around Histones

Transcription

Genetic information packaged as genes

3 types of RNA in human Body

① mRNA (RNA poly II)
② tRNA (RNA poly III)
③ rRNA (RNA poly I) (ribosomes)

STORY I

Nucleotides: Purines (A, G), Pyrimidines (C, T, U)

DNA — Transcription — RNA — RNA polymerase II

Still pre RNA at this point

CAP — Intron — Exons — PolyA tail (many Adenosine) — AAA

Splicing — Keep Exons, Remove Introns

CAP — AAA — now its mRNA

STORY II

pre tRNA

Transcript — RNA poly III — pre tRNA — splicing — tRNA

STORY III

making of Ribosomes (must go into nucleolus)

pre rRNA — RNA poly I — splicing — will splice into big and small Subunits

Nucleolus — Nucleus — small, large — Still pre rRNA

Nucleus — cytoplasm — Large, small — will attach to CAP end of mRNA

Transcription (make RNA from DNA) + Translation

mRNA polymerase

Nucleus — cytoplasm

mRNA leaves nucleus goes into cytoplasm

The mRNA Serves as a Template to make proteins

20 Amino Acids (hang out in cytoplasm)

In the cytoplasm is Aminoacyl tRNA synthetase and it binds an Amino Acid to tRNA

will bind A.A. to tRNA

Each tRNA will attach to an AA one

Note: The tRNA will now fold up differently

remember: tRNA is made up of nucleotides, not AA

So now in cytoplasm we have

The ribosome connect tRNA to mRNA

AUG = Methionine

Ribosome — Large, small — moves — CAP — mRNA — AAA

The Ribosome has 3 special sites w/n it.

E = exit
A = Attaches incoming Aminoacyl tRNA

The ribosome will move towards Poly A

E P A — CAP — AAA

Codons — E P A — match mRNA-tRNA — anti-codon

If its a match the tRNA will bind to A site and mRNA will move to the right, then next will be P site

the tRNA will now move to P site where it will release its Amino Acid

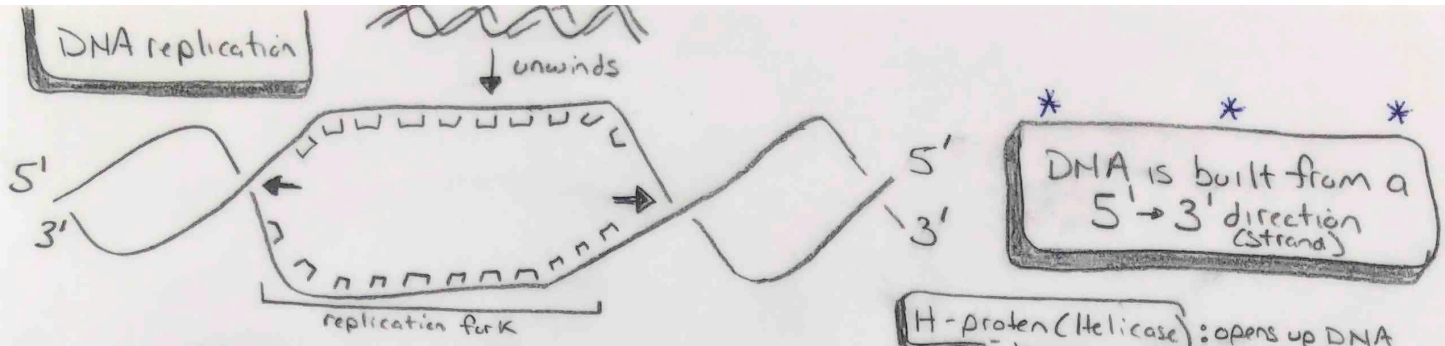
Join AA — Amino Acid chain (protein) grows.

A = Attach
E = Exit

anti-codon (tRNA)
codon (mRNA)
match (Translation)

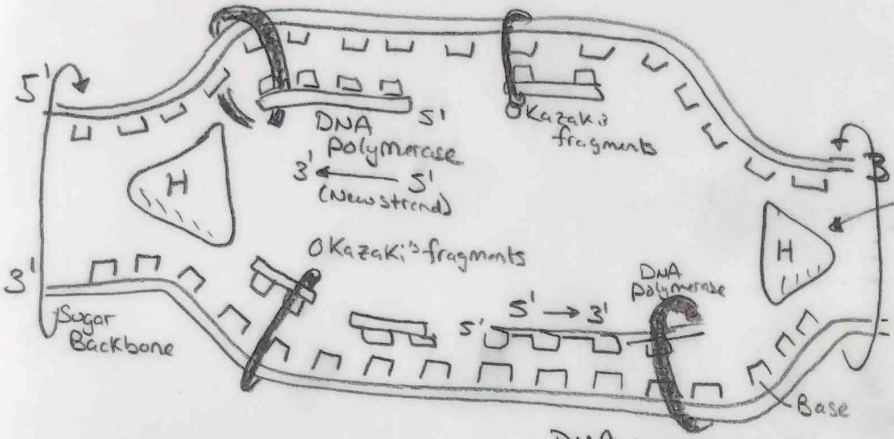
Ribosomes are Translating the mRNA to make proteins

DNA replication



DNA is built from a 5'→3' direction (strand)

H-protein (Helicase): opens up DNA breaking the Hydrogen Bond "unwinds"



cant start 5-3 here b/c it is blocked.

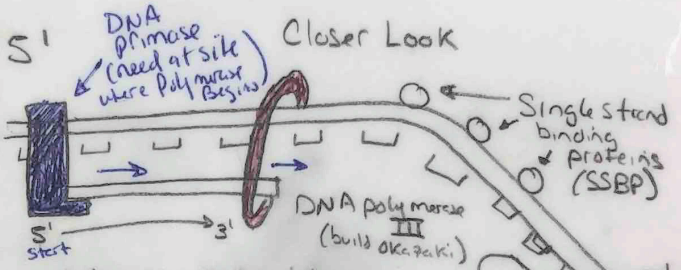
So how does DNA get built from where the H-protein is, since it is always moving.

Answer: DNA is built in small fragments together with RNA

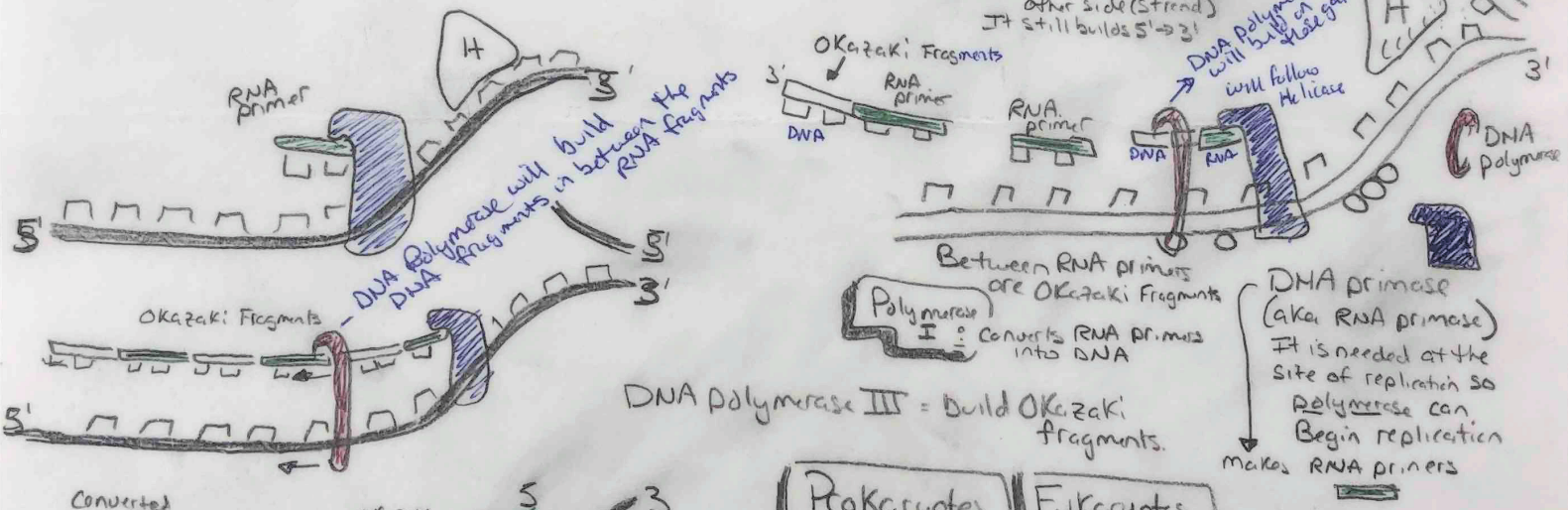
DNA doesn't like to stay straight. ∴ we use Single Strand Binding Proteins to prevent recoiling

Lagging Strand

Closer Look



So how can DNA build on other side (strand) It still builds 5'→3'



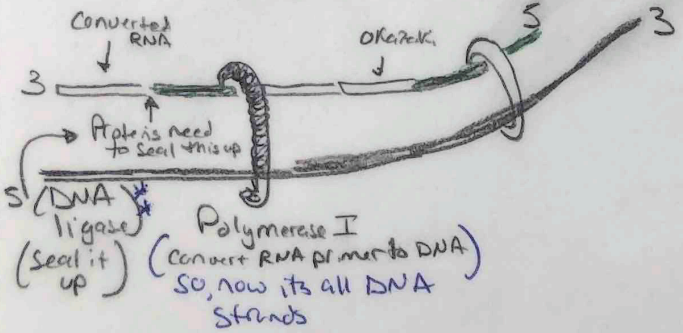
Between RNA primers are Okazaki Fragments

Polymerase I: converts RNA primers into DNA

DNA primase (aka RNA primase) It is needed at the site of replication so Polymerase can Begin replication

makes RNA primers

DNA polymerase III = build Okazaki fragments.



Prokaryotes	Eukaryotes
Helicase	= Helicase
Polymerase III	= Polymerase δ (Delta)
SSBP	= Replication factor A (RPA)
DNA primase	Polymerase α
Polymerase I	MF-1
Ligase	Ligase I

Mutations in DNA

DNA → RNA → Protein

3'-CTC-5' 5'-GAG-3' Glutamic Acid

mutation

3'-CAC-5' 5'-GUG-3' Valine

Mutations are mistakes in cells DNA

- Mutations occur at the DNA level, but have their effects at protein level.
- Can be classified by either their effect on DNA or Protein

Severity:

nonsense > missense > silent

Types of Mutations

1. **Point Mutation** - one base is replaced w/ another - "base substitution"

3'-GTCCTCCTC-5' DNA → 5'-GAGGAGGAG-3' RNA → Glu-Glu-Glu (Glutamate) (normal)

3'-CTCCTCCAC-5' → 5'-GAGGAGGUG-3' → Glu-Glu-VAL (mutation)

In this scenario a Thiamine Base was replaced by Adenine. (This ex. is Sickle Cell when Glutenic acid is replaced by Valine at the sixth position.

Point Mutations can be subdivided

Silent

If you changed the 3rd base pair, it would not change the A.A.

normal: DNA TTC / mRNA AAG / Lys

Protein level: TTT / AAA / Lys

Nonsense (worse than missense b/c you loose everything after the stop)

Change results in early STOP codon

ATC / UAG / STOP

Missense

Conservative

The new A.A. is like the old one. ie Glutamate → Aspartate (both acidic) or

TCC / AGG / Arg

non-conservative

Serine → Phenylalanine (small, polar) (large, non-polar)

TGC / AGG / Thr

Examples of Point Mutations: Cystic Fibrosis, Cancer, Neurofibromatosis, Sickle-Cell anemia, Tay Sachs, Color Blindness.

2. **Frameshift** - Insertion or Deletion to the Sequence ∴ more significant than point mutation b/c more changes affected.

DNA 3'-CTCCCTC CTC-5'

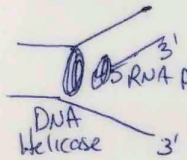
RNA 5'-GAGGGAG GAG-3' An extra Guanine

Glu-Gly-Gly

now all the codons after it will read differently.

ex's: Cancer, Chrons, Cystic Fibrosis, HIV, Tay Sachs, Hypertrophic Cardiomyopathy

DNA Repair



DNA Polymerase III

1. Replicates
2. Proofreads

Thymine, cystine - Pyrimidine - 1 ring

adenine, Guanine - Purines - 2 rings

If wrong pairing, then DNA Poly. III will remove incorrect base and replace w/ correct one.

DNA damages the backbone and then can be recognized.

DNA polymerase III

Exonuclease Activity (3'→5') - remove nucleotide at the end of the strand

remove nucleotide

Endonuclease Activity - can remove a bad nucleotide in the middle.

DNA Polymerase I (5'-3' direction)

- also has exonuclease activity
- It will remove RNA Primer

DNA I, III → Can repair

But, what if mistake not corrected during replication:

This happens after replication

Mismatch repair Mechanism

- Composed of Proteins

1st task: Is there a problem.

yes, b/c there is an error in the backbone.

1. Proteins mark area w/a cut. (mark)
2. Exonuclease will remove incorrect nucleotide
3. DNA Polymerases (I, III) will insert correct nucleotide
4. DNA ligase will connect them together.

(Backbone is now prepared)

How does Mismatch repair mechanism know which base is correct and which one is incorrect?

In Bacteria Adenine and cytosine will be methylated.

In Eukaryotes, we are unsure.

UVB rays → UV damage causes pairing of Pyrimidine dimers (now the Backbone sticks out and makes the surrounding bonds weaker)

We can fix this by nucleotide excision repair.

1. endonuclease will remove pyrimidine dimers
2. DNA Polymerase will bring in good nucleotides
3. DNA ligase will "glue it"

Note: exonuclease can only repair if at beginning or end.

DNA Repair

Single Strand Repair

- nucleotide excision repair (UV light)
- Base excision repair caused by chemicals leads to deamination repair by Glycosylation
- Mismatch Repair (unmethylated strand) ie. HNPCC

Double Stranded repair

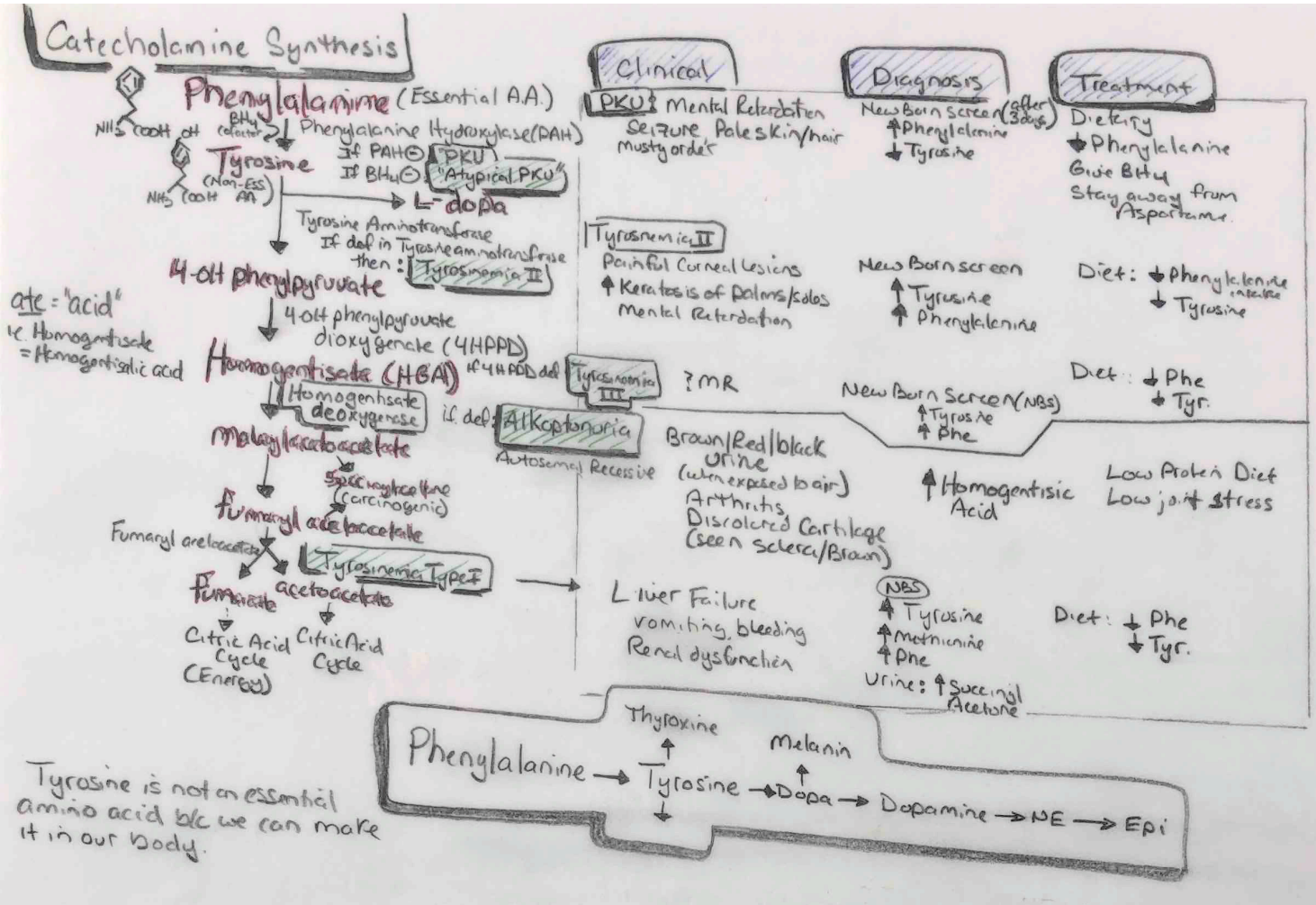
- non-homologous end-joining: ligase (just binds them together)
- Microhomology: the overhanging area is removed.
- Homologous recombination

DNA damage

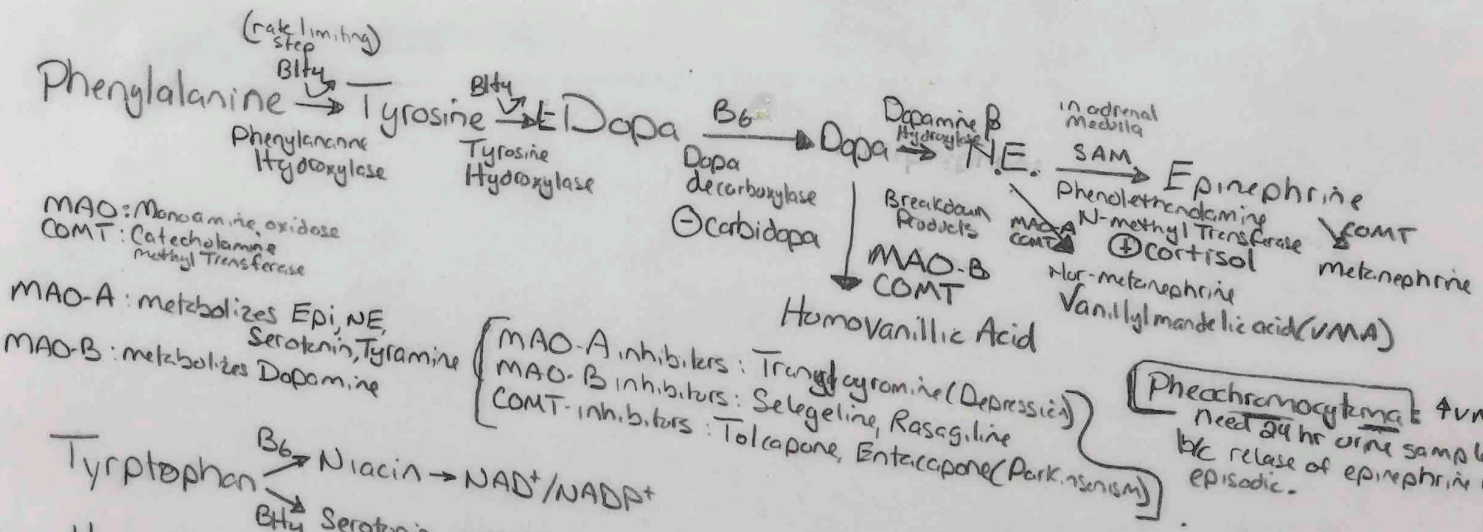
Endogenous	Exogenous
• Reactive O_2 species	• UV rays
• Peroxides	• Gamma Rays
	• X Rays

If cell can't get repaired the bad cell will:

1. Senescense "sleep"
2. apoptosis
3. Divide uncontrollably (cancer)



Tyrosine is not an essential amino acid b/c we can make it in our body.



Tyrptophan → (B6) Niacin → $NAD^+/NADP^+$
Tyrptophan → (BH4) Serotonin → melatonin
Histidine → (B6) Histamine
Glycine → (B6) Porphyrin → Heme
Arginine → Creatine
Arginine → urea
Arginine → Nitric oxide
Glutamate → (B6) GABA
Glutamate → Glutathionine