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Lecture 1

9/5

- professor has a math PhD???
- other professor does chemistry??? Wanted to study drama too

Important central idea of this class: unifying principles. Not facts only!

Important stuff

- Read the course website.
  - → Course policies, recitation sections, etc.
  - → Office hours, recitations start next week
- Fill out the mock submission survey!
- MITx?
- 4 exams + final. (This is a change!) Also, you can drop an exam.
  - → You DO need to show up.
- Problem sets cannot be late. Collaboration is fine, but not copying.
- Different styles of learning
  - → Paper textbook (Life), series of videos (see MITx), online textbook (!)
  - → Choose what you want for your own learning

What is this class?

- Medicine rapidly developing in the present
- Cancer therapy, etc.
- DNA fingerprinting → then privacy issues, ancestry, etc.
- Constantly changing & driving forward? Things like genome editing!
- "Diversity of life" — what's the common operating system?
- Different "levels" — biospheres, ecosystems, organisms, organs, tissues, cells, organelles, molecules (big to small)
  - → We focus on the small things because they are common.
- Evolution — we won't talk much about it. 60 seconds:
  - → 4.5 billion years — Earth forms
  - 4 billion yr — cools
  - 300 mil / 3.7 billion — life forms, somehow (prokaryotes)
  - ! 2200 mil / 1.5 billion — first nucleated cells (eukaryotes)
  - → 0.5 billion yr — multicellular organisms!
  - 0.005 billion yr — human-like lineage
  - 0.001 billion — Homo sapiens
  - 0.0000002 billion — MIT founded.

- Cell biology — covered a bit.
  - → Important: prokaryotes ~1-2 μm vs eukaryotes ~40 μm.
  - → There is no "the cell," but it doesn't matter that much.
    - General abstract principles!
- Organization of class
  - → Biological functions — biochemists (components w/o organism) vs geneticists (organisms w/o components)
    - ↳ results: look at proteins and genes, respectively.
    - ↳ Then in the 20th century: DNA → molecular biology
      - → Idea: RNA from DNA is sent off to a ribosome and creates proteins!
      - → New scientists: recombinant DNA
      - → Then came genomics: study the entire human genome
      - → Cost of human genome: $3 billion → $500
- Basically, we are always doing more and moving forward
  - → Pertubation, not observation
  - → Data scientists
  - → Continual improvement!

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Lecture 2

9/7

- Change recitations now!
- P-set posted on Monday (see instructions on Stellar)
- Fundamentals of life = fundamentals of matter.

Periodic Table

- Important to life: H, C, O, N, S, P
  - → Also, Se, Si, I
- Cations: K^+, Na^+, Ca^{2+}, Mg^{2+}
- Anions: Cl^-
- d-orbital ("d-block") atoms: Fe, Mn, Co, Ni, Cu, Zn, etc.
  - → ~1/3 all proteins are metalloproteins
  - → Good for catalyzing reactions, etc.

Chemical bonding

- Goal is to have a complete valence shell of (e^-)(electrons)
  - → "Noble gas" configuration
- Who gets the (e^-)? Depends on electronegativity! (χ)
  - → ability of atoms to attract (e^-) from other atoms
  - → [On a scale of ~0–4.] Low near bottom right, high near Fluorine

Ex

Na (1 val.) Cl (7 val.)

→ transfer of electron.
= ionic bond; this gives electrostatic attraction.

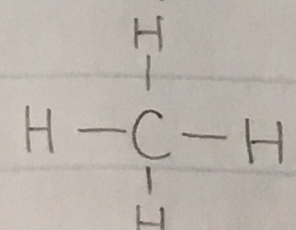
Since $\chi_{Cl} \gg \chi_{Na}$, Cl "takes" an electron from Na.

Ex

H (1 val.) C (4 val.)

⇓

Methane:



Need a lot of electrons → "sharing".

☆ Carbon likes to have 4 bonds.

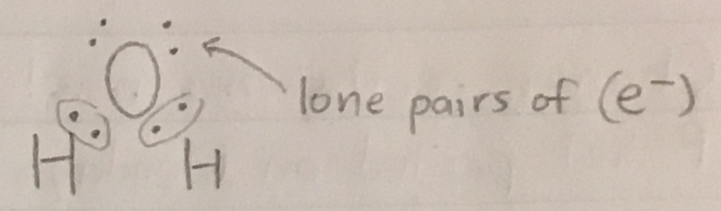
Covalent bond; pairs are shared.

- Nonpolar ≈ equal sharing
- vs
- Polar → unequal sharing

Since $\chi_H = 2.2$, $\chi_C = 2.55$, the difference $0.35 < \underline{0.4}$ (critical value) so nonpolar.

Ex

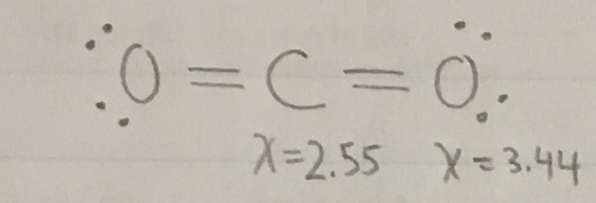
H (1 val.) O (6 val.)



$\chi_H = 2.2$, $\chi_O = 3.44$ → polar.

H_2O = water is polar molecule! (important)

Ex

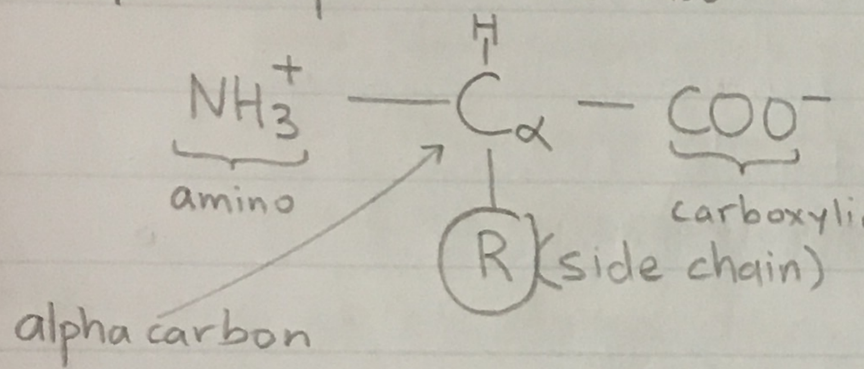


CO_2 = carbon dioxide has double bonds.

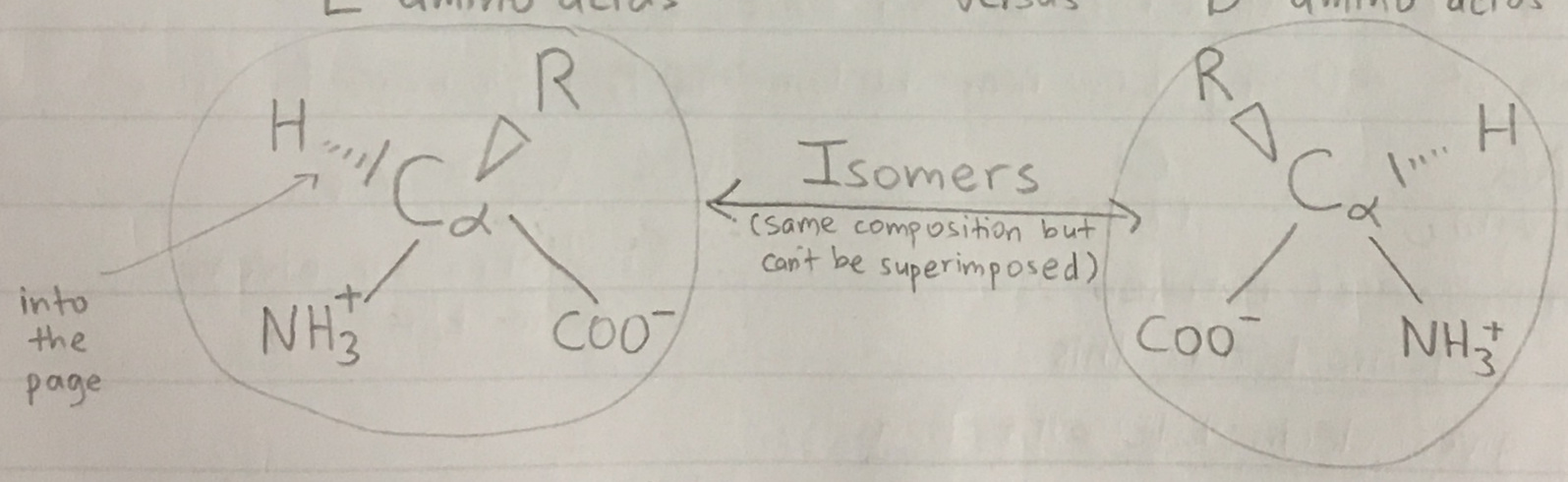
Bonds are polar, but by symmetry, the molecule it is nonpolar.

Molecules of Life

- Peptides / proteins are made of amino acids.



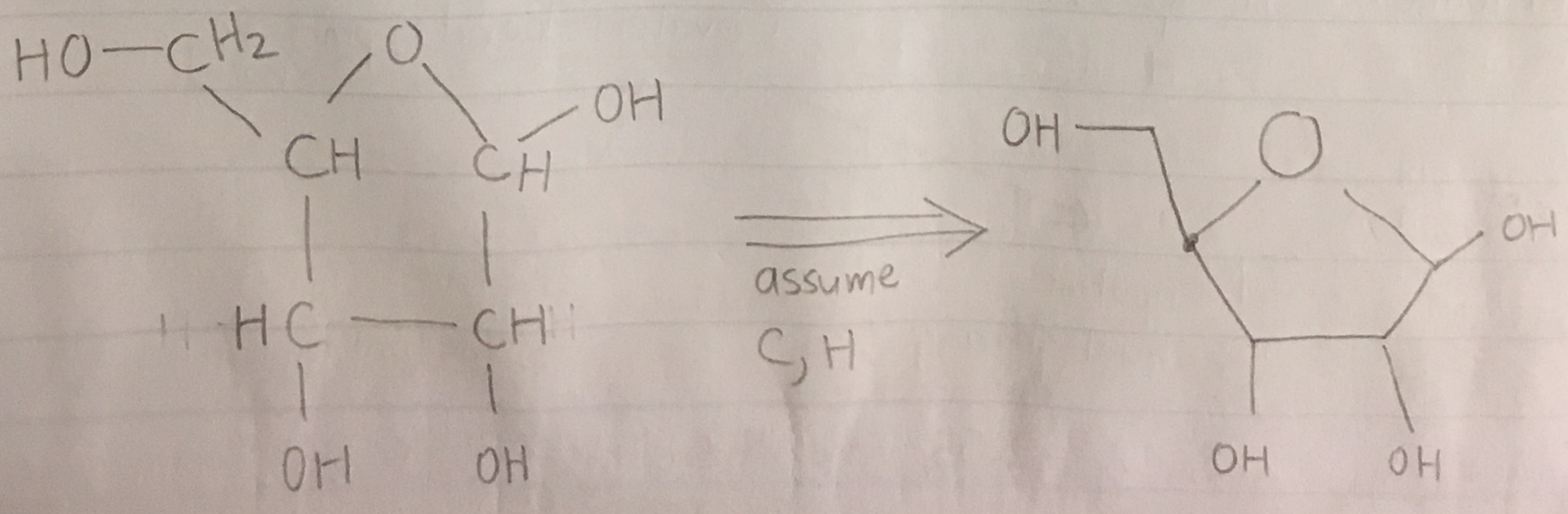
→ L-amino acids versus D-amino acids (mirror image)



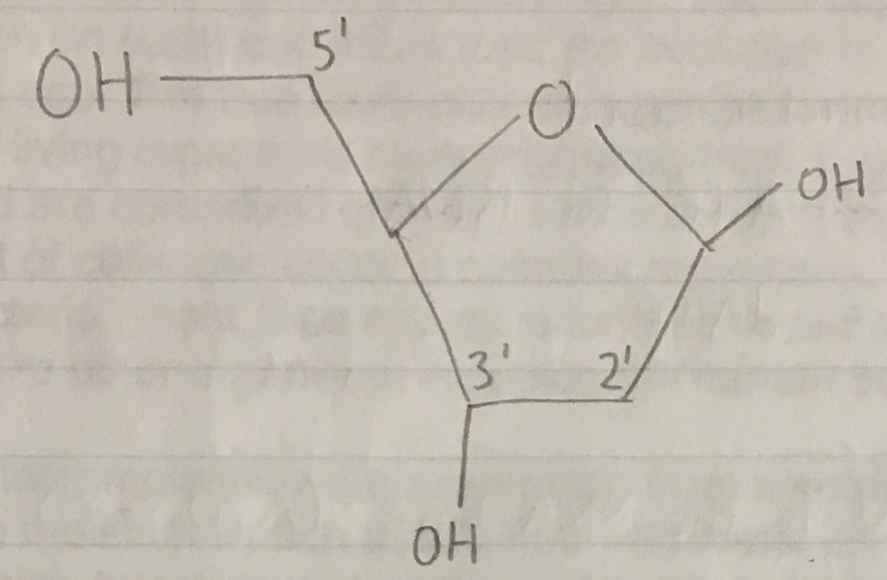
→ Peptide bonds attach amino acids together.

- Carbohydrates (sugars)

Ex Ribose



(Ex) Deoxyribose (missing OH at 2').



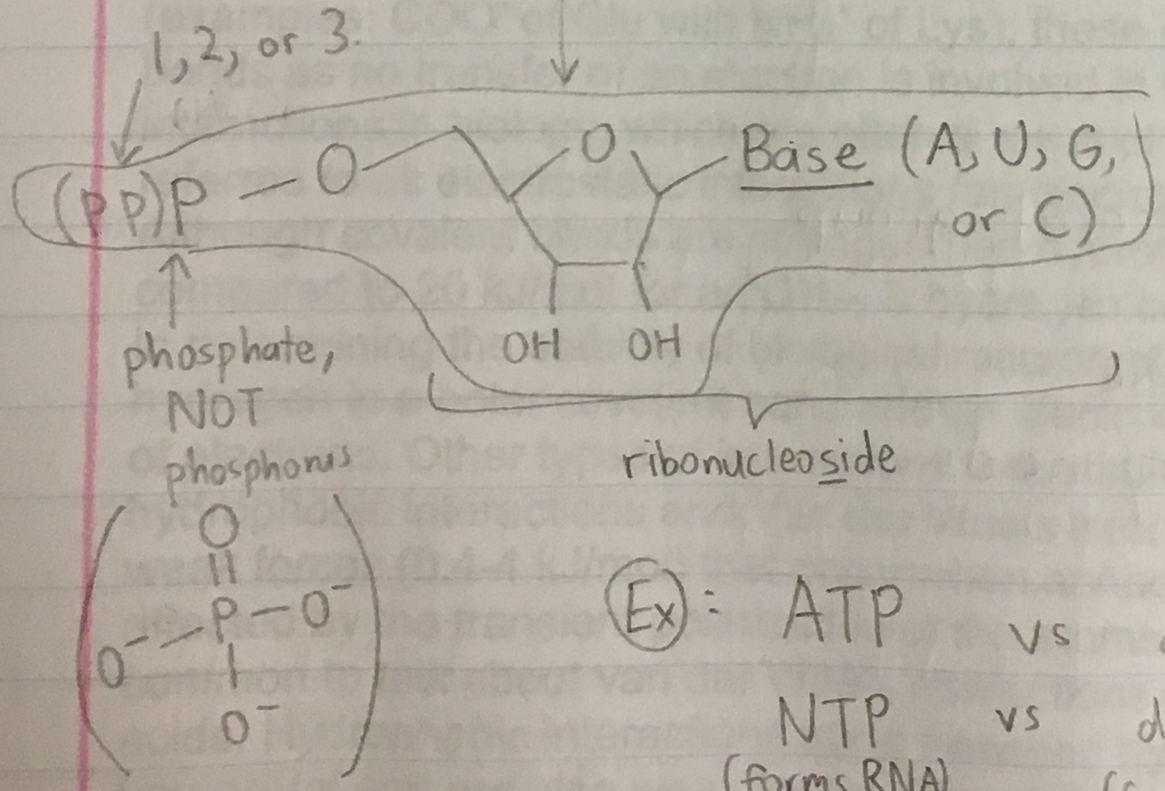
(Ex) Glucose ($C_6H_{12}O_6$)

→ Monosaccharides (single sugars) join and form oligosaccharides;
e.g. starch, cellulose, etc.

→ <u>Glycosidic</u> linkage between sugars.

- Nucleic acids

→ <u>Ribo</u>nucleotides form RNA; <u>Deoxyribo</u>nucleotides form DNA.



Same as RNA, but U becomes T, and missing 2' OH.

(Ex): ATP vs dATP

NTP (forms RNA) vs dNTP (forms DNA)

→ <u>Phosphodiester</u> linkage.

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Lecture 3

- RNA has extra hydroxyl group over DNA
- Mono-, di-, or tri-phosphate

(Ex) If base is A (adenine), the nucleic acid

adenosine triphosphate = ATP for RNA, and

deoxyadenosine " = dATP

- In general, call it NTP where N is the RNA base (A, U, G, C)
  or dNTP where N is A, T, G, or C. (DNA bases)
- NDP is diphosphate, etc.
- DNA stores info; RNA does more stuff
  ⇓ more stable ⇓ catalytic/reactive
- Nucleic acids linked at 5' and 3' carbons by phosphodiester

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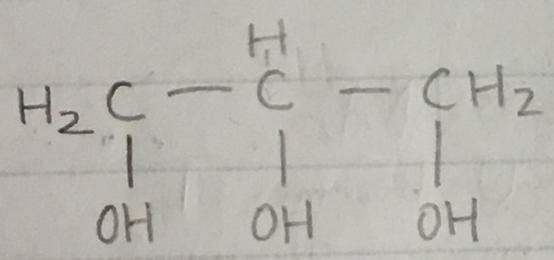
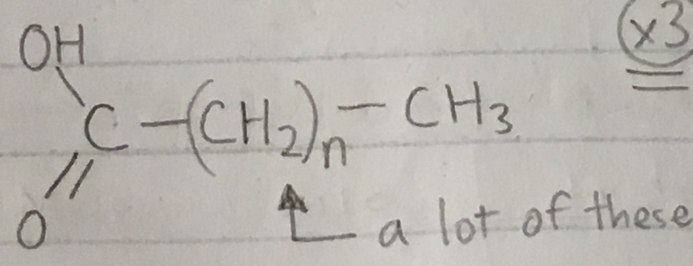
- Lipids (aka fats)

(Ex) triglycerides



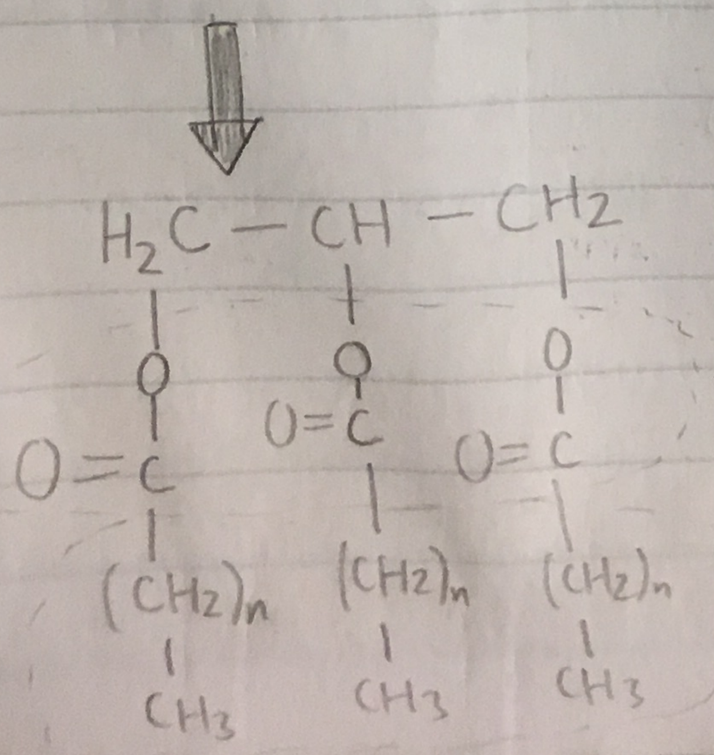
= glycerol group

+



(x3) of these = fatty acids

a lot of these

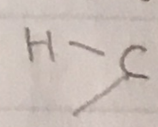


polar

nonpolar; called a hydrocarbon chain

Two kinds

Unsaturated has ≥1 double bond.

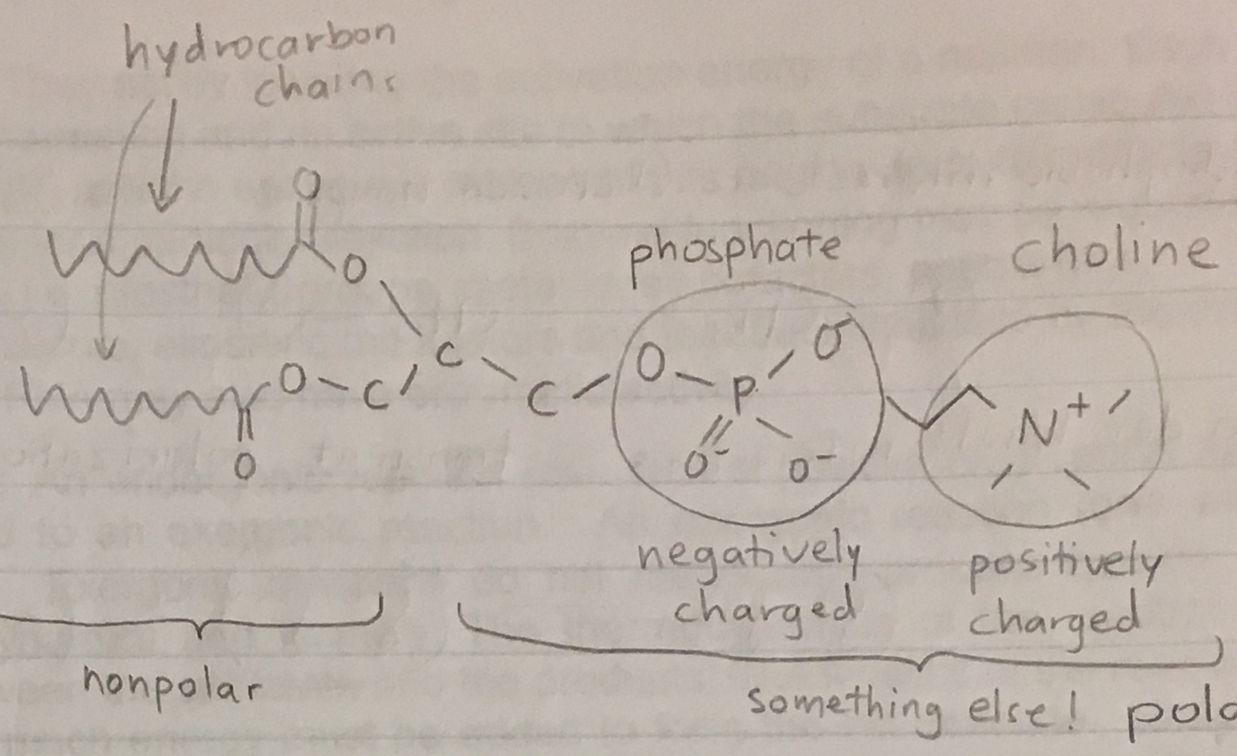


Saturated has none.

Types of Lipids

- Phospholipids.

(Ex) Phosphotidylcholine:



(recall C are not marked)

nonpolar

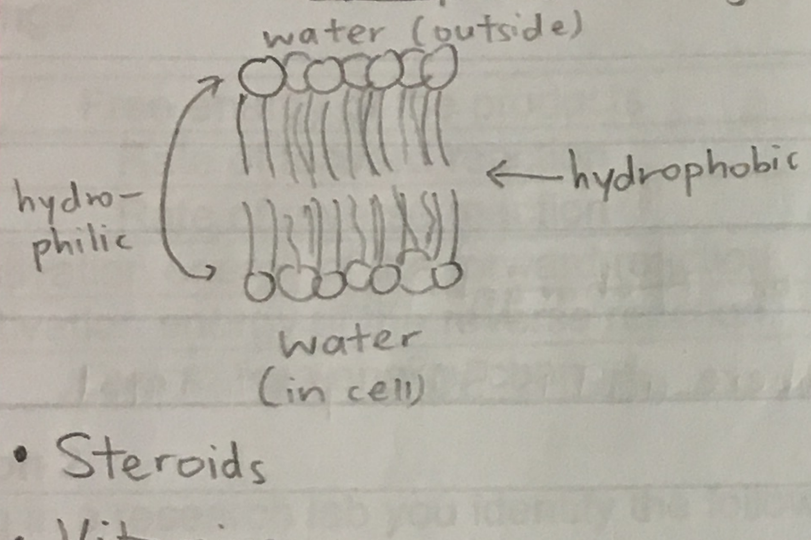
something else! polar.

→ this is called amphipathic.

→ Polar wants to be near water, nonpolar does not.

hydrophilic head group

hydrophobic tail



called the phospholipid bilayer.

This process is driven entropically.
(thermodynamically favorable)

- Steroids
- Vitamins

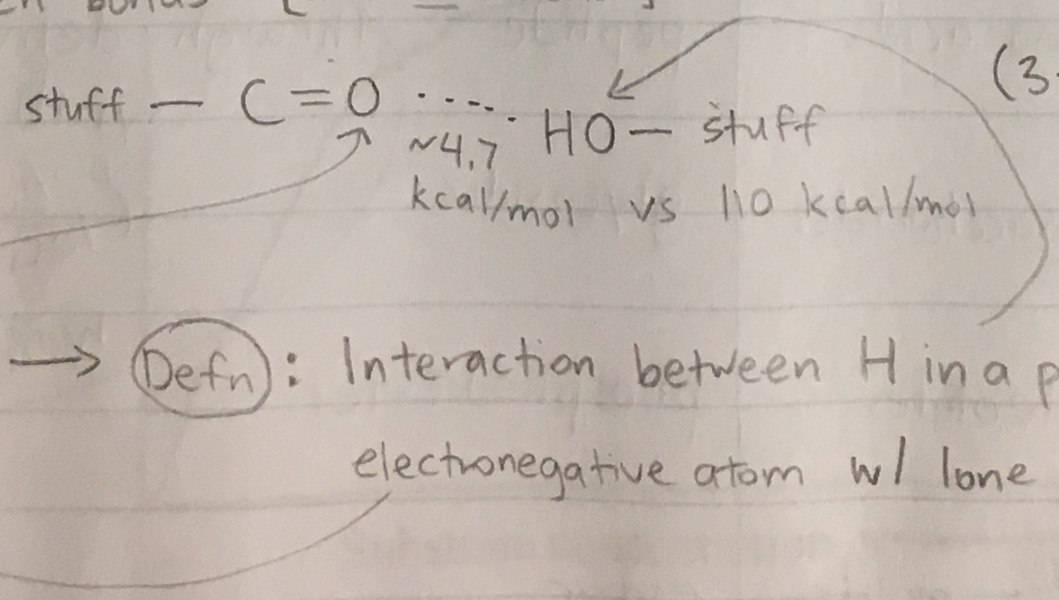
→ (Ex) Vitamin A, D, E, K fat soluble [more nonpolar bonds]
Vitamin B9 water soluble

Interactions that hold molecules together, from strong to weak

- Covalent bonds (C–C, O–H, etc.) (80–110 kcal/mol)
- Electrostatic interactions ($NH_3^+ - COO^-$) (3–8 kcal/mol) [Formal charges]

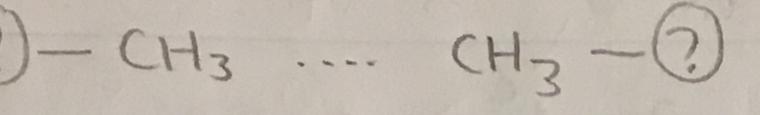
→ Note: $Na^+ - Cl^-$ ionic bonds are much stronger! >100 kcal/mol

- Hydrogen bonds [are not bonds]



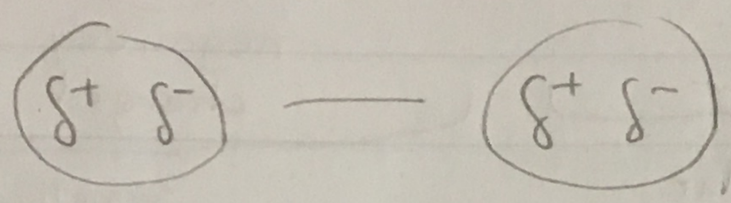
→ (Defn): Interaction between H in a polar bond and some electronegative atom w/ lone pair of (e^-).

- Hydrophobic interactions (between nonpolar substances) in the presence of water



(~1-2 kcal/mol)

- van der Waals interactions — transient polarization.



(~0.1-1 kcal/mol, distance-dependent)

There are also unfavorable interactions:

- Electrostatic repulsions
- Repulsion due to "buried charge" in the middle

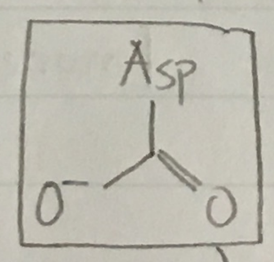
(Ex) Protein structure

→ Amino acids can be polar, nonpolar, + or − charged too.

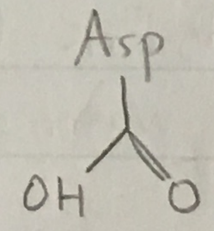
→ Some dependent on pKa → the pH where acid is 50% protonated.

$$\left(pH = pK_a - \log\left(\frac{[\text{protonated}]}{[\text{deprotonated}]}\right)\right)$$

(Ex) Aspartic acid.



or



$pK_a = 3.7$ so if pH < 3.7, more protonated and vice versa.

So at neutral pH ~7, this is dominant species.

Macromolecule	made of	Bond formed
Proteins	amino acids	peptide
Carbohydrates	monosaccharides	glycosidic
Nucleic acids	nucleotides	phosphodiester
Lipids	fatty acids	ester

through dehydration synthesis (loss of water)

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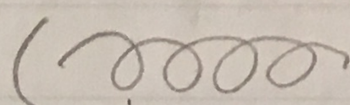
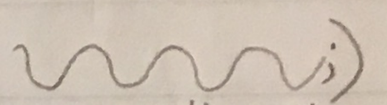
Lecture 4

9/12

Different types of structure

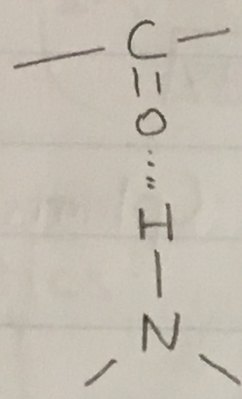
1° (primary) = sequence of amino acids (most proteins have many)

→ connected by peptide

2° (secondary) = α-helix or β-strand ( vs )

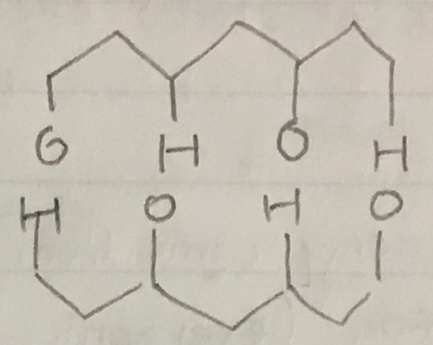
how the amino acids arrange themselves

very stable, hydrogen bonds



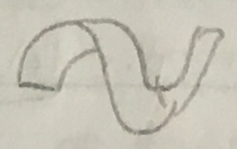
between backbone atoms

hydrogen bonds here too



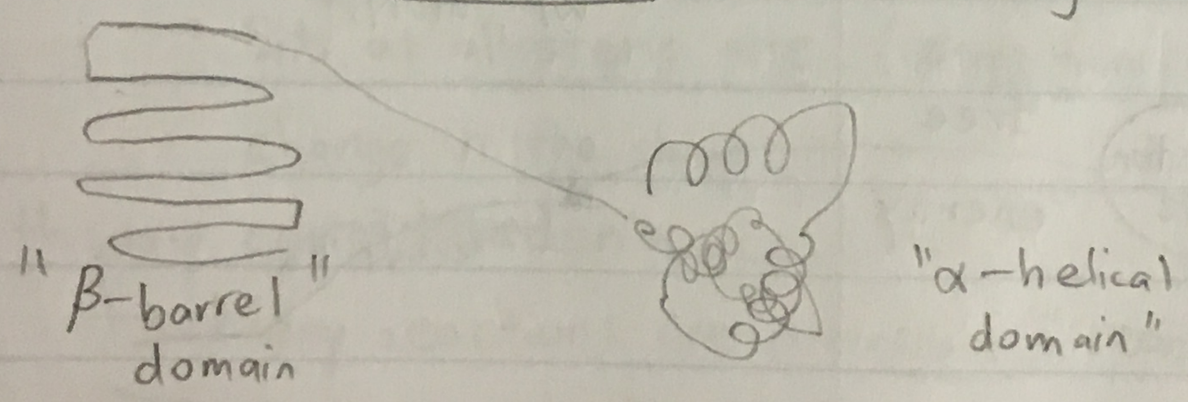
→ parallel or antiparallel

3° (tertiary) = protein fold (the 3D arrangement of the secondary structures)

→ α-helix referred to (ribbon drawing) 

→ β-strands shown as ⇄ or ⇉

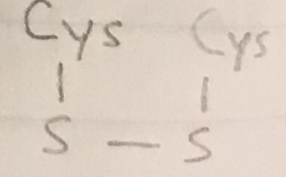
(Ex) chain w/ two domains (self-folding section)



4° (quaternary) = essentially number / arrangements of chains.

→ 1 chain = monomer, 2 chains = dimer, 3 chains = trimer,
4 chains = tetramer. "homo" = same amino acids, "hetero" = different

Factors that stabilize protein structure



covalent bonds → 1° (peptide bond), 3° (disulfide bonds), 4°

electrostatic interactions → 3° (side chains, e.g. Glu, Arg), 4°

hydrogen bonds → 2° (from backbone atoms), 3° (some backbone interaction), 4°

hydrophobic interactions → 3°, 4°

van der Waals → 3°, 4°

Why do proteins fold?

- Hydrophobic effect (entropically driven)
  → "Bury" hydrophobic, nonpolar atoms inside hydrophilic, polar atoms

Chemical reactions of life

$C_6H_{12}O_6$ [has lots of stored energy] + $6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ + energy
(glucose)

→ Where does energy come from? Need high availability (plants), favorable reaction (exergonic 👍 vs endergonic 👎)

[spontaneous] [nonspontaneous]

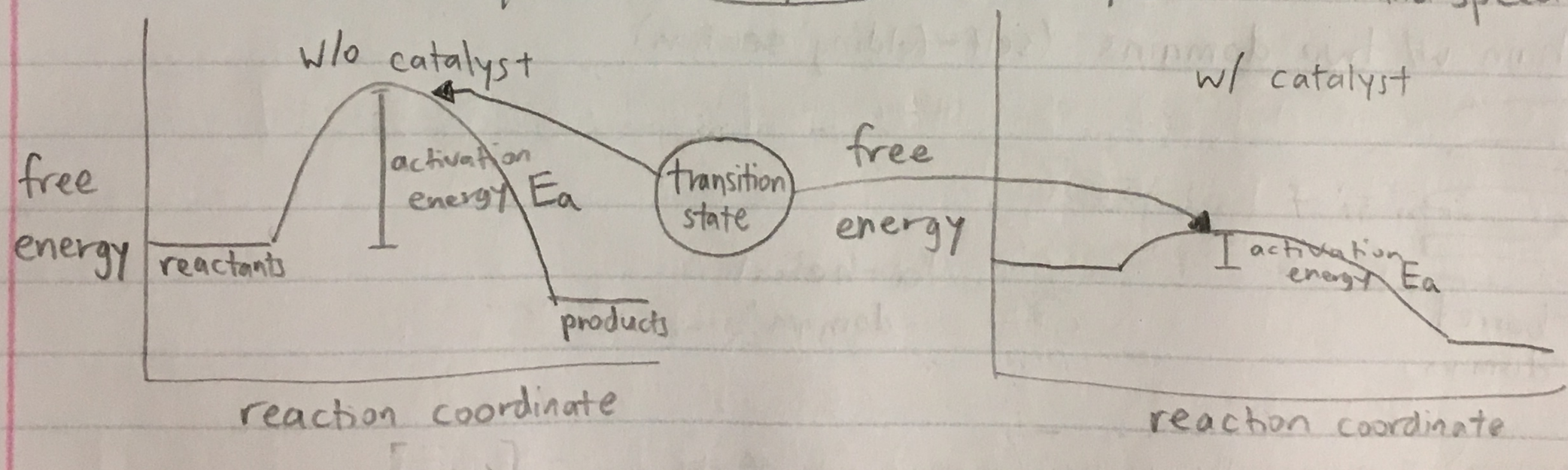
$\Delta G^\circ < 0$ $\Delta G^\circ > 0$

Standard state

→ ΔG° = difference in Gibbs free energy

→ For cellular resp., $\Delta G^\circ = -673$ kcals/mol

But rate of reaction (kinetics) is a factor too. So it is pretty slow unless you use enzymes to catalyze reaction and speed it up



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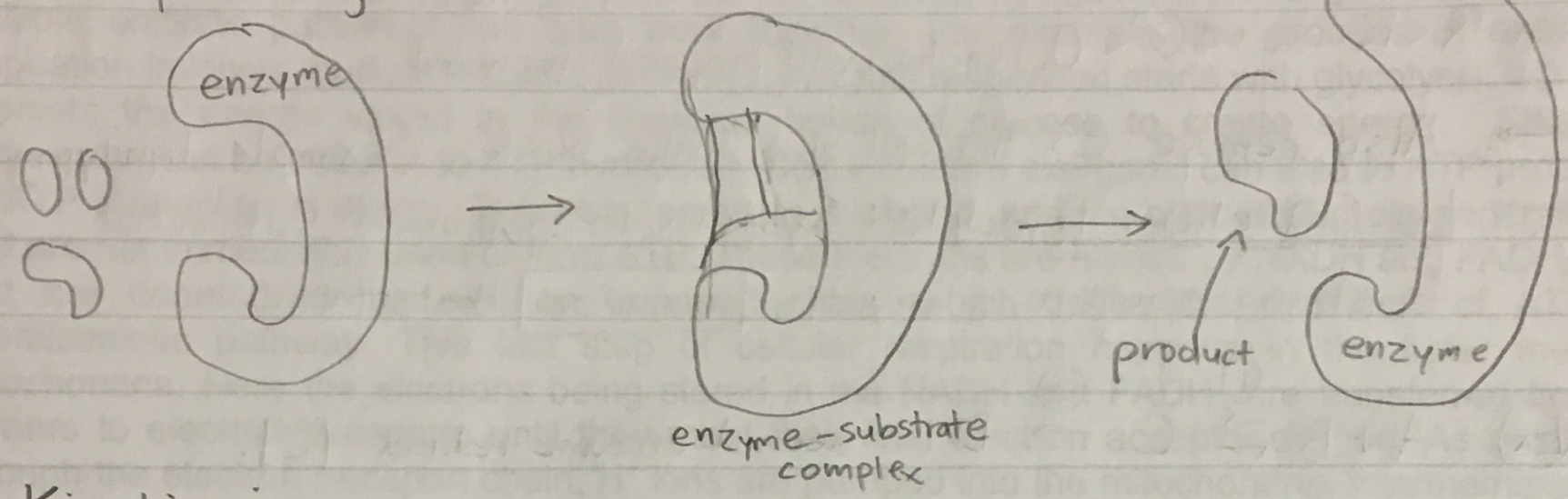
Lecture 5

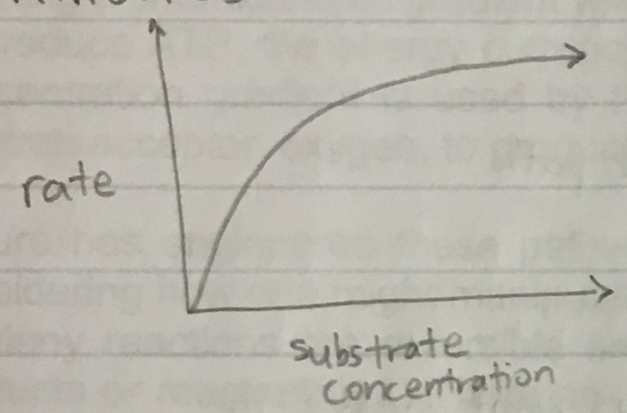
9/14

Enzymes / their pathways

- Bind reactants [= substrates] at an <u>active site</u>
- "Helpful diagram:



- Kinetics:



  - At low [A], rate increases quickly
  - At some point, active sites are full. Known as V_{max}; dependent on amt. of enzyme

- Also bind to <u>inhibitors</u>, which slows reaction
  - → Sits in active site to prevent binding, or
  - → Sits at <u>allosteric site</u> (remote from active site); causes a change in the shape.
- Pathway considerations
  - → Many reactions are <u>reversible</u>; reach equilibrium

$$A + B \rightleftharpoons C$$

↓

$$r_{forward} = r_{back}$$

→ Equilibrium constant $K = \prod_{products} [A]^{a_k} / \prod_{reactants} [B]^{b_k}$ at equilibrium.

where a_k, b_k constants = coeff. in front of chem. molecule

→ If $\Delta G^{o\prime}$ ($pH=7$) < 0 [exergonic, spontaneous], $K > 1$, and vice versa.

- Why? $\Delta G^{o} = -RT \ln K$, where R is the ideal gas constant and T is temperature in Kelvin.

→ In not-standard state,

$\Delta G = RT \ln(Q/K)$, where Q is like K but with arbitrary concentrations.

Shifting a reaction at equilibrium

- $A + B \rightleftharpoons C$; make more C by adding more A or B.

Then $Q < K$ so $\Delta G < 0$, reaction will proceed forward

→ Also remove C; this will also make $Q < K$. (Example: liquifying)

→ Le Châtelier's Principle: System in equilibrium subjected to stress reacts in a way that minimizes that stress.

(Ex) $A + B \overset{①}{\rightleftharpoons} C \overset{②}{\rightarrow} D$ second enzyme reduces [C].

Pathways — rate-limiting step

- Governs overall reaction rate based on slowest path

(Ex) $A + B \underset{①}{\overset{}{\rightleftharpoons}} C \underset{②}{\rightarrow} D \underset{H}{} \overset{③}{\rightarrow} E \overset{④}{\rightarrow} F \underset{⑤}{\overset{}{\rightleftharpoons}} G$

if this is slow, others won't evolve to get faster

⑥ (for example)

Branch points / feedback inhibition / etc.

- Might create J, a branching path for a different product
- For efficiency — no reason to have too many pathways that make the same thing
- Human body does this a lot, but sometimes want to cut off / direct flow
  - → Solution: increase rate. or use inhibitors
  - → Ibuprofen, aspirin examples of inhibitors
- Reagants (e.g. H) can also be inhibiting

(Ex) Aerobic (O_2) cellular respiration

$$\underbrace{C_6H_{12}O_6}_{\text{eat}} + \underbrace{6\ O_2}_{\substack{\text{breathe}\\ \text{in}}} \rightarrow \underbrace{6\ CO_2}_{\substack{\text{breathe}\\ \text{out}}} + \underbrace{6\ H_2O}_{\text{pee}}$$

→ Multiple pathways, each with multiple enzymes

- ↳ Some endergonic, driven by ATP cleavage; others exergonic, generate ATP
- ↳ Some of 32 ATP indirect (NADH transfers electrons; makes ATP later)

Payoff during electron transport / ATP synthesis (28)

☆ enzyme that makes ATP is ATP synthase

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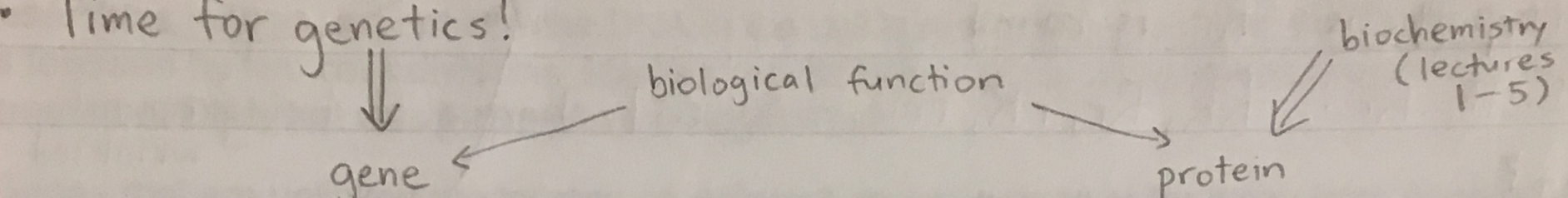
Lecture 6

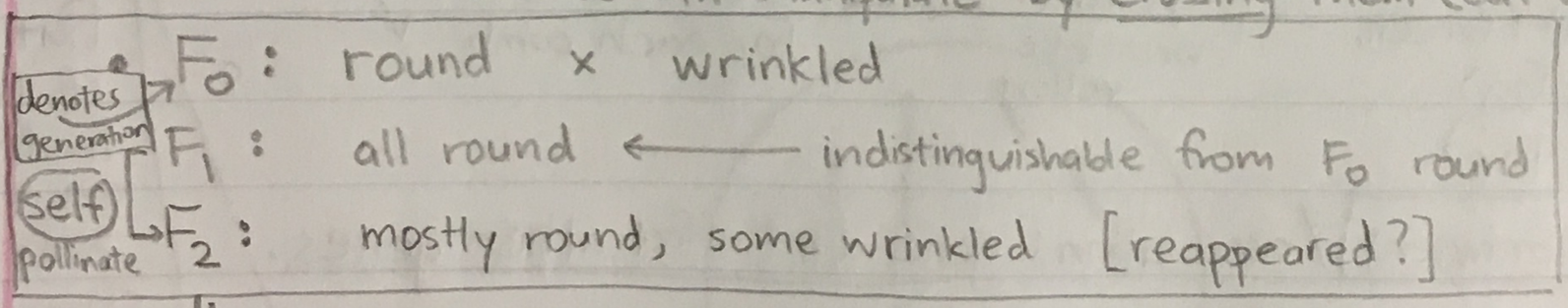
9/17

- 9/20, time TBD: chemistry review
- Time for genetics!



- History
  - → Europe going through changes
  - → Economic changes: better product
  - → How to produce better sheep? (textiles)
    - → Study of heredity (?) in Mauravia (?)
    - → Mendel — monk hired to do this stuff

- Mendel's experiments
  - Pick a plant (strain) — why peas?
    - → Can cross them; reproduce quickly; easily available in market; small
    - → Mendel found 34 varieties; kept breeding to guarantee they were pure (children look like parents).
    - → 2 years → down to 7 pure strains; the controls (1)
  - Plants can produce / self-pollinate (have both pollen / ovial "organs")
    - → But we can manipulate by crossing them (cut off pollen organs, use a brush)! (2)



★ F_1 rounds must be different from F_0. (3)

★ But Mendel counts: he gets 5474 round, 1850 wrinkled. He does this with more and more traits: unfortunately, statistics is invented by biology.

So he just says "it's 3 to 1!" (4)

- He makes a model!
  - → Roundness controlled by some factor: each plant has two. (RR vs rr) are the pure ones.

- Cross = take one from each parent at random. (Mendel's first law)
- Hypothesis: Rr is round.
- Self Rr: RR, Rr, rR, rr ⟶ 3:1 chance of being round: wrinkled

What next?

- Mendel sends a paper ⟶ peer review time!
  - ⟶ Model was made after results
  - ⟶ Need prediction power. (?)

- Why not self F_2?

RR	Rr	Rr	rr
↓	↓	↓	↓
4/4 round	3/4 round	3/4 round	0/4 round

⟶ turns out to be true!

↳ can't tell which is which; we can't see genotypes.

- Backcross / testcross: F_1 (Rr) × (rr) = 50/50 round/wrinkled.
  - ⟶ this works too!

- More complicated crosses (two-factor crosses)

F_0: Round + Green (RRGG) × Wrinkled + yellow (rrgg)

⇓

F_1: Round + Green. (RrGg)

Testcross: wrinkled + yellow ⟶ 25% chance for each comb ✓

(Mendel's second law)

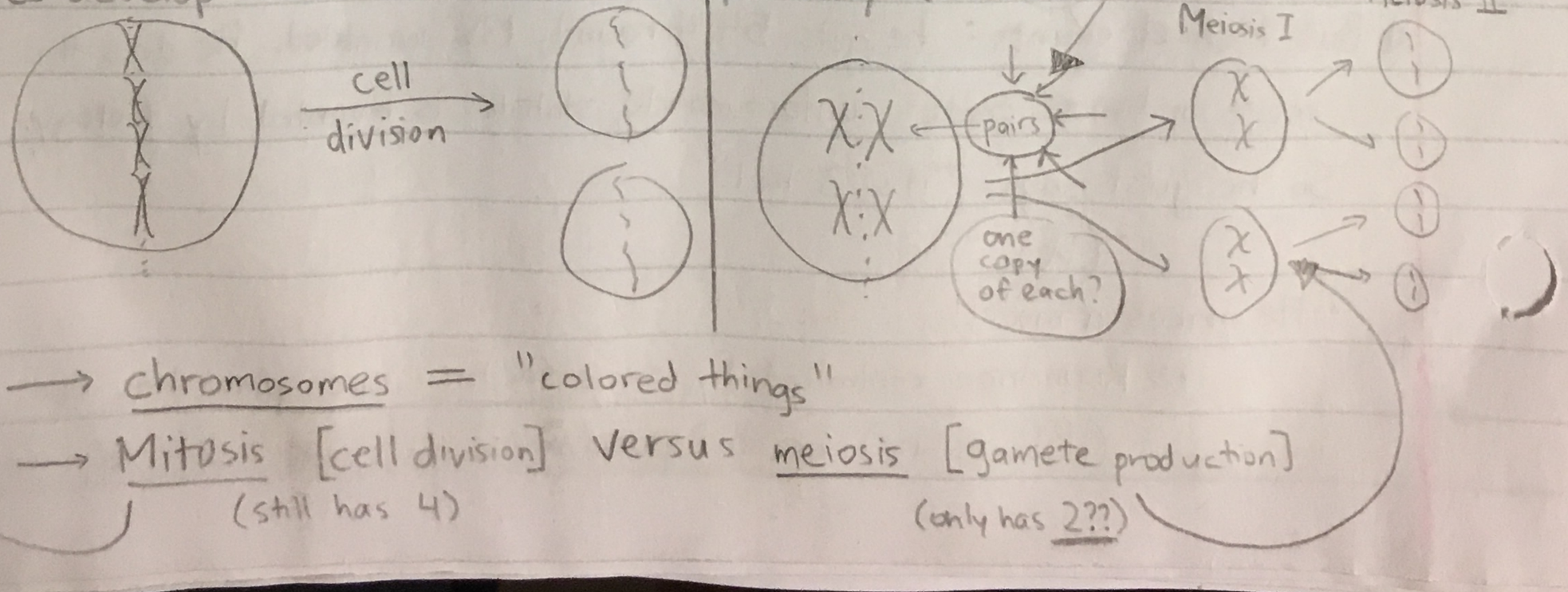
- Independent assortment / association: genes don't affect other genes.
- No one pays much attention, but...

~1900: "interesting..."

But what if two traits are on the same chromosome?

????

- Entirely different field: the cytologist
  - Dyes develop: some cells absorb dyes very well



⟶ chromosomes = "colored things"

⟶ Mitosis [cell division] versus meiosis [gamete production]

(still has 4) (only has 2??)

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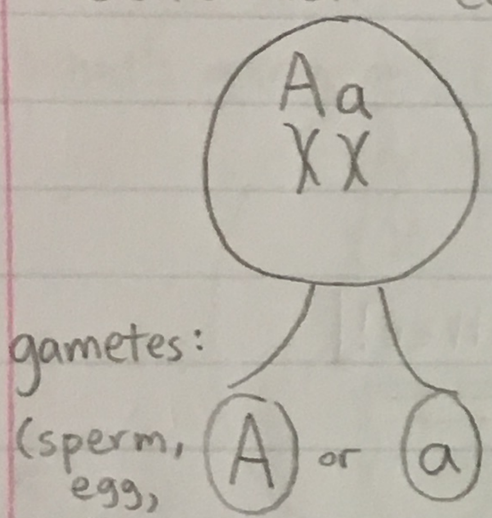
Lecture 7

9/19

- Chem review tomorrow
- Practice test posted!
- Gene = sequence of bases (genotype = RR, Rr, rr, etc.) ← allele
- Phenotype = manifestation (physically)
  ↳ it is these that are dominant or recessive.

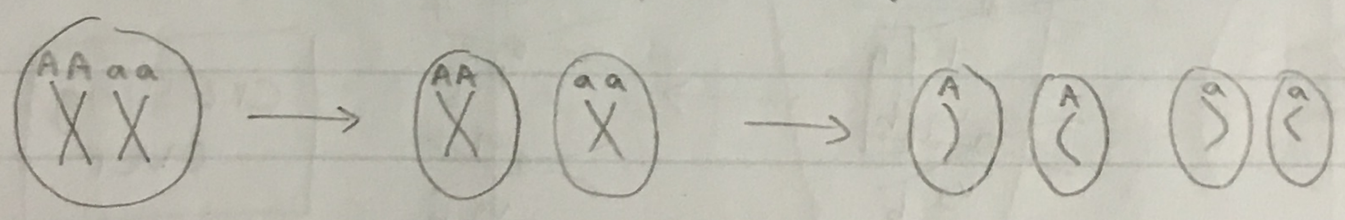
Chromosome theory

- Gene "factors" carried by chromosomes.

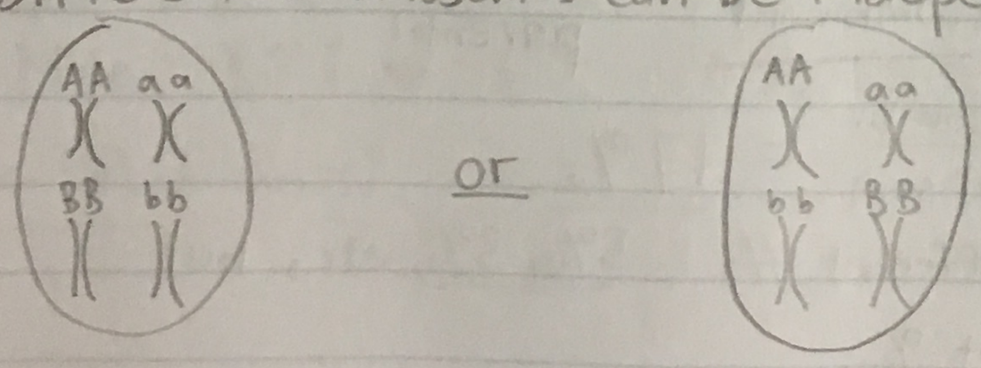


← alleles different = heterozygous
same = homozygous

→ In meiosis, first duplicate your DNA, then do two divisions.



- But... what about multiple factors carried?
  → Different chromosomes can be independent!



→ But it seems that some genes can be on the same chromosome?

- Thomas Hunt Morgan – worked with fruit flies (Drosophila melanogaster)
  dew loving black bodied fly
  → Found mutant flies

- Body: wild type vs black (mutant
- Wing: wild type vs vestigial (mutant)

F_0: body $\frac{+}{+}$ wing $\frac{+}{+}$ ♀ (wild type) × $\frac{b}{b}$ $\frac{vg}{vg}$ (all mutant) ♂

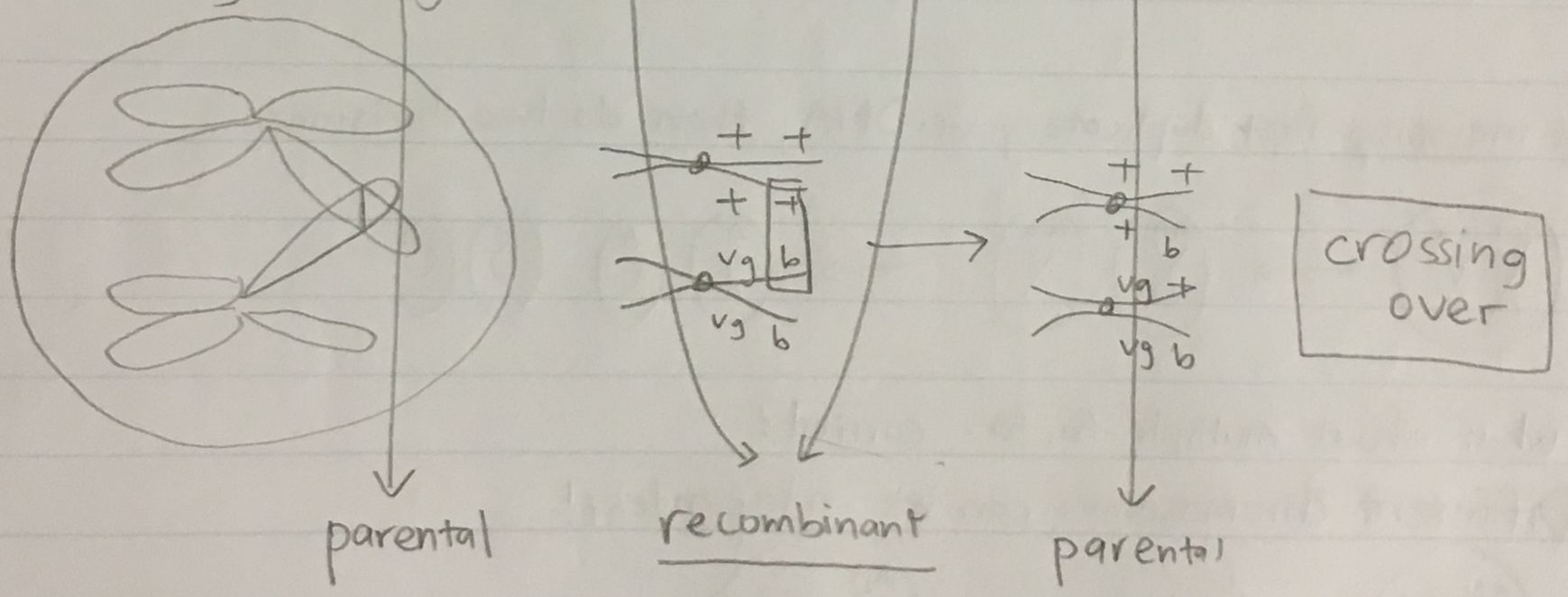
F_1: all $\boxed{\frac{+}{b}\ \frac{+}{vg}}$ (wild type phenotypes)

→ Now testcross F_1 with $\boxed{\frac{b}{b}\ \frac{vg}{vg}}$:

	$\frac{+}{b}$ $\frac{+}{vg}$	:	$\frac{+}{b}$ $\frac{vg}{vg}$	:	$\frac{b}{b}$ $\frac{+}{vg}$	:	b vg / b vg
by Mendel's second law,	1	:	1	:	1	:	1
if same chromosome:	1	:	0	:	0	:	1
actual:	965	:	206	:	185	:	944

[neither!]

Is info being exchanged? Yes!



crossing over

→ Freq of recombination = $\frac{\text{recomb.}}{\text{total sum}}$ = 17%.

→ Other genes had different #s: 5%, 3%, etc, but same pairs = consistent %.

What now?

b —— vg 17% recomb.

cn —— b 8% recomb.

⇒ also, test cn —— vg 9% recomb

→ So cn gene is between b and vg!

→ This is called linkage mapping

↳ disease traits, etc.

But one cross was weird..

F_0: white eye ♂ (recessive) × wild type ♀

→ F_1: wild type. ♀ testcross (×) wild type ♂

⇓

all females wild type
all males: 50% wild, 50% white. ???

What's going on? Look at humans!

XX
XX
⋮
XX
X × }

→ XX versus XY chromosomes!
Same w/ fruit flies.

So,

F_0:

Cross white-eye male w/ normal female:

$X^wY \times X^+X^+ \rightarrow X^wX^+, X^wX^+, YX^+, YX^+$ — F_1: wild type

F_1: $X^wX^+ \times X^+Y$

Female: X^wX^+ or X^+X^+ (all normal)
Male: X^wY or X^+Y (½ white eye!)

☆ sex-linked traits!

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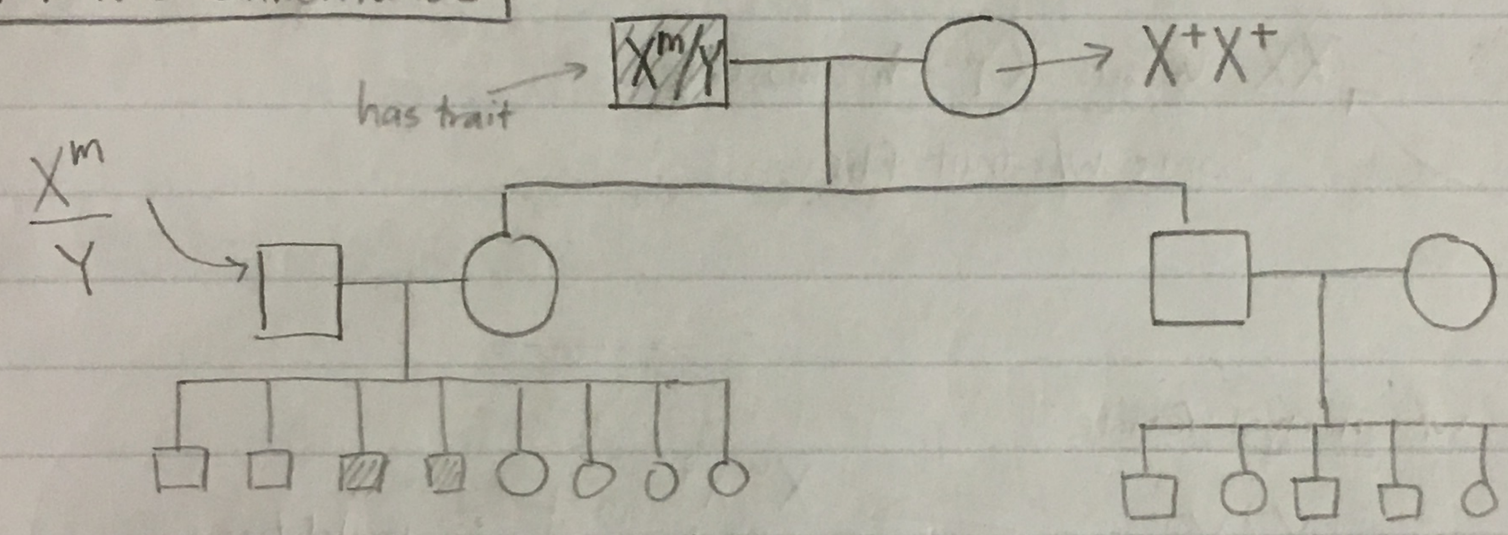
Lecture 8

9/24

- Exam on Wednesday (check whether to go to Walker or 26-100)
  - → Review Monday night
  - → Ice cream social on Wed.?
  - → Pset released Wed.

- Recombinant DNA — map genes before DNA
- Now look at humans!
  - → Charles Davenport → found trait "love of the sea"?!
    - ↳ Sea captains?... maybe there's other reasons...
  - → Genetics no longer the only reason... look at <u>pedigrees</u>!

X-linked Inheritance



→ <u>X-linked</u> recessive disorder?

→ Let X^m be mutant, X^+ "normal". Male grandfather $\frac{X^m}{Y}$, grandmother $\frac{X^+}{X^+}$

→ gen 2 must be $\frac{X^m}{X^+}$, $\frac{X^+}{Y}$.

→ Only colorblind if $\frac{X^m}{Y}$, $\frac{X^m}{X^m}$. $\frac{X^+}{X^m}$ called a <u>carrier</u>.

Rules: ① More common in <u>males</u> than <u>females</u> (p vs p^2)

→ ex: colorblind female has colorblind father

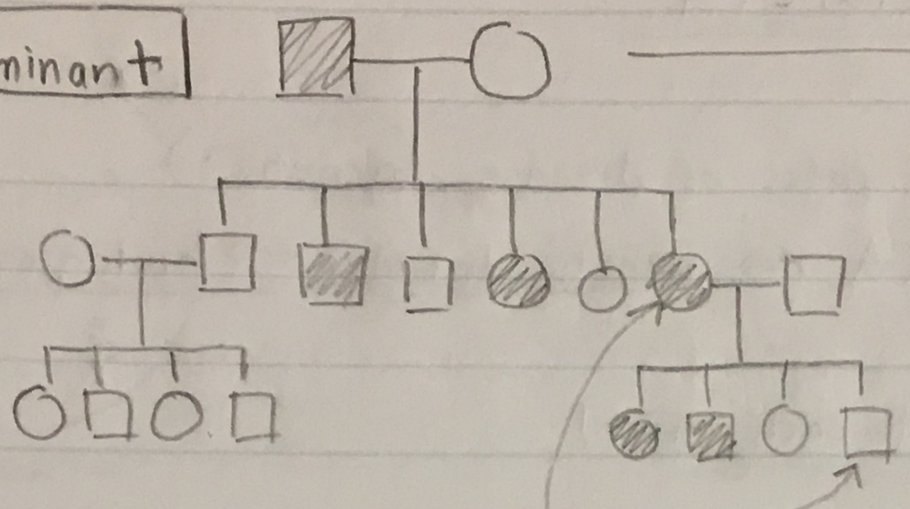
② Carrier ♀ → 50% affected sons

etc.

Fact: ~8% males colorblind. Look at <u>population genetics</u>

→ Square this to get ~0.64% of females colorblind

Autosomal Dominant



$\frac{m}{+} \times \frac{+}{+}$

Rules:
1. Never skips generations (if affected, then a parent has it)
2. ~Equal male/female
3. Transmit ~1/2 of the time.

→ Not X-linked: male here has affected mother

→ Equally affects all independent of sex

→ (Ex) Huntington's disease.

Look at population genetics: p + → p^2 $\frac{+}{+}$

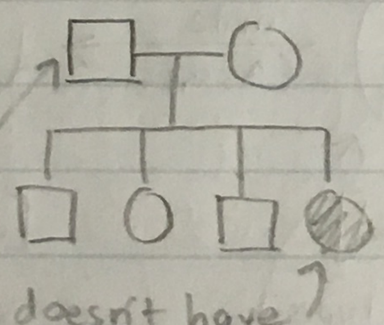
$1-p$ m → $2p(1-p)$ $\frac{+}{m}$

$(1-p)^2$ $\frac{m}{m}$

↳ $(p^2, 2pq, q^2) \approx (1, \sim 2q, \sim 0)$

↳ 1/500 has disease ⇒ ~1/1000 chromosomes carries it

Autosomal Recessive



Rules:
1. Equal male/female
2. Usually parents unaffected
3. 1/4 transmit iff carrier parents

→ Not X-linked: father doesn't have

→ Not autosomal dominant: skipped a generation

But cystic fibrosis → ~1/3?

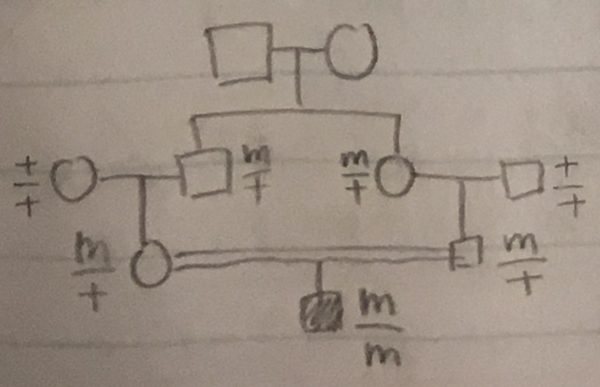
↳ Sometimes never have kids w/ cystic fibrosis! You're only counting those biased already w/ cystic fibrosis.

☆ Human genetics is different b/c you observe

Incomplete penetrance

- Only show trait ~70% of the time (e.g. breast cancer in women)

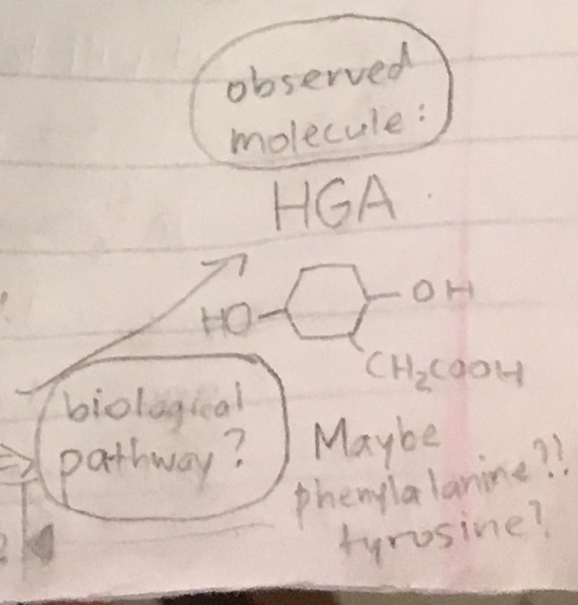
One last case: **what about the biochem?**



"black urine" — alkaptonuria (when washing in highly basic soap)

☆ Majority from first-cousin marriages!

↳ easier to homozygose(?) w/ inbreeding!



Real question: Connecting biochem/genetics

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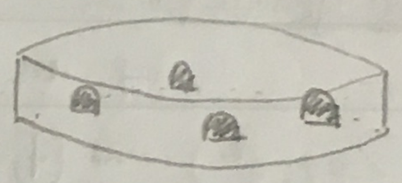
Lecture 9

Recall 1908 — inbreeding = higher rates of disease (genetic?)
↳ Also noticed excess HGA ⇒ genetic disorder ↔ biological pathway

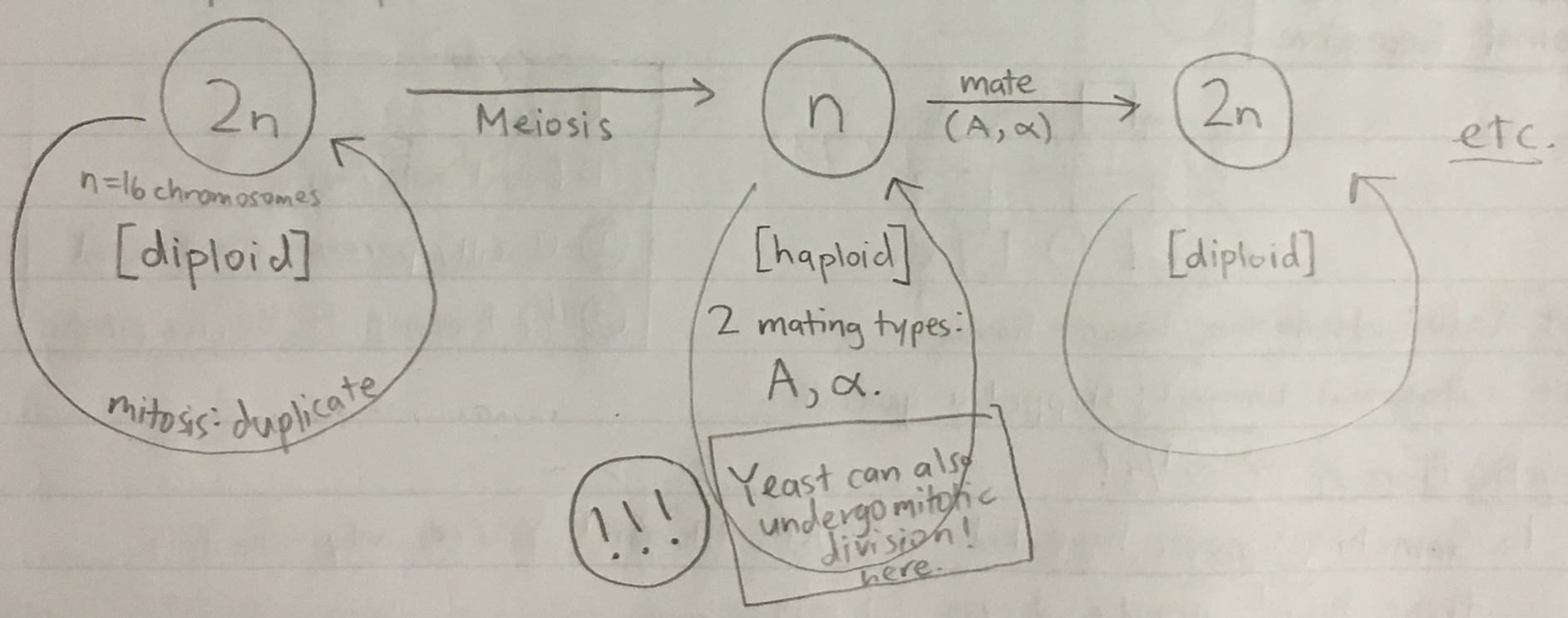
Biochem & genetics started to talk to each other!

Biochemical genetics

- Tried fruit flies
- Instead, used different organism similar (?) to yeast
  → Single-cell eukaryotic fungus
  → To grow it, use a petri dish
  ↳ Mitotic division: colony from yeast each cell.

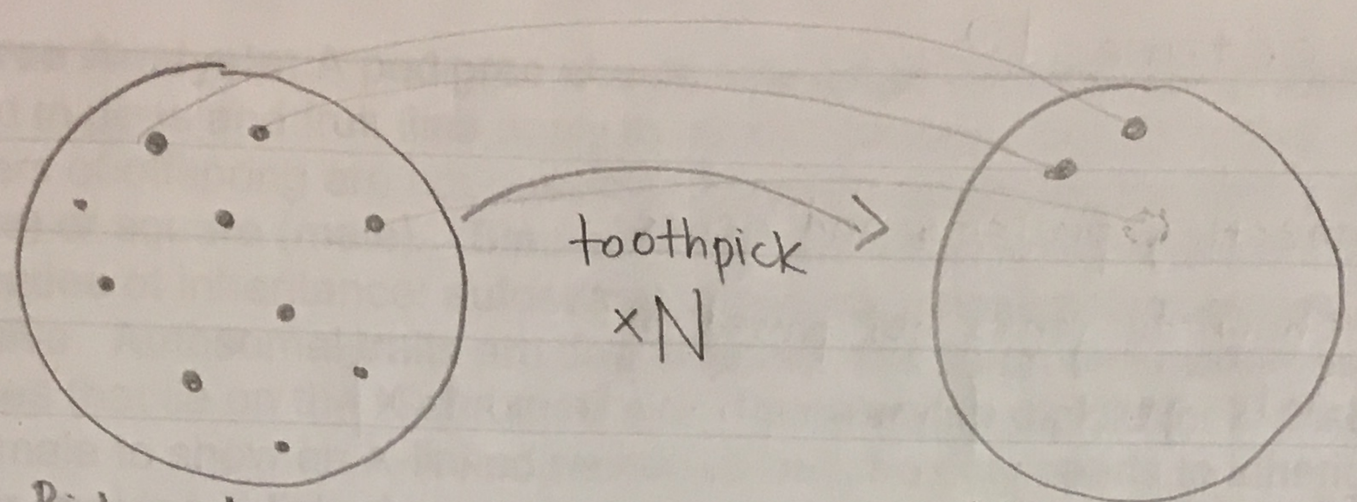


- Life cycle of yeast:

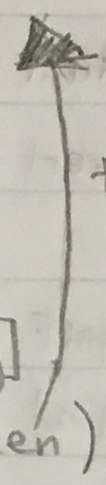


- There are some growth requirements, though... it needs a carbon source (e.g. sugar), + N, P_i salts. Nothing complicated.
  → Yeast doesn't need that much. But if you give it a rich medium, it will take those in, too.
  → regulated biosynthetic enzymes

How do you find mutants?
→ e.g. find yeast that can't make Arg amino acid?



- **Prototrophs** = can grow on minimal media.
- **Auxotrophs** = need something else (e.g. auxotrophs for Arg)



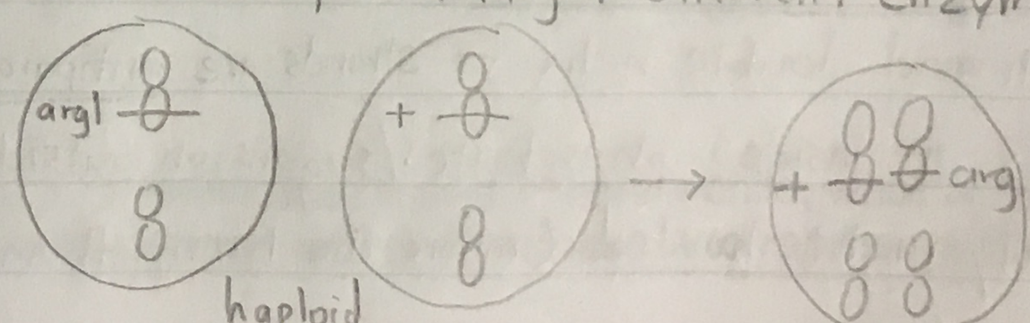
Faster ways? Better transfer instrument?

- Use a piece of velvet! Transfers in the same locations [replica plating]
- Better to have mutants that gain an ability (selection vs screen)
  → The one that grows is (e.g.) antibiotic-resistant
- Diploids or **haploids**? Pick the one w/ only 1 chromosome copy
  → if mutation is recessive, much easier to find those!
  ↳ most mutations likely recessive since 1 working copy is generally enough.

How to characterize these auxotrophs?

- Start with arg1, arg2, ...
  → Probably genes that affect this ability will target different enzymes
- 3 main tests:

→ **Test of recessivity / dominance**
→ If diploid grows, it's recessive. Else, dominant.

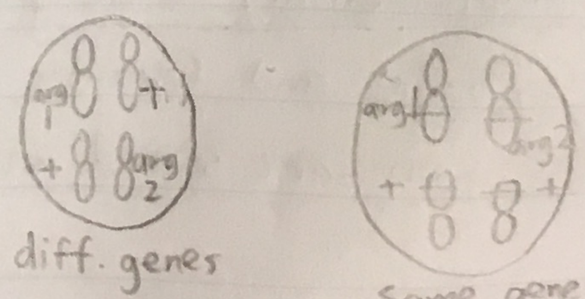


→ **Test of complementation**
→ Recombination says nearby, but not same gene...

	a1	a2	a3	+
a1	X			✓
a2		X		✓
a3			X	✓
+	✓	✓	✓	✓

→ Instead, cross $\frac{arg1}{+}$ with $\frac{arg2}{+}$!
→ Make a table. If (a_i, a_j) = ✓, diff. genes
= X, same genes

Sort into genes!



→ **Test of epistasis** $\alpha \rightarrow \beta \rightarrow \gamma \rightarrow$ Arg (We're given some pathway)

Ask: what if we just give α? Or β? Or γ? **Feed different steps!**

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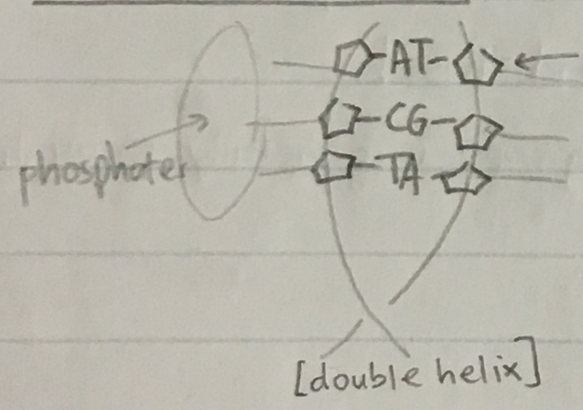
Lecture 10

Molecular biology — connecting proteins and genes
- → DNA replication (how do genes get passed on?)
- → How to read / translate genes [transcription / translation]
- "The central dogma" ....

Review
- Individual nucleotides connected (3′ → 5′) o w/ phosphodiester linkage
- Different bases

History
- Chargaff rules: A and T = same # bases; same with C and G
- Rosalind Franklin: X-ray diffraction
  - → helical structure of DNA, other measurements
- → Watson and Crick's model — sort of used data w/o permission...



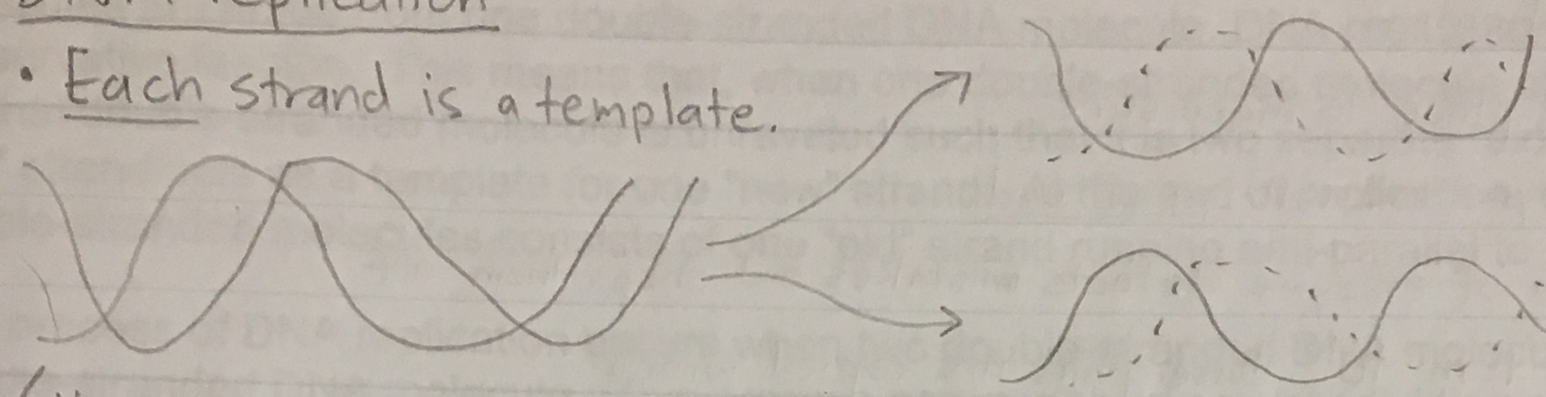
[Model is consistent w/ data.]

Structure of DNA – features
- 2 strand double helix — strands are antiparallel
- Bases on inside; phosphates/sugars on outside
- Usually right-handed (mimic the turn, thumb pointed up)
- Major/minor grooves
- Complementary base pairing — Adenine, Thymine form 2 hydrogen bonds, Cytosine and Guanine form 3 (at standard pH)
- Bases on same strand will stack on each other (van der Waals)

Why is DNA structure essential for function?
- Complementary base pairing = copying mechanism
- DNA can encode lots of info with long chains
- Adaptation — can change bases w/ mutation.

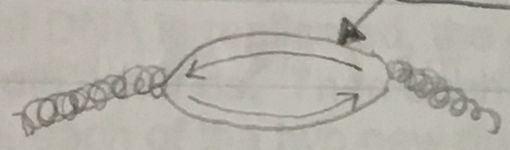
DNA replication

- Each strand is a template.



("semi-conservative" replication. Conservative = preserve original molecule)

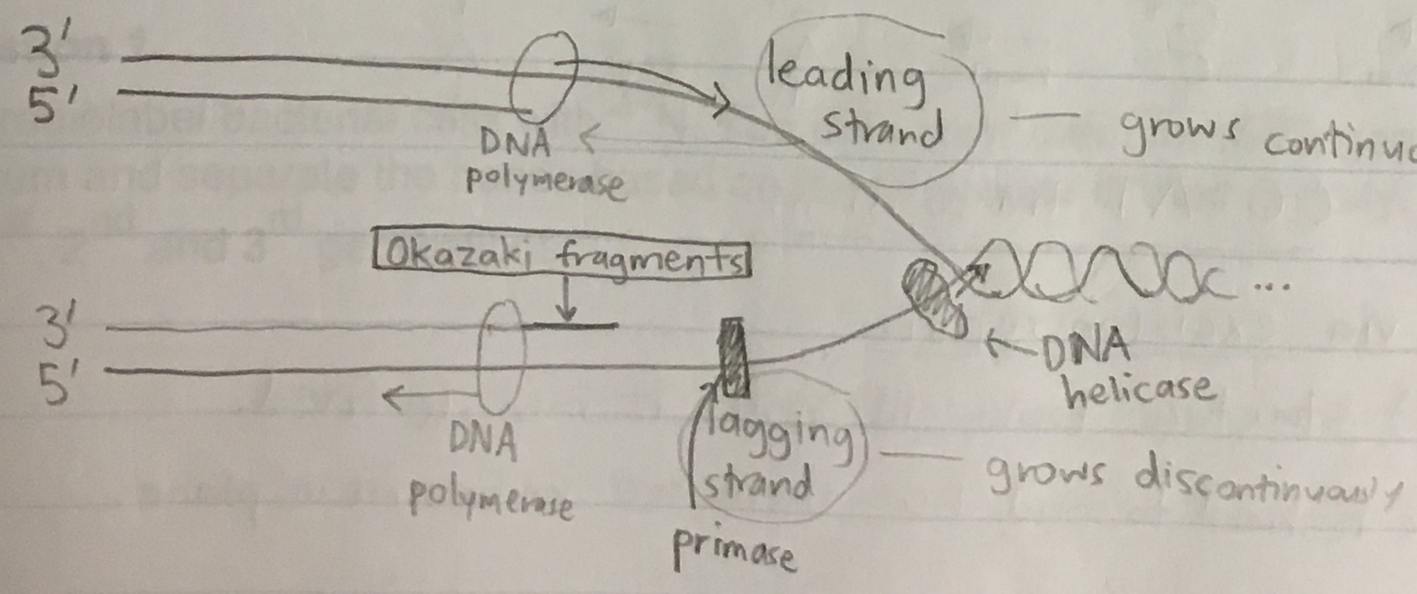
- Start at origin of replication (ori)
  - → DNA unwinded there; this results in a replication fork
  - → Enzyme [DNA helicase] does the job



- Use a primer (a short starter strand) created by [primase]
  - → Complementary to template
  - → Provides 3' OH group so [DNA polymerase] can start.
- Polymerization reaction catalyzed by this ↑
  - → dNTPs added to 3' (new bases added, 5' → 3' direction) for new strand
    - deoxy
    - A, C, G, T
    - triphosphate

★ travel 3' to 5' wrt template

- Pyrophosphate (Ⓟ—Ⓟ) is lost; this cleavage of phosphate groups is favorable
- How to deal w/ opposite directions?



- ~~Lagging strand~~ needs lots of primers — it grows a bit at a time
- [DNA ligase] links those fragments together.

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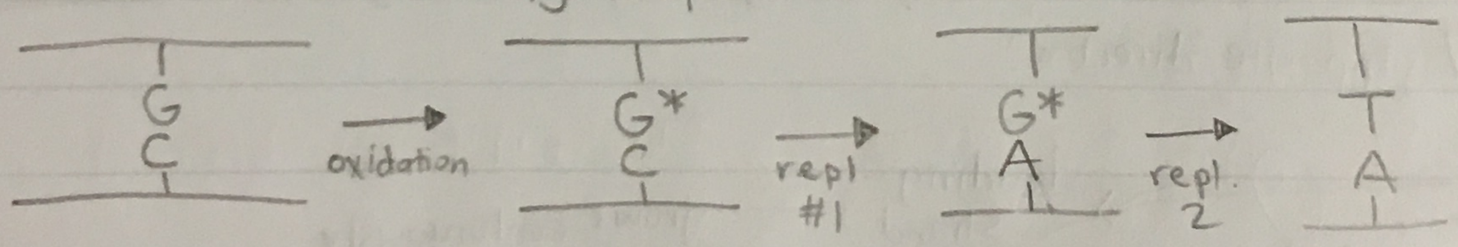
Lecture 11

10/3

- "Fidelity" of DNA replication?
  - Mechanisms in place to make sure.
    - Most DNA polymerase will proofread
      - ↳ Region of enzyme detects mistakes and chops them off
      - → Only 1 in 10^{10} base pairs are copied wrong
    - Others aren't; they're error-prone DNA polymerases

    use: (e.g.) directed evolution
      - ↳ essentially, transform/mutate enzymes & see if it works better?
      - → Nobel Prize in Chemistry awarded today
  - Mismatch repair — repair enzymes scan DNA to look for / fix mistakes.

- How about DNA repair (due to damage?)
  - Bases (A, C, G, T) can be chemically unstable
  - Oxidation [loss of electrons vs actual oxygen atoms?]
    - Oxidative damage = accumulated damage
  - (e.g.) Guanine → 8-oxo-G
  - Double-bonded O means 8-oxo-G can bond to A (?)
  - ROS - reactive oxygen species



this is a CG:AT transversion.

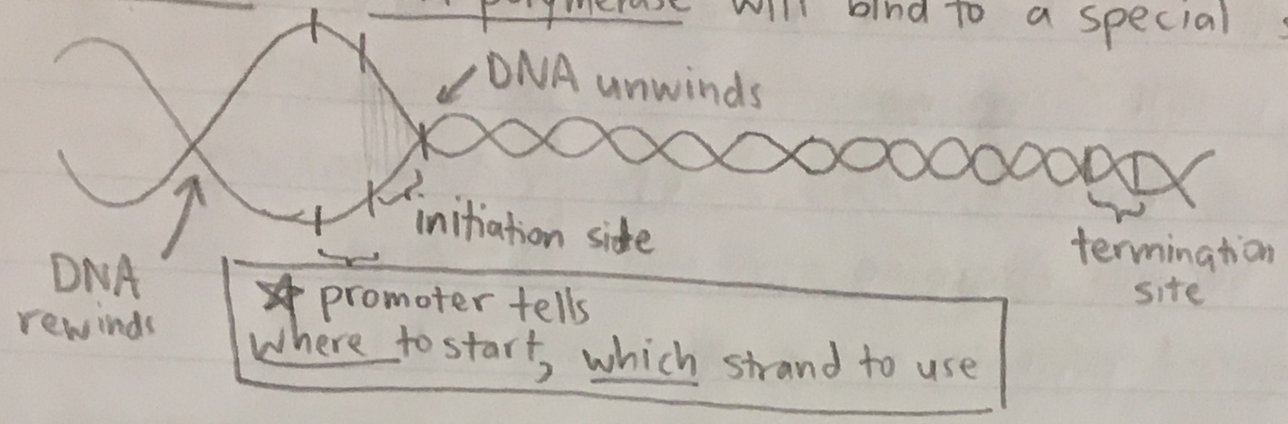
  - Can be repaired via excision repair.
    - → If DNA structure looks different, it can be fixed.
    - ↳ Damage is hard to find, but there are mechanisms in place.

- DNA → Protein sequence?
  - Transcription sends DNA → RNA (DNA copied to complementary seq.)
  - Translation sends RNA → protein

↓
messenger RNA
(mRNA)

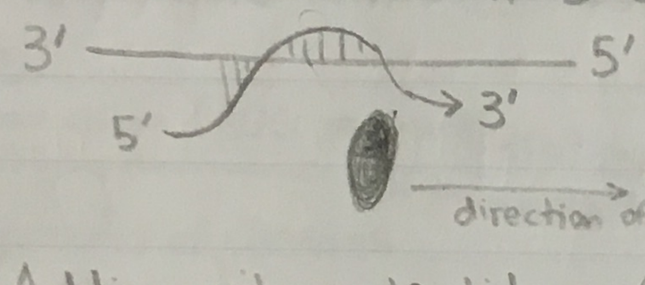
Transcription:

- Initiation: RNA polymerase will bind to a special sequence, the promoter



  - Polymerase binds to promoter w/ help from transcription factors
  - Only ~10 base pairs unwinded

- Elongation: RNA polymerase reads the template from 3′ to 5′
  - Primers are not needed — first base added (nucleotide) is 5′ end of mRNA.
    - ↳ Next ones added at 3′ end of the mRNA



  - Adding ribonucleotides (NTPs) = ATP, CTP, GTP, UTP
    - Uracil
  - 2 phosphates cleaved (just like last time), process becomes favorable.
  - No proofreading here; mRNA has a short lifespan.
  - DNA being rewinded during this as well.

- Termination:
  - RNA polymerase reaches termination site
    - ↳ transcript & polymerase released. mRNA now comp. to DNA template strand
      - → nontemplate strand has same sequence = coding strand.

(e.g.)			
	coding strand	5′	ATG
	template strand	3′	TAC
	mRNA	5′	AUG

→ T → U, otherwise same.

Genetic code — specifies the amino acids used to build protein.

☆ codon = sequence of 3 bases. Each one = amino acid.

↳ ~20 standard bases, $4^2 < 20 < 4^3$. Most amino acids > 1 codon.

- Also special start / stop codons.
- Importantly, no ambiguity, almost universal among all organisms.
  - → Can make proteins in E. coli instead of humans! But viruses...

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Lecture 12

10/5

Single-base mutations (of the genetic code)

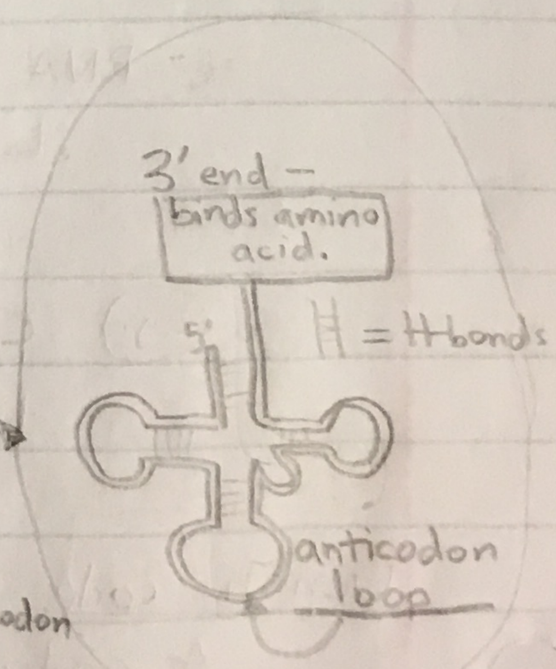
- Silent mutation — change of base does not change amino acid
  - (e.g.) UGU → UGC is still Cysteine. → forms disulfides
- Missense (?) — amino acid is changed (pretty different)
  - (e.g.) UGU → CGU becomes Arginine → positive charge
- Nonsense mutation — truncated protein, since an amino acid is changed to a stop codon.
  - (e.g.) UGU → UGA becomes stop.
- Frameshift mutation — a base is added or deleted, so amino acid gets changed drastically.
  - (e.g.) AUG UGU GAU ··· → AUG GUG UGA U ···
    Met Cys Asp Met Val stop (?)

Translation of the genetic code [RNA → protein]

- We need:
  - → message (mRNA) ①
  - → translator (transfer or tRNA) ②
  - → machinery for translation (the ribosome) ③

  ① ② ③ → 3 types of RNA
  - Ribosome contains ribosomal or rRNA, as well as protein.
- 3 kinds of RNA all made of ribonucleotides, but have different jobs and (3D) structures.

- tRNA — ~70-90 ribonucleotides
  - → structured (sort of like an L) →
  - → H-bonds stabilize it!
  - → represented sometimes w/ cloverleaf representation →



- ★ fun fact: RNA has lots of modifications
  - → Anticodon bases — important for matching/complementing codon
    61 codons, but cells don't have exact #.
    (Depends on organism)
- Wobble position — some times tRNAs can recognize multiple codons.
- Naming: $tRNA_{ala}$ = responsible for ala amino acid, etc.

Charging of tRNA

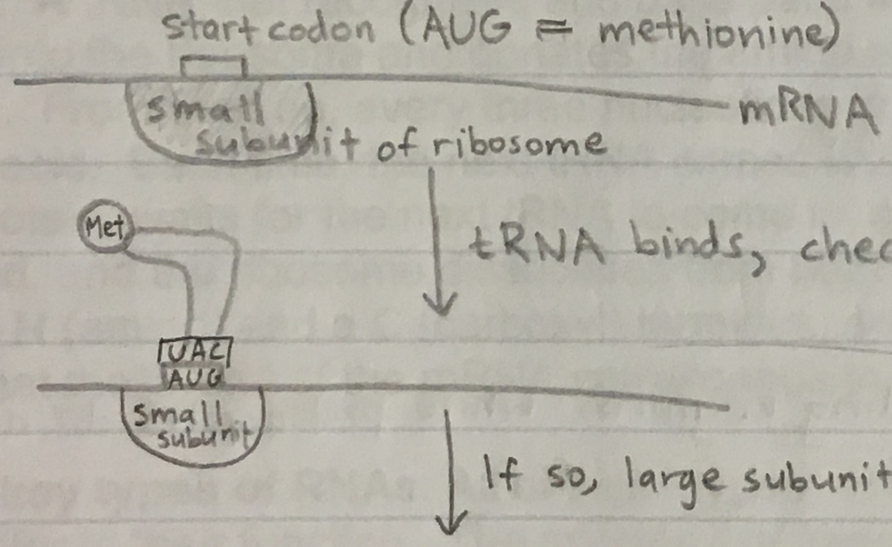
- Enzymes put amino acid onto tRNA (at the 3' end?)
- Form high-energy bond between tRNA, amino acid using ATP
  → Enzymes are specific: $tRNA_{ala}$ activated by ala only, etc.

name: aminoacyl (rel. to amino acids) – tRNA synthetase (req. ATP)

Translation in the ribosome

- Cells have lots of ribosomes that can be reused.
- 2 subunits in the ribosome: large and small.
  ↳ prokaryotes have ~1500–3000 ribonucleotides, ~20–30 proteins
  → More for eukaryotes. Fact: ribosomes are different for pro/eukaryotes, so (e.g.) antibiotics can target bacterial ribosomes only.

- **Initiation**



- **Elongation**
  → Next tRNA binds at the A site
  → break bond b/w tRNA and current amino acid in P site
    ↳ Methionine at N-terminus of chain.
  → Form a peptide bond w/ next amino acid; first tRNA ejected at E site
    ↳ Growing amino acid chain at P site.
  → mRNA read 5' → 3'
  → rRNA is the catalyst for forming these peptide bonds.

repeat until stop

- **Termination**
  → Stop codon → protein release factor enters A site and cleaves polypeptide off.

3 sites: A site – amino acid
P site – peptide
E site – exit

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Lecture 13

10/10

- P-set due Friday
- Exam next Wednesday (10/17)
  → Genetics & molecular biology

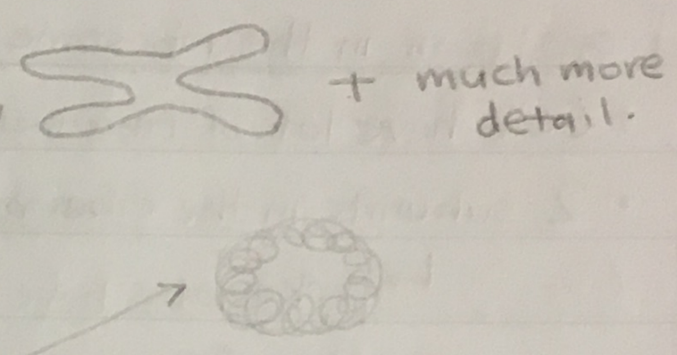
☆ Main idea: lots of variations on the main idea (central dogma)

Differences in genetic material

- Eukaryotic (e.g. human) vs prokaryotic (e.g. bacterial) cells!

Where is the material stored?
→ Nucleus vs cytoplasm (everywhere)

What is the structure?
→ Double-stranded for both (dsDNA)
→ Linear (wrapped around proteins) vs circular + much more detail.
↓
this is highly organized and very complicated



Telomeres?
→ Repeat sequences at ends of chromosomes
→ Only in eukaryotic cells

How compact is the genome?
→ Eukaryotes have introns = non-coding regions, while prokaryotes do not

More about telomeres

- "End parts"
- Repeat sequences at ends of chromosomes: $(TTAGGG)_n$ ← thousands
  → With each replication, chromosomes get shorter
  → Serves as buffer region so essential genes are OK
  → Nothing useful in genetic code
- Enzyme telomerase adds buffer region
  → Active in cancer cell (rapidly dividing)

Transcriptional differences

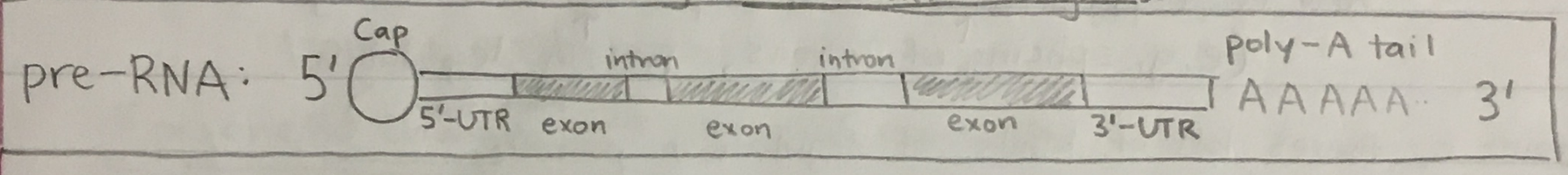
- Location: again occurs in nucleus vs cytoplasm
- mRNA is processed in eukaryotic cells (e.g. introns), not in prokaryotic cells

→ precursor mRNA (the initial transcript) in eukaryotes are not the same as mature RNA.
→ the introns are spliced out

RNA splicing — removal of introns; connection of remaining exons

- Intron — non-coding region between exons, removed by splicing
- Exon — coding regions, remain in transcript
  → both protein-dependent.

☆ Not all non-coding regions are introns!
5′ and 3′ UTR → noncoding untranslated regions



→ catalyst of splicing = spliceosome (-osome = big)
↳ made of many proteins / RNA

- Why splice?
  → If introns were translated, might get different / nonfunctional protein.
  → Also, you can get multiple proteins from the same transcript!
    ↳ alternative splicing; genetic variation
  → So eukaryotic cells = more complicated

Other modifications (in eukaryotes)
- 5′ cap: facilitates mRNA binding to ribosome
  ↳ protects from degradation
- Polyalenine tail for stability of mRNA
  ↳ (?) important for export of mature mRNA (nucleus → cytoplasm). for translation

Translational differences
→ In both types, takes place in ribosome.
  → But in eukaryotes, attached to ER = endoplasmic reticulum. (Free in prokaryotes)
  → Bigger in eukaryotes (good for antibiotics)
  → Ribosome binds to 5′ cap in eukaryotes vs specific seq. in 5′ UTR during initiation
    → 5′ UTR longer in eukaryotes.

→ Eukaryotes — typically monocistronic vs polycistronic in prokaryotes
  1 mRNA, 1 polypeptide
  more starts/stops = multiple polypeptides!
→ More efficient in some scenarios. (e.g. same pathway)

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Lecture 14

10/12

- Review session for exam: Monday (10/15) 7–8:30 in 10-250
- Cryo-EM tour: 10/17 5–7 pm (?)

Gene regulation — related to previous material

↳ regulation of gene transcription / expression

What does it do?

Regulate the amount of active protein!

genes DNA → mRNA → Protein

transcriptional regulation – ↑ vs translational regulation ↑

Also exists post-transcriptional or post-translational regulation

(e.g. splicing of introns, protein degradation / inhibition.)

→ The point is, there's lots of kinds of regulatory processes.

So... why regulate the amount of protein in the cell?

- In eukaryotes, cell differentiation.
  - → Don't need liver cells for other uses, etc.
  - → Prokaryotes are single-cell, don't need to worry about this
- Prokaryotes respond to a change in environment
  - → e.g. availability of food

Kinds of proteins

- Inducible — made only in certain conditions
- Constitutive — always made
  - → e.g. "housekeeping"

Main methods for gene regulation

1. "Reversible modifications of DNA / histone proteins

(this is what DNA is wrapped around)

→ Both prokaryotes & eukaryotes

(e.g.) Methylation of DNA (usually on cytosine), occurs in both types

- DNA methylase puts on a methyl group, DNA demethylase does opposite.
- Heavily methylated genes are NOT expressed (silent)

What are some functions of this?

→ Protection from foreign DNA — cell will methylate it.
  ↳ So it doesn't transcribe data from a virus.
  ↳ Causes some frustration w/ introducing enzymes though...

→ In prokaryotes, identify strand to repair in mismatch repair.
  → Old strand methylated first = template strand
    ↳ Fixes mismatch problem! Only in prokaryotes though

→ But in eukaryotes, we have cell differentiation!
  → Heavily methylated → genes not needed. (eg skin cells have others methylated)

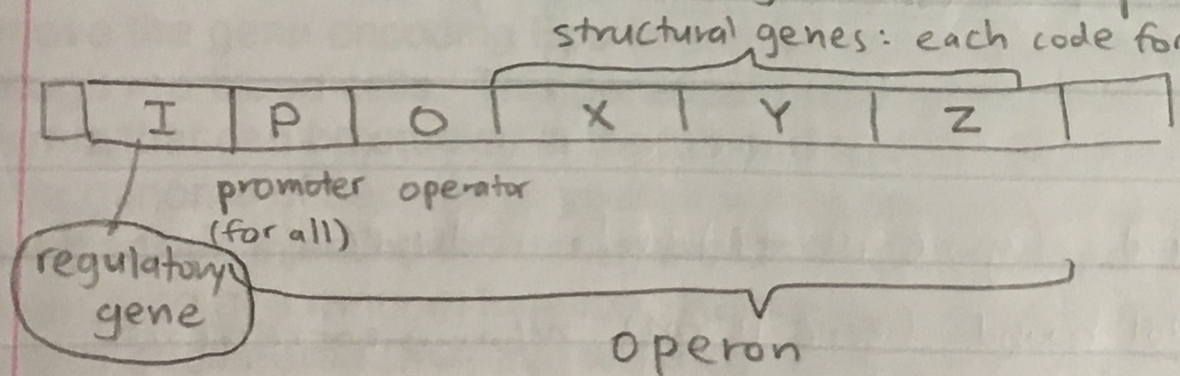
☆ telomerase active for cancer cells — any way to fix regarding methylation?

Epigenetic change → area of research.
does not change in DNA sequence

2. Using DNA binding regulatory proteins

Turn on/off regulatory pathways!

- Organization of genes in prokaryotes → whole pathways can be turned on/off.
- Operon = a cluster of genes w/ one promoter.



→ Promoter is where RNA polymerase binds.
→ Operator is a short sequence b/w promoter & structural genes.
This is where regulatory proteins bind! Sometimes binds to block / allow RNA polymerase from/to binding.

What are types of regulation?

- Negative — a repressor blocks RNA polymerase.
  ↳ if repressor is bound, no transcription.
- Positive — no/weak affinity for RNA polymerase unless in presence of activator.
  ↳ Activator stimulates transcription.

Example of a LAC operon: inducible system for metabolizing lactose

↳ Only need this if lactose is present!

- If lactose absent, operon is repressed by an active repressor.
- When lactose is present, there is induction of gene expression
  → Allolactose, an inducer, binds to the repressor and inactivates it.
  → Now transcription does occur!
- Also, if there is low glucose & lactose is present, further activated transcription w/ activator protein.
  → cAMP increases, which binds to CRP, which helps RNA polymerase bind more efficiently.

- Some repressors only bind w/ a co-repressor. bound to them
  → (e.g.) NikR repressor for nickel uptake in E. Coli only binds in presence of Ni^{2+} ions
  (i.e. avoids too much nickel in cell)

☆ Most co-repressors and inducers work by a conformational change / change in oligomeric state

- Inducible systems — repressor is bound (gene is off) w/o inducer.
- Repressible system — repressor is not bound (gene is on) w/o a co-represior

How to find out how much protein was made?

- Gel electrophoresis! Compare bands to see how much there is w/ or w/o. an inducer.

7.012
Lecture 15

10/15

Exam review: tonight 7-8:30pm

- Molecular biology connected genes ↔ proteins
- But even after all of this, we still can't read any genes?

Why is this hard?

- You can purify enzymes easily - different sizes, other properties
- But DNA all looks the same?!

→ No physical property — only what it encodes.

- Today is the start of Recombinant DNA: how to find genes?

Overview

- 3×10^9 bases in a human genome
- 3×10^3 bases in a gene
- 1 base in a mutation

} ? how?

→ Cloning! tl;dr:

- Cut DNA at specific points
- Paste to vectors: DNA propagating in cell
- Transform into cell
- Select for cells w/ this DNA.

Cutting DNA

- Let's say you want to cut out "GAATTC"

→ Use bacteria! 20 min generation time vs 20 years.

↳ Bacteria have enzymes to cut these out! EcoRI can do this!

- Why would bacteria even have this?

→ Protects it from foreign DNA (e.g. viruses) as an immune system

G|AATTC
CTTAA|G

"reverse palindrome"

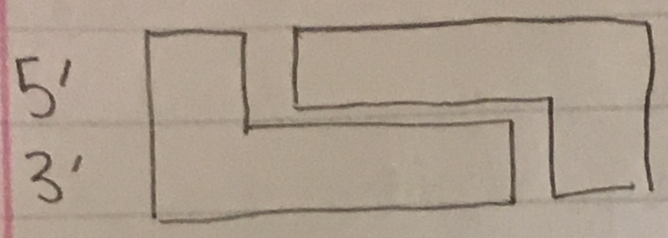
→ What if you cut your own DNA?

↳ Every ~4000 bases, GAATTC should occur. Seems hard to avoid it!

→ But E. Coli can tell the difference between the foreign / own DNA!

☆ Enzyme puts a CH_3 methyl group

↑ restriction enzyme (restricts viruses) is the cutter,
methylation enzyme (puts CH_3) is the protector.

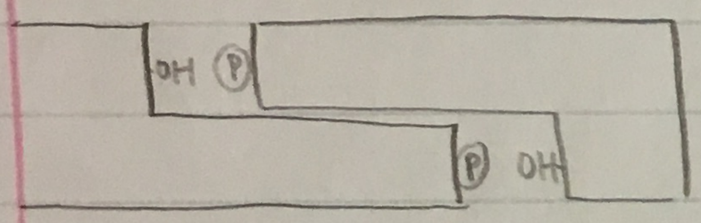


GAATTC, GGATCC, AAGCTT all cut like this

GATC / CTAG , CAG|CTG / GTC|GAC , etc... many restriction enzymes!

- How to find / obtain restriction enzymes?
  - → Used to purify by growing E.coli.
  - → Now order online! e.g. $59 for 10000 units of EcoRI
- So what happens if we put this in human DNA? It'll cut, avg. length ~4000.

Pasting DNA

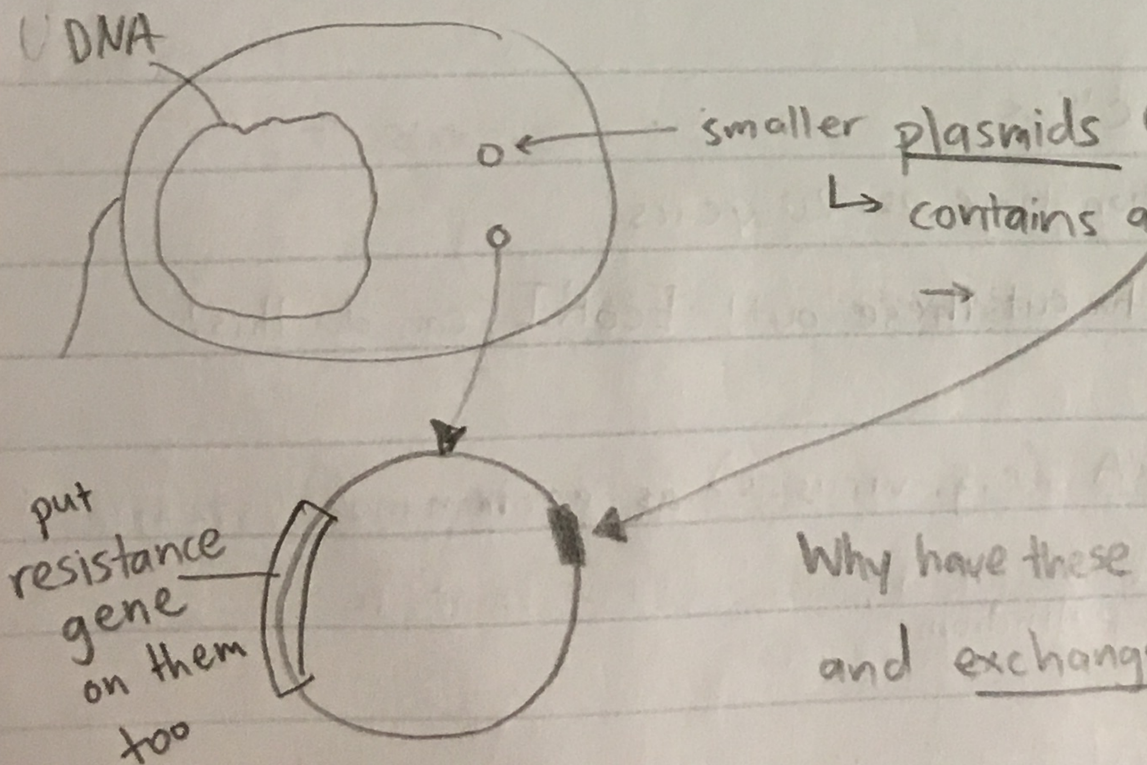


To paste, need to repair sugar-phosphate bonds.

→ that's ligase's job! which you can also buy online

→ You can even glue DNA from different organisms.

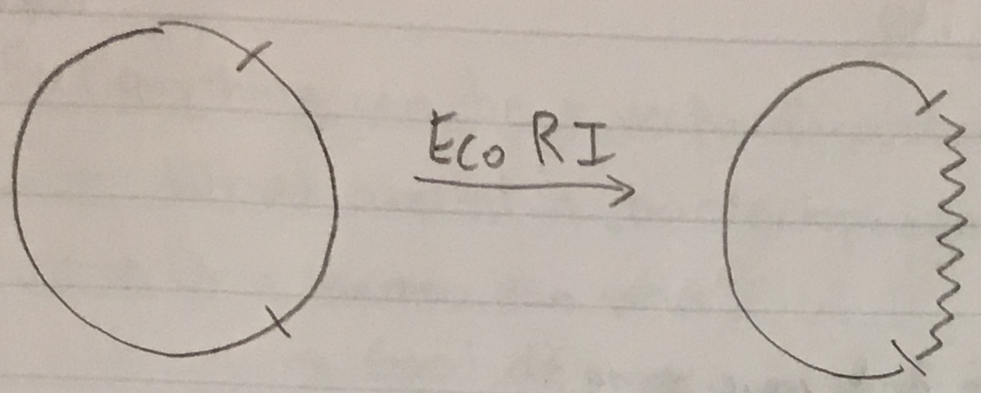
- But we want it to propagate — replicate in E. Coli!
  - ↳ How? Ask the bacteria!



Why have these circles? Transmit information and exchange these! Or do horizontal transfer

(Pen^R, Kan^R)? ≠ ↳ Defense mechanism, again. Antibiotics — penicillin secreted by fungus! So bacteria wants enzyme to degrade it.

- Let's say we use this. Cut plasmid w/ restriction enzyme.



Get a plasmid w/ human DNA!

Transform the DNA

How to get bacteria to take up circles of DNA?

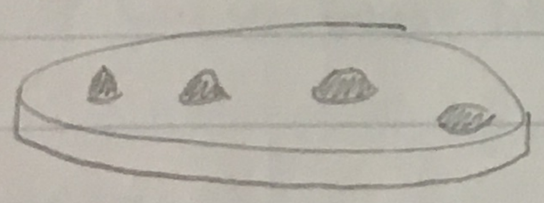
- Do a lot of possible things (e.g. electric shock), and they'll "slurp it up!" by chance.

Select for those with your plasmid

- Pour onto a petri dish; only the ones w/ the resistance gene will survive!

But how do you identify things now? (Random library)

- Many colonies, each w/ single different piece of human DNA



Purified by randomly sticking these in bacteria!

✡ This is only possible b/c DNA goes down to a single molecule & grows it back up!

- But still — how do you find the hemoglobin gene?
  → Transcribe it!
  → Needs plasmid to tell enzymes to do this, but this is possible!
- Early on, though, look at red blood cells, which have lots of RNA containing hemoglobin
  → Reverse transcriptase! Turn back into DNA & clone it
  → Much turns out to be hemoglobin.
- Doesn't work in general though.
  ↳ Try to go from random library to finding your gene

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Lecture 16

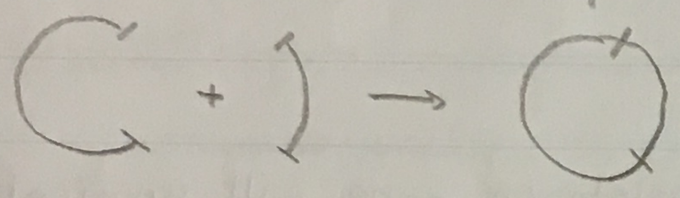
10/19

- Red Sox are in the World Series!
- Pset 4 posted...

Last time — cloning genes. Now — how to find your gene?

Steps

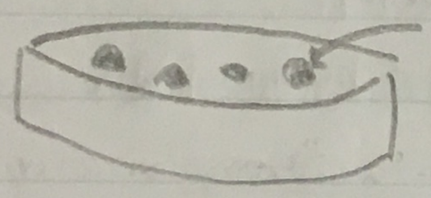
- Cut DNA w/ restriction enzymes @ specific spots
  - ↳ they're an "immune system" for single-celled organisms
  - ↳ end up w/ pieces of DNA
- To make it grow, use vectors to propagate
  - ↳ e.g. bacteria's plasmids (circular dsDNA)



use ligase to stitch back together

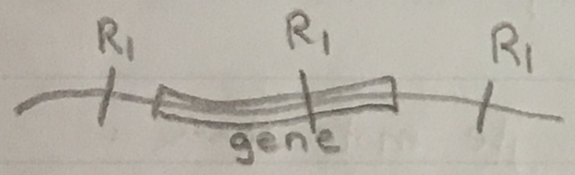
- Select for antibiotic resistance.

So now



different human genes (but purified)

What if...



the gene gets broken in half?

→ Randomly throw in methylase's only cut (on avg) some proportion
↳ Partial digestion (!)

- Alternative: "shred" the DNA randomly w/ sonic waves
  - ↳ Can't match to a plasmid though... they might stick out
  - → Polish the ends by getting rid of single strands
  - → Many variations!

Cloning vectors

- plasmid (5000 base pairs)
- What if we wanted to clone in eukaryotic cells? They have plasmids too!

- But anything can be a vector if it replicates! / propagates
  - → What about a bacteriophage?
  - → Or a mammalian virus?
    - → Good delivery system
  - → Artificial chromosomes? (harder b/c big)

Can we clone RNA? Not quite.. it's single-stranded.

↳ But you can convert it back (reverse transcriptase)

RNA ~~~~ AAAA ← poly-A tail

DNA ~~~~ TTTT ← poly-T starter (called a cDNA)

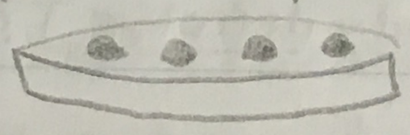
Why is this cDNA cloning good? No introns!

So now, how do we find the gene?

With yeast, we found auxotrophs for arg.

↳ labeled an arg1, arg2, etc. mutant.

- Make a library! (Cloning by complementation)
  - → Cut up yeast DNA, put it in a yeast plasmid. Transform into yeast cells!

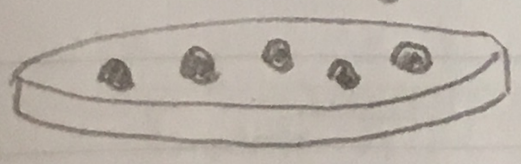


☆ But use wild type DNA, mutant yeast cells. (arg1⁻). Grow on min. media

↳ Only plasmids with arg1⁺ will survive! ↖

- What if I want to clone (human) hemoglobin? not fatal like here...
  - → Way to recognize hemoglobin. Antibodies (grow by injecting mouse)

Steps of (cloning by expression):

→  colonies of human cells (that are defective)

→ use expression plasmids so it has a promoter before the hemoglobin gene

↳ Wash antibodies to see which produce hemoglobin!

- What about diseases that (e.g.) cause brain degeneration?
  - → can't use either of the methods above ↑
  - → 3rd / different way!

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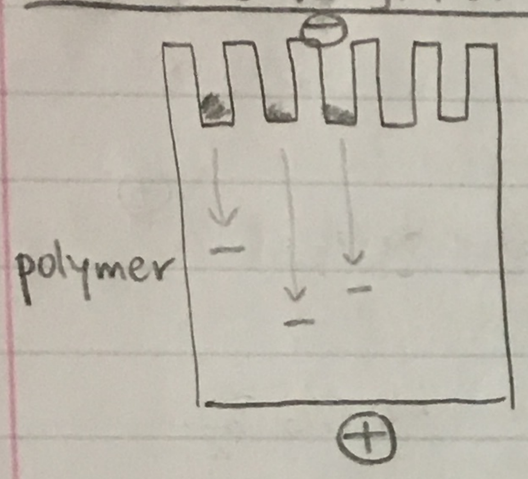
Lecture 17

Recall

- Chop up DNA, put them in plasmids
- Purify plasmids — now we have a test tube w/ DNA in plasmids
  - ↳ Restriction enzymes → we have 1 piece of DNA

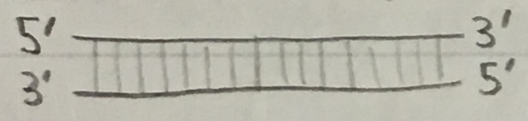
What do we want to know about it?

Measure length of DNA: gel electrophoresis



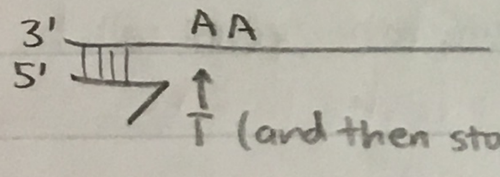
- DNA can wiggle through polymer, but only if there's a current (DNA (−) charged)
- Smaller moves faster
- Compare against known fragments.

Sequencing the base pairs



what is this sequence?

① What if I undo the double helix and give DNA polymerase A, C, G, and a defective T?



↳ this would tell us what the next base is

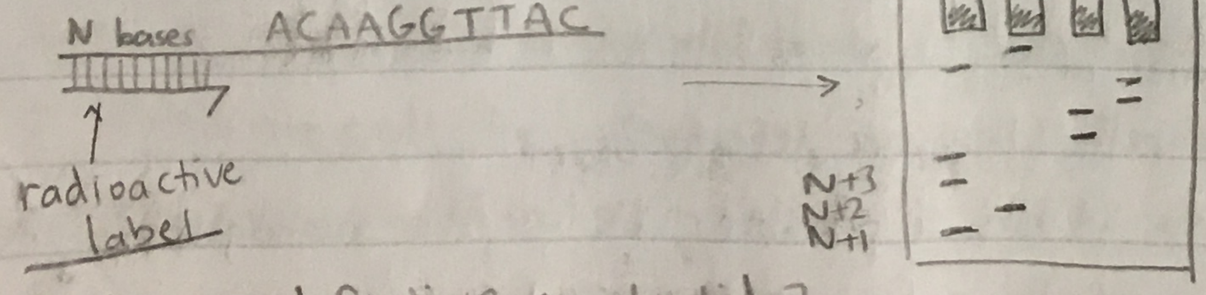
② What if I only add some defective Ts?

↳ we have many copies of the DNA, and each stop happens w/ some probability

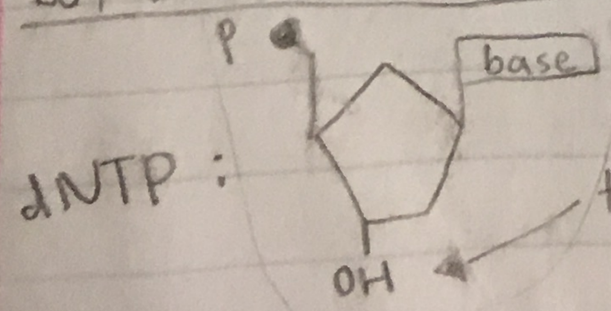
→ Now measure lengths = locations of Ts!

→ Do same w/ A, C, G.

e.g.



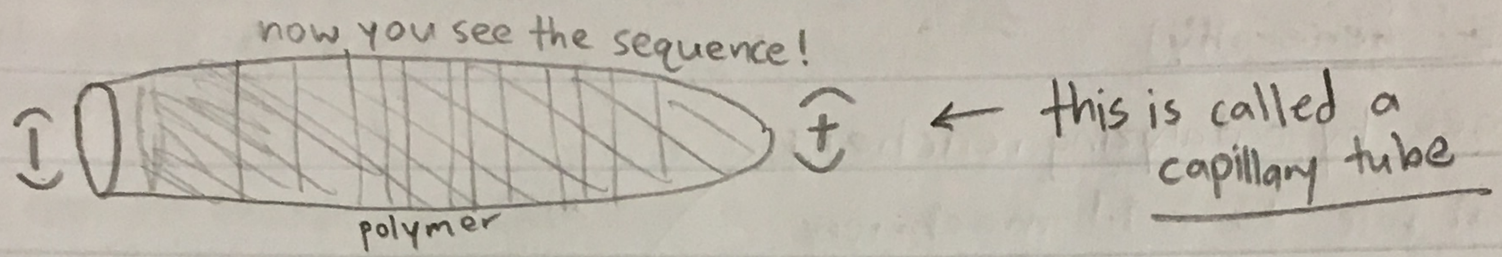
But what is a defective nucleotide?



dNTP:

this 3′ hydroxyl is important — it attaches to the next nucleotide. Remove it!

So this creates 2',3'-dideoxyribonucleotides.
Doing this is tedious though... any way to make it more efficient?
Add a fluorescent dye to defective T! maybe different color w/ A,C,G.
→ Now lanes will light up diff. colors! Now we can throw them all in together, and we can even do the reactions together.

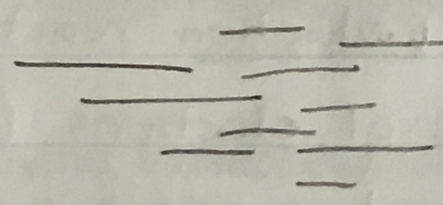


← this is called a capillary tube

Scale this up → machines can do 100000s of base pairs to read DNA.

But how do you assemble this?

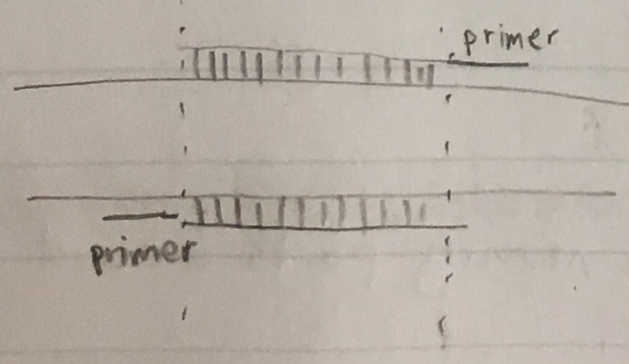
Used to do primer walking - after getting N bases, chop it off and keep going
Instead, start at random points (random subclones)
↳ We can sequence each one individually
→ Now fit the pieces together! Look at overlaps


this works for bacteria

→ BUT humans have really repetitive DNA — it's not random.
↳ handwave handwave it was hard.

So now... how to get your hemoglobin gene if I have mine?

- Throw in a copy of the hemoglobin primer?
  → But we need to amplify this with a polymerase chain reaction



2 copies of the double helix

Keep repeating this!

1 million copies of genes after 20 rounds.

7.012
Lecture 18

10/24

- Another p-set is due on Friday! Make sure you're using the corrected version.

Today we're talking more about proteins — molecular biology tools for studying structure and function

Protein Functions (generally)

→ Act as enzymes by catalyzing reactions
→ Play structural role to hold machinery
→ Transport stuff
  - e.g. hemoglobin
→ Regulation

we'll talk about this now

What are the types of enzymes?

- Oxidoreductases → catalyze oxidation and reduction reactions (usually happen together in redox reaction)
  - ↳ this means loss & gain, respectively, of electrons.
  - (e.g.) Aerobic respiration → pyruvate oxidation!

- Transferases transfer functional groups (where you have to form/break bonds)
  - ↳ CH_3, OH group, etc.
  - (e.g.) DNA methylase adds CH_3 to DNA.

- Hydrolases uses water molecules to cleave bonds
  - (e.g.) Proteases cleave peptide bonds!

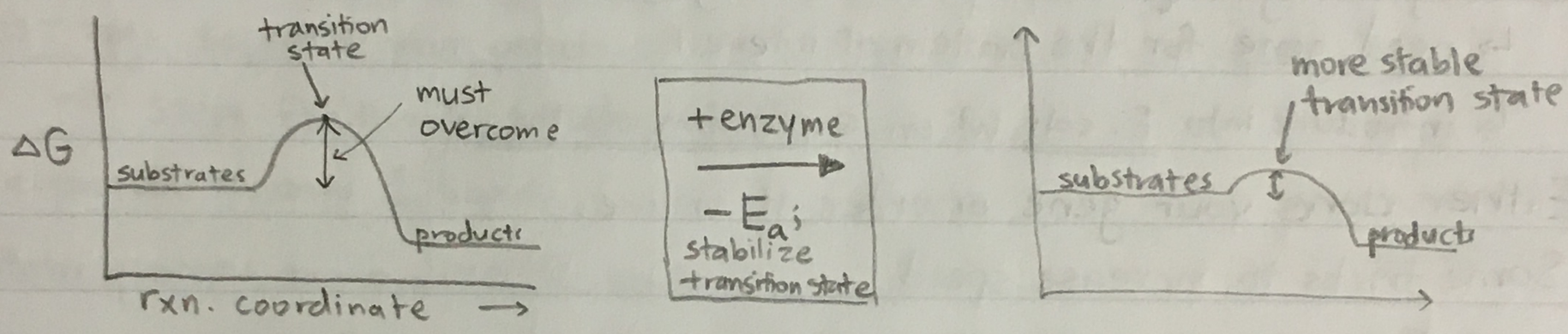
- Isomerases change structure of a molecule by moving parts around
  - (e.g.) TIM — triosephosphate isomerase

- Lyases remove something in an elimination reaction
- Ligases form bonds, but this is "expensive"
  - ↳ so it's coupled to ATP hydrolysis

When we're characterizing enzymes...

- What do we want to know?
  - → Reactions in play — what are the substrates and products?
  - → What is the enzyme mechanism — how many steps? what type?
  - ☆ You can show that mechanisms are inconsistent, but not that they are true — they are just models.
  - → Rate of reaction / enzyme kinetics; how well do molecules bind?

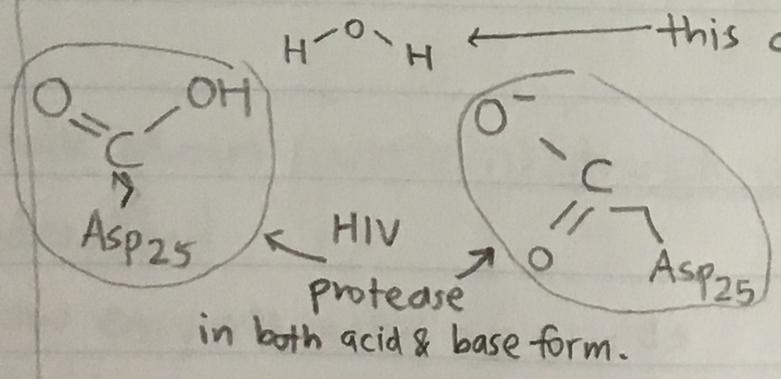
- Common enzyme tricks — a review



→ Acid-base catalysis: stabilize transition state w/ proton acceptance/donation

- Acid donates a proton (H^+), base accepts one

(e.g.) HIV protease: helps water attack carbonyl carbon



→ end result: peptide bond is broken

→ Covalent catalysis — substrate forms a transient covalent bond w/ the enzyme.

(e.g.) Chymotrypsin is a serine protease. Forms a tetrahedral intermediate as well.

→ Metal ion catalysis — metal ions can stabilize transition state by activating H_2O for hydrolysis reactions

(e.g.) zinc protease contains Zn^{2+}.

- **Why do we want to know it?**
  - Design enzyme inhibitors
    - (e.g.) HIV proteins not dangerous in immature form
      - ↳ mature form created by having protease chop up its proteins
    - → So we inhibit HIV protease [to make it not dangerous] → basically, resemble tetrahedral trans. state.
  - See if human enzymes are different from viruses / pathogens
  - Make faster enzymes.

- **How do we do it?**
  - Getting enough "quality" protein—using recombinant DNA methods.
    - ↳ need gene for the protein of interest
    - ↳ introduce into E. coli w/ an expression vector
  - Either clone your gene or order it online.
  - Some tricks to increase speed
    - → Put gene in vector after strong, inducible promoters.
    - → Modify end of gene w/ a purification tag.
      - (e.g.) His-tag → strong affinity for nickel, so now we can separate ones w/ tags from ones without.
    - → Evaluate purity w/ gel.

6 histidine residues at the end

7.012

Lecture 19

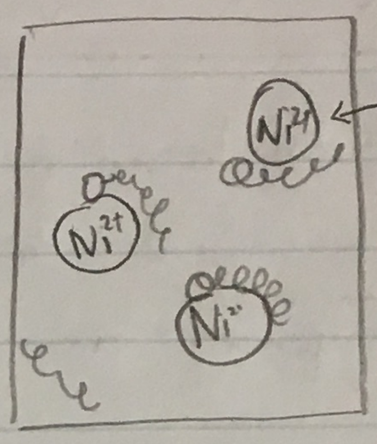
10/26

More about gels from previous lecture!

SDS-PAGE gel electrophoresis

- Separates proteins by mass
  - → confirm expression
  - → evaluate purity of protein
- Proteins not as charged as DNA
  - → SDS covers protein (after first denaturing them)
  - → Uniform charge/mas ratio
  - → So larger proteins travel slower
- His-tags separate your protein of choice
  - → steps: flowthrough, wash, elute
- Pure protein = only 1 band.

☆ Stain proteins to visualize the bands.



Site-directed mutagenesis – test pathways using introduced mutations.

- Use synthesized DNA primer w/ necessary mutation
- E. coli does the rest → now you can see enzyme mechanism w/ your necessary mutation.

Next, we'll talk about (protein) structural biology tools.

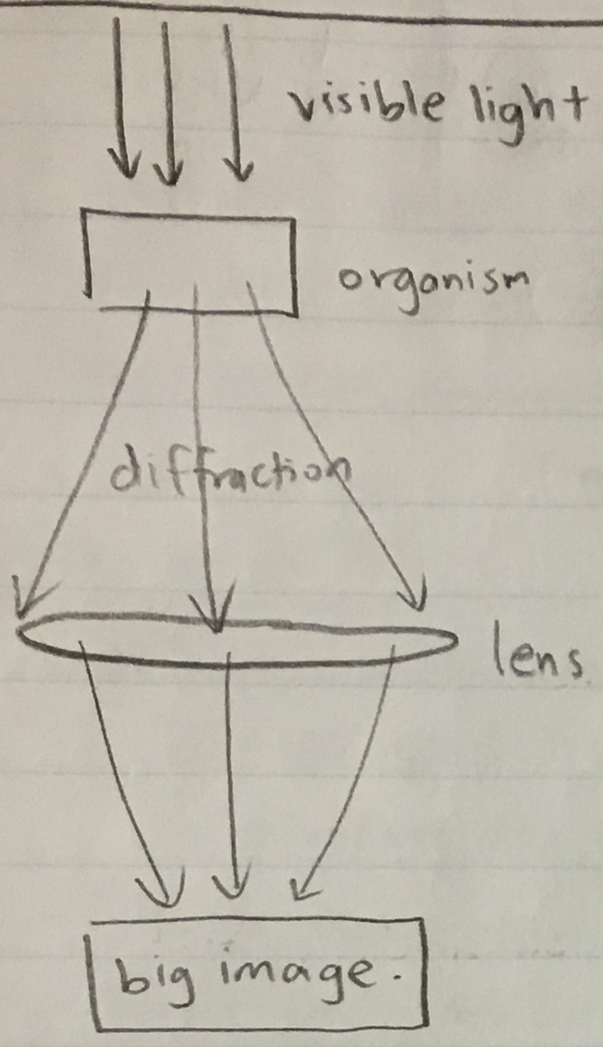
- We can understand
  - → How enzymes make molecules
  - → How to design inhibitors
  - → Basis of genetic disease
  - → Changing enzymes to make new products/chemistry.

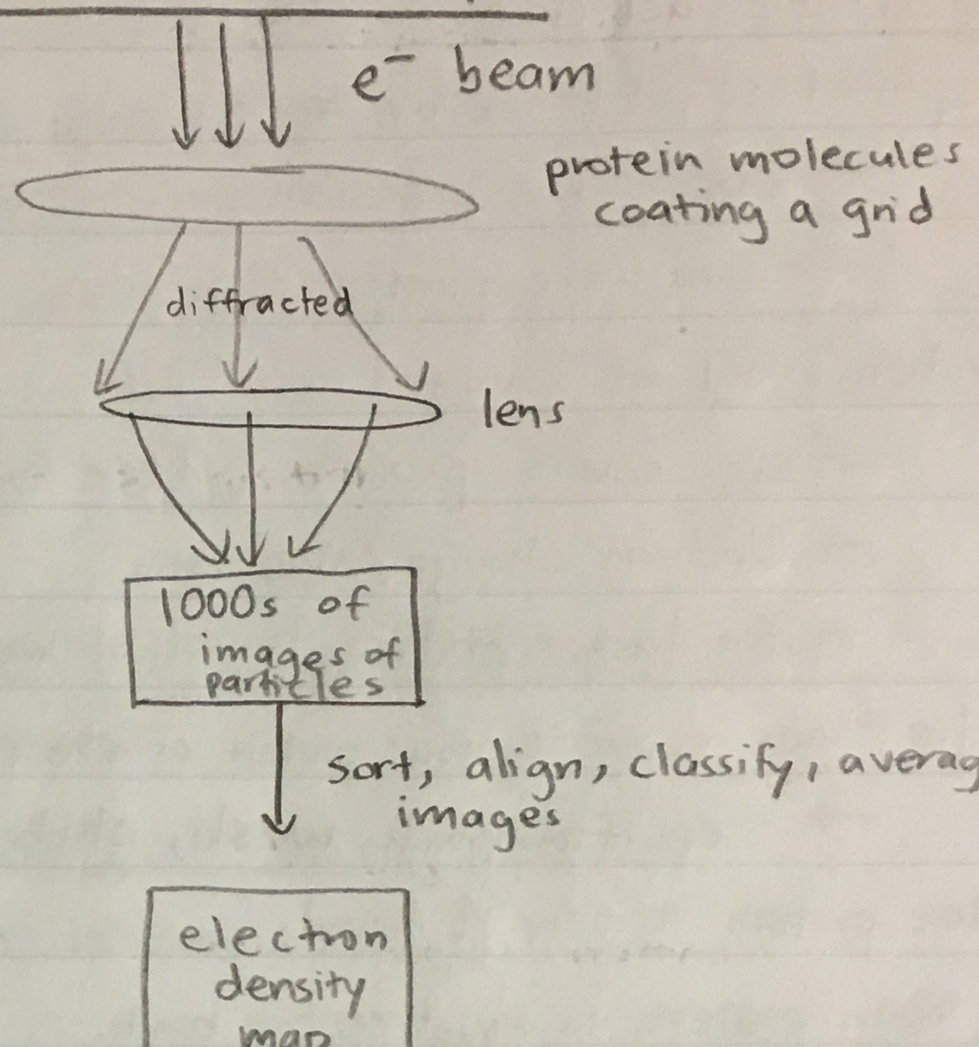
3 main methods for determining structure:

- One of them is NMR (nuclear magnetic resonance spectroscopy), but we're not talking about it.
- Others: X-ray crystallography and electron microscopy

Currently, 145,690 structures on the protein data bank.

Light microscope vs Electron microscope





A few more details...

- Need to preserve specimen in some way
  → Stain specimen or use cryo-EM (frozen-hydrated)
- Have to align all particles and avg. to get detailed info.
- But the particles might have different conformations or orientations.
  → Classify before averaging
- If 3-D, we look at different projections from different angles.
- But it's hard to see: what's a particle? what's noise?
  → wow mAcHiNe LeArNiNg!

Resolution — how precisely we know atomic positions

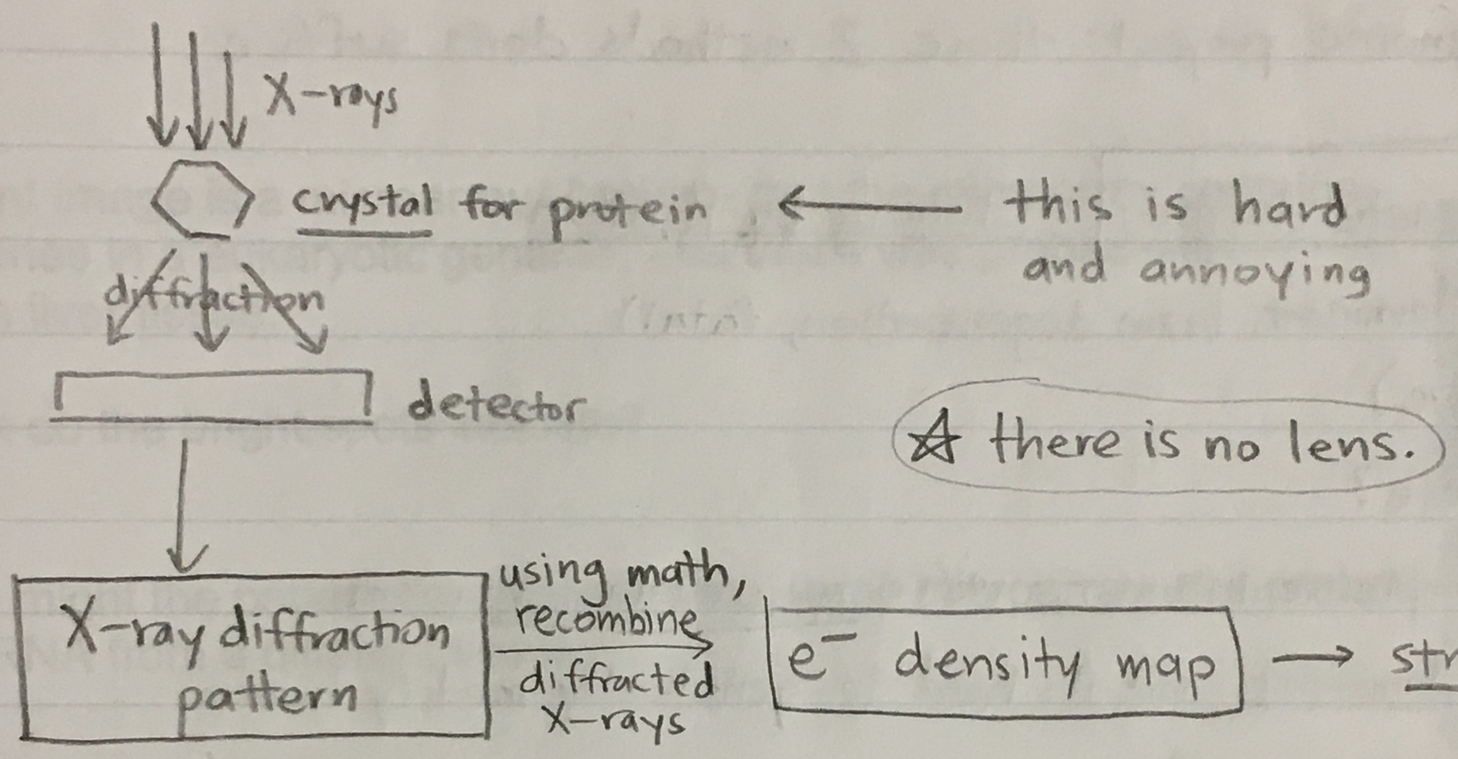
- 15-20 Å (← 1 angstrom = 10^{-10} m) tells us the overall shape.
- 6 Å – start to model α-helices
- 4-5 Å – β-sheets
- ≤3.5-4 Å – side chains.
- ≤2 Å – atomic positions (w/ precision)

* low-resolution can still be accurate, but not precise

Cryo-EM – resolution revolution
→ e^- more easily & efficiently detected w/ aligned / averaged frames,
→ Microscopes going from 19 Å resolution → ~3–4 Å.

compare to...

X-ray crystallography



- Sometimes hard to crystallize proteins in a regular repeating pattern, or only in one conformation.

Pros and Cons

✓	X
• Cryo-EM doesn't need crystals, while X-ray does. ↳ better for membrane proteins (e.g. need lipid bilayer) ✓	
• Cryo-EM can show multiple conformations in one grid ✓, while X-ray may not be able to always do this.	
• In X-ray crystallography, easier to validate crystal structure. (check it back w/ the data).	
• Still higher resolution w/ X-ray at the moment.	

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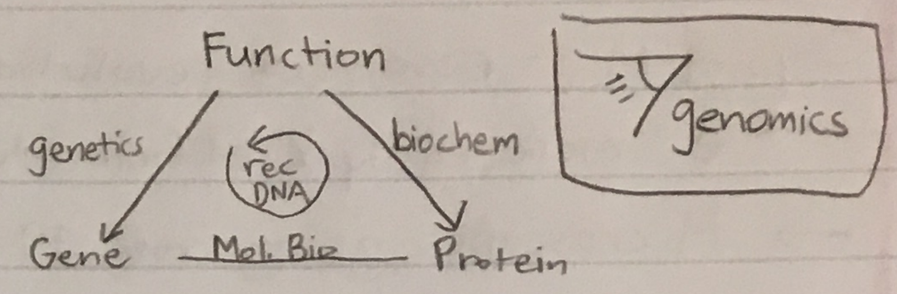
Lecture 20

10/29

(Missed the first 5 minutes, sorry)

Look at human genome @ larger scale.



Recall: make library by transforming into plasmids

→ To find those

→ Origin of human genome project: those 2 methods don't suffice

How to find gene for a genetic disease? – Cloning by Position

- Huntington's disease (dominant, brain degeneration, fatal)
- Cystic fibrosis (recessive)

→ How to do these?

→ Can't do cloning by complementation — we're multicellular organisms.

Plus, Huntington's is dominant, and it's hard to genetically modify humans

→ What about cloning by expression?

→ We have no idea what proteins caused this! So that doesn't work either

☆ Maybe find linked genes by mapping them.

In flies: (e.g.) cross

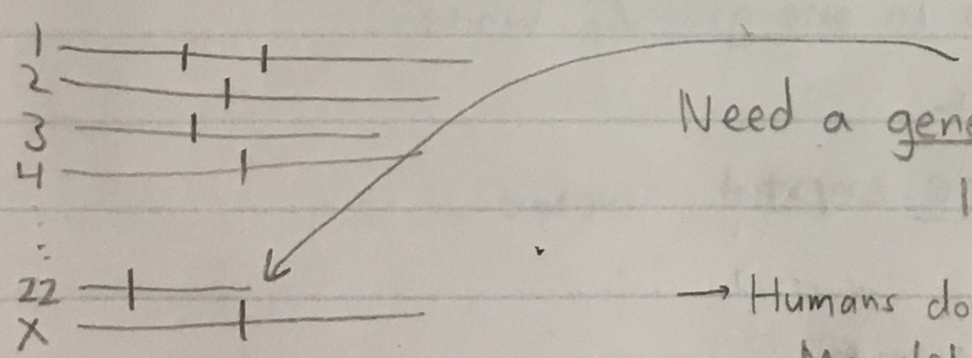
b	cn	vg
+	+	+

x

b	cn	vg
b	cn	vg

and see how linked the genes are.

How to do this in humans?



Need a genetic marker; can't use something like vestigial wings

→ Humans don't have a lot of single-gene Mendelian inheritance, either.

→ Also, we can't cross humans ethically

Key idea

Human DNA has spelling difference b/w 2 copies of the human genome

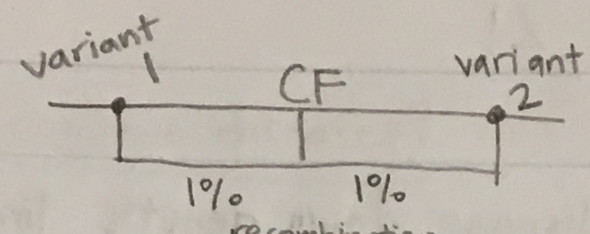
↳ "natural heterozygosty" ~1/1000 bases (e.g. G vs A in one spot)

→ Trace them in families! Keep track of each one. More likely to find specific markers of interest

* Single-nucleotide polymorphism

- Markers near the disease will be very likely inherited (low rec. %)
- Use multiple families across generations.

(e.g.) cystic fibrosis



→ Chromosome walking — put gene over library, find adjacent genes that overlap. → make a primer based on sequence of your marker → sequence ~N base pairs, repeat

→ Takes a very long time

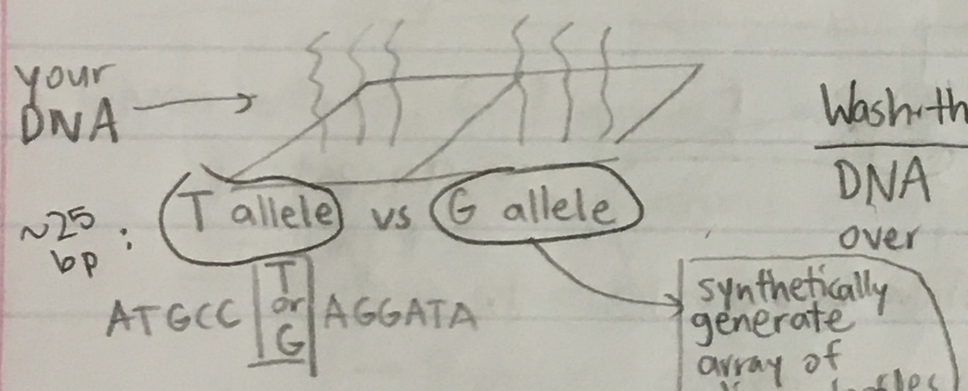
What's a better way? Human Genome Project (flex)

→ Instead of boring genetic maps, find all in parallel.

→ Do lots of sequencing w/ computers

Then what? Improvements?

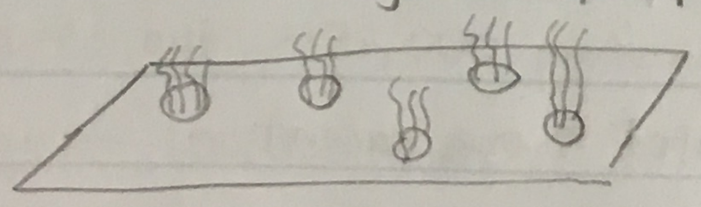
- Genetic variants? Which is more likely?



- How about sequencing?

→ Wash random pieces of DNA of you

→ Do a PCR reaction to get many copies



→ Recall: we used dideoxyribonucleotides and we'd stop.

→ But we can use a chemically reversible reaction!

Reversible blocking groups.

→ we can now do 7 billion spots of this × 150 bases/spot × 2 sides of a chip
~ 2 trillion bases = 24 human genomes! for ~$700./genome

Review

7.012

Lecture 21

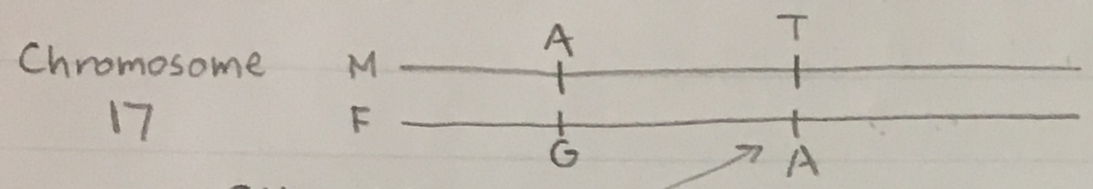
10/31

Last time — how to map strange trait / disease down genetic line

→ Modern biology — how do we do this?

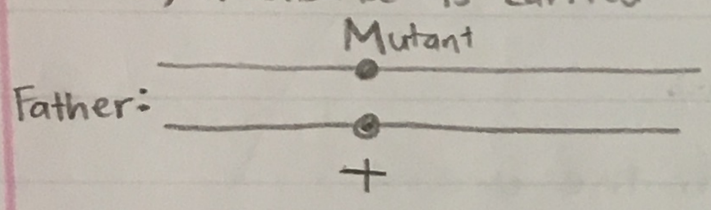
- Human genome finished in April 2003
- Today, all we do is just observe

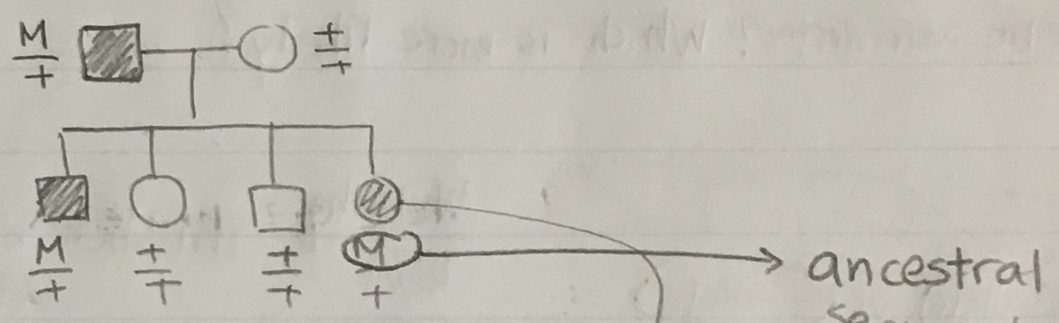
Mapping disease genes



→ Site of heterozygosty b/w the two chromosomes (1 per 1000)

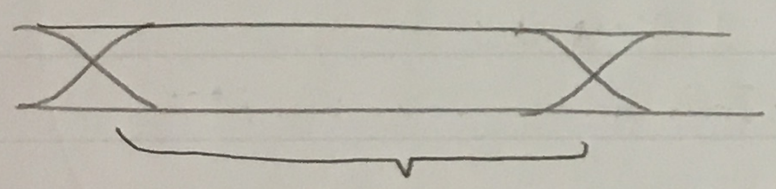
So, if disease is carried:





→ 1cM = 1% crossing-over chance ~ 1 million bases.

↳ on average, ~ 50 cM = first cross-over.

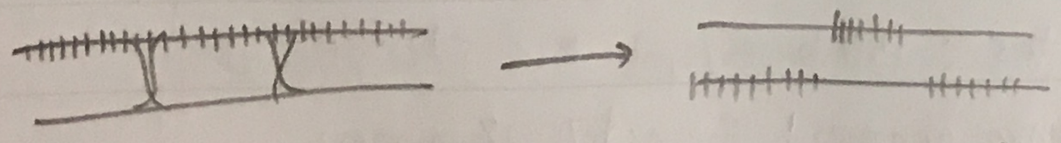


→ 50,000 polymorphisms in dad.

→ Look for ~50k genetic markers attributed to one parent

(e.g.) recessive monogenic traits

→ Inbreeding often leads to homozygosity



segments get smaller:

$\text{in cM} = \frac{50}{N}$ for N generations

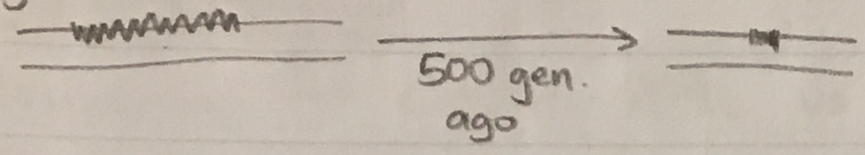
→ But in inbreeding, you don't just inherit mutation:
the whole chunk of mutant DNA is the same!

→ Homozygosity mapping — find large chunk that is identical!

→ (eg) Finland has higher variance in disease alleles
b/c of founder bottleneck. We can find the 1000 common genetic markers from 100 generations ago!

What about polygenic common diseases?

(e.g.) if a gene increases risk by even small factor (2x)



→ You can still see the common region! No need for Mendelian inheritance.

(e.g.) we can map schizophrenia! (at MIT)

→ 3000 w/, 3000 w/o were mapped: no statistical significance

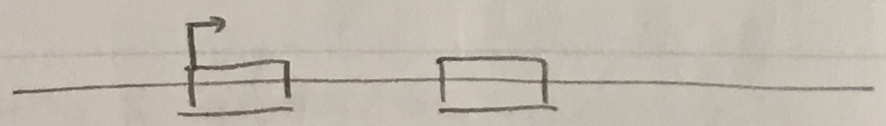
→ increased size to 20,000 → 50,000 → 110,000

↳ Found lots of excess sharing in 100+ spots!

Next, reading genomes

genome ~~~~~~~~~~~~~~~~~~~~ 3 billion nucleotides

- Given letters, find genes? Where are proteins made?
  - → Expect stop codons every ~60 letters on average
  - → If long stretch has no stop codons, highly significant.
  - → Problem: coding sequence has introns; we don't know where they are. (they're ~180 bases)
  - → Solution: read & sequence the cDNA from spliced mRNAs! Now unsplice them!
- → figured out ~21,000 protein-coding regions.
- Transposons — 100,000 copies!



promoter → mRNA → protein = reverse transcriptase copies RNA → DNA.

This one only recognizes itself. So it puts itself somewhere else in the genome!

→ it's been spreading since ~1 billion years ago.

→ We can even map them over time.

- Compare humans & chimpanzees — common ancestor → similar DNA sequences.
  - → genomes ~98.5% identical.
  - → We can still see alignments from millions of years ago!

✱ Important DNA (functionally important) has slower rates of mutation (natural selection!)

↳ so exons = less mutation. Same (ish) with promoters

→ Compare human, mouse DNA

↳ 6x DNA show slower signs of mutation

→ <u>Regulatory</u> systems!

→ Human vs Neanderthals

↳ there was interbreeding, only in Europe/Asia

☆ can find children that are hybrids!

Gene Expression

2 patients with leukemia / blood cancers

→ what if we <u>compare</u> patterns of <u>RNA</u>?

- 20,000 genes in a genome
- To describe, put them in a vector of length 20,000.

Thinking: every cell has some RNA per gene. It's a point in $\mathbb{R}^{20000}$.

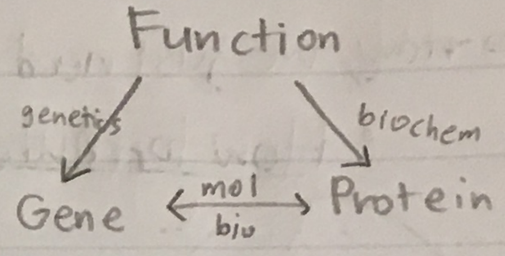
What can you do?

7.012

Lecture 22

11/2

We're going to complete the triangle today:



Global view of RNA Expression

→ Study leukemias → classify AML vs ALL (looked at nuclei, etc.)

↳ different treatments = respond differently

→ Slowly found more & more differences, but it took ~40 yrs to classify

☆ Instead, question: express it as "how much each gene on/off"

↳ tumor 1 = $(x_1, x_2, \dots, x_{21000})$, x_i denotes level of expression

→ Get gene expression by reverse transcription OR a chip

in the last 3-4 years: sequence & count.

oligonucleotides for each gene

→ Now you can easily match by classification: clustering per group!

→ Last 5 years — human cell atlas

↳ Get gene expression cell by cell

- 18 cells → 12.5 million cells!

☆ Discover lots of new cell types by doing this

→ We can't quite finish the triangle yet. How to go gene → function?

→ If we don't have a mutation, make one — interfere with genes!

Adding & subtracting genes

→ Add DNA to bacteria in form of plasmids

→ What about a mouse?

- We could inject a virus
- Or take a fertilized egg and inject DNA there!

→ Hard to be exact, but 1st step to transgenic mice

→ What if we use embryonic stem cells? (for mice)

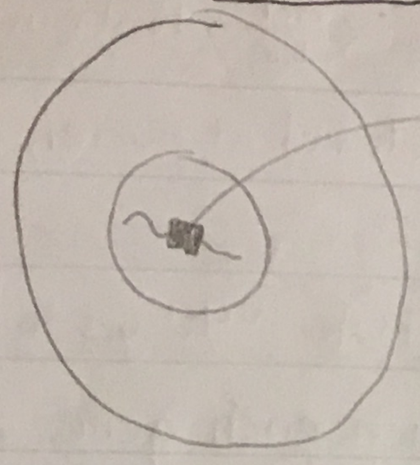
- Early stage of embryo — they have an inner cell mass
- Grow those cells → inject into new embryo

↳ they'll become part of new mouse

→ To recognize, use different fur color! → chimera

→ So now, we just need to be able to change those ES cells! (embryonic stem)

- How to mutate a gene? w/ cells growing on a plate



let's say I want to remove YFG, "your favorite gene"

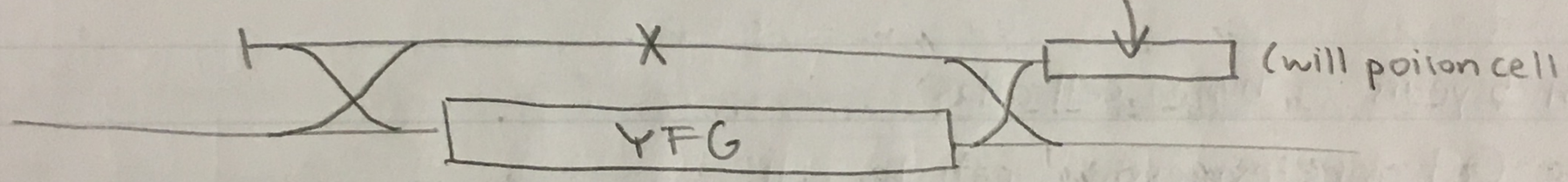
Transfect cell w/ damaged DNA w/ mutation in YFG gene.

→ Then hope for recombination?
only happens 1/million cells

☆ Select for those cells! Give cell an advantage: antibiotic resistance. (positive selection)

→ But we also need negative selection so only rec. at YFG is good.

→ Put negatively selectable marker gene!



→ After ~1 year, could get mouse of choice!

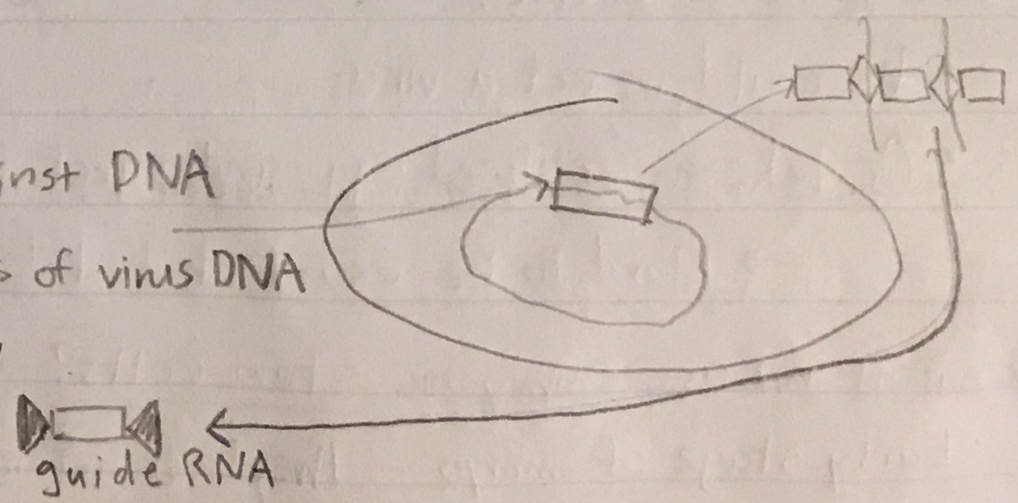
→ There's other modifications we can make (tissue/organ-specific)

Next method: RNA interference

- What if we destroy RNA corresponding to gene?
  - → Cells have sensor system that sees double-stranded RNA
    - ↳ search & destroy: find anything that matches it & destroy it
  - → Defense against dsRNA viruses!

CRISPR

- Bacteria also have bacteria mechanism against DNA
  - → library of memories: 20-30 bases of virus DNA
  - → this is called a CRISPR array
- Bacteria copies into CRISPR RNA
- Cas9 scans DNA/genome and cuts.



→ Works in human cells! (January 2013) → now you can introduce DNA and cut

7.012
Lecture 23

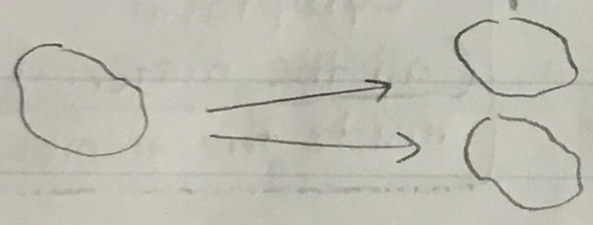
11/5

- Exam on Wednesday
- Pset 6 will be posted a bit earlier
- Vote tomorrow!

Today we'll take a closer look at <u>cell structure</u>

<u>"Cell Theory"</u>

- Fundamental unit of life – (all?) living things are or have cells
  → Cells are made from preexisting cells ; evolved from a common ancestor



cell division

<u>Size of a cell</u>

→ Typically ~1–100 μm ($1\ \mu m = 10^{-6}\ m$)
→ Can't get much larger b/c <u>SA to V</u> ratio $\propto \frac{1}{r}$.
★ Activity in cell scales w/ volume, so we need more and more nutrients / produce more and more waste
★ But transport scales w/ surface area.

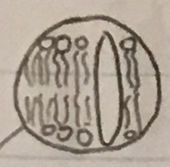
<u>Classifying cells</u>

- Prokaryotic cells – found in form of bacteria, archaea
- vs
- Eukaryotic cells – animals, plants, fungi, <u>protists</u>, other eukaryotes?

Differences:

① Eukaryotic cells have membrane-enclosed internal <u>compartments</u> called organelles (e.g. nucleus)
② Eukaryotic cells ~10–100× bigger than prokaryotic cells

Prokaryotic cell



cytosol (intracellular fluid)

cell membrane (outer boundary)

- consists of phospholipid bilayer, (hydrophobic tails in the middle)
- proteins. Acts as selective barrier (transport in/out), maintains homeostasis, cell signaling, communication.
  - constant internal environment

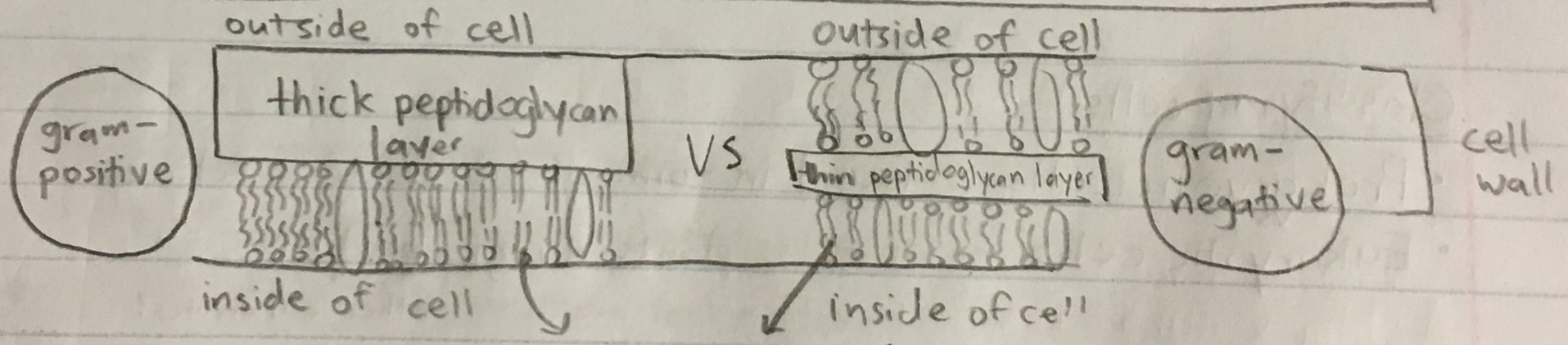
ribosomes (for protein synthesis)

cytoplasm: (all the material inside the membrane)

nucleoid (region in which DNA is located)

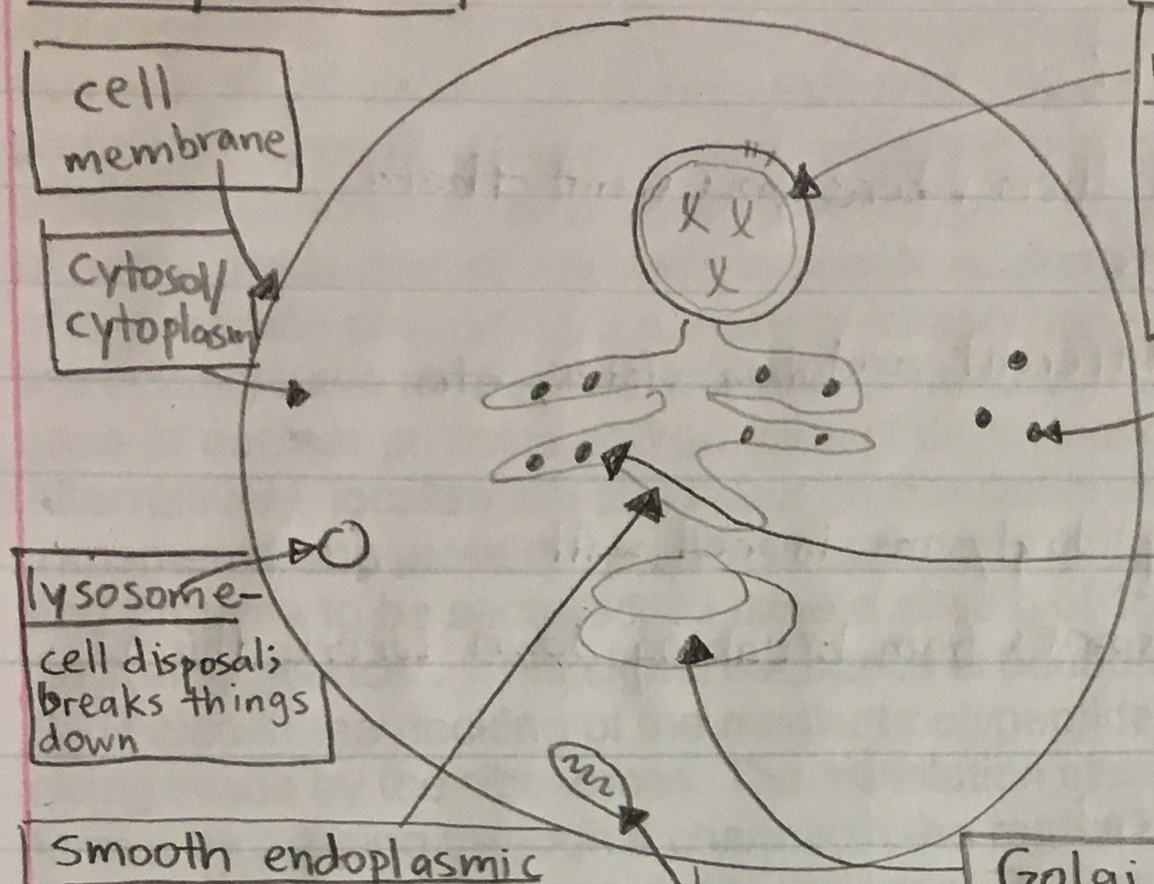
cell wall (found in most prokaryotes)

- consists of peptidoglycans. (peptides, sugars) 2 basic types: (in bacteria)
  - gram-positive vs gram-negative. (retaining stain after washing)



→ antibiotics generally target cell walls - easier for gram-positive

Eukaryotic cells



nucleus w/ **nuclear membrane**

↳ contains most of the genetic material.
site where gene regulation, transcription occur.

free ribosomes – make proteins used in the cell.

rough endoplasmic reticulum (RER)
contains **ribosomes** as well.
→ makes proteins to go **into** membrane, outside cell.

Golgi apparatus – modifies, sorts, and packages proteins that will be used @ membrane / outside cell

mitochondria – powerhouse of the cell, site for ATP synthesis. contains its own DNA, ribosomes, can even make its own proteins. (prokaryotic-like cell inside?)

7.012
Lecture 24

11/9

Protein localization — why is it important?

→ "Everything in its place" — only put them where you want them
(e.g.) DNA polymerase in wrong spots
→ Micro-environments: different pH, different oxidation states, etc.
→ Protein folding, modification
↳ sugars are not flexible — peptidoglycans in cell wall
↳ glycosylation — prevents lysosomes from breaking down wrong things

Natural next question: how does this happen?

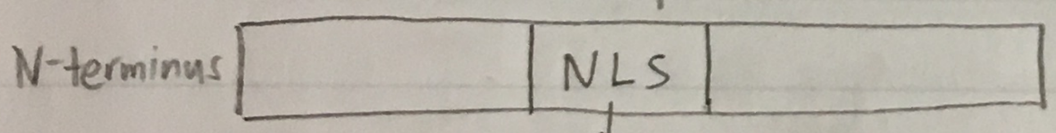
How do proteins get to their cellular location?

- Cytoplasm is where the proteins are made (by the ribosomes)
  ↳ If proteins are cytoplasmic, easy
- Other proteins for lysosome, plasma membrane, Golgi, ER, outside the cell...
  ↳ need a signal — seq. of amino acids that fold so other proteins can bind (for import)

(1) Post-translational import → after proteins are made in cytoplasm

(e.g.) Mitochondria is "given" some proteins aside from its own; also nuclear proteins (in the nucleus)

↓ here's the protein



→ proteins go to nucleus

☆ nuclear localization signal

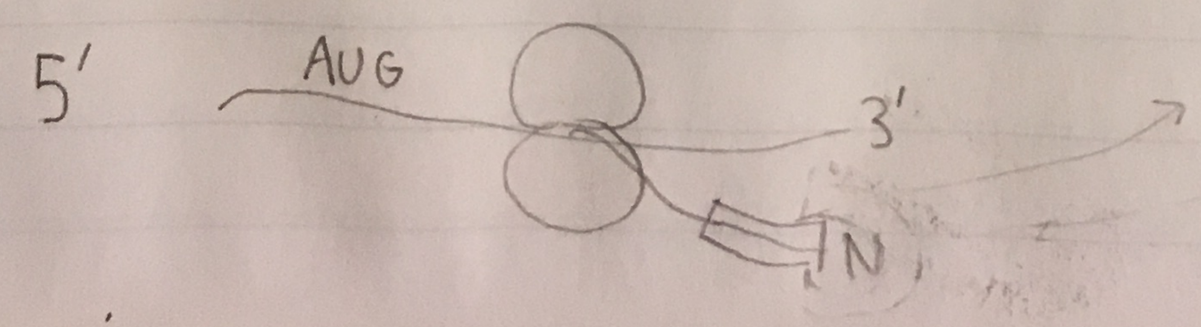
✓ → to check if it is necessary, knockout the gene → proteins go to cytoplasm

✓ → check if sufficient: take cytoplasmic protein, add NLS → went to nucleus

(2) Co-translational translocation → during translation

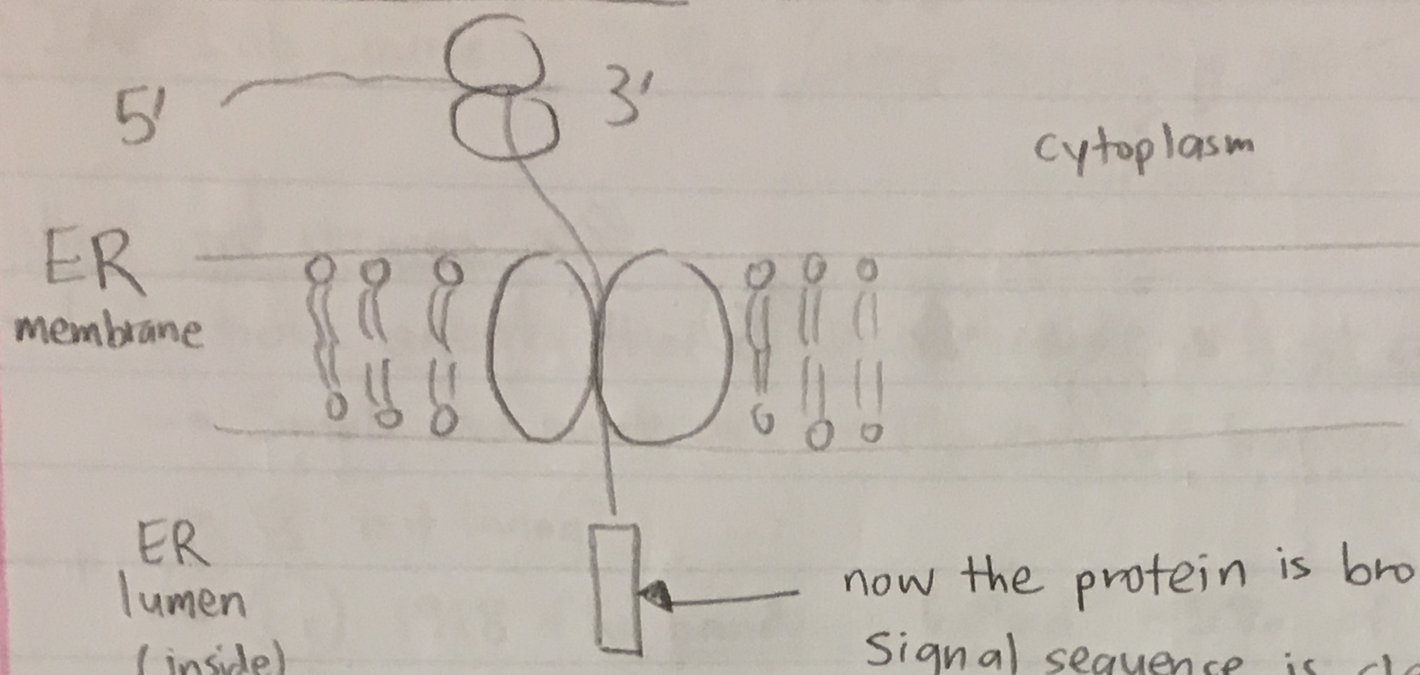
(e.g.) [ER, Golgi, lysosomes, plasma membrane, outside cell] proteins

- All proteins had signal sequence of ~15-30 amino acids at N-terminus only

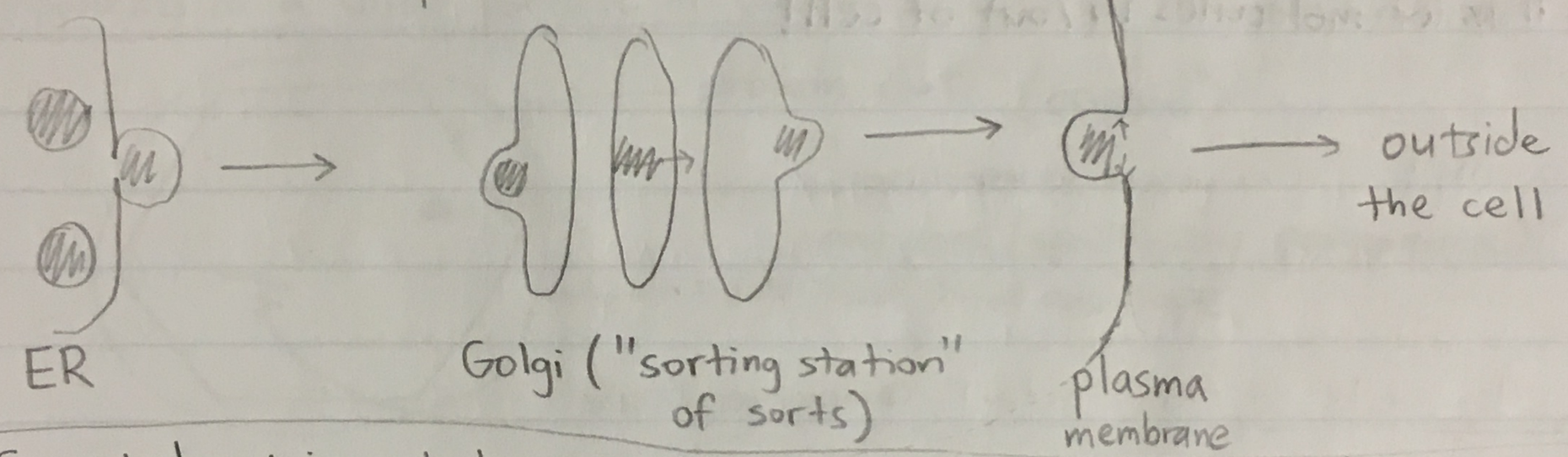


once this is made, protein called Signal Recognition Particle (SRP) binds; stops translation

- Next, SRP brings nascent peptide, mRNA, ribosome to a <u>translocation channel</u> @ ER.



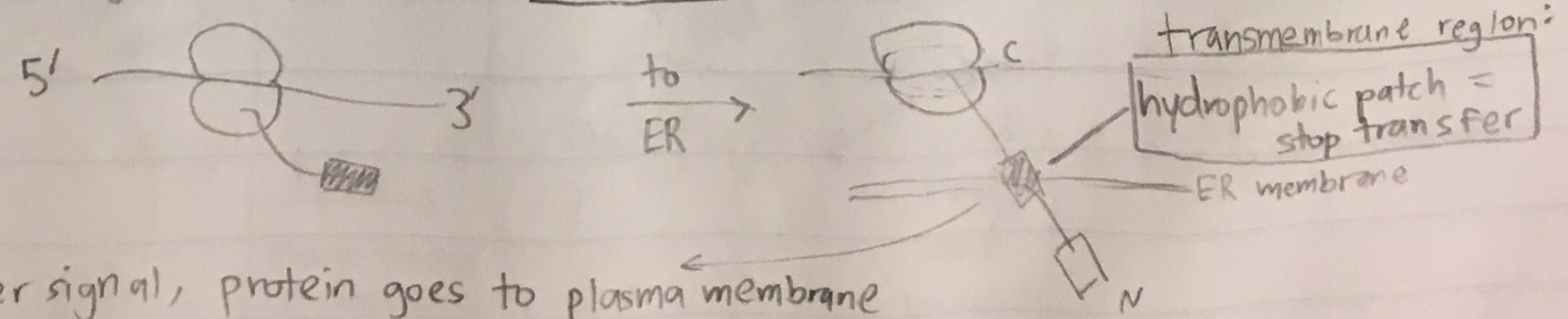
- <u>Vesicle</u> will (often) pinch off from ER



- Secreted proteins only have a signal sequence
- Proteins of <u>ER</u>, <u>Golgi</u>, <u>lysosome</u>, etc. have additional signals
  → ER retention, Golgi localization, Lysosomal localization

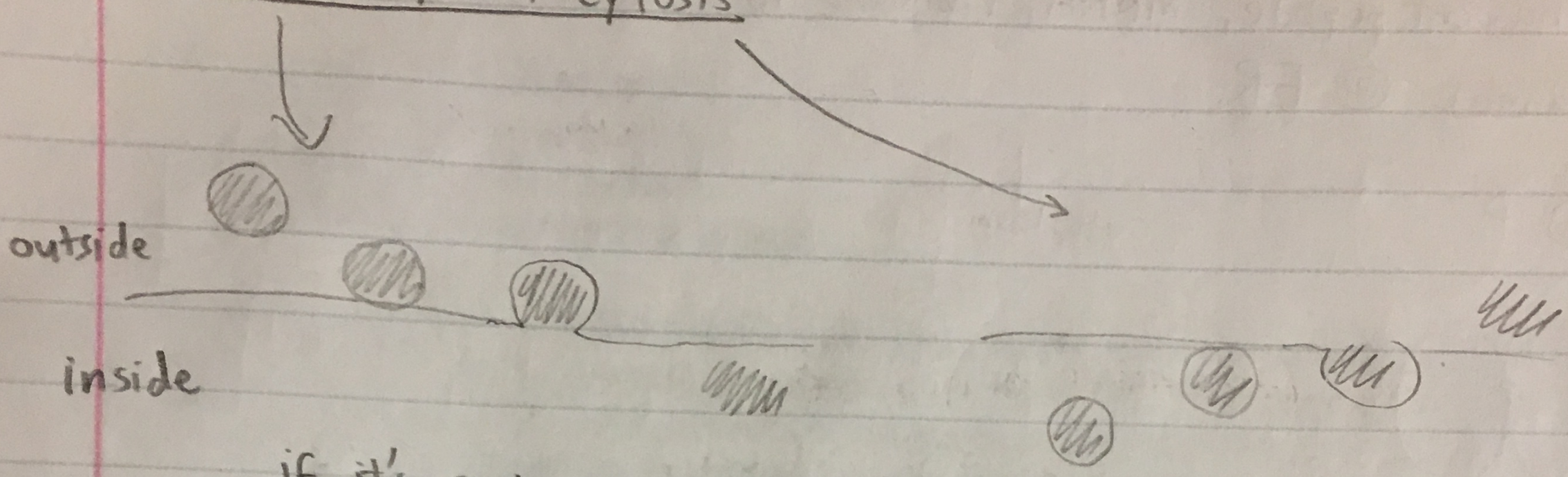
* remember outside of cell is different!
  → so inside of ER, Golgi, vesicles ≅ outside of cell, <u>not</u> inside.
  → inside is a <u>reducing</u> environment, outside is <u>oxidizing</u>

- So now — what about <u>integral membrane proteins</u>?
  → Also have signal sequence + <u>hydrophobic patches</u>



If no other signal, protein goes to plasma membrane
  ↳ N terminus on outside (since ER lumen has N terminus)

Endocytosis / exotocytosis



if it's really big, it's called phagocytosis (e.g. engulfing a bacteria)

→ Helps transfer molecules in / out of cell!

7.012

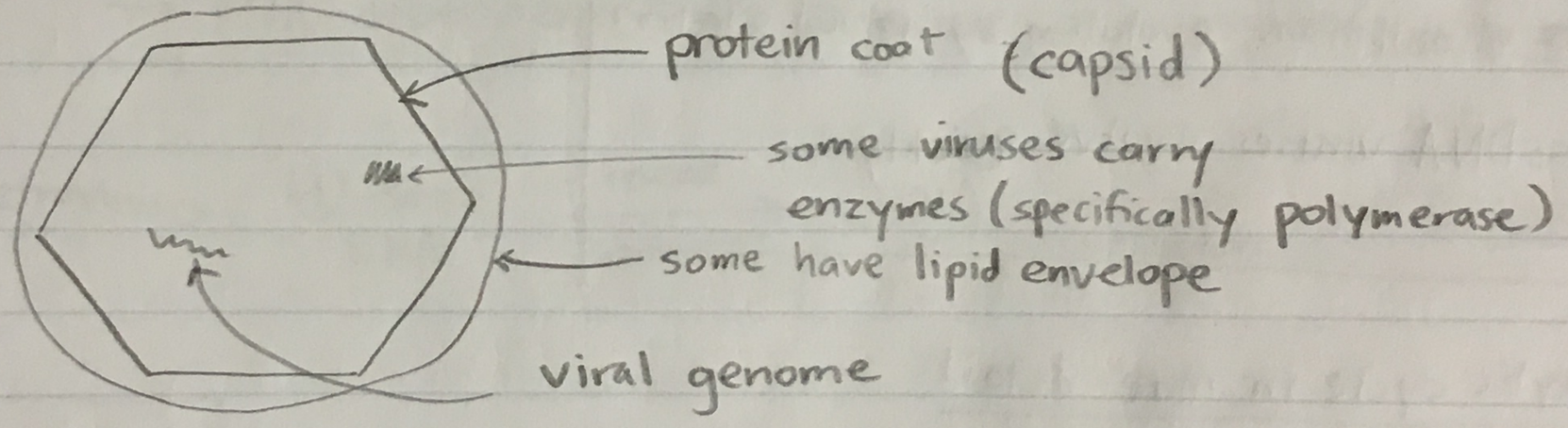
Lecture 25

- P-set 6 posted today — due 11/28
- Exam 3 is posted as well
- IAP Lab Course — 7.102 (Intro to mol. bio techniques) is apply by 12/7

What are viruses?

- Infectious agents that replicate inside a host cell
  - → Small and numerous: ~1% size of bacterial cell, ~10^{31} of them. (10 million / drop of seawater)
  - → Q: is it living?
  - → (Ex) 1918 flu pandemic killed ~5% of world population
  - → (Ex) ~36.7 million worldwide (~2.1 million children) with HIV/AIDS.

Components of a viral particle or virion

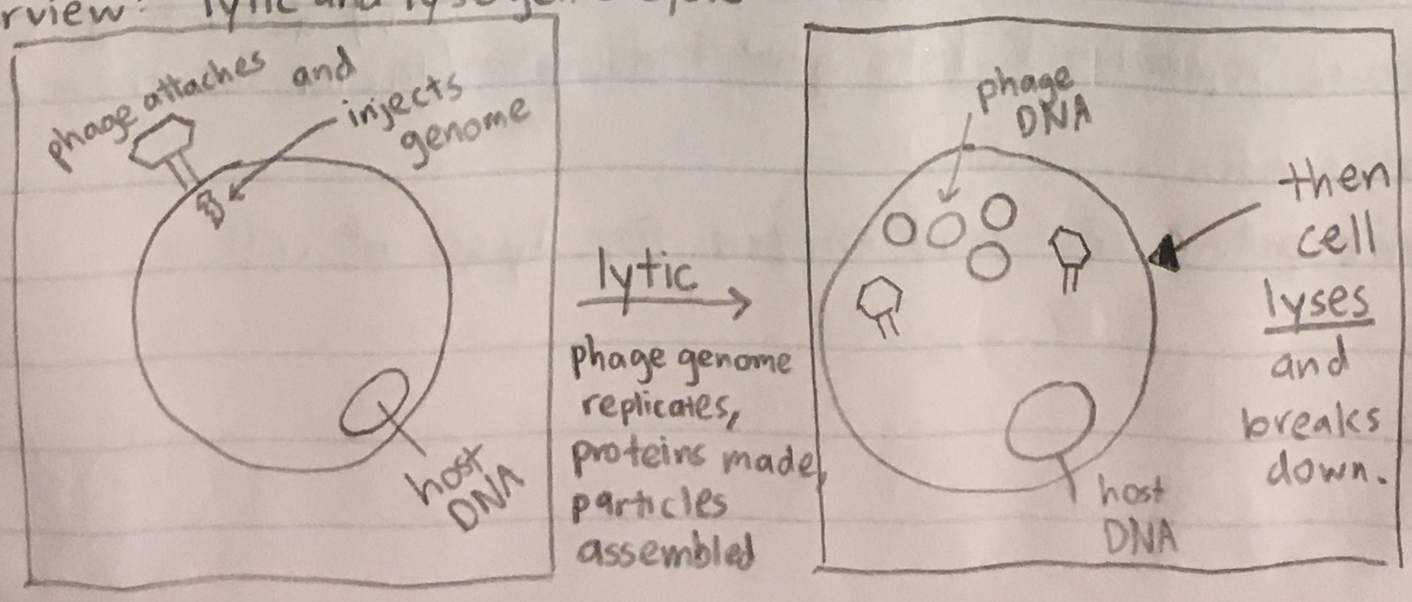


- Viral genome encodes ~2–4 proteins (small, ~2000 bases)
  - → DNA or RNA
  - → sometimes overlapping genes — frameshift to get start, stop, or ordinary codons!

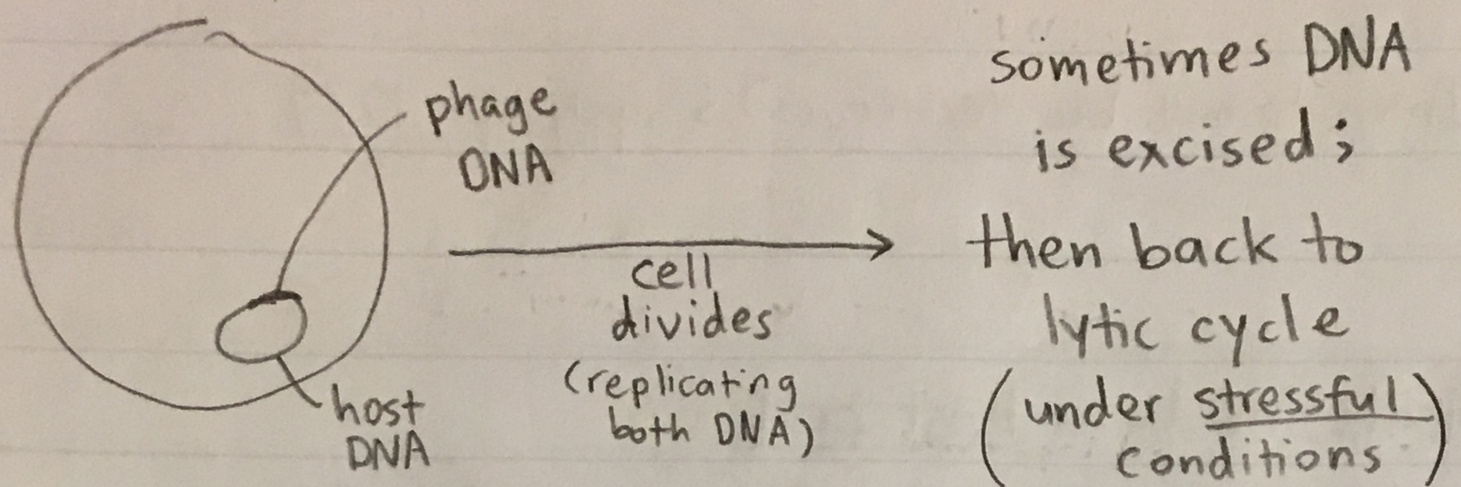
How does a virus replicate?

(Ex) bacteriophage

Overview: lytic and lysogenic cycle



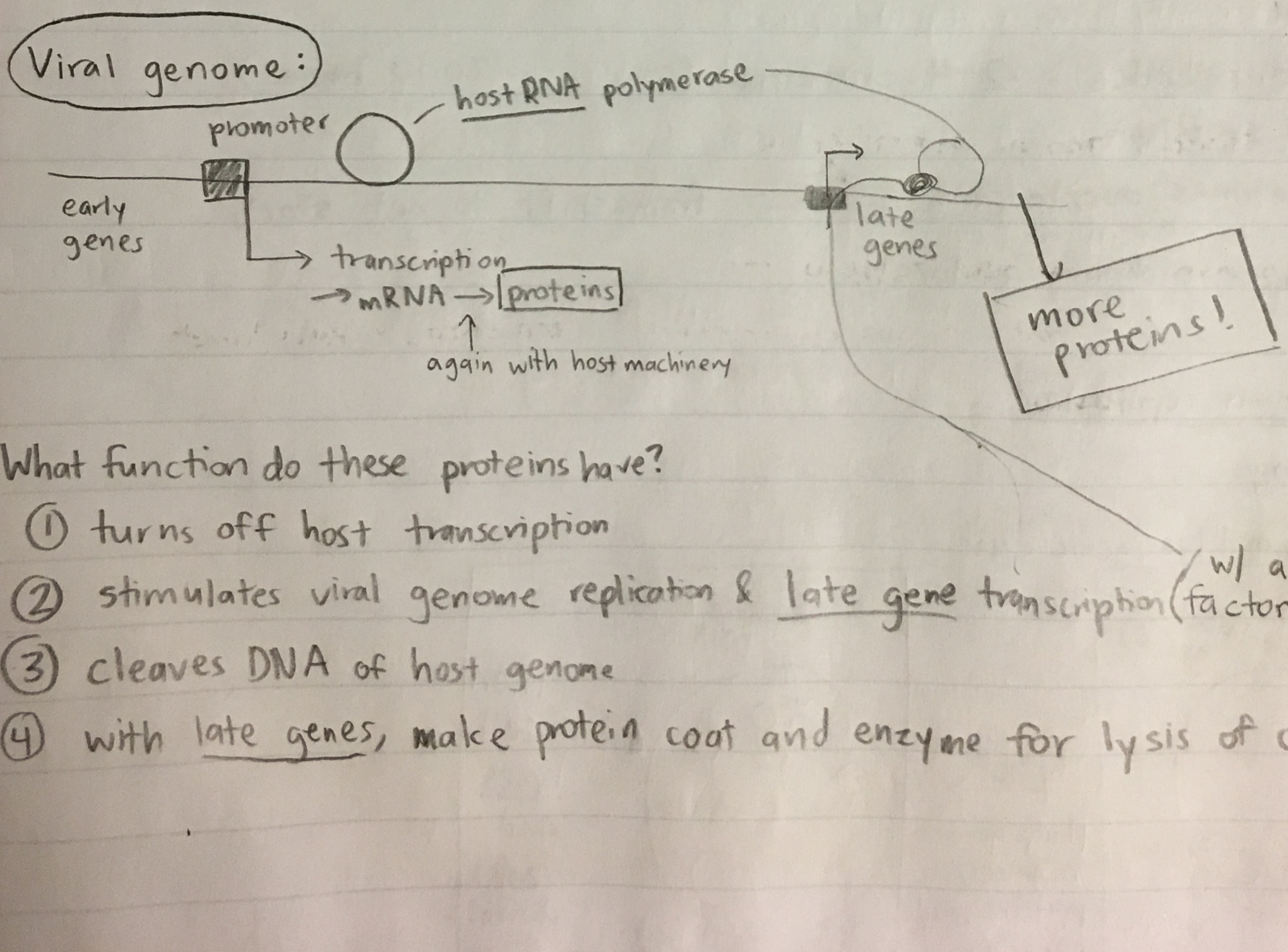
Alternative: combine host and phage DNA.



Key points

- This is a source of horizontal gene transfer
  - ↳ increases genetic diversity
- RNA viruses — replication is more error-prone b/c RNA less stable
  - → lots of mutations, evolution by natural selection
- However, ssDNA viruses evolve quickly

Looking at lytic cycle in more detail



What function do these proteins have?

① turns off host transcription
② stimulates viral genome replication & late gene transcription
③ cleaves DNA of host genome
④ with late genes, make protein coat and enzyme for lysis of cell

Types of viruses

- DNA viruses — ssDNA (parvo) vs dsDNA (herpes)
- RNA viruses — ⊕-sense (cold, SARS) vs ⊖-sense (influenza, rabies, ebola) vs dsRNA (rotavirus)
- Reverse-transcribing viruses — ⊕-sense (retrovirus HIV) vs dsDNA (herpes)

★ ⊕-sense is ready for translation
⊖-sense must be converted to ⊕-sense w/ enzyme first
→ RNA-dependent RNA polymerase - encapsulated in virion

★ Retroviruses: ⊕-sense RNA —reverse transcription→ cDNA —integration→ host DNA

Types of hosts

- Bacterial, archaeal, animal, plant cells
- Some viruses have many hosts — generalists
  and others have only a few — specialists
- High barrier to switching hosts — usually infection is too strong & kills host or too weak and does nothing

7.012

Lecture 26

11/16

What are bacteria?

- Prokaryotic cells
- There are many ; ~10 million / grain of soil
- Estimates vary — ~120k to ~1 trillion species.
  → In particular, genetic diversity mostly unknown
- Nomenclature: Genus species, (e.g.) *Escherichia coli*, *E. coli* for short
  (capitalized ↑ lowercase)
  (e.g.) *Bacillus cereus*, *B. cereus* for short
  ↳ subcategory of species = <u>strain</u>
  (e.g.) *E. coli* K-12 (lab strain), *E. coli* O157-H7 (bad)
- Live basically everywhere in/out of hosts.
  → free living : your gut, lettuce, acidic hot springs, radioactive waste (Uranium-eating)

Friend or foe?

- <u>Symbiotic</u> bacteria — mutually beneficial w/ host
- <u>Commensal</u> bacteria — gets nutrients from host, host not harmed
- <u>Parasitic</u> — (ditto above but) host is harmed.

most fall in this category

☆ Most bacteria aren't parasites, and many parasites are not bacteria!

What do parasites do?

- Produce toxins (poisons)
  → Protein-based or small molecules made by proteins
  → (e.g.) *B. cereus* makes <u>cereulide toxin</u>, causes stomach distress
  → (e.g.) *C. difficile*, *C. botulinum*
  Botox in small amounts, but 400g would kill all people
- Infect parts of body where they shouldn't be
  → <u>normal</u> : gut, mouth, nose, skin
  <u>bad</u> : internal tissue (e.g. *H. pylori*)

Bacteria aren't "born to be bad" though

- **Virulence** — pathogen's ability to damage host
  → Not all pathogens are virulent all the time
  → Controlled by **virulence factors** (proteins / small molecules made by proteins) which increase destructive nature of pathogen.

How to fight bacterial / viral infections?

- **Immune system**:

	viral	bacterial
natural response from body	✓ works	✓ works
to elicit immune response, vaccines can be used	major method !	don't work as well

  → what's in a vaccine?
  → Live-attenuated, killed viruses, viral proteins
  → No vaccine for HIV, Zika yet

- **Drugs**:
  - anti-viral drugs successful sometimes, but not primary method
  - antibiotics — primary treatment for bacterial infections
  → both typically small molecules
  - Anti-viral drugs target viral enzymes (e.g. virus polymerase, reverse transcriptase, HIV protease)
  - Antibiotics target differences between human/bacterial cells
    → e.g. cell wall biosynthesis, ribosome
    → Kill bacteria or keep them from reproducing

☆ Blunt instruments — kill both good / bad bacteria
☆ **Antibiotic resistance!**
  → Bacteria adapt b/c there are not enough new antibiotics
  → Current ones being used too often.

CDC levels of concern.

7.012
Lecture 27

11/19

- P-set due 11/28

"The bacteria within us" — topic for today.

Antibiotic resistance, con't

Defn — when a species or strain becomes resistant to antibiotic.

↳ multi-drug resistance = multiple antibiotics.

- Where did this come from?

→ We keep using the same ones.

↳ Lack of new drugs — discovery void 1986–now.

↳ particularly b/c of cost to bring to market.

→ We use them too often

↳ Humans and animals

↳ allows for natural selection, spread of AR bacteria.

→ Natural selection ⟶ evolution is fast when selection pressure is high.

→ Blunt instrument — kill both good/bad bacteria, untargeted can also develop resistance.

- How big of a problem is this?

2013 US: 2 million sickened by AR bacteria

↳ 23,000 died; cost in this year ~ $20 million.

CDC has threat level for bacteria — urgent, serious, concerning

(ex) N. gonorrhoea — 246,000 drug-resistant infections.

↳ most resistant to tetracyline

(ex) Vancomycin-resistant enterococcus — 20000 drug-resistant / 66000 infections per year.

☆ Resistance has developed for all classes of antibiotics.

- How does resistance develop?

① Inactivation — enzymes able to reduce/inactivate drug

(e.g.) penicillinase — cleaves the molecule

② Modification — alter target enzyme so molecule (antibiotic) cannot bind.

③ Change in ability to get in / stay in cell.
- (e.g.) impermeability — change in cell wall protein
- (e.g.) Active pumping (efflux of drugs)

How is resistance transferred?

① bacteria multiply; take over new hosts?

② bacteria can pick up a resistance gene.

→ Horizontal gene transfer. Take genes and give them to other bacteria. Sometimes involves bacteriophage.

Human Microbiome Project (HMP)

- microbiome — combined genetic info of microbes from particular environment.
- Learn about bacteria that is not easily cultured
  - → culture = grow bacteria under defined conditions
  - → >99% bacteria cannot be easily cultured; we want to know more
    - ↳ instead, culture them in our body! Isolate total DNA from microbes in/on body. (metagenomics)
      - ↑ study of genes from whole communities
      - → over 1000 microbes from diff. body parts
  - New tools developing for DNA sequence analysis

Results of HMP: 10:1 ratio of bacterial : human cells

→ (e.g.) you have 5000 times more bacteria than # people on Earth

☆ Microbiomes are unique for each person!

- 1 million (human) microbiome genes!
  - ↳ 80% not assigned specific function, 50% no annotation.

BLAST search — find against others in data bank.

Alternatively, biochemical characterization or look at operon.

Idea: manipulate microbiome

7.012
Lecture 28

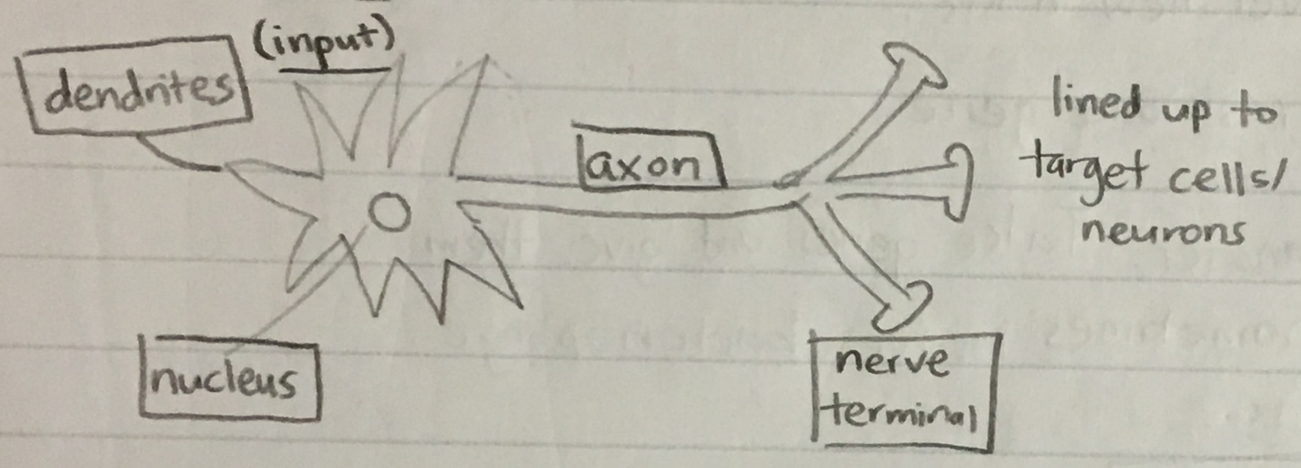
11/21

Neurobiology — the engineering of life

What are neurons?

- Nerve cells ($\sim 10^{12}$ in body)
  → make $\sim 10^3$ connections to other cells (synapses)
  (e.g.) photoreceptor in eye → axon → muscle fiber
- Many types of receptors: light, sound, touch, temperature

Here's one neuron:



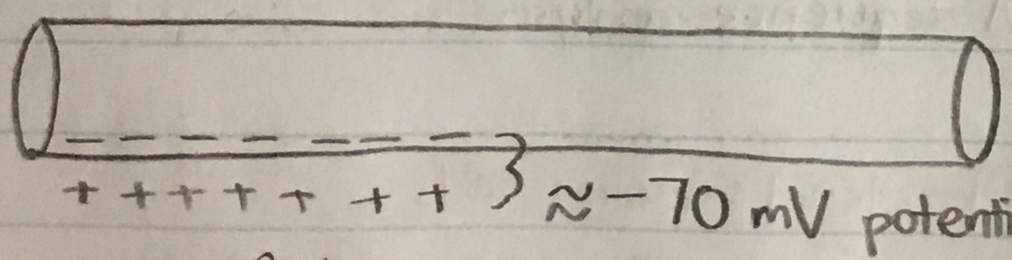
Fact: while the cell body may be ~10 μm, axons can go up to 1 m (brain → spinal cord!)

What are some questions to ask?

- How do receptors transfer signals? How sensitive are they?
- How are signals transmitted along an axon?
- What about across synapses?
- ☆ How do patterns of connections give rise to computations? How do they change for learning? → What about consciousness?

Transmission along an axon

- Take squid axon and cut off the ends — look at electrical properties

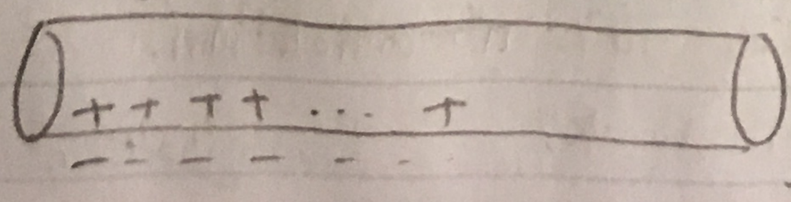


≈ -70 mV potential difference.

But thickness of cell membrane = 3 nm

→ Electric field = $\frac{-70\text{ mV}}{3\text{ nm}}$ = 200000 V/cm

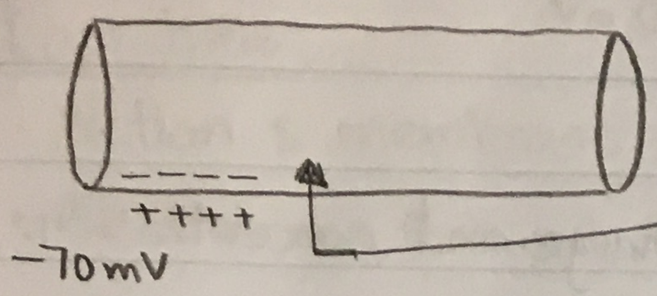
→ This changes when a neuron fires:



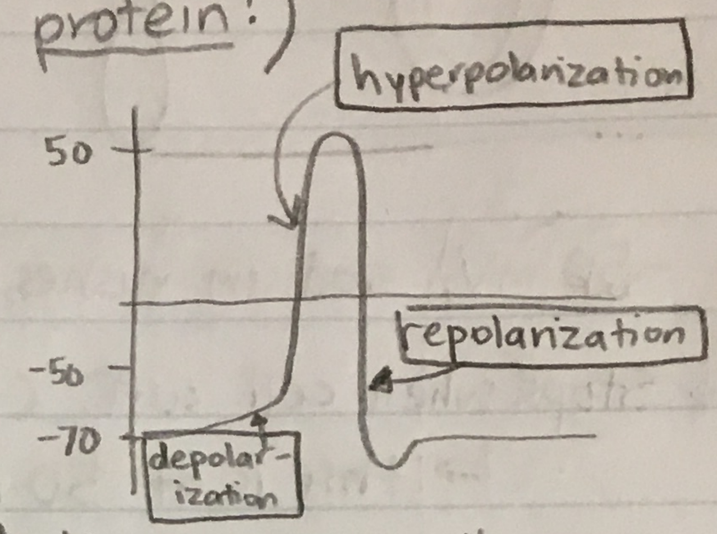
+50 mV.

Large diff. in things w/ dipole moment or charge!

(How to transmit information? Try affecting a protein!)

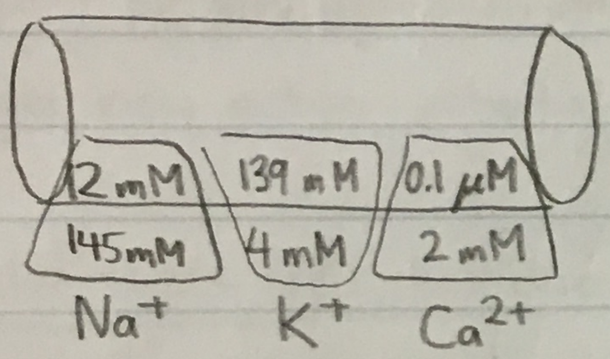


Let's say we gently introduce charge. This happens at −50 mV:



"Action potential"

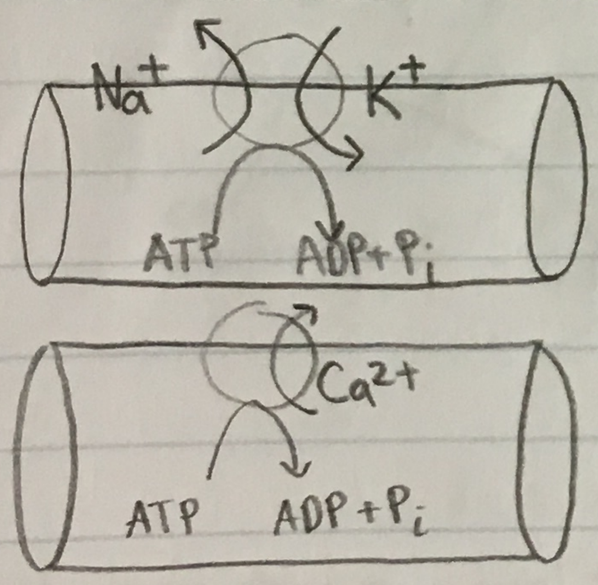
Where do charges come from? Ion concentrations!



Ionic gradients somehow shift!

How?

Let's look at the membrane proteins.

① 

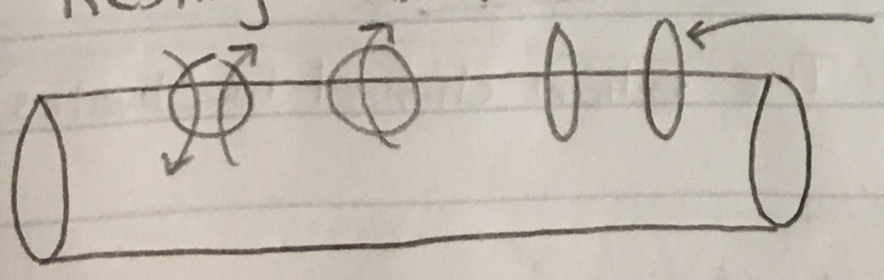
Process requires energy: ATP → ADP + organic phosphate

(Na^+-K^+ ATP-driven pump)

(Ca^{2+} ATP-driven pump)

These are called (obviously) ATP-driven pumps

② Resting Channels



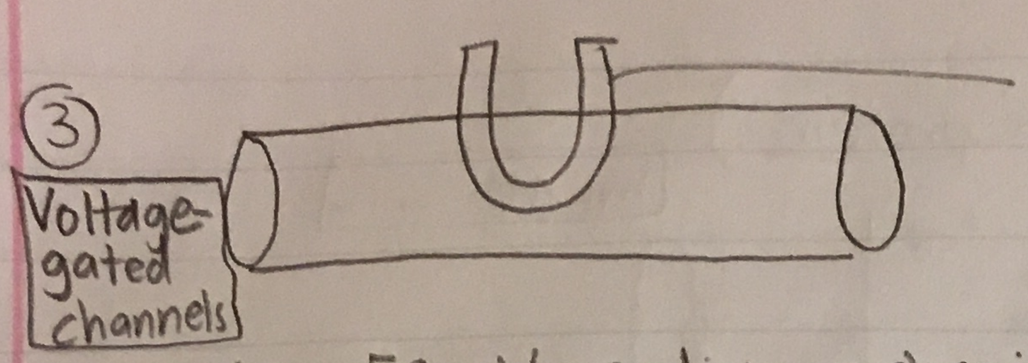
resting K^+ channel; only permeable to Potassium. So general flow back into cell.

Let's say initial membrane potential is 0 V. K^+ flows out, so outside of cell becomes higher potential. Favorable wrt concentration but not charge!

→ concentration / electrical gradients counteract at equilibrium position.

Happens around −70 mV!

Fact: Only 1/100000 of K^+ move, so no veal change in concentrations.



Ion channel for Na^+ closed <u>until</u> cell goes from −70 mV to −50 mV

→ At −50 mV, sodium rushes in. Favorable for both charge and concentration.

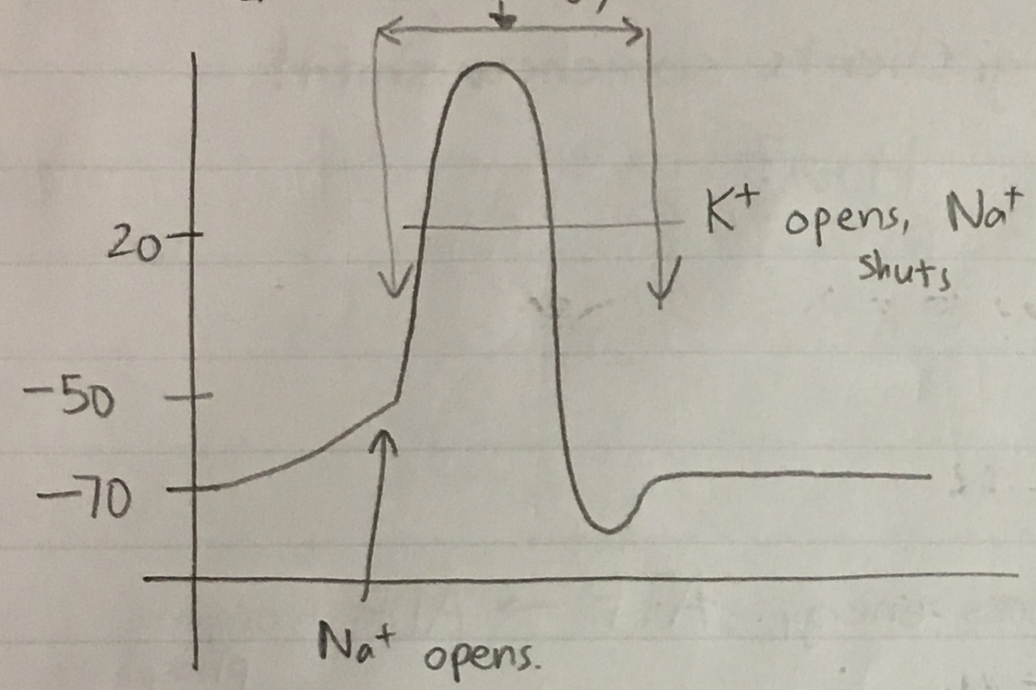
↳ Stops when cell suff. (+) that it's electrically unfavorable

↳ this is at 50 mV!

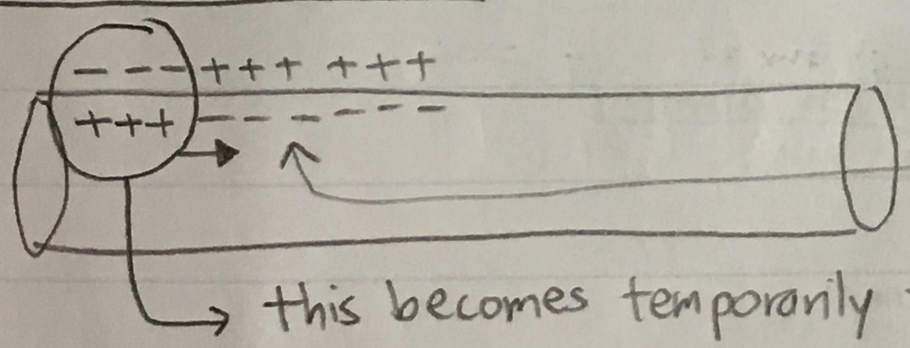
<u>Now what?</u> Around ~20 mV, K^+ voltage-gated channel opens; K^+ rushes out.

→ Na^+ shuts, remains inactive for ~1 ms.

So in 1 millisecond,



<u>So how is this transmitted?</u>



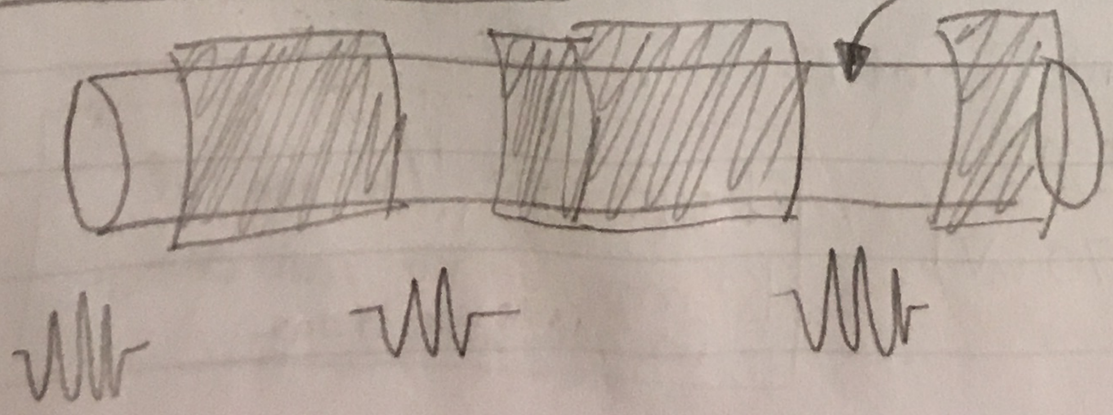
→ this becomes temporarily +50 mV. That moves this, which gets the charge up on the next patch. → <u>action potential!</u>

<u>Question</u> - why doesn't this happen in reverse? The sodium channel inactivates!

→ <u>Unidirectional propagation.</u>

Often axon has an insulator → <u>myelin</u> wraps itself around.

<u>faster propagation!</u>



Transmission leaps ~100 times faster!

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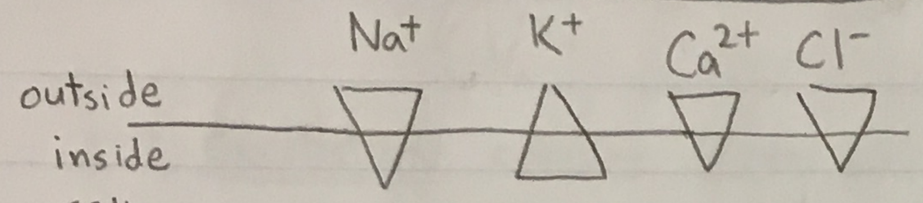
Lecture 29

11/26

Last time:

- Action & membrane potential
- To balance this out, ionic concentration gradients

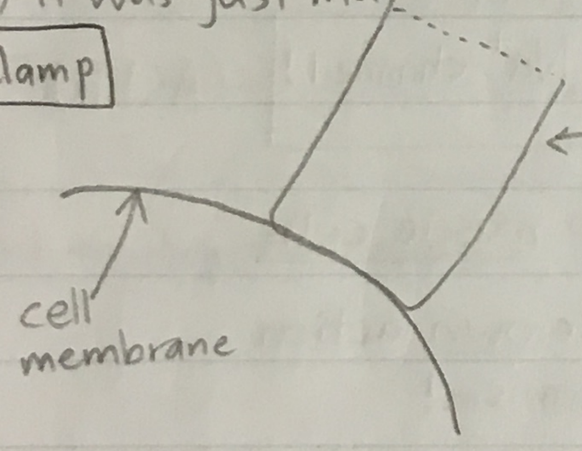


- Resting K^+ channel
- To start action potential, take voltage-gated Na^+, K^+ channels
- Trigger new action potential by dragging charge.

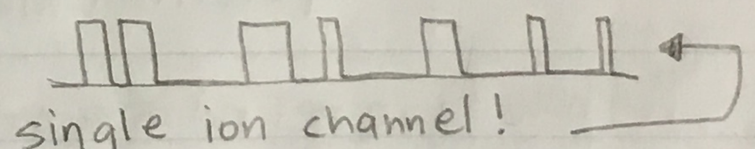
How did these first get discovered?

At first, it was just math, but... what's a way to actually observe the channels?

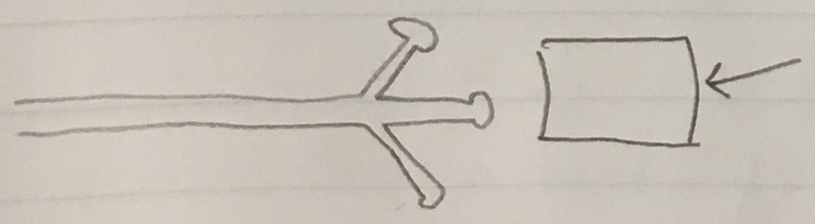
Patch clamp

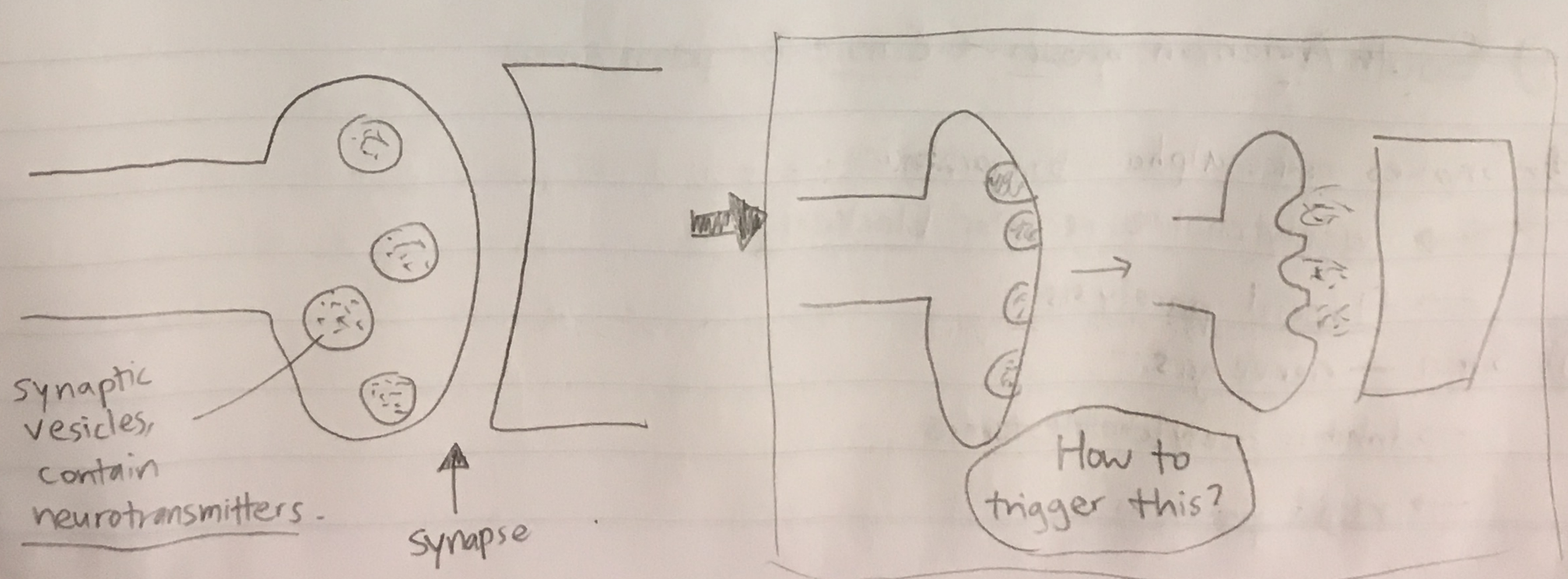


★ rip it off — see current through this piece.



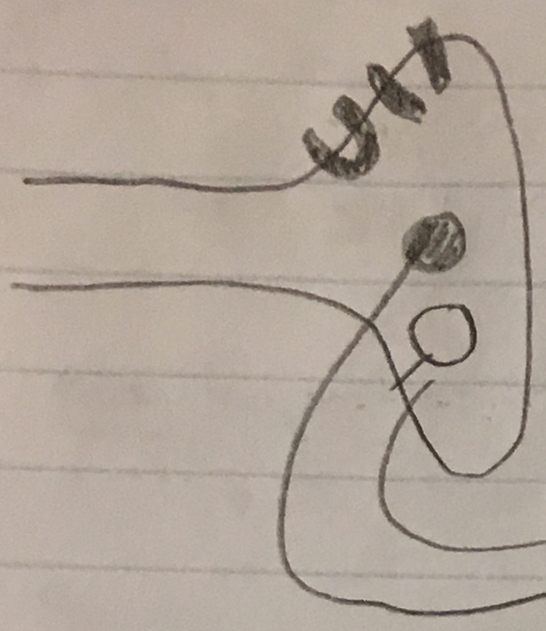
Next, how do we signal to the next cell?





- Use a voltage-gated channel.

At +50 mV:



★ Ca^{2+} has 2000x higher concentration on the outside.

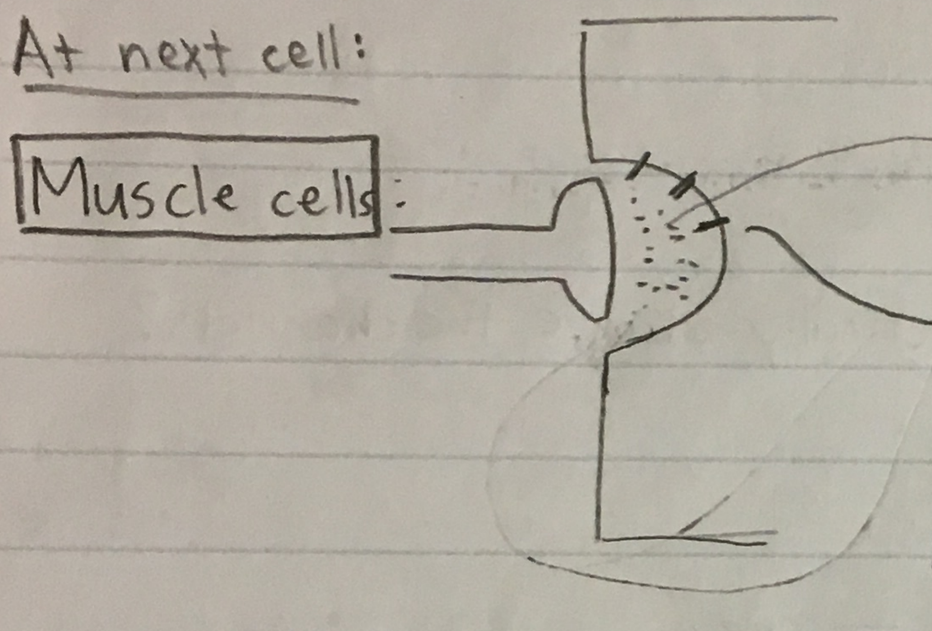
→ this vesicle is tethered by synapsin

→ here's a Ca^{2+}-dependent protein kinase

once Ca^{2+} binds, that tether is let free!

At next cell:

Muscle cells:



the neurotransmitter is acetylcholine (ACh).

→ cell has ACh receptors.

→ ligand-gated Na^+ channel! (AChR)
(ACh)

So Na^+ flows into muscle cells.

→ these have their own action potential mechanism!

But how do we get rid of a molecule?

Break it down w/ enzyme acetylcholinesterase.
(AChE)

What about toxins?

(e.g) Puffer fish → tetrodotoxin.

→ blocks voltage-gated Na^+ channels.

(e.g) South American groups - Curare for poison arrows

(eg) Snakes make alpha-bungarotoxin

→ acetylcholine receptor blockers

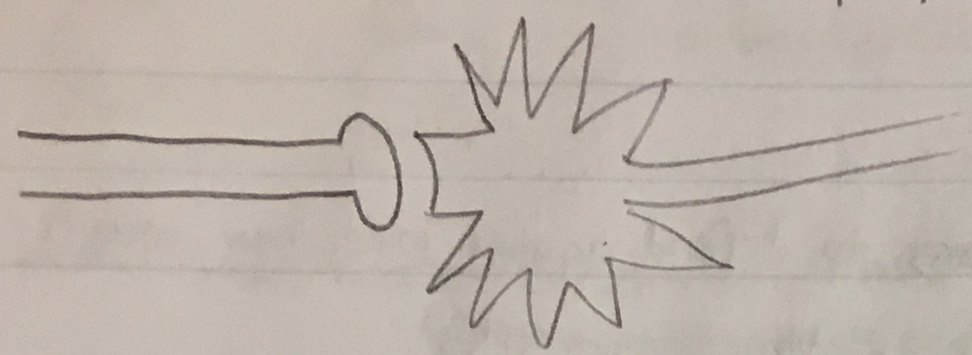
→ flaccid paralysis

(eg) Sarin — nerve gas.

→ Inhibits acetylcholinesterase

→ rigid paralysis

What about neuron–neuron synapses?



Different neurotransmitters this time.

→ <u>excitatory</u> causes V to go up, <u>inhibitory</u> causes V to go down.

↓ e.g. glutamate (Na^+ flows in)

↓ other chemicals (Cl^- flows in)

→ Causes V to change by ±3 mV, but there's no action potential mechanism in the dendrites.

→ Instead, our goal is to shift axon to -50.

→ Tree of dendrites is integrated

↳ charge changes in some way. Analog computer of thousands of signals!

Hard to describe even 1 dendritic tree.

7.012
Lecture 30

11/28

- Exam 4 on 12/3 (Monday)
- P-set 7 due next Friday

Immune system — network of cells, tissues, & substances to defend against infectious agents
→ pathogenic bacteria — cause disease, viruses, parasites.

- Goals: ① keep out, ② destroy, ③ neutralize, (④ coexist)

Lines of defense

in order

- Skin and mucosal barrier
  → both physical and chemical
  → (eg.) stomach acid, enzyme (lysozyme) in bodily fluids

- Innate immunity — cells that initiate early, generalized response.

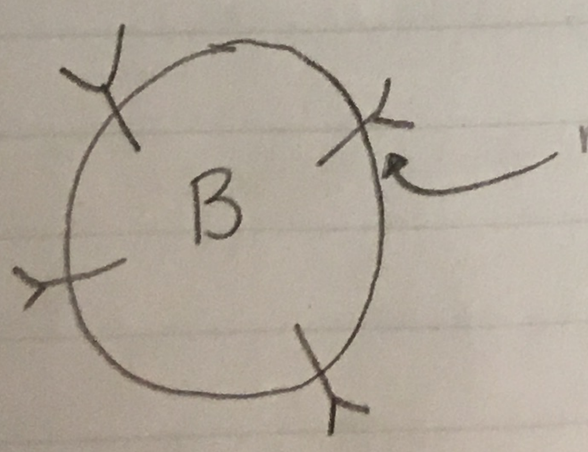
- Adaptive immunity — cells that initiate stronger and more specific response
  → Humoral response — consists of soluble substance → **B cells**
  → Cellular response — T cells

★ Bone marrow contains hematopoietic stem cells
  ↳ turn into all kinds of blood cells!

main focus

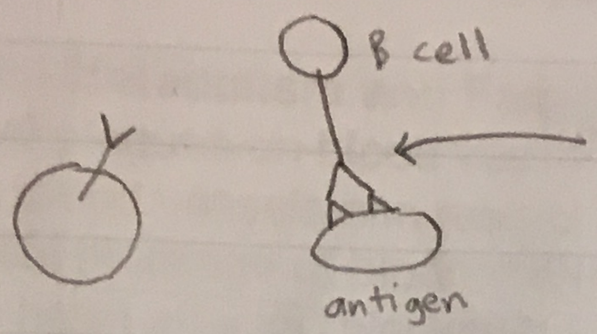
B cells

- make antibodies that bind to antigens. ← foreign substance



Each B cell makes one type of antigen.

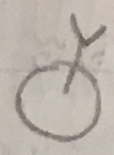
- Blood cells have lymph nodes — where cells meet to activate.



activation when antibody binds to a bacterial protein

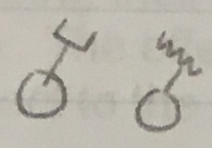
→ Make more B cells of this type (division, proliferation =

↳ lots of ER, secrete soluble antibodies



differentiate into **plasma cells**.

↳ or into **memory cells** stays for years in bone marrow

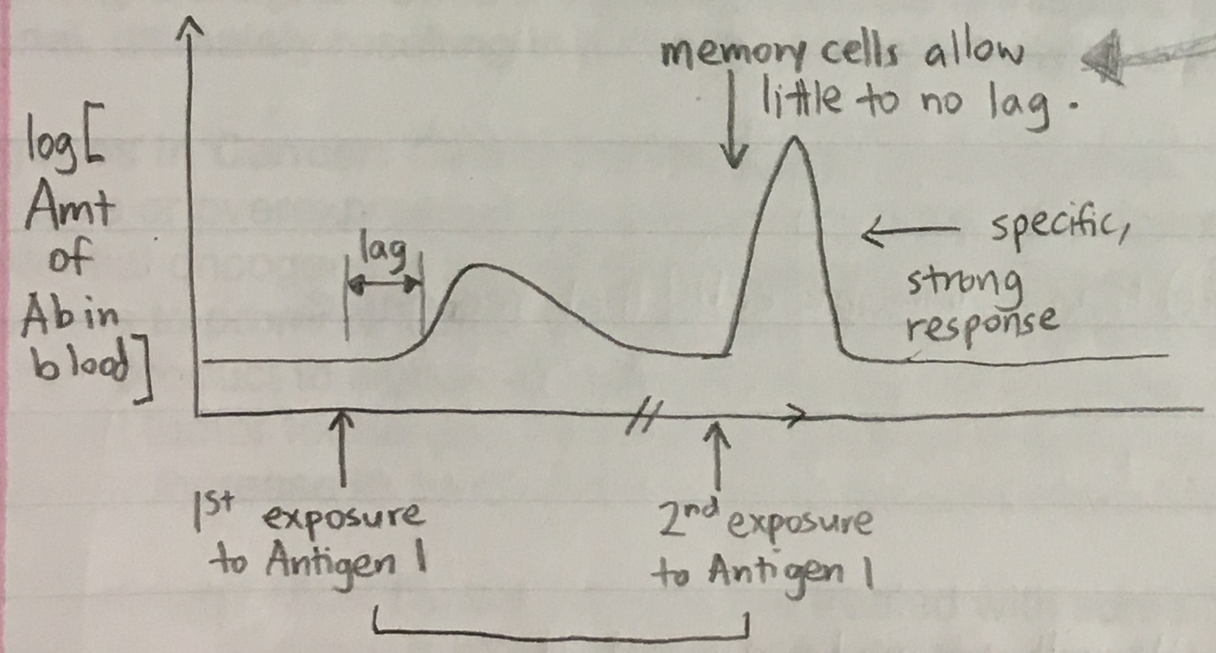
→ Other types  called naive B cells.

What happens when antibody (Ab) binds?

→ Bacteria can't bind, so target is neutralized

→ Alerts phagocytic cells to engulf bacteria

→ Macrophage recognizes antibody.

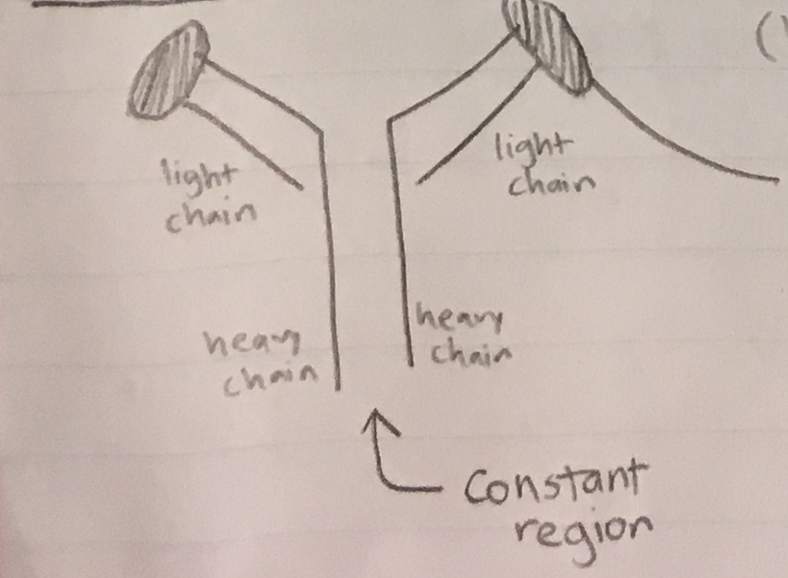


this is why we use vaccines — injecting dead or attenuated virus/protein to get memory cells.

* Why flu vaccine every year?

→ it mutates!

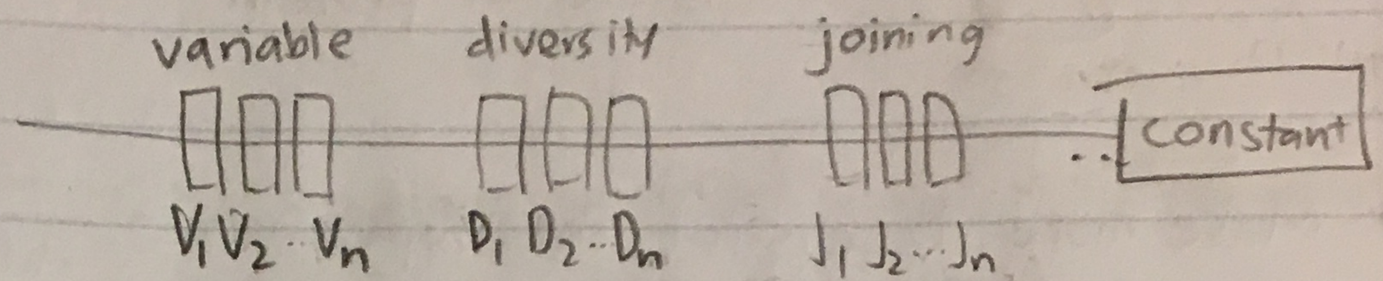
Structure of antibody — 2 heavy, 2 light chains (heterotetramer)



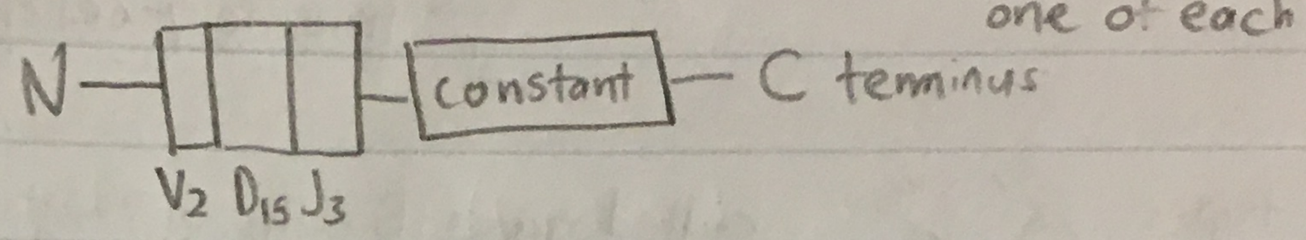
this is where antigen binds: variable region

Antibody diversity — only 20000 genes? How to get mill

(e.g) heavy chain locus (in genome)



① During development, DNA rearrangement — bring together one of each



★ similar happens w/ light chain (VJ)

② Also junctional diversity. — sequence variations introduced.

③ Somatic hypermutation — enzyme does more mutation

→ $\underline{10^{14}}$ combinations — recognize molecule of <u>any</u> type.

What about self vs non-self?

- During early development, <u>clonal selection</u> - immune cells that recognize self are eliminated.

In research and medicine

- Inject any protein into organism (rabbit or mice) → T cells will be made against it.
  - → collect blood serum, contains polyclonal antibodies
    - ↳ monoclonal antibodies from B cells fused to immortalized B cell = hybridoma

7.012

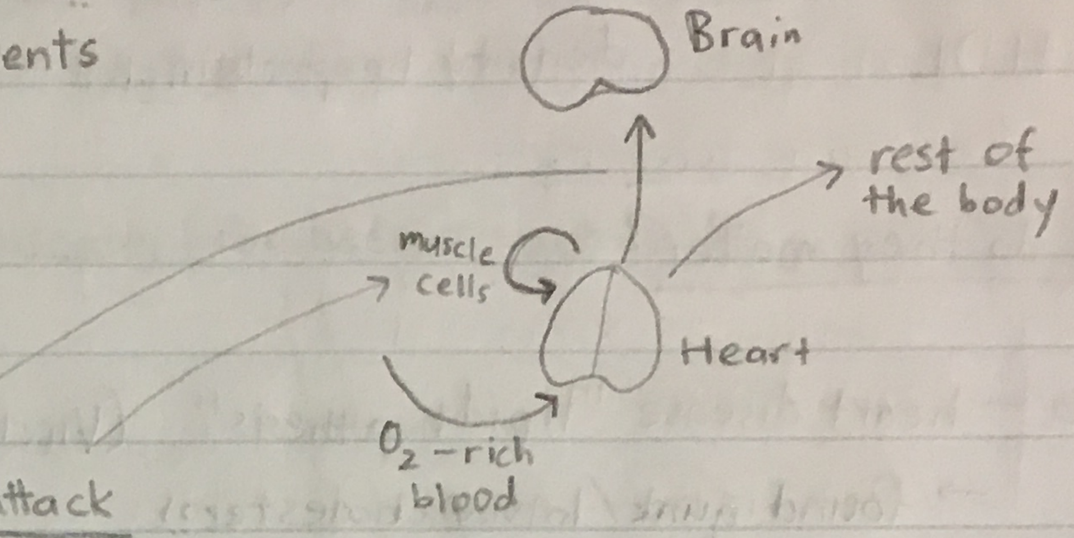
Lecture 31

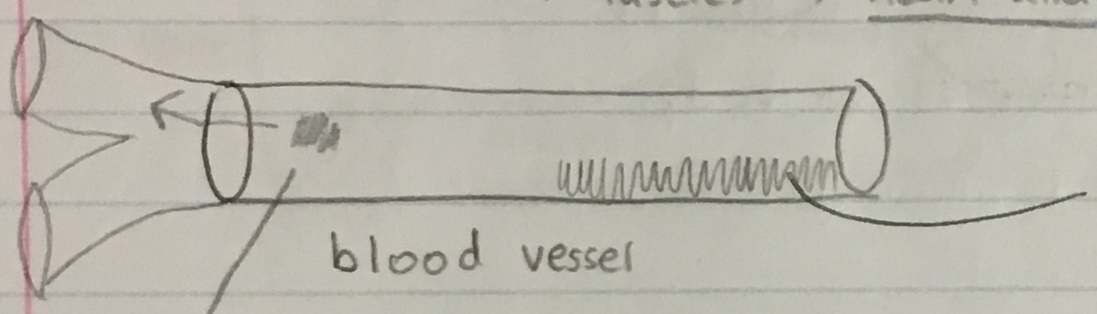
11/30

Title: Rational Medicine: Familial Hypercholesterolemia

Heart Disease

- Purpose of heart: to pump blood
  - → Provides O_2, hormones, nutrients
  - → Pumps red/white blood cells
  - → Pumps away waste
- If blood vessels are blocked, bad.
  - → @ brain → called a stroke
  - → If @ heart muscles → heart attack

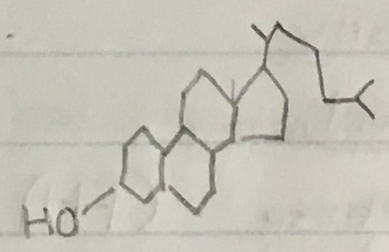




Atherosclerotic plaque:
contains cholesterol / proteins / lipids

→ bad if chunk breaks off and clogs a smaller vessel (or if main one clogs)

What is cholesterol?



- Hydrophobic molecule
- Often attached to phospholipid tail
  - → cholesterol ester (CE)
- Purpose:
  - → Makes up half of cell membrane!
    - ↳ Provides fluidity
  - → Biochemical precursor
    - → Steroid hormone
    - → Vitamin D
    - → Bile acids – emulsify fats
- Source:
  - → Partially from diet.
  - → Also synthesis in body!

general pathway:

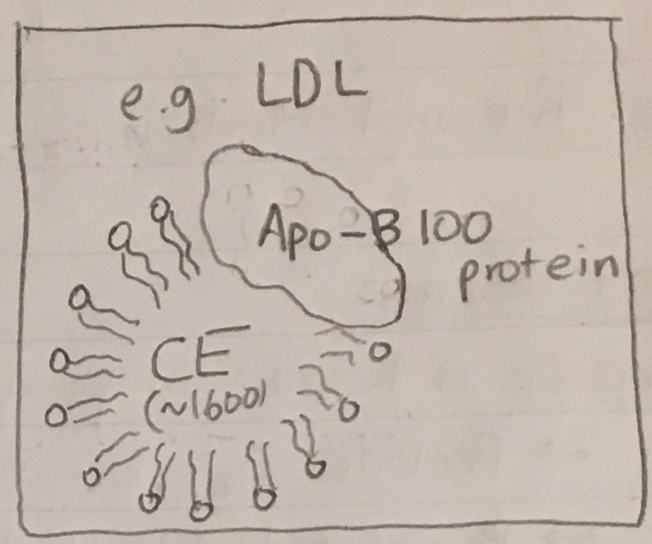
acetic acid

$CH_3COOH \longrightarrow$ [HMGCoA → Mevalonate] → Cholesterol

HMGCoA Reductase
(first committed step, regulation point)

Lipoprotein particles

VLDL
LDL
IDL → high, intermediate, low, very low density lipoprotein particles
HDL



transport system

Why do they matter?

1856 – heart disease "lipid hypothesis" (Virchow)
→ found gunk/lots of cholesterol
1913 – fed rabbits high cholesterol
→ symptoms ~ atherosclerosis
1950s – LDL vs HDL tracked
↓ correlated w/ risk of heart attack
↓ negatively correlated!

Genetics of Cholesterol Levels

Familial hyper (lots of) cholesterol emia (in blood), (FH)

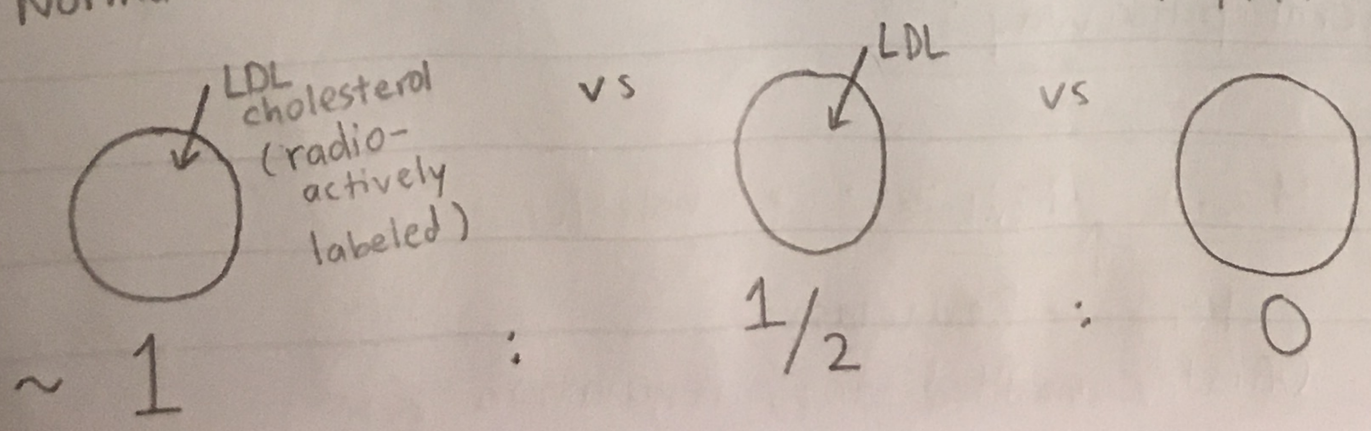
Look at	normal (+/+)	heterozygotes (FH/+)	homozygotes (FH/FH)
LDL	100 mg/dL	250 mg/dL	>600 mg/dL
Age of heart attacks	normal age	10–20 years earlier	teenagers

1 in a million people

so allele freq. is 1/1000
→ freq of heterozygotes = 1/500.

Fibroblasts:
Look at skin cells in petri plate.

Normal cells vs FH/+ vs FH/FH

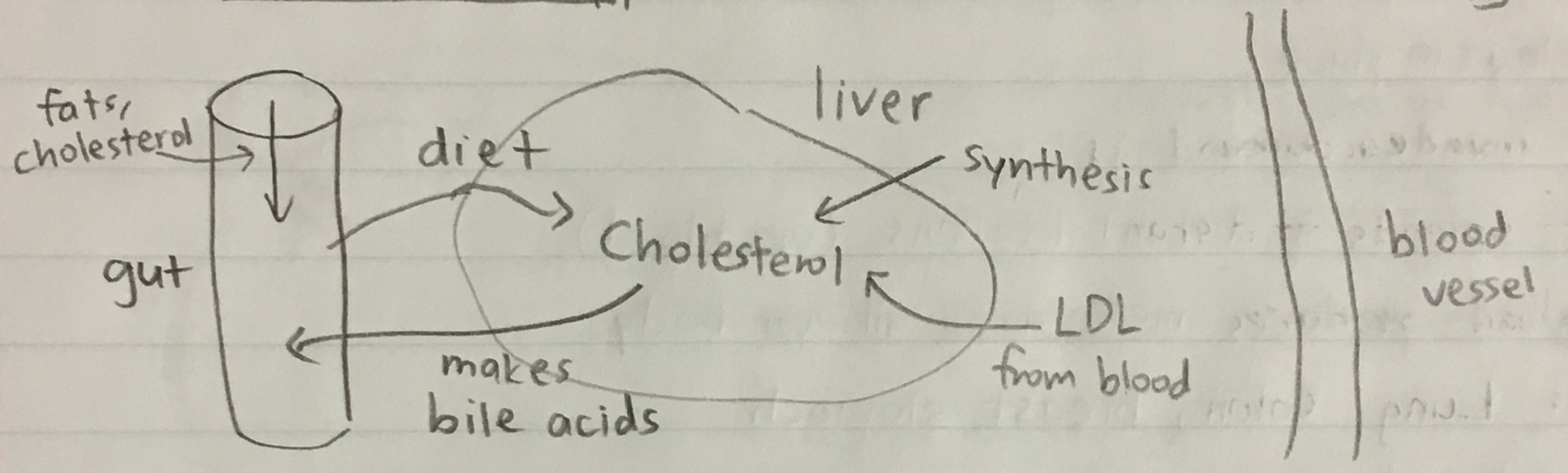


there exists
↓
Hypothesis: ∃ LDL receptor. Helps take up LDL (e.g. liver)!

Biochemical Regulation

If LDL levels are down: → make more HMGCoA reductase
→ more LDL Receptors.

→ So rational therapy for heart disease (for heterozygotes):



Strategy 1 Eat less cholesterol.
→ Has some effect: LDL ↓ ~10%

Strategy 2 Try to deplete cholesterol.
→ Bile acid binding resin → forces body to make more bile acid
→ Still only ↓ 20%

Strategy 3 HMGCoA reductase inhibitor → called statins
→ Decreased LDL by 60%!
→ Taken by 35 million people.

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Lecture 32

11/5

Title: Rational Medicine: Cancer Targeted Therapy

(By the way, exam 4 posted, p-set due Friday)

What is cancer?

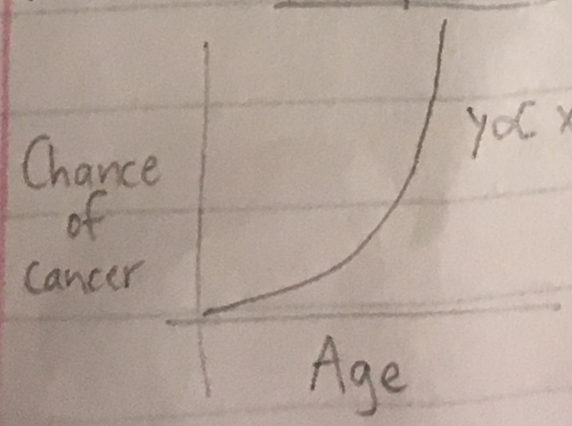
- Cells that grow and divide <u>withaut limit</u>
  - ↳ create tumors
  - → "Neoplasm" = name for abnormal growth of tissue
    - → "Benign" – stays in place
    - → "Malignant" – invades surroundings
    - → "Metastatic" – spreads to distant locations (via blood)
      - ↳ this is bad: produces <u>metastases</u> all over body.
- <u>Common cancers</u>: Lung, Colon, breast, stomach
  - ↳ also skin, prostate, ovarian, etc.
  - → ~1.2 million new cases per year; half cause death.
    - ↳ account for ~1/4 of all deaths
- <u>Cancer grows exponentially</u>
  - ↳ first time you can see on X-ray: 10^8 cells
  - → Palpable: 10^9 cells
  - → Death ~ 10^{12} cells

~1/2 the time it took to get to first diagnosis

Why does cancer happen?

- Mutations.
  - → 10^{16} cells, $3 \cdot 10^9$ bases in genome, 10^{-8} mut/base
  - ↳ 30 mutations / base
- Increase chance by UV (sunlight), smoking, charred beef, working at Chernoby!
  (skin) (lung)

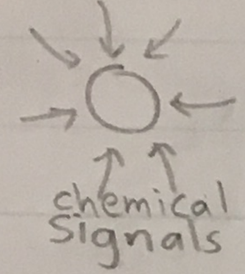
★ Takes <u>multiple mutations</u> to become cancer cell.



↳ <u>Also</u>, can inherit alleles that are likely to lead to cancer

Why don't all cells grow like this?

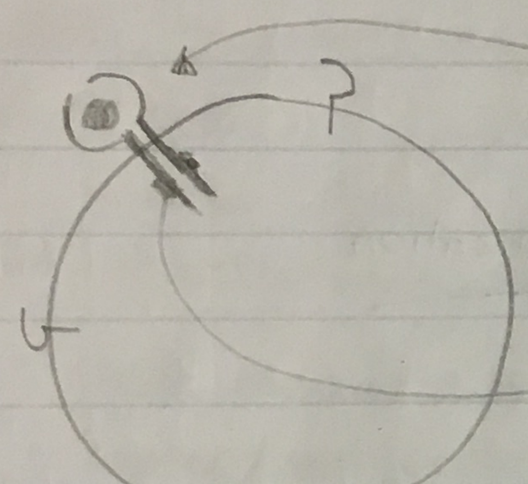
Regulation



growth factors tell a cell to grow

(EGF, FGF, NGF, PDGF, etc.)

How does a cell detect these? Receptors!



presence of growth factor makes it favorable to dimerize.

↳ they're close to each other inside the cell, too.

→ enzyme activity:

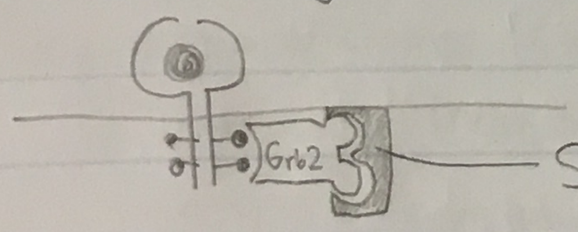
protein kinase phosphorylates Tyr residues.

↓

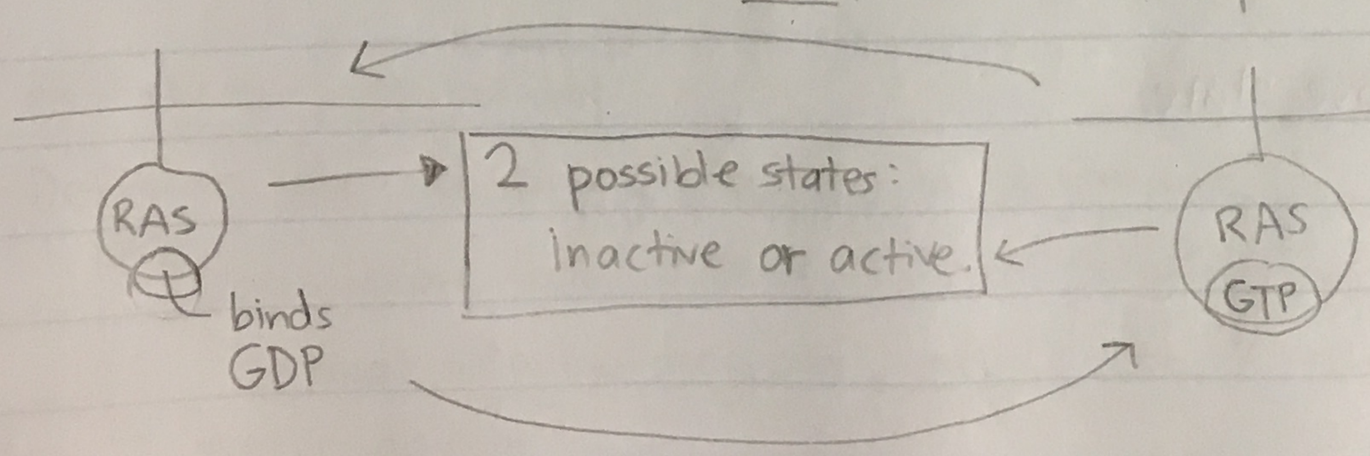
they can phosphorylate each other!

→ Phosphate groups change shape of protein.

↳ changes the binding patterns — adapter protein Grb2.



Sos protein → then binds to / affects RAS, a membrane protein.



RAS is a GTP-ase: catalyzes reaction to make itself inactive.

☆ There are proteins that can speed up GDP → GTP.

↳ GEF (guanine exchange factor); e.g. Sos!

(Also GAPs: GTPase activating proteins competing)

So active RAS undergoes conformational changes

↳ binds to RAF, which becomes phosphorylated.

→ RAF also protein kinase → phosphorylates MEK.

↳ MEK activates ERK, which phosphorylates transcription factors, other things

↳ cell growth!

☆ Reason for this: amplification.

So what mutations cause cancer?

- Dimer stays together w/o GF (growth factor) — constitutive dimerization
- Cell produces too much GF

☐ Knocking out RAS or Sos won't work

☆ But we could have RAS stuck in active state. Very common!

↳ unable to hydrolyze

- Kinases become constitutively active

How to cure?

- Inhibitors for enzymes that are always on. (e.g. RAF)
- Why not inhibitor for RAS?

↳ It would be stuck in active state.

7.012

Lecture 33

12/7

- Final is on Wednesday 12/19 at 9am
- 5.302 – Intro to Experimental Chemistry during IAP!

Title: Using Biology to Save the Planet

Superbugs – today, helping save the planet

What does the planet need to be saved by?

- Climate change / greenhouse gases
  - Risk by 2040
  - ~100000x weight of Empire State in warming emissions
    - Oil in Alaska → spills
    - Water / soil contamination

Microbes can "eat" / metabolize greenhouse gases!

- Also hydrocarbons from crude oil (e.g. toluene)
- Hazardous waste (e.g. perchloroethylene = PCEs) → manmade dry-cleaner solution.

- Key idea – microbes / their enzymes replace chemicals in industrial processes / replace energy-consuming processes!

Let's review **metabolism**.

- Defn – life-sustaining processes in organisms.
  - (eg) conversion: food → energy to run cellular processes
  - Also made into building blocks of life
  - Waste disposal.

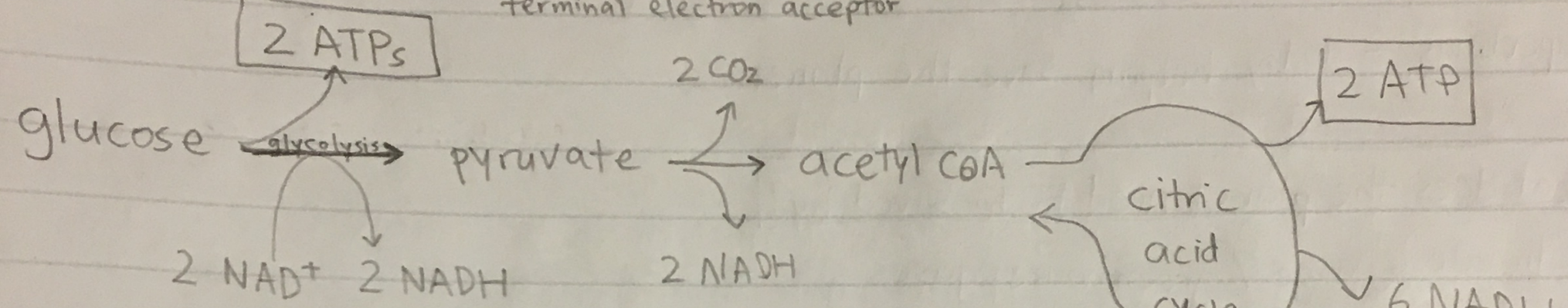
2 types:

Catabolic = breaking down (to make energy),

Anabolic = synthesis, makes energy

Review: Metabolism of glucose with O_2 to make ATP

- Glucose has stored energy.
  - ↳ Redox reaction: O_2 reduced, glucose oxidized
    - O_2 ↑ terminal electron acceptor

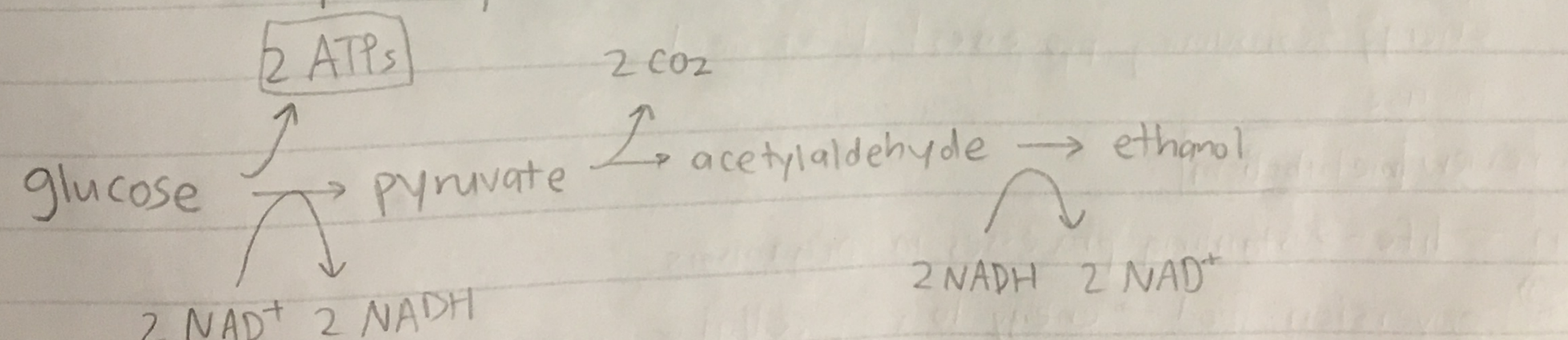


- There is a charged gradient!
- What if there is no mitochondria?
  - ↳ use cell membrane!

⟹ electron transport chain w/ proton gradient make ATP (×28), $O_2 \rightarrow H_2O$.

What if we don't have O_2 as terminal electron acceptor?

- Fermentation to ethanol
  - ↳ use acetylaldehyde.



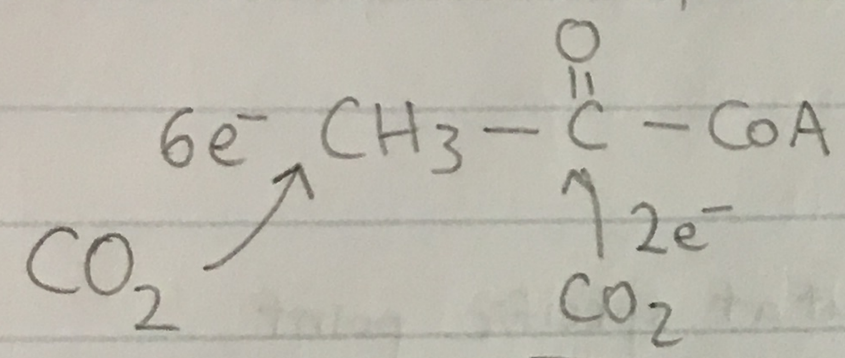
Net of 2 ATPs instead of 32!

Remember: NADH is an electron carrier!

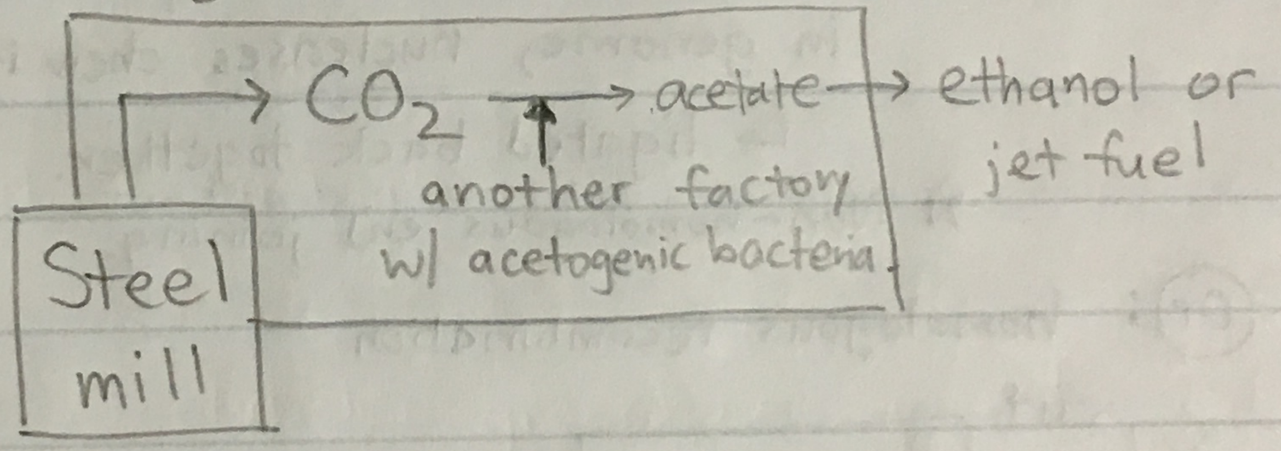
* Glucose also source of carbon!
  → stop at acetyl CoA, make fats instead
  → Can also make nucleic acids, etc.

Absence of glucose + O_2

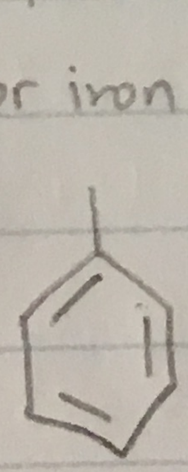
- Acetogenic bacteria (e.g. M. thermoacetica)
  → oxidize H_2 gas; use CO_2 as terminal electron acceptor.
→ Make acetate or acetyl CoA (~10^{11} tons removed / year)!



- LanzaTech



- Anaerobic hydrocarbon metabolizing bacteria (e.g. T aromatica)
  → oxidizes hydrocarbons. (e.g. toluene)
  ↳ use NO_3^- or iron or sulfur as terminal electron acceptors.



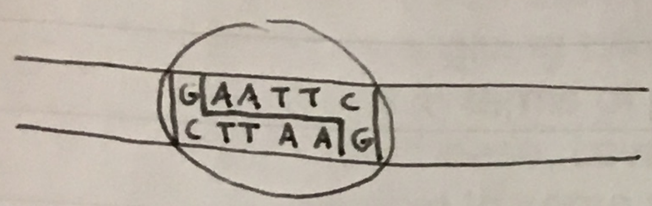
← how to metabolize this?
"unprecedented C-C metabolism"?

7.012

Lecture 34

Today and Wednesday: Science in Society

Recall: Restriction Enzymes

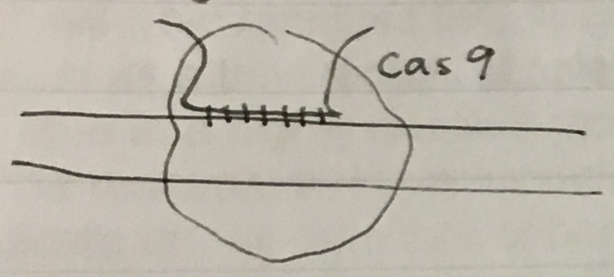


- EcoRI – cuts specific sequence
- come from bacteria
- defend against viruses

→ To protect itself, methylation.

Recall: CRISPR is a programmable restriction enzyme!

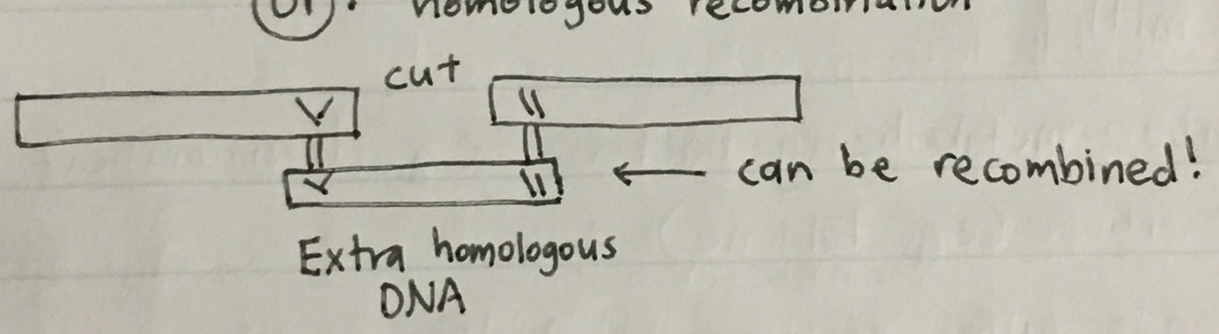
- Uses guide RNA to cut @ ~20 bases.



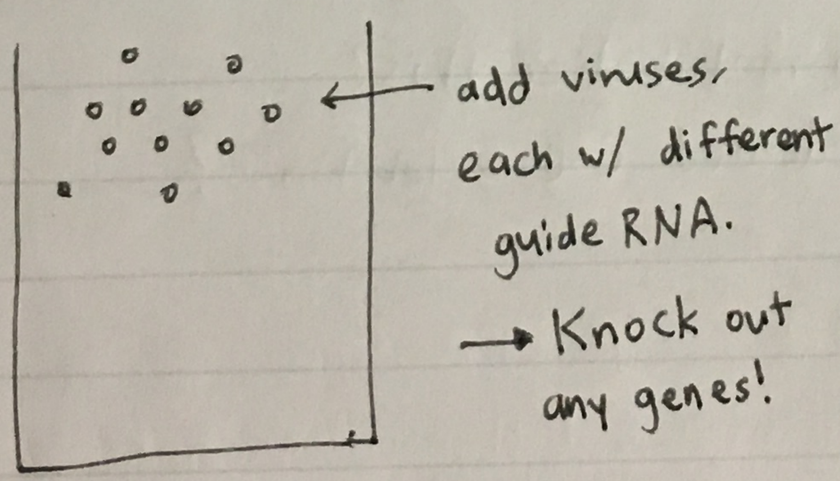
- Useful – if cut at specific point in genome, nucleases chew it back
  ↳ ligated back together.

★ Non-homologous end joining.

(Or): homologous recombination



★ This allows us to add any sequence we want!



Medical applications?

① Somatic gene editing

e.g. Dominant Retinitis Pigmentosa

→ if chew back is a multiple of 3 bases, won't knock out the gene.

→ But could we have an off-target cut? Cancer? etc

② Germline editing — done to fertilized embryo.

e.g. ~~2%~~ of Europeans have a cystic fibrosis allele
≈4%

↳ ~8% of pregnancies?

e.g. norovirus has receptor. If you're mutant for Fut2, you don't get it.

↳ Is this OK? does it do anything else?

→ increases risk of inflammatory bowel disease

7.012
Lecture 35

- Recommendation @ CRISPR babies.

Facts – what happened?

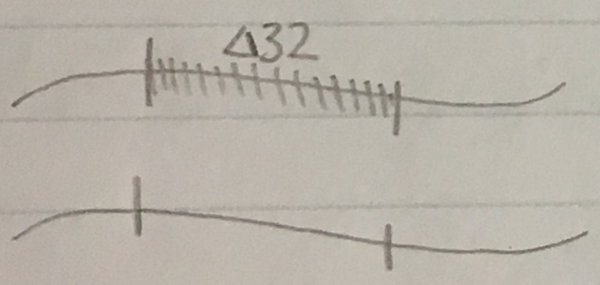
- Jiankui He – took fertilized eggs, tried to edit CCR5 gene.
  - ↳ Lulu & Nana, 2 twin girls – result of reimplanted embryo
- CCR5 – relevant to immune system (cell surface protein). HIV can use as receptor.
  - ↳ Some have Δ32 mutation (frequent in pop.)
    - Fact) HIV can't use homozygote mutatied CCR5, so it's pretty strong. Not absolute protection though
- HIV can transmit w/ father's sperm, but you can wash it
  - ↳ if mother has HIV, there are also ways to drive down HIV infection.

→ So why?

- ↳ Other effects of this gene deletion? Enhancement of some kind?
  - → Lack of CCR5 increases severity of inflammation in brain.

→ Better genes could have been chosen

How well did he do, anyway?



Lulu – heterozygote
wild type / 15 base deletion

Nana – heterozygote
1 base / 4 base deletion

This doesn't even give protection against HIV. Also 15 bp is in frame!

→ Parents were not properly informed (medical jargon)

This is a +1 instead of a -1 frameshift. No way to know effect.

- Done out of own pocket, unclear @ IRB

Another gene: PCSK9. Degrades LDL receptor

↳ Mutations in this gene are present @ Afr.-Am. population

ΔPCSK9 – homozygote → very low LDL levels

⟶ ∃ antibodies to deplete PCSK9 gene.

- Jiankui He has also made deletions in this gene. Also enhancement

Broad societal consensus?

The thing about 7.012 – gets us to the frontier of biology!