Likely q's on SA BIO206 test

① **Properties of genetic code?** - Non overlapping - Successive triplets read in order (each nt only part of one codon)
- Comma-free
- Degenerate - some a.a's have multiple codons,
- triplet - 3nt code for 1 a.a.
- unambiguous - every codon specifies only 1 particular a.a (or Stop/start codon)
- nearly universal - only diff's in Mitochondria and some protozoans genetic codes,

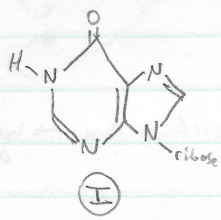
Genetic Code
↳ translates nucleic acid sequence of mRNA into a.a's of proteins.

ATG > AAG (M > K)
wild codon | mutant codon | a.a. change

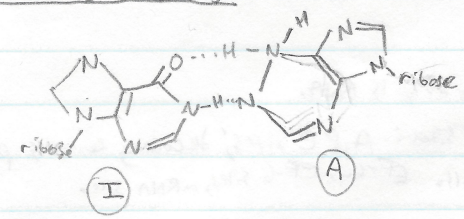
(M>K)

② **Degenerate code minimizes mutation effects?**
- point mutation can cause aa substitution - ex - Try, Met → only 1 codon, since Try = biggest, Met = Start codon, So most deleterious aa substituted accidentally.
- Codon: 1st a.a → Similar amino acids coded
- 2nd a.a → purine → purine = Mostly polar a.a's; pyrimidine → pyrimidine = mostly hydrophobic a.a's; if purine → pyrimidine then will get big change.
- 3rd a.a → degeneracy - even w/ change many still code for same amino acids.
- mutations often "silent"

③ **Draw inosine, draw non complimentary base pairing?**



I

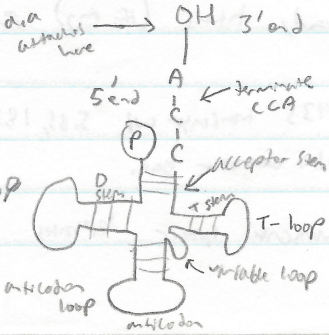


I A

④ **3 types of RNA**
3 req'd for protein synthesis:
- ~~mRNA~~ → codes for proteins
- rRNA → structures in ribosomes
- tRNA → carry a.a from cytoplasm → ribosome bound to polypeptide.
- Small RNA → pre mRNA splicing,

1st base in anticodon = wobble.
1, 2 = watson/crick
3 = wobble (sometimes)

⑤ **tRNA: Draw it, describe it**



(iso-accepting tRNAs)
↳ bind to multiple codons w/ wobble base pairing.
20 a.a's → 61 codons → 31 tRNAs → 20 aaRS (one per a.a.)
↳ degenerate
3 Start/Stop
↳ UAG - amber
↳ UAA - ochre
↳ UGA - opal

- has modified bases in it,

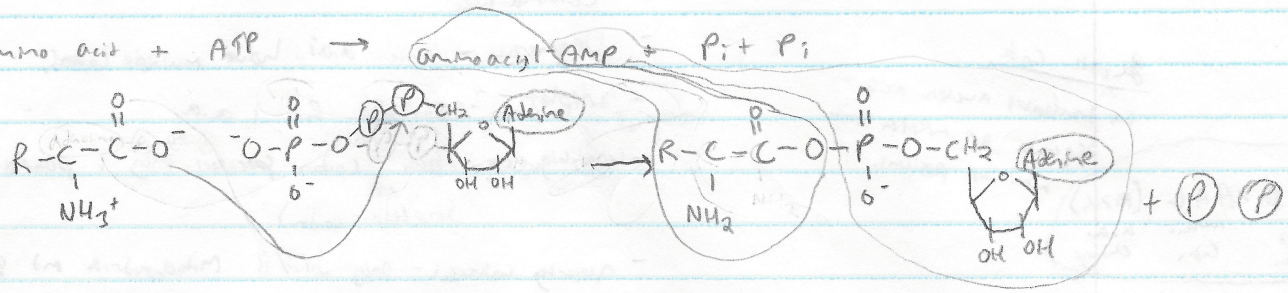
⑥ aaRS charging rxn...

↳ 2 classes... Class I → aminoacylates at the 2'-OH
II → " " 3'-OH

* reversible, Anticodon is only link to mRNA sequence.

2 steps

1) amino acid + ATP → aminoacyl-AMP + P_i + P_i (from aminoacyl synthetase)

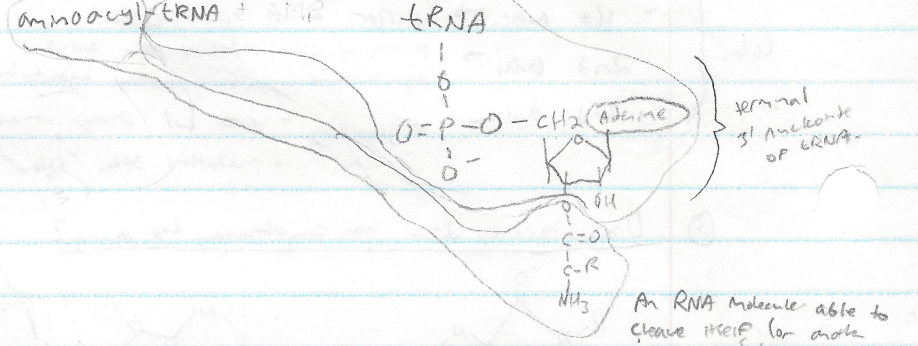


2 step rxn catalyzed by aminoacyl-tRNA Synthase...

+ tRNA

+ AMP

2) aminoacyl-AMP + tRNA → aminoacyl-tRNA (charging tRNA)



Ribozymes - 23S, 28S, snRNA

An RNA molecule able to cleave itself (or another substrate) w/o a protein. ↑ Catalytic RNA at active sites.

⑦ Ribosomes - 2/3 mass + 85% is RNA.

- 5 active sites: A, P, E sites; decoding centre; peptidyl transferase centre → have at active sites.

* also EF-Tu, EF-G sites, mRNA site.

Prokaryote	Eukaryote
- 70S ribosome	- 80S ribosome
- 30S ribosome subunit	- 40S ribosome subunit → matches tRNA to codons
↳ 16S, 21 proteins	↳ 18S, 33 proteins
	* Cavity btw subunits fits 2 tRNAs.
- 50S ribosome subunit	- 60S ribosome subunit → Catalyzes peptide bond formation (23S, 28S)
↳ 5S, 23S, 34 proteins	↳ 5S, 5.8S, 28S, 49 proteins

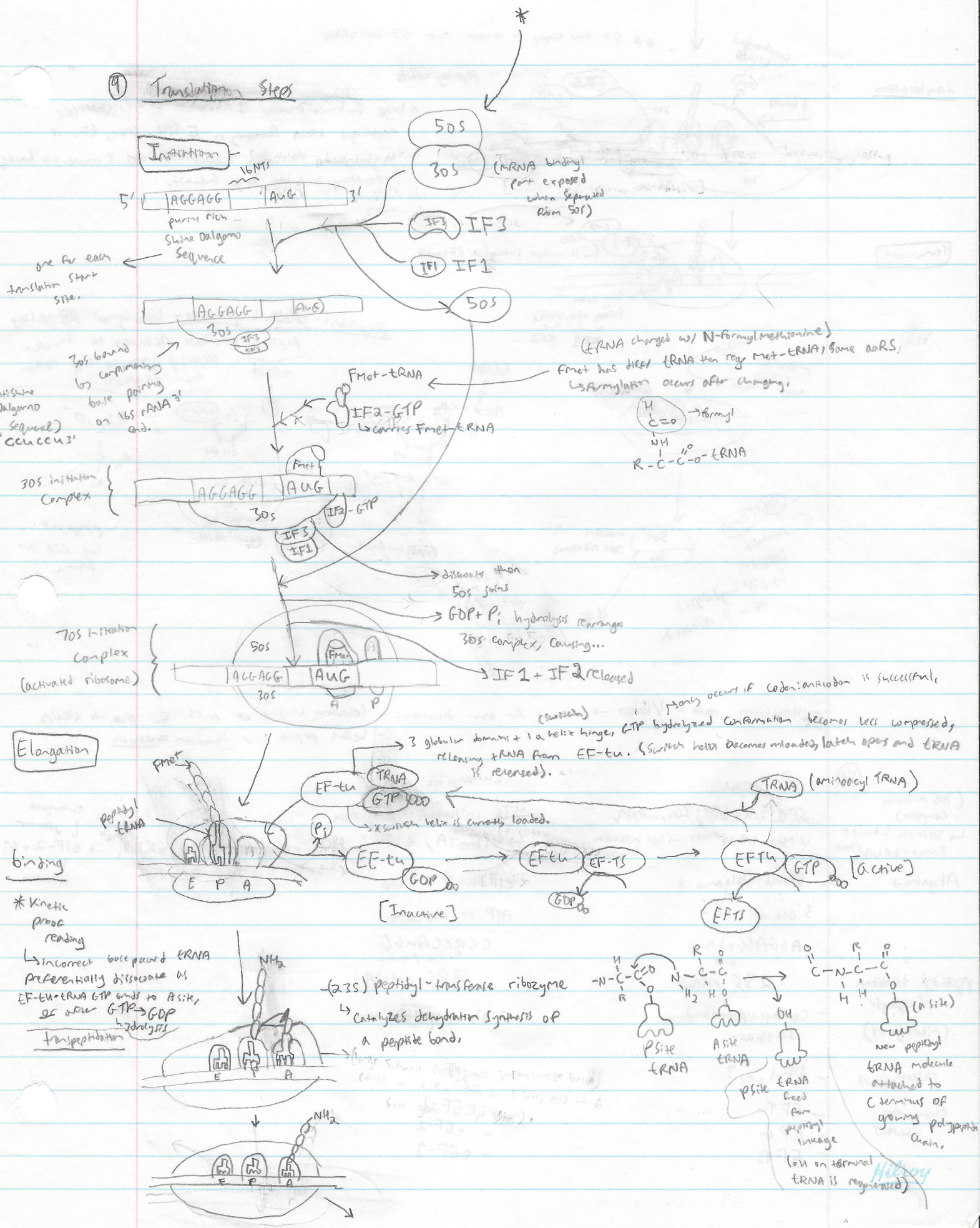
⑧ tRNA, rRNA processing?

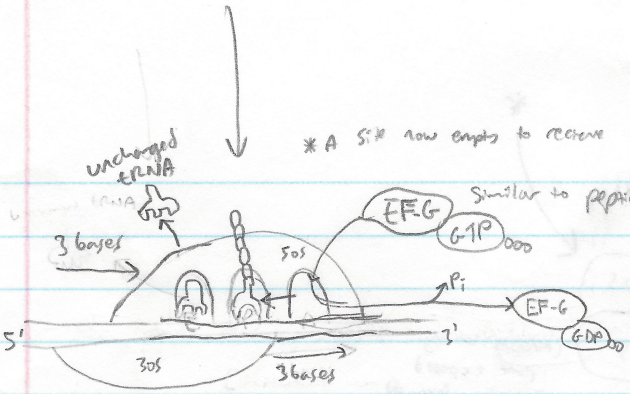
tRNA → RNAP III, 5', 3' ends removed, CCA added to 3' end, introns (if any) removed by mitochondrial bound endonucleases.

rRNA → rDNA genes in nucleolus, 2 genes: - 1 hnRNA 43S transcript w/ 5.8S, 18S and 28S
↳ RNAP I - 5S coded on other gene.

hnRNA 43S → 5', 3' ends removed, 18S cleaved, 5.8S anneals to 28S then loop. * a non-transcribed spacer separates transcription units.

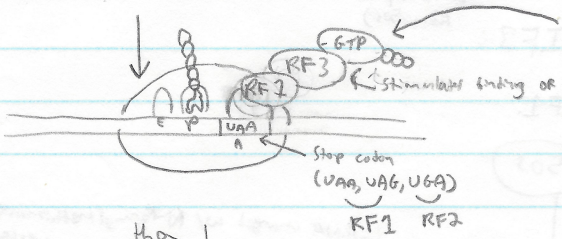
⑨ Translation Steps



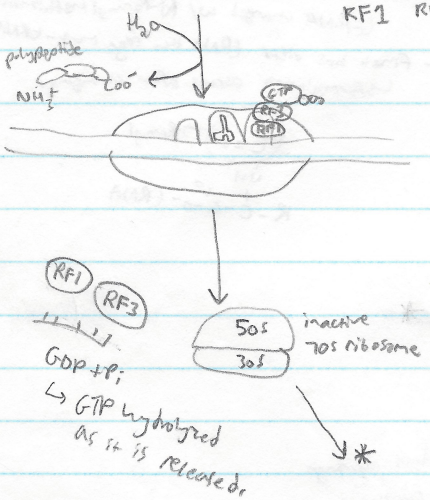


translocation

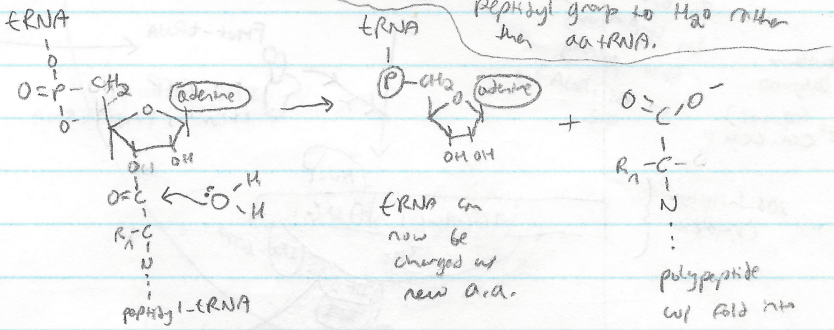
* large subunit moves 3 nucleotide bases, releases uncharged tRNA formerly in E site. Once, EFG is hydrolyzed, the small subunit shifts 3 nucleotide bases, restoring it with large subunit.



Termination



Peptidyl-tRNA hydrolysis - binding of RF allows peptidyl transferase activity to transfer peptidyl group to H_2O rather than aa-tRNA.



- misTranslation rate = 1/10000 → 2 means for error detection: - Selective binding of aaRS for a.a → tRNA
- before peptide bond formation = kinetic

(10)	Prokaryotic	Eukaryotic
(70S initiation Complex) ↳ 50S+30S Subunit + $FMet-tRNA^{FMet}$	- IF1, 3, 2+GTP; 30S, mRNA, $fMet-tRNA^{FMet}$ → 30S initiation Complex	- 43S initiation complex ↳ eIF-1A, eIF-3, 40S ribosome, mRNA, $tRNA_i^{Met}$ + eIF-2+GTP (eukaryotic factor; initiation) ↳ also: eIF4A, G, E.
Alignment	- Shine-Dalgarno + 3' end of 16S (AGGAGGN AUG, 8-15)	- eIF4E+G + 5'Cap ATP to Kozak CCRCCAUGG (R is a purine)
peptidyl transferase (Catalyst) (ribozymes)	23S rRNA	28S rRNA
	- Co-transcription and translation	- No co-transcription
release factors	- RF-1, 2, 3	- eRF
Elongation factors	- EFTu - EFG - EFTs	- eEF-1 - eEF-2 - eEF-1

CC1090 5:15

(11) - After translation — Chaperonins may assist in folding

- post translational modifications: - Cleavage of initiator Met
  - Cleavage of Signal Sequences
  - addn of Cofactors
  - glycosylation, acetylation, phosphorylation
  - multiunit proteins assemble
  - transport to site of action.
  - tagged w/ ubiquitin attached to lysine side chains of proteins.
    - ↳ areas on proteins meant to be destroyed damaged w/ are exposed.
    - → if tagged then degraded by proteasomes.

AACACNNNNNNNN
WNNNNNNNCAGAA

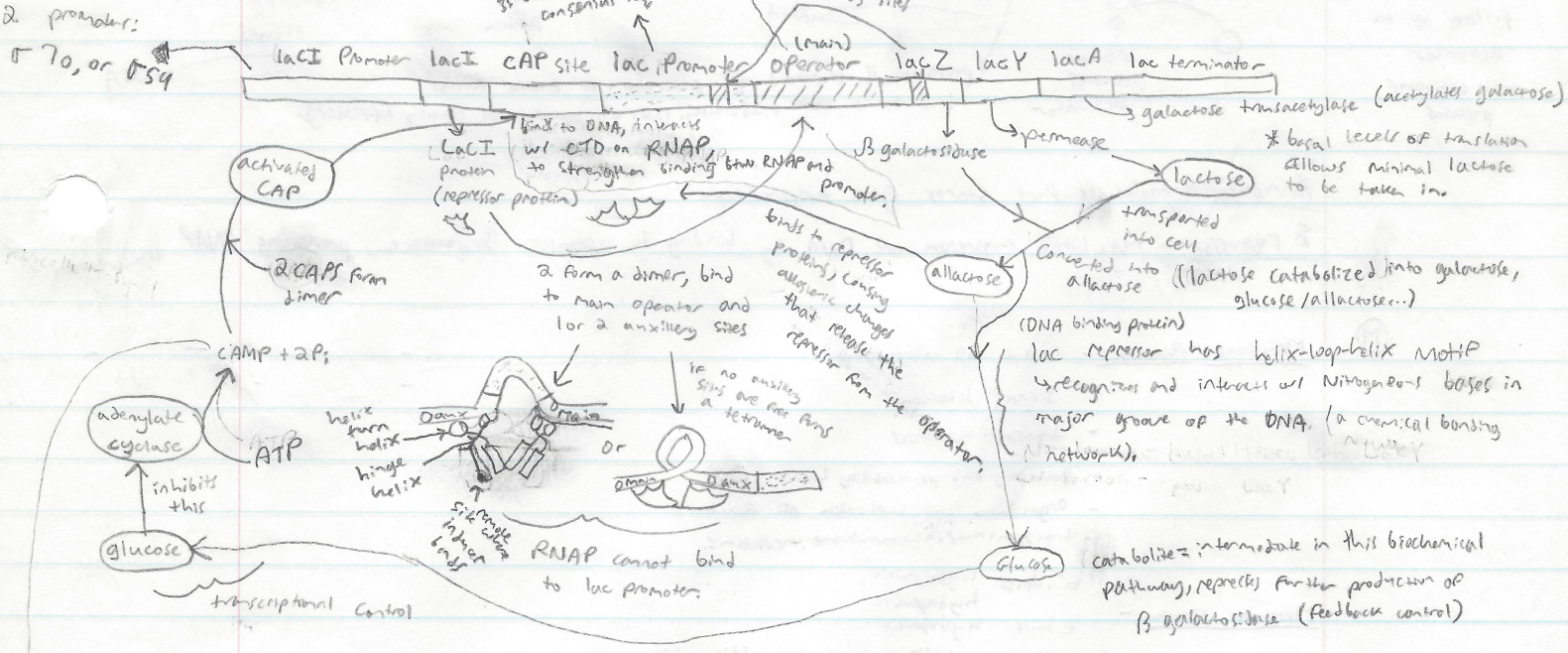
O_1 → sequence has 2 fold rotational symmetry.

(12) Lac Operon

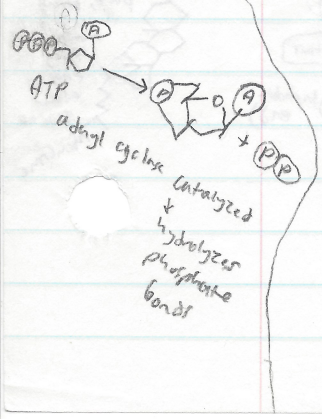
2 promoters: σ^{70}, or σ^{54}

very weak promoters, -35 box very unlike consensus sequence.

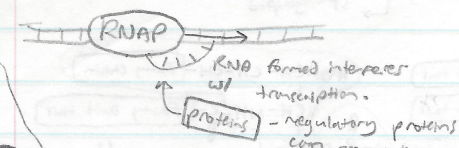
auxiliary sites



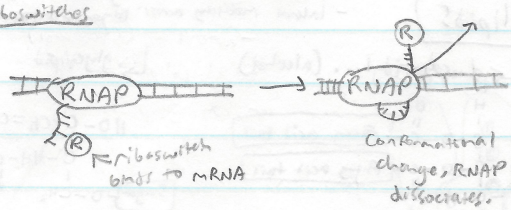
intracellular signal (↓ glucose → ↑ cAMP)



Attenuation



Riboswitches

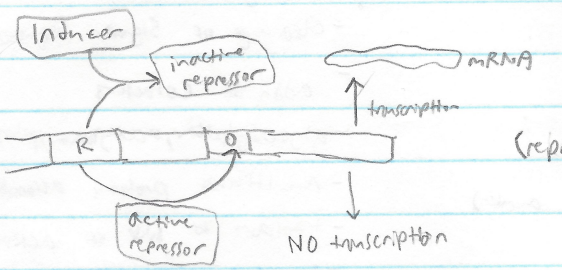


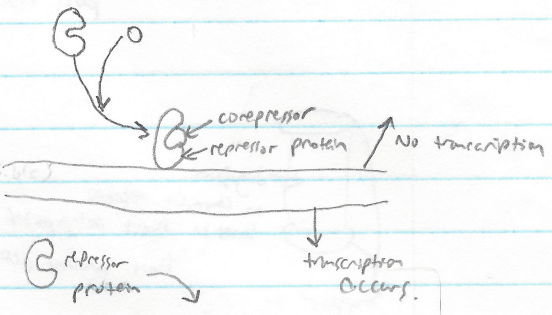
(13) Positive vs. negative Control

-ve Control

(inducible)

- expression normally blocked
- uses repressor proteins

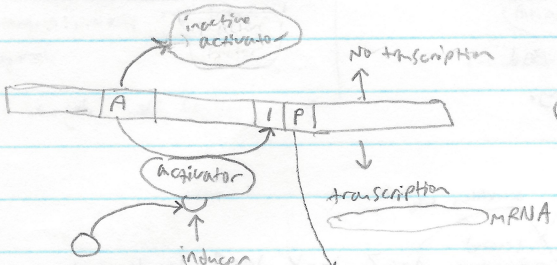


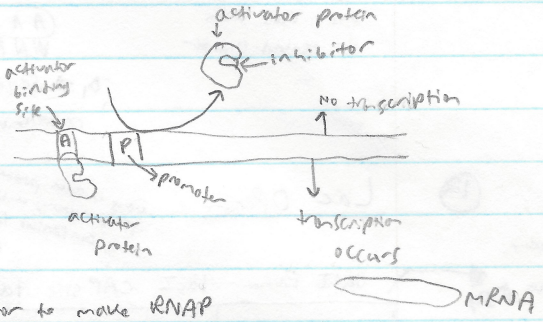


+ve Control

(inducible)

- expression requires presence of an activator
- uses activator proteins





*inducer - Molecule that starts gene expression

*repressor - regulates expression of DNA by binding to operator sequence, preventing RNAP transcription

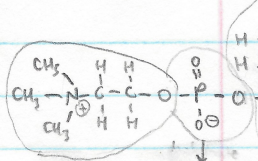
(14) Membrane functions - Cell to cell interactions
- Signal detection
- transport processes
- Boundary and permeability barrier
- Organization and localization of function
- transmembrane/intermembrane reactions.

Membrane structure - e⁻ dense hydrophilic
e dense hydrophobic
e dense hydrophilic

- Composed of lipids and proteins 4:1 → 1:4 ratio.
- membrane lipids are asymetrical (polar head/nonpolar tail) so bilayers. bilayer are stablized by hydrophobic interactions.
- lateral mobility across layer, flexibility, rotation, Flip Flop (must be enzyme catalyzed)

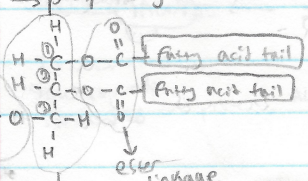
(15) lipids

↳ phosphotidyl... (alcohol)



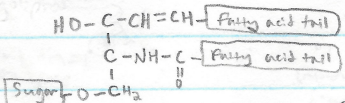
choline (alcohol)
↳ hydrophilic
↳ can introduce charge to lipid
↳ variability to phospholipid.

phosphate



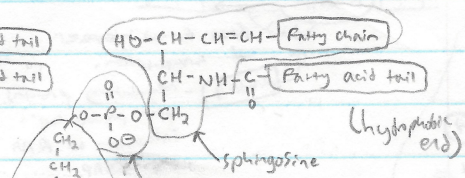
common Alcohols → Choline
ethanolamine
Serine → has net + charge
glycerol
inositol

↳ glycolipid



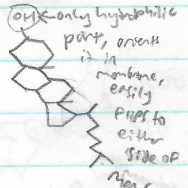
* C2 usually longer and more double bonds

↳ Sphingolipid



(sphingomyeline)
↳ has a choline head)

↳ Sterol



properties - hydrophobic tail, hydrophilic head → amphipathic (polarity, directionality)

- Spontaneously form micelles, liposomes, bilayers → thermodynamically favorable - minimize hydrophobic interactions w/ H_2O.
- tails differ in saturation, length.
- Membranes

Fluidity depends on: - temp → ↓ = less rotation and flexation motions = ↓ Fluidity
- Composition ↔ ↑ cholesterol = ↓ Fluidity
- Length of fatty acids → ↑ = more hydrophobic interactions = ↓ Fluidity
- Saturation of fatty acids → ↑ = less kinks, more compact = ↓ Fluidity

lipid rafts - specialized membrane microdomains compartmentalize cellular processes. ordered together but float freely in the membrane bilayer.

- Membrane synthesized in ER, inserted into cytosolic face, flippase catalyzes transfer of phospholipid molecules to outer face, of plasma membrane

*Scramblase flips phospholipids from cytosolic half of ER to ER lumen half of bilayer. the membrane

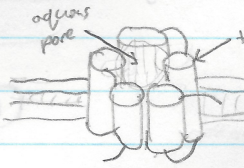
↳ req. detergent to solubilize the membrane
↳ integral proteins, ~25 a.a domain (in bilayer)

(16) Proteins - Membrane proteins - 2 classes: → integral → transmembrane
→ peripheral → Membrane associated
GPI anchors proteins by → lipid linked
Covalently linking carboxyl terminus of protein to glycolipid → protein attached.
head group

Functions → transporters
→ anchors
→ receptors
→ Enzymes

using prenyl group → palmitoyl anchor → thioester
myristoyl anchor → amide
- GPI anchor - cytoskeleton anchor

Channel formation



transmembrane α helix, amphipathic, a.as on sticking out on outside are hydrophobic, ones facing aqueous pore are hydrophilic

16 strand β-barrel porin

- relatively non selective, molecules < 600 daltons pass, larger ones do not.
- Side groups facing inward → hydrophilic.
outward → hydrophobic.

(17) Cytoplasm - Peripheral membrane proteins → filamentous proteins (intermediate, actin, microtubule) and accessory proteins.
- reinforced w/ protein Meshwork → cytoskeleton (cell cortex)
↳ scaffold to coordinate intracellular events.
- asymmetric
- coated w/ glycoproteins (proteins post-translationally modified to add glucose) + glycolipids.
↳ oglycosylated exterior surfaces only.

- Organelles - Single membrane - ER, golgi, lysosome, endosome, peroxisome
- Double membrane - nucleus, mitochondria, chloroplast

↳ mutually incompatible chemical reactions must be able to take place simultaneously in same cell, need segregation.

(18) Signal Sequence for nucleus: Pro-Pro-lys-lys-lys-Arg-Lys-val (any of these charged a.as)
P P K K K R K V
↳ can have multiple, none ↳ stay in cytosol
↳ usually removed at final destination

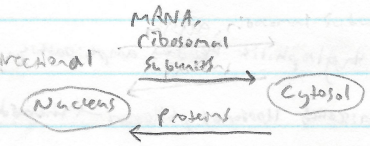
Hilroy

(perinuclear space)

outer nuclear membrane continuous w/ ER, → lumen in ER continuous w/ intermembrane lumen in nucleus

19

Nuclear Pore complexes – bidirectional

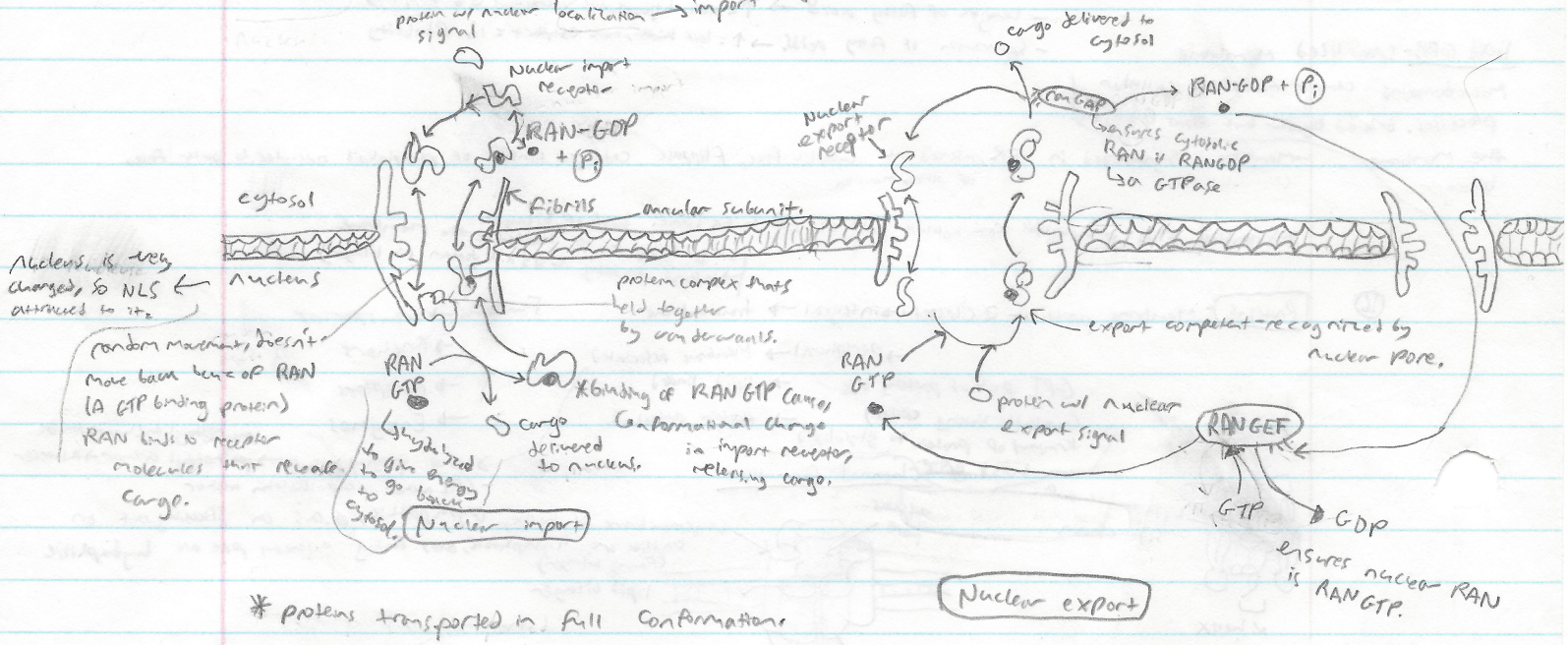


↳ small = water filled channels

↳ large = gated channel

- 300 distinct Nuclear pores (specificity)
- 3000-4000 npc per cell, ~125 mil daltons, 500 molecules, 120 nm, 100 diff proteins

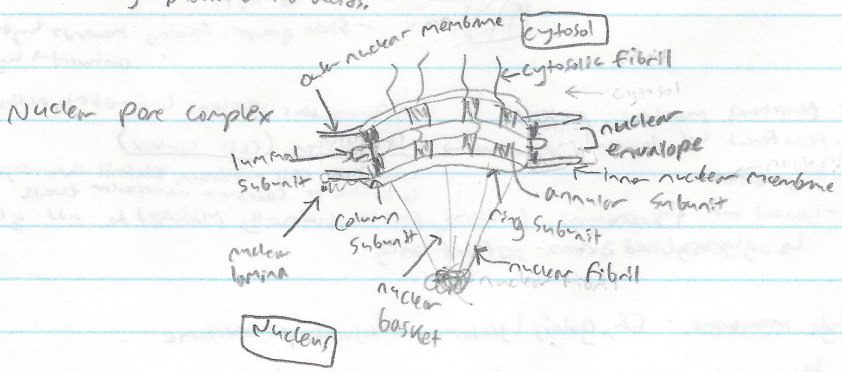
protein w/ nuclear localization → import receptor binds to positively charged NLS



* proteins transported in full conformation.

NES – nuclear export sequence. LX1-3LX2-3LXL X=any amino acid.

↳ rich in hydrophobic amino acids.



EF-tu → brings charged tRNA with GTP to mRNA and ribosome complex in the A site

→ If anticodon matches w/ codon, switch helix will release tRNA via hydrolysis of GTP → GDP + P_i, latch opens

→ EF-tu then leaves and gets phosphorylated and recycled, binding to a new charged tRNA

→ If anticodon doesn't match, GTP is not hydrolyzed and switch helix stays closed not releasing the tRNA.

Protein Sorting and transport