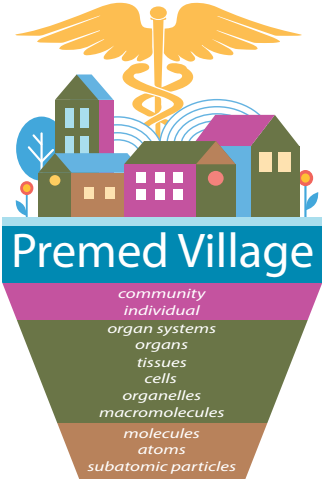


Molecular Bio Notes

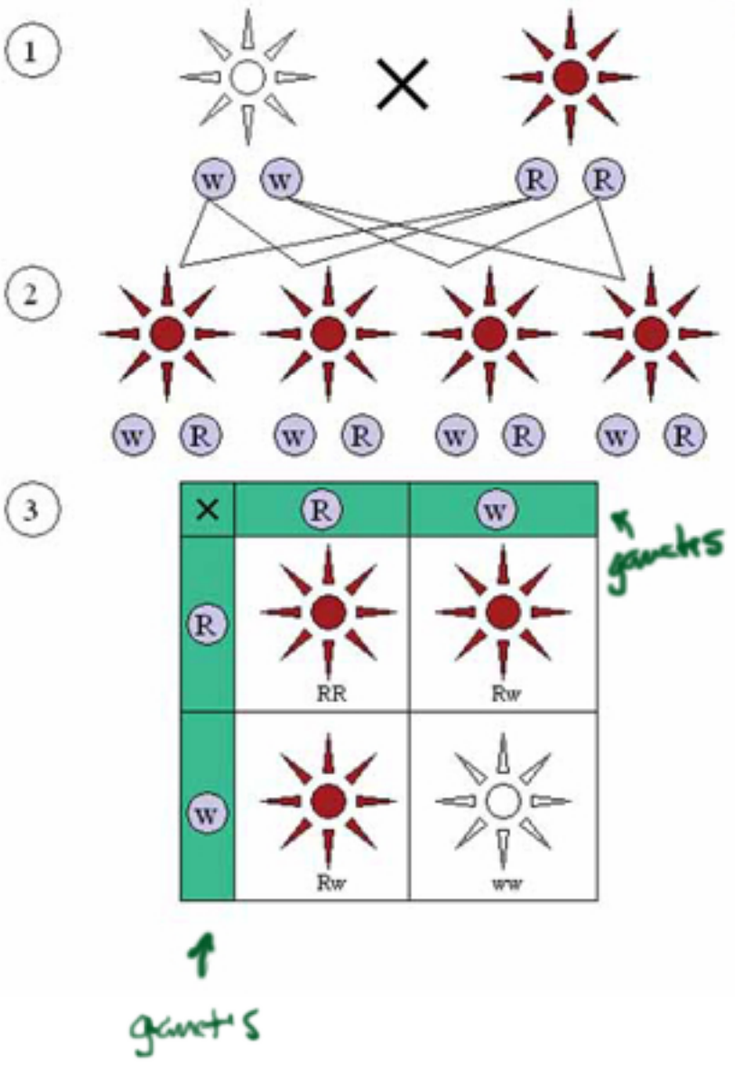
premedvillage.com

Share this book!

Mendel

- allele - alternative forms of genes
- organism inherits one allele from each parent

- Law of segregation
- Law of Independent assortment



parental
- true breeding
- homozygous

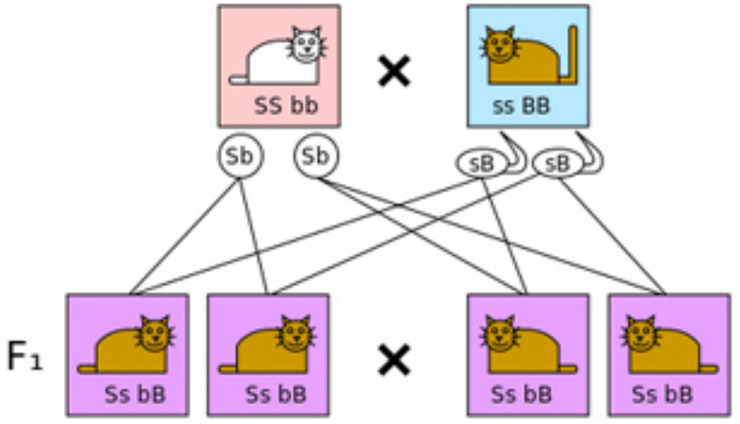
F1 - first filial

F2 -

3:1 phenotypic ratio

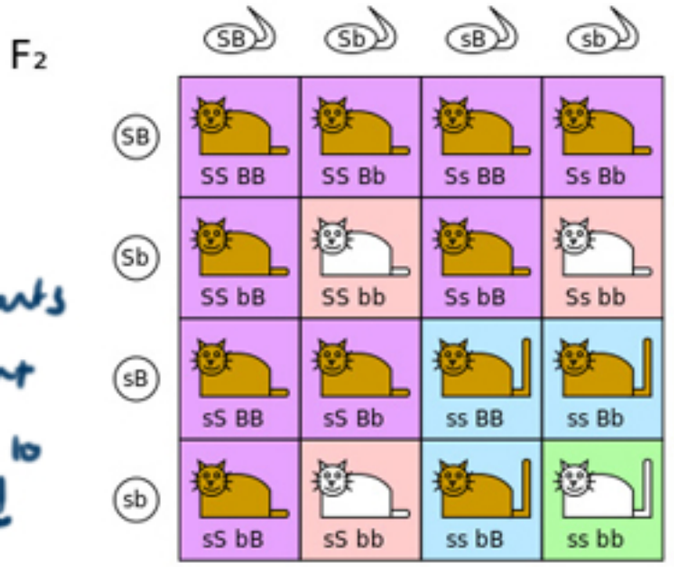
demonstrating independent assortment

- exception
- linkage



Dihybrid cross.

← dihybrids

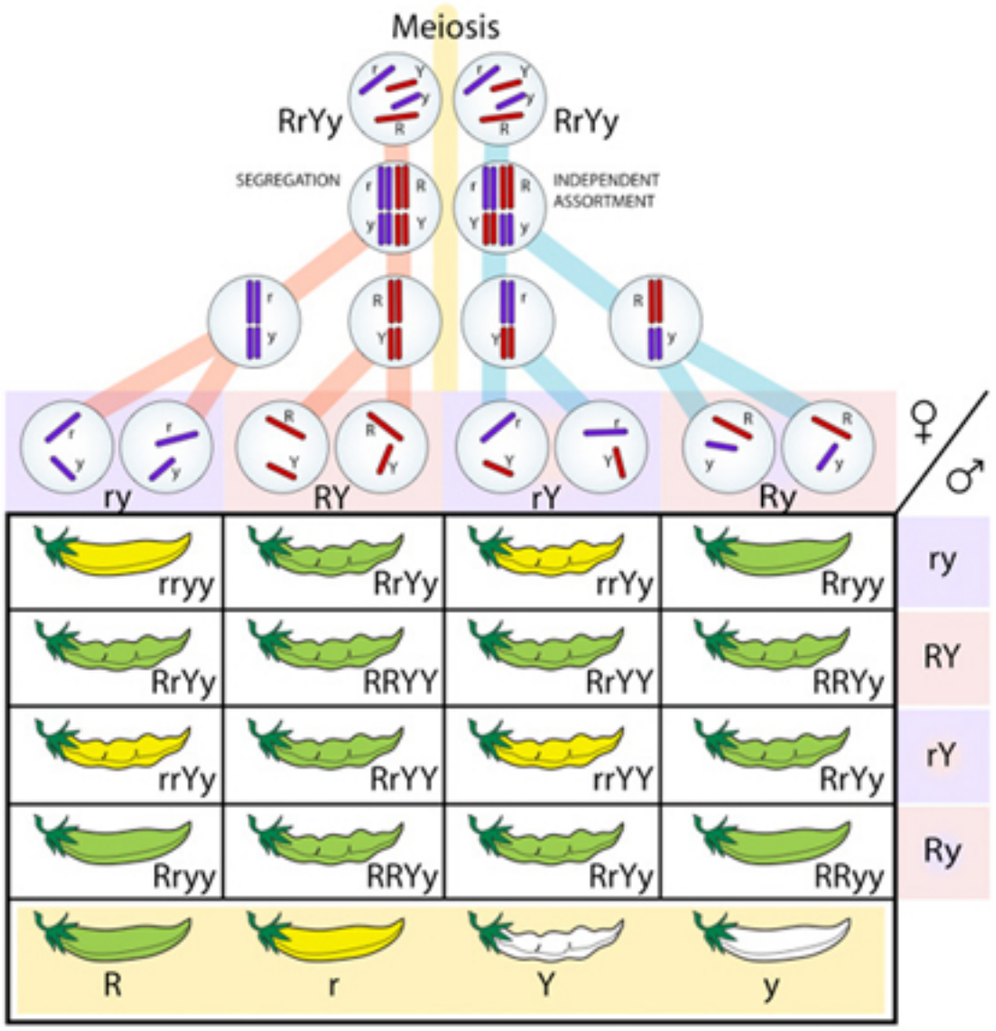


9:3:3:1

phenotypic ratio

NEAT!

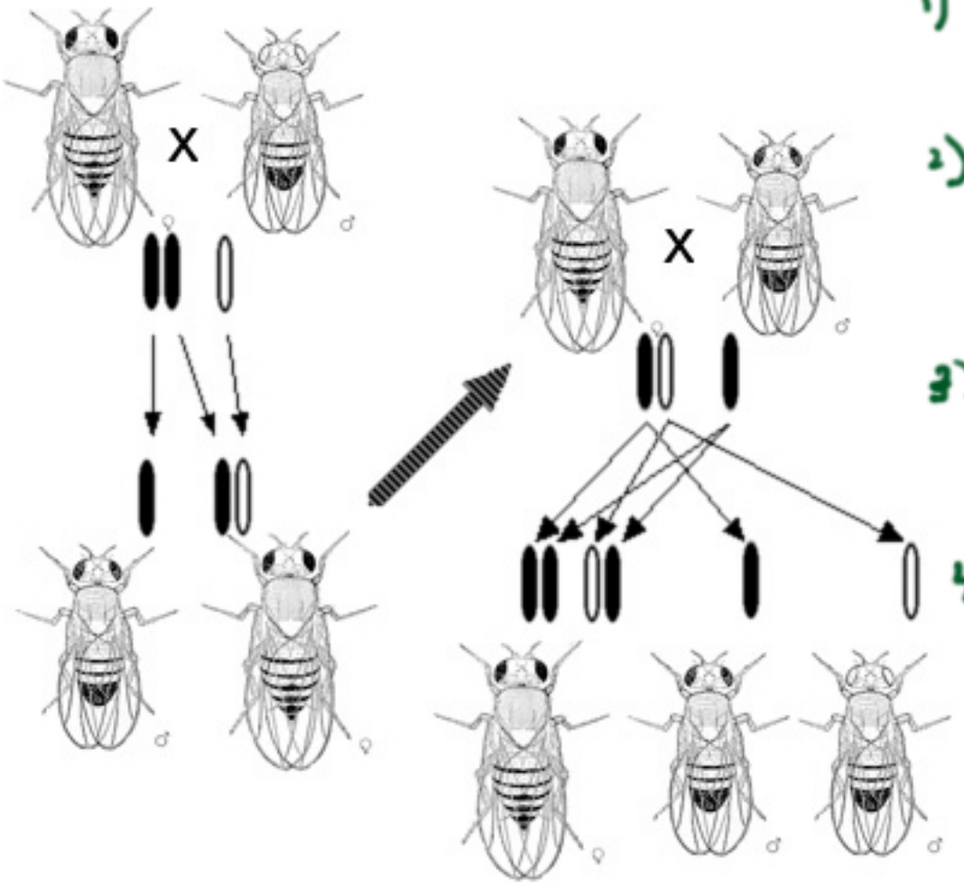
- genotype of parents will be different
- often don't have to fill them all in!


Meiosis
RrYy
RrYy
SEGREGATION
INDEPENDENT ASSORTMENT
ry
RY
rY
Ry
♀
♂
rryy
RrYy
rrYy
Rryy
RrYy
RRYY
RrYY
RRYy
rrYy
RrYY
rrYY
RrYy
Rryy
RRYy
RrYy
RRyy
ry
RY
rY
Ry
R
r
Y
y

Thomas
Hunt
Morgan

- Sex linked trait on X chromosome

- Demonstrate of Chromosomal theory of inheritance



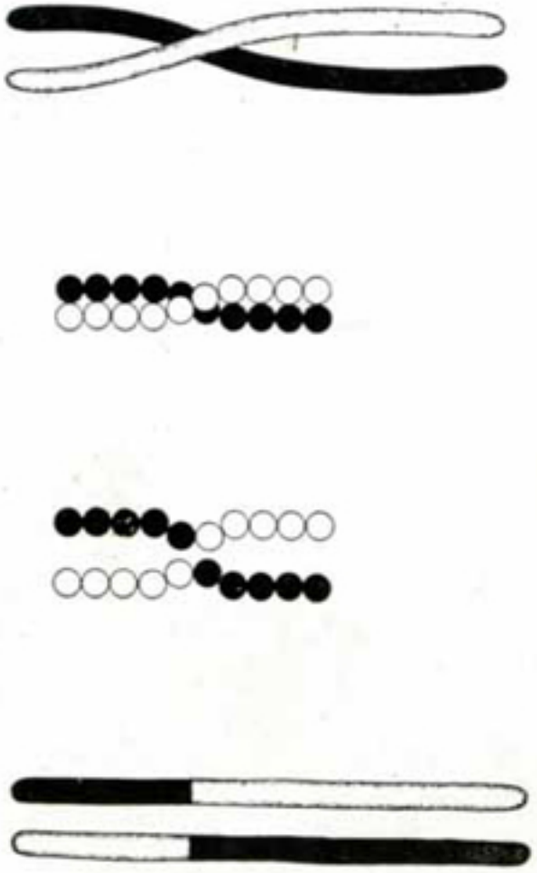
1) Noted white eyed mutant males

2) Mated them with true breeding red eyed females

3) F1 - Red eyed males and females

4) F2 - ¼ white eyed males

Crossing Over



Morgan identified

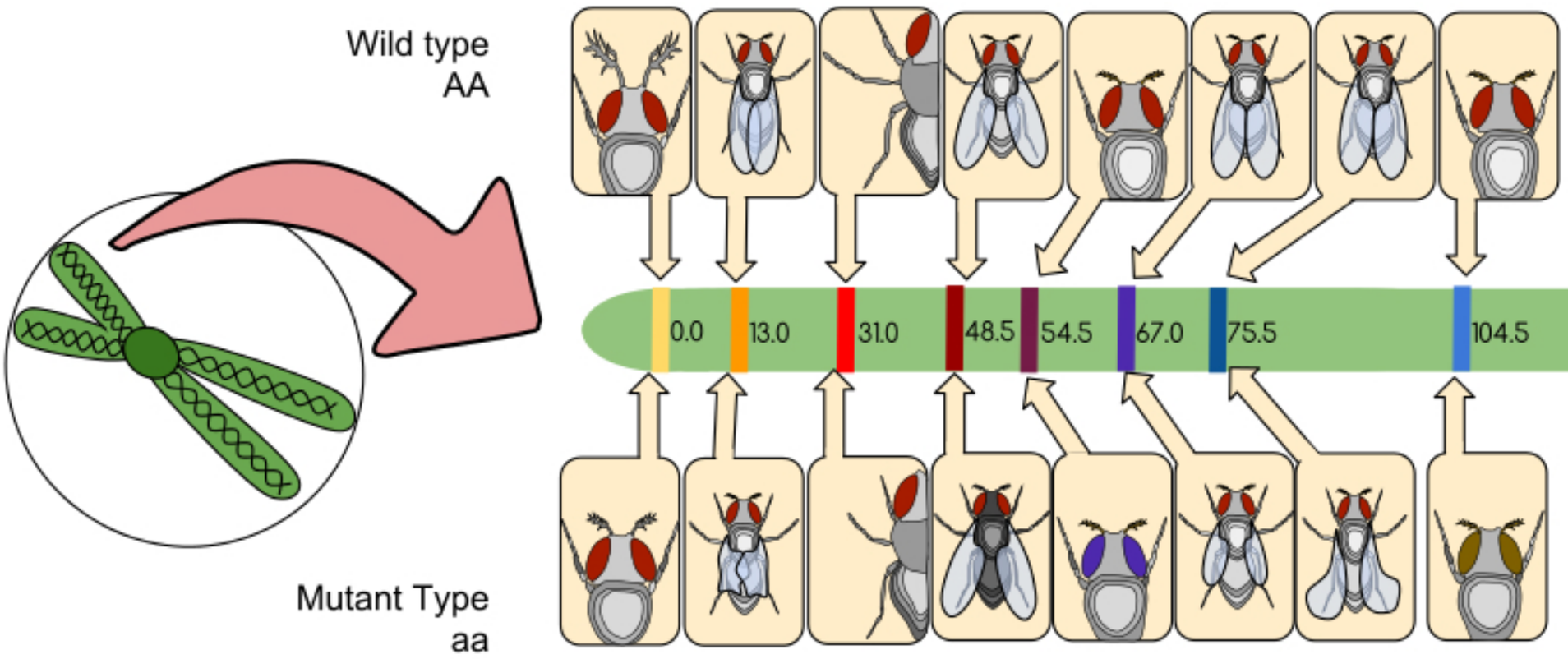
- Miniature winged mutant - also sex linked
- isolated miniature winged whited eyed male
- repeated original experiment.
- expected 25% miniature winged white eyed males

However - the two traits often assorted independently

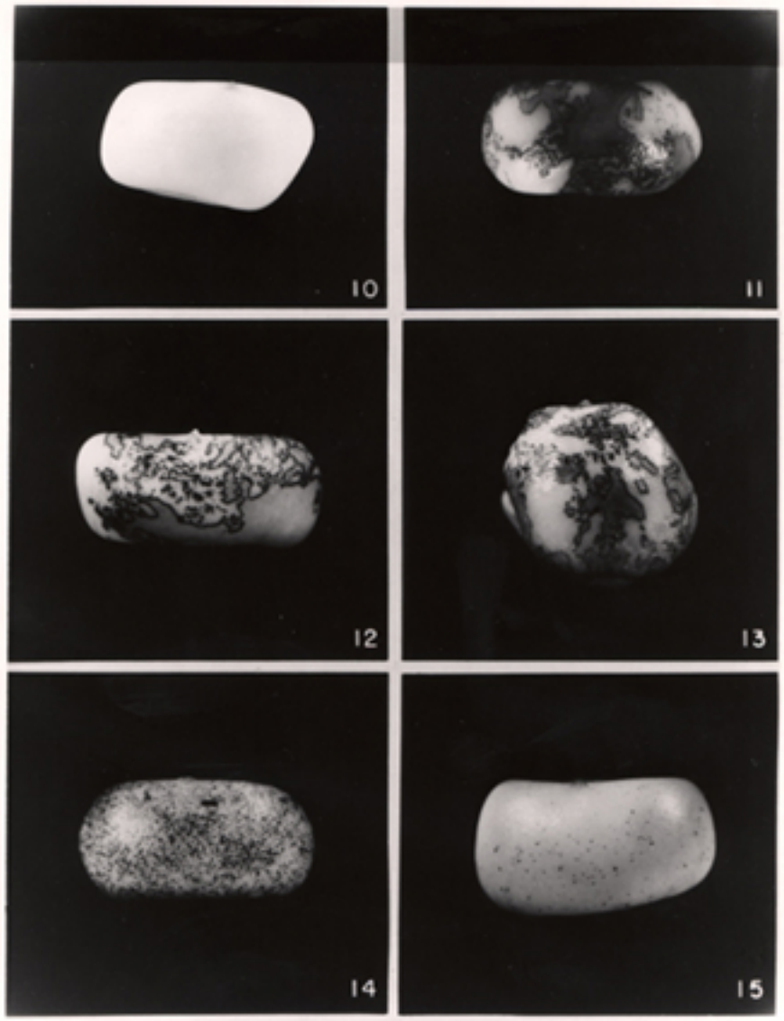
FIG. 64. Scheme to illustrate a method of crossing over of the chromosomes.

Stuyvesant

gene mapping



recombination frequency
of 1% = 1 centiMorgan



Barbara McClintock

Activator (Ac) - gene for synthesis of anthocyanin pigment

Dissociator (Ds) - disrupts activator

↑

transposon

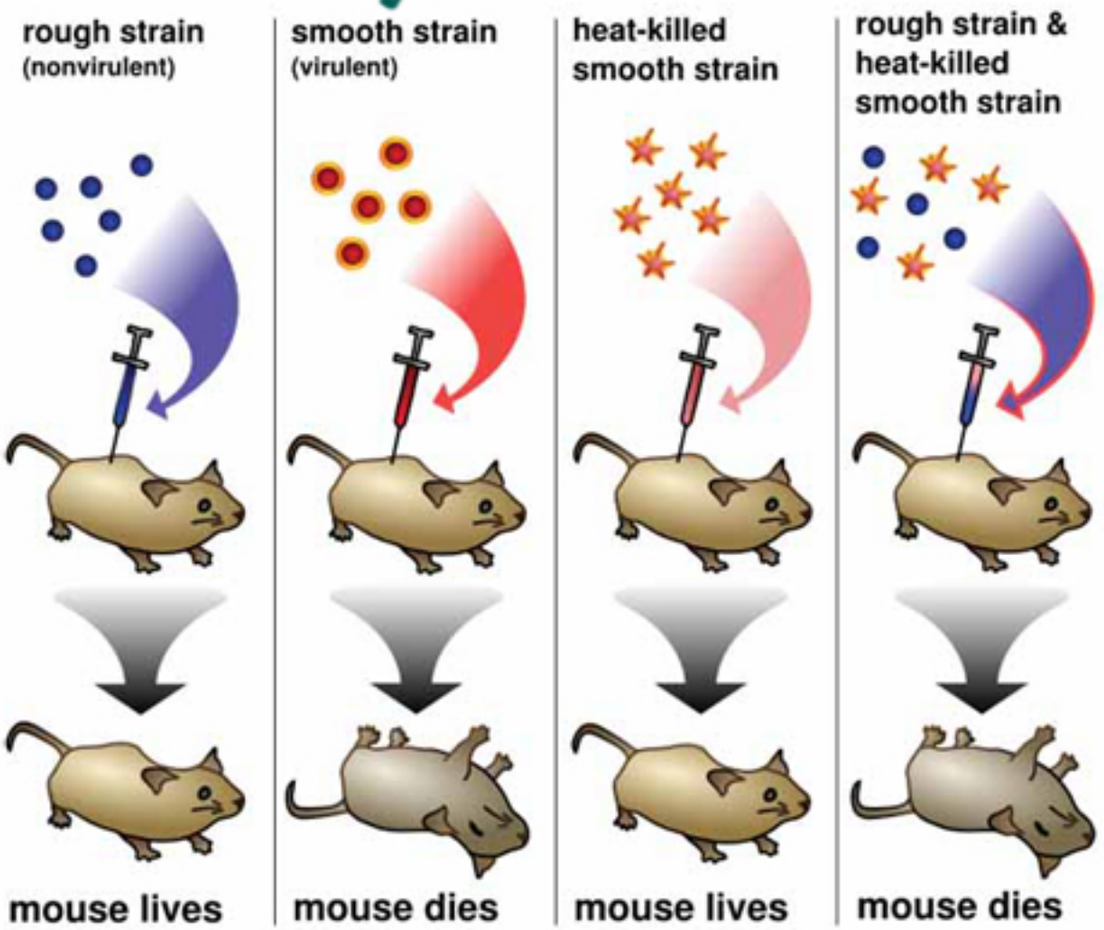
Mobile genetic elements

1) transposons
2) virus
3) plasmids - origin of replication

Friedrich Miescher

- alkaline extraction of nuclei
  - source of pus -
- produced nuclein
  - high in phosphorus + sugars + bases

capsule



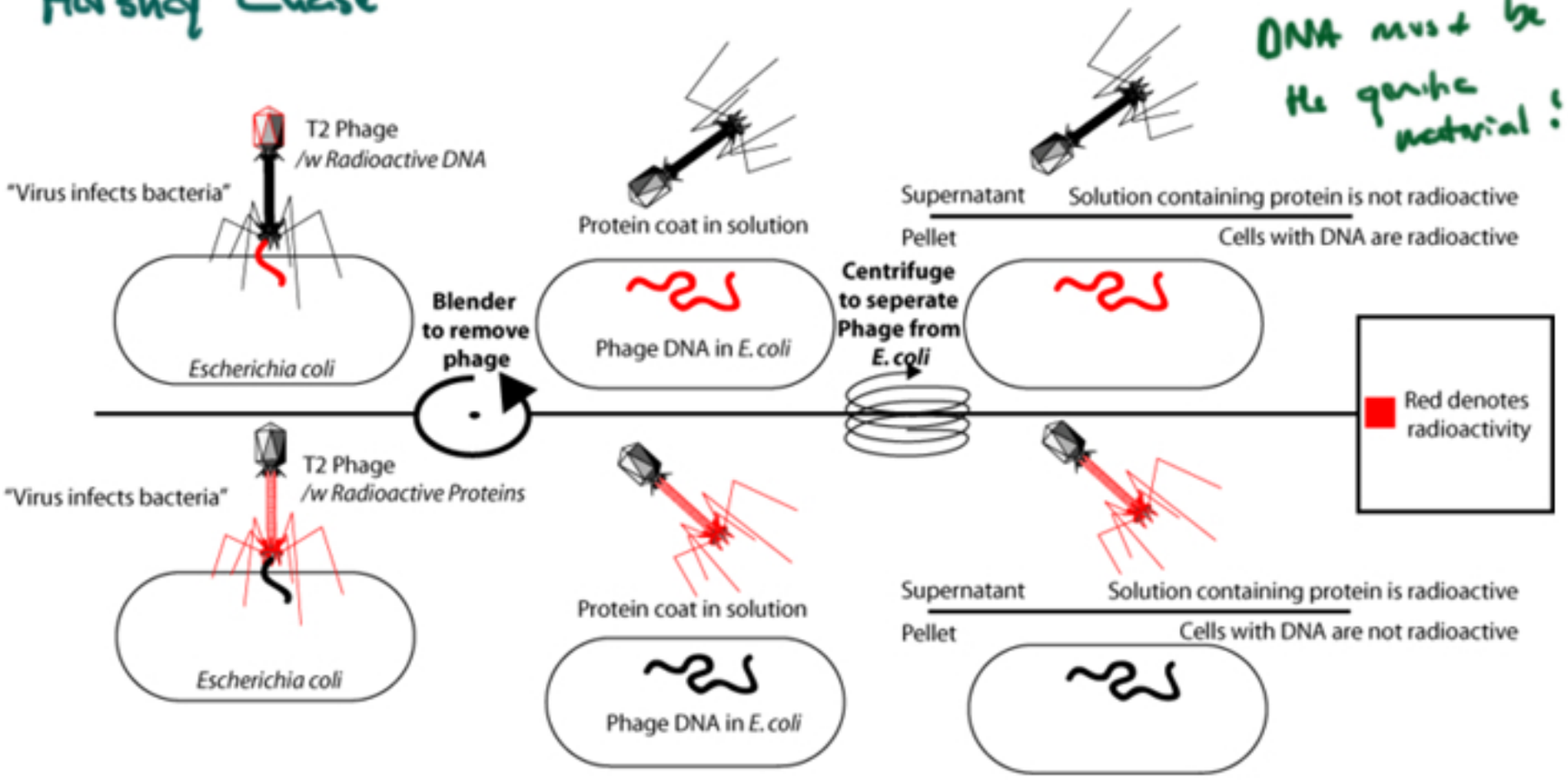
Griffiths

transformation

Avery · showed that the genetic material survived trypsin, RNase but DNase destroyed it.

DNA must be the genetic material !

Hershey Chase



DNA must be the genetic material!

Two experiments - Incubated the virus

1) ^{32}P - Follow DNA

2) ^{35}S - Follows Protein

β^- emitters

β^- emitters

^3H, ^{14}C, ^{32}P, ^{35}S

Dark memorize

Baltimore System

I - dsDNA

II - ssDNA

III - dsRNA

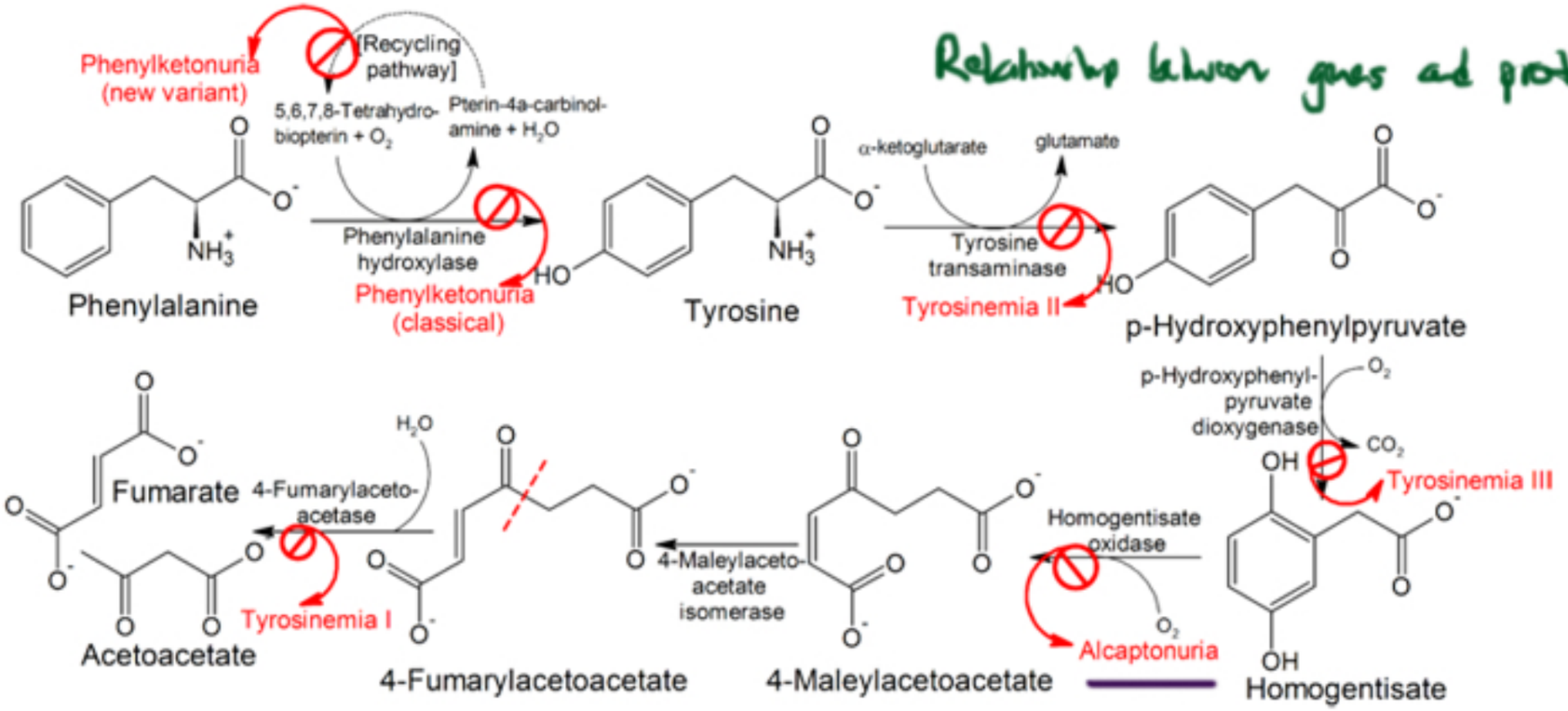
IV ssRNA (+) → ss (−)

V (−) ssRNA → viral polymerase

VI ssRNA-RT → dsDNA

VII dsDNA-RT

Relationship between genes and proteins

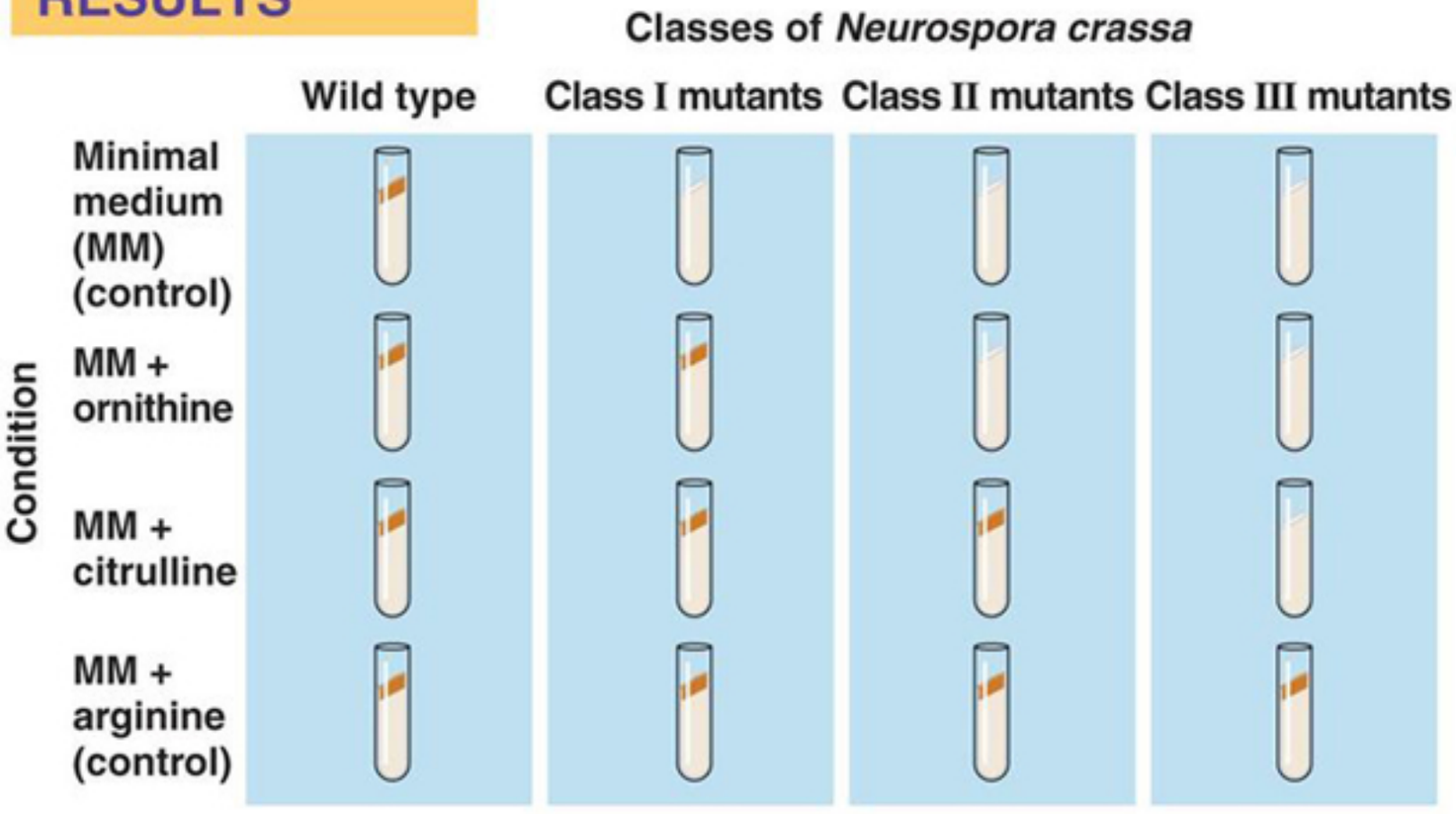


- Alcaptonuria
  - trait that obeyed Mendelian inheritance

Archibald Garrod

- One gene one enzyme hypothesis
  - not quite right
    - multisubunit enzymes
    - splicing variants
    - some genes encode RNAs

RESULTS

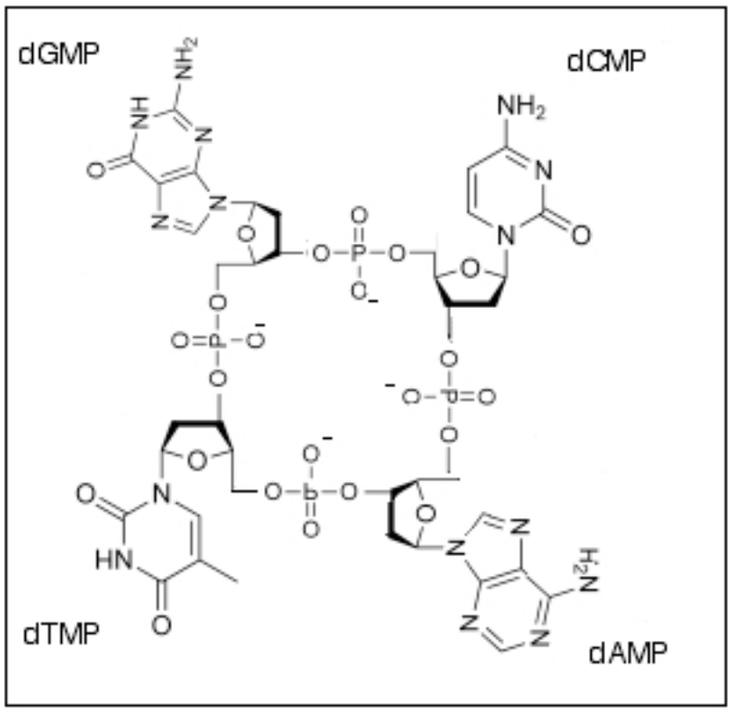


Beadle and Tatum

- Irradiating neurospora to produce mutants (most fungal tissue is haploid)

precursors $\xrightarrow{\text{I}}$ ornithine $\xrightarrow{\text{II}}$ citrulline $\xrightarrow{\text{III}}$ arginine

What is the structure of DNA



Not this!

- Diagram by Charge.

Chargaff's Rules

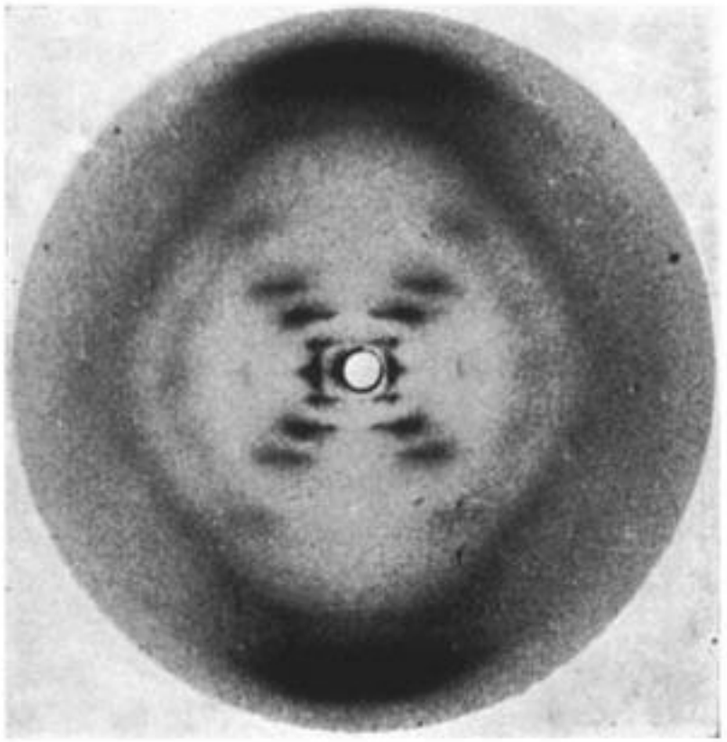
Through careful experimentation, Chargaff discovered two rules that helped lead to the discovery of the double helix structure of DNA.

The first rule was that in DNA the number of guanine units equals the number of cytosine units, and the number of adenine units equals the number of thymine units. This hinted at the base pair makeup of DNA.

The second rule was that the relative amounts of guanine, cytosine, adenine and thymine bases varies from one species to another. This hinted that DNA rather than protein could be the genetic material.

$[A] = [T]$ $[G] = [C]$

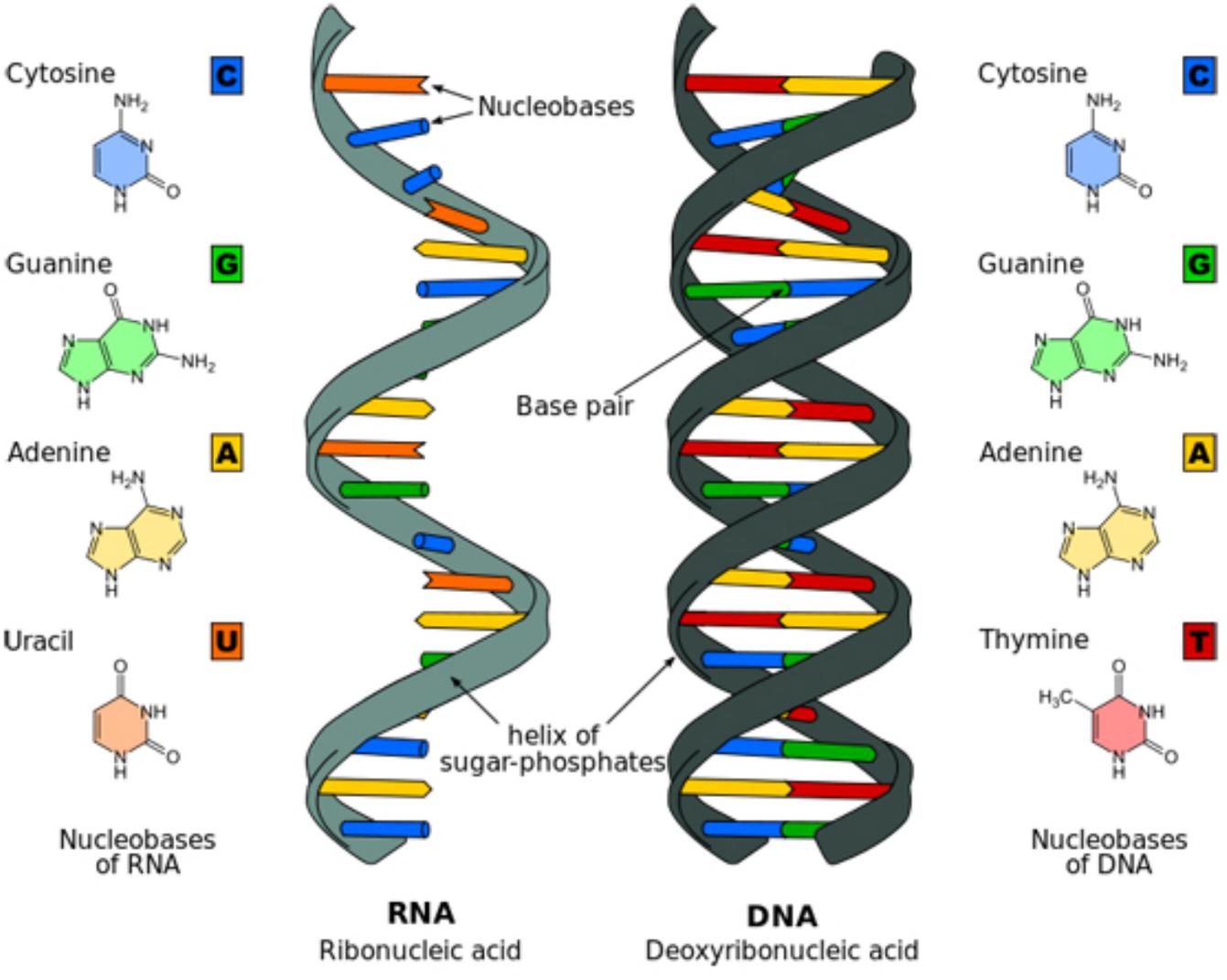
Rosalind Franklin

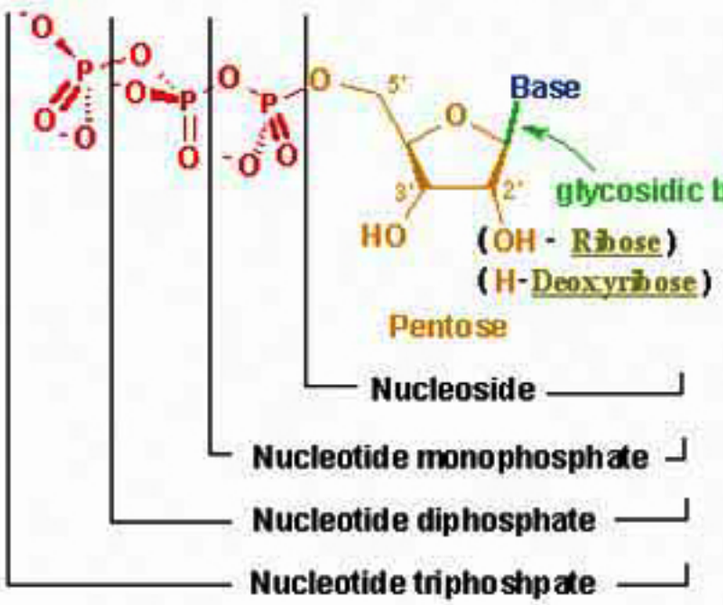


$2d\sin\theta = n\lambda$

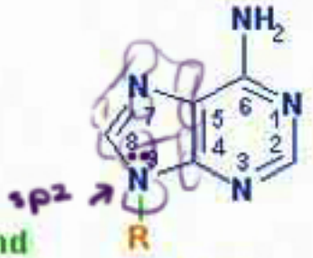
Bragg's Law

Watson & Crick





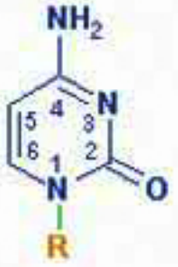
Purines



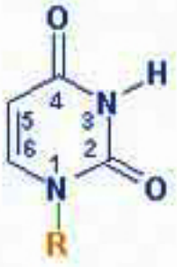
Adenine

Guanine

Pyrimidines



Cytosine

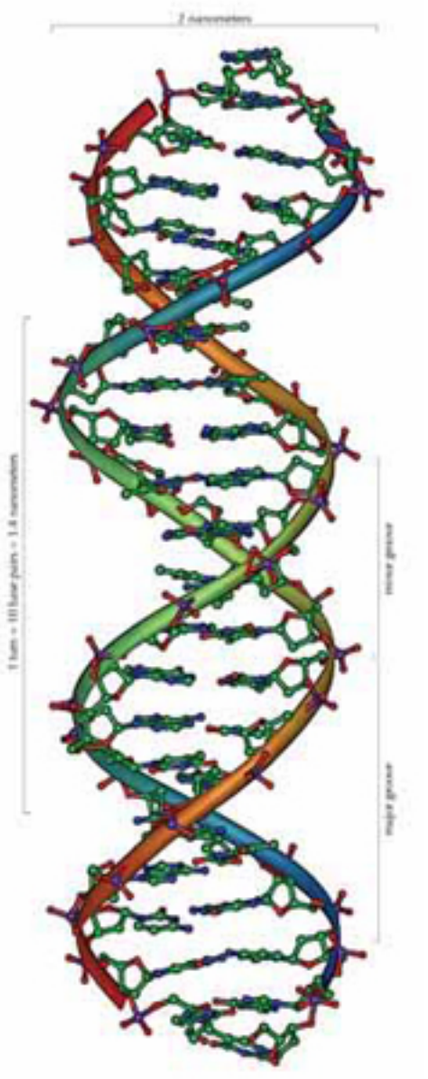


Uracil

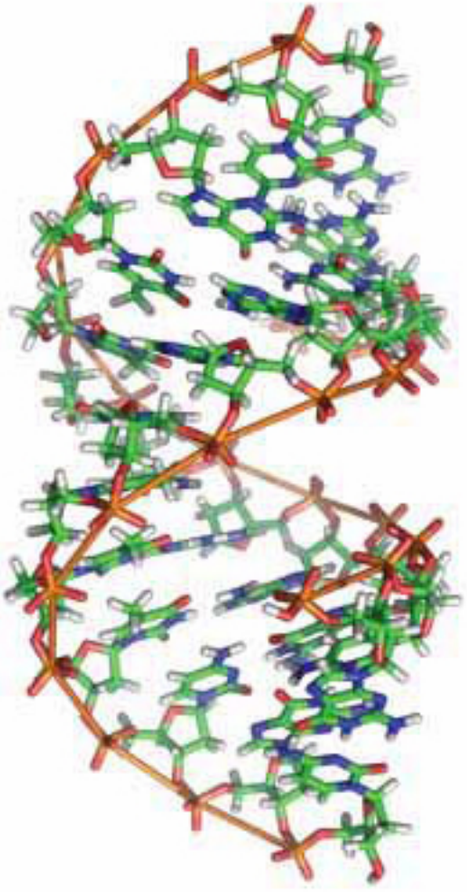
Thymine

(methylated uracil)

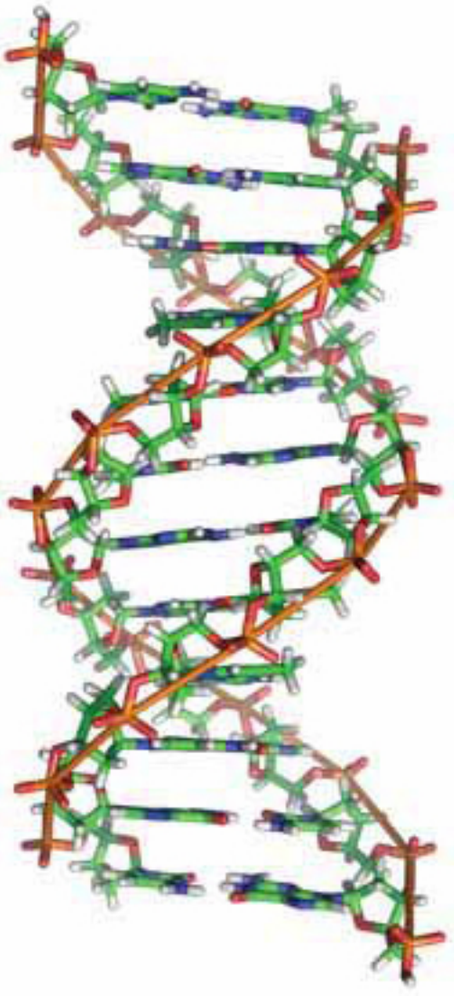
All rings are aromatic


2 nanometers
1 turn = 10 base pairs = 3.4 nanometers
minor groove
major groove

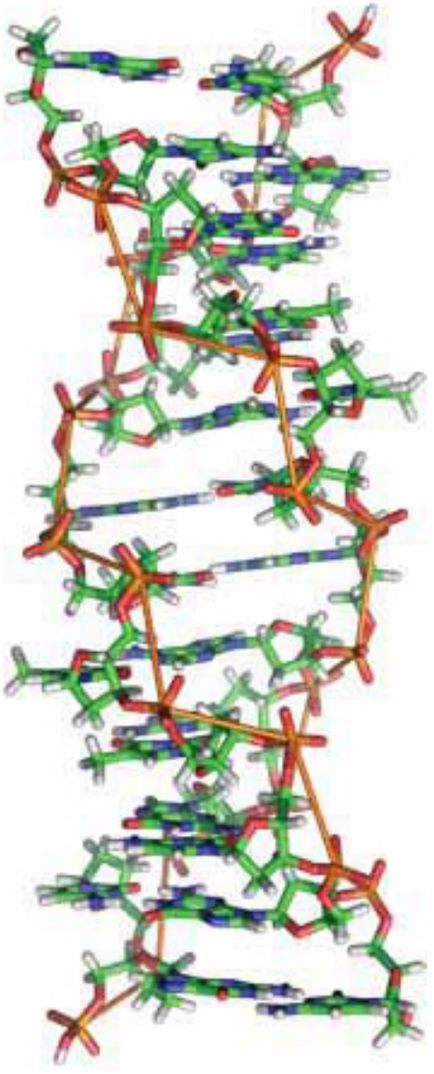
B Form



A Form

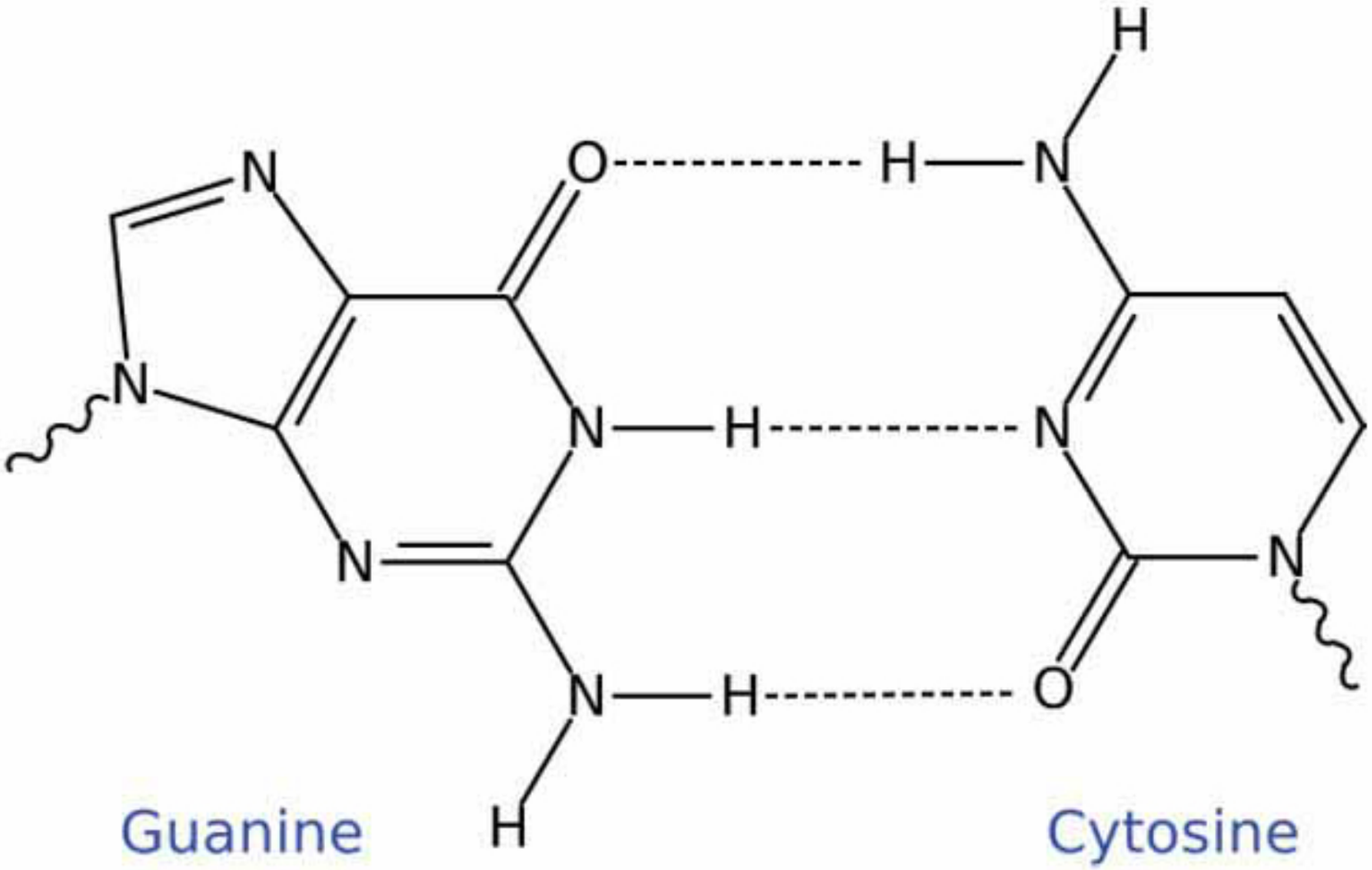


B Form



Z Form

(left handed)

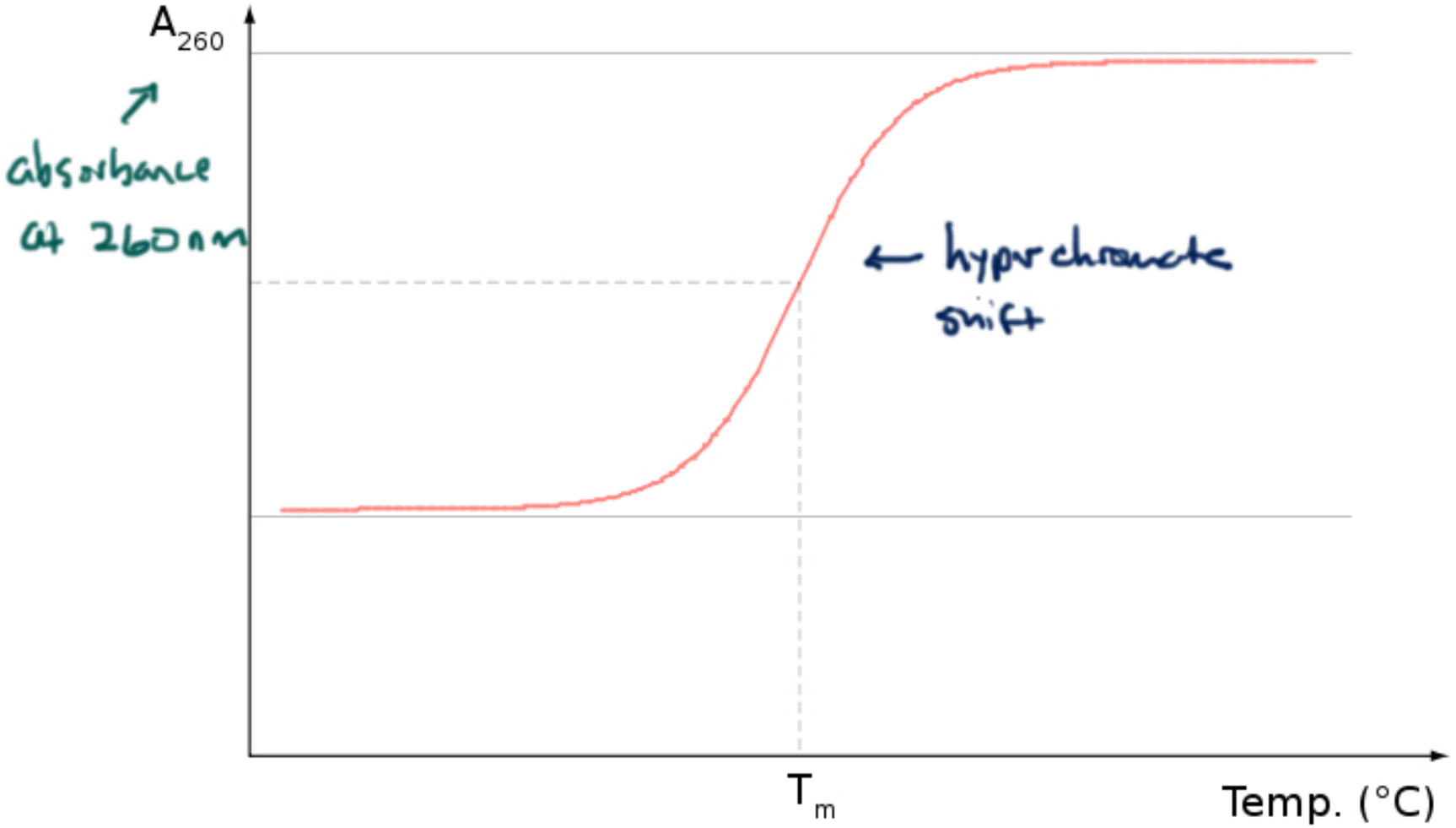


Guanine

Cytosine

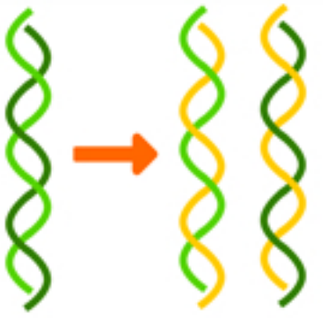
G ≡ C

A = T

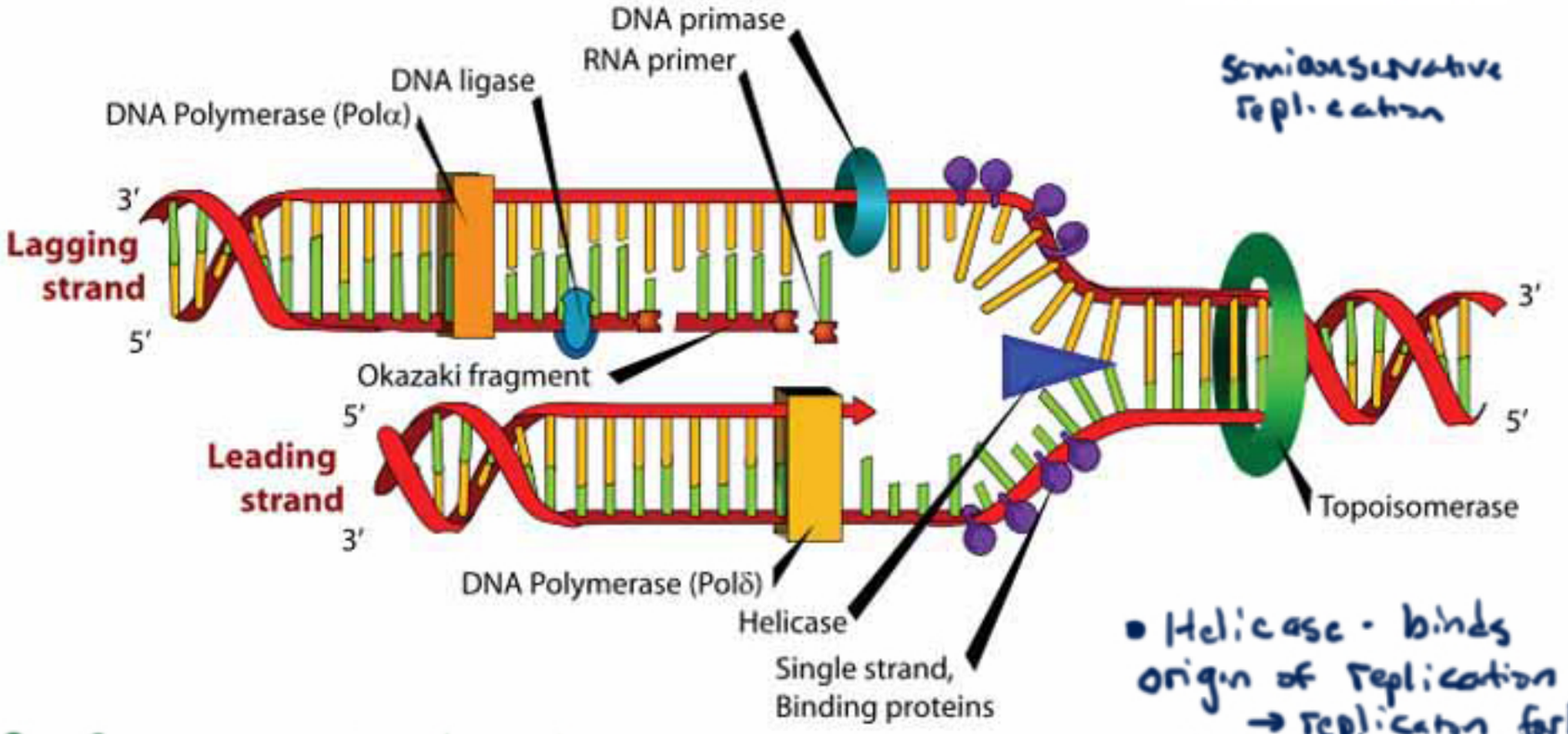


Melting DNA is synonymous with denaturation (unzipping)

DNA Replication



Semiconservative replication



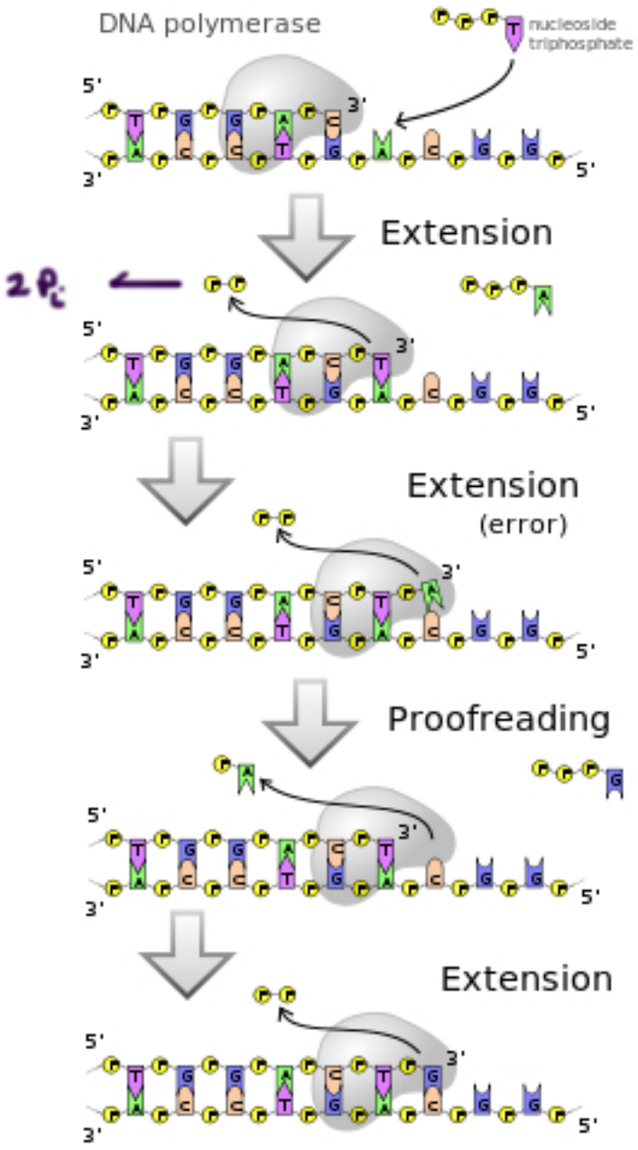
- Helicase - binds origin of replication → replicaton fork (make binding proteins)
- Primase - short complimentary RNA

- Pol δ has proofreading - 1 mistake per billion
  - steric forces shift from the polymerase domain to exonuclease domain.
  - misses 1 in a billion of these - these require mismatch repair

- topoisomerase
- specific exonuclease remove RNA primer pol δ fills in.

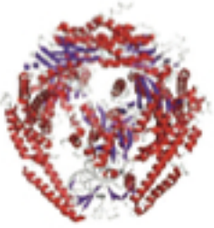
- DNA polymerase reads 3′ → 5′ writes 5′ → 3′
- leading strand and lagging strand (multiple primers and Okizaki fragments - DNA ligase

Note - In elongation 3′ -OH is nucleophile for phosphoryl transfer attacks α - linkeages pyrophosphate.



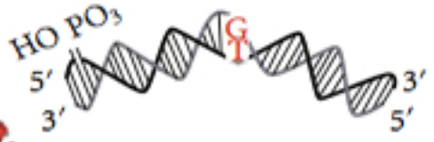
Proofreading

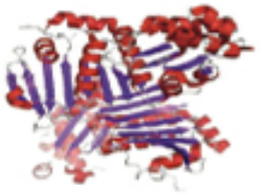
Mismatch Repair



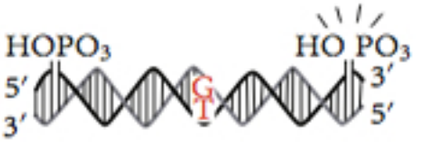
MutSα ↓ Recognition

DNA clamp binds





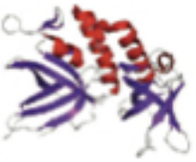
MutLα ↓ Incision



EXO1

↓ Removal

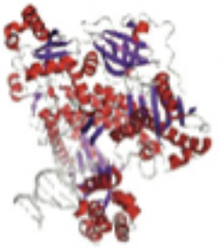
Exonuclease



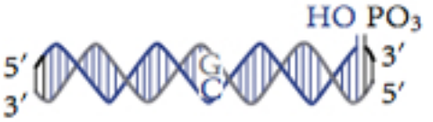
RPA

and

- homologous and nonhomologous end joining

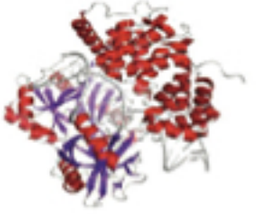


DNA polymerase δ ↓ Resynthesis



also:

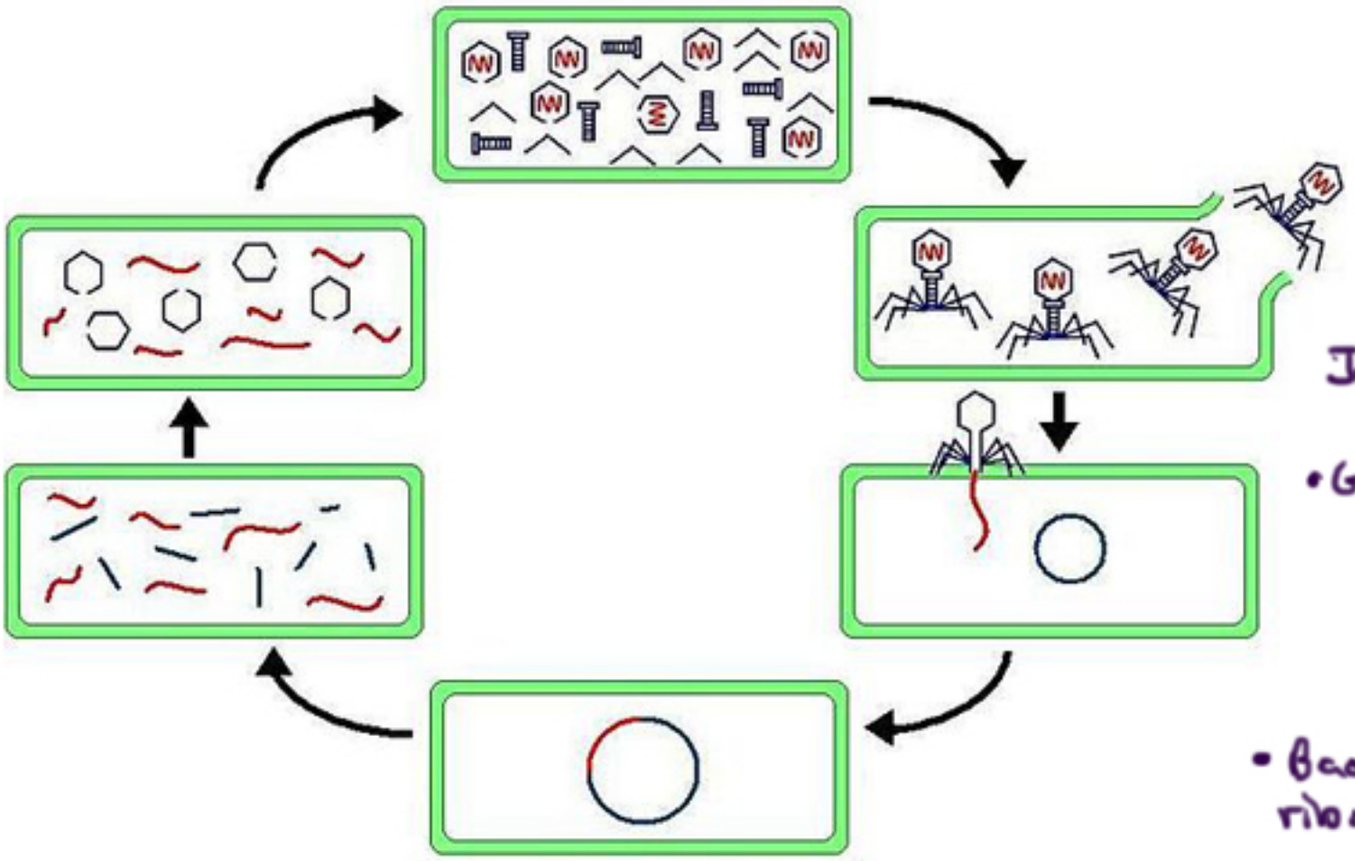
- nucleotide excision repair - for pyrimidine dimers from UV



DNA ligase ↓ Ligation

- base excision repair - for things like deamination of cytosine → uracil

←



Discovery of mRNA by disproving the ribosome hypothesis.

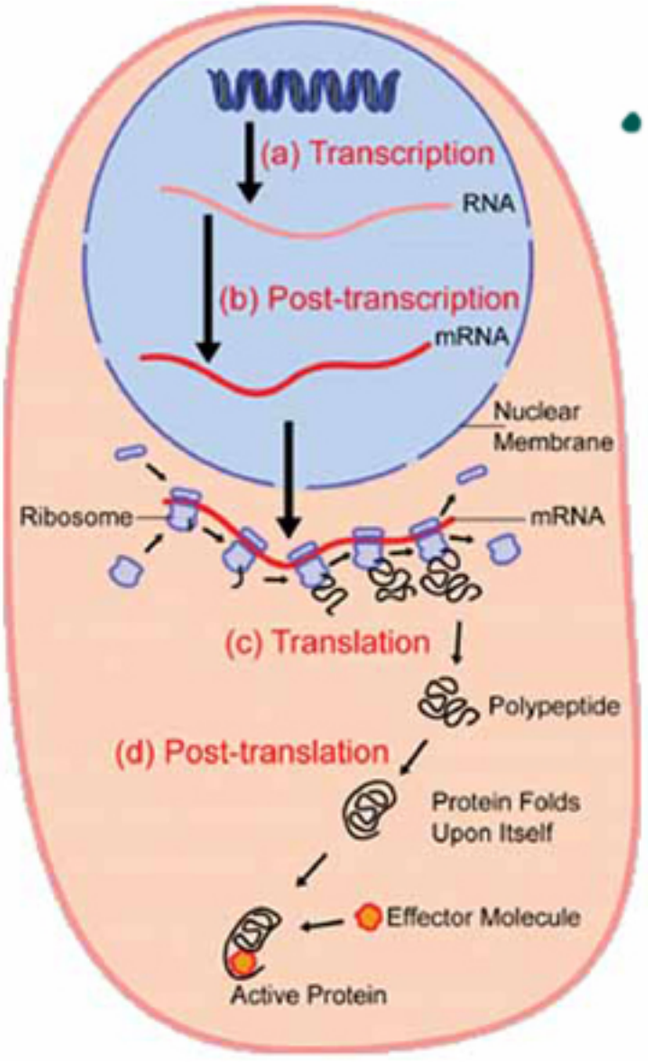
Jacob, Brenner, et al

- Grew bacteria in $^{15}NH_4Cl$ + ^{13}C glucose
  - ↑ heavy
  - ↗
- Bacteria formed heavy ribosomes (old ribosomes)
- Infected with T4 phage and transferred to light media.
- Did the cells make new ribosomes?
- Only found old ribosomes

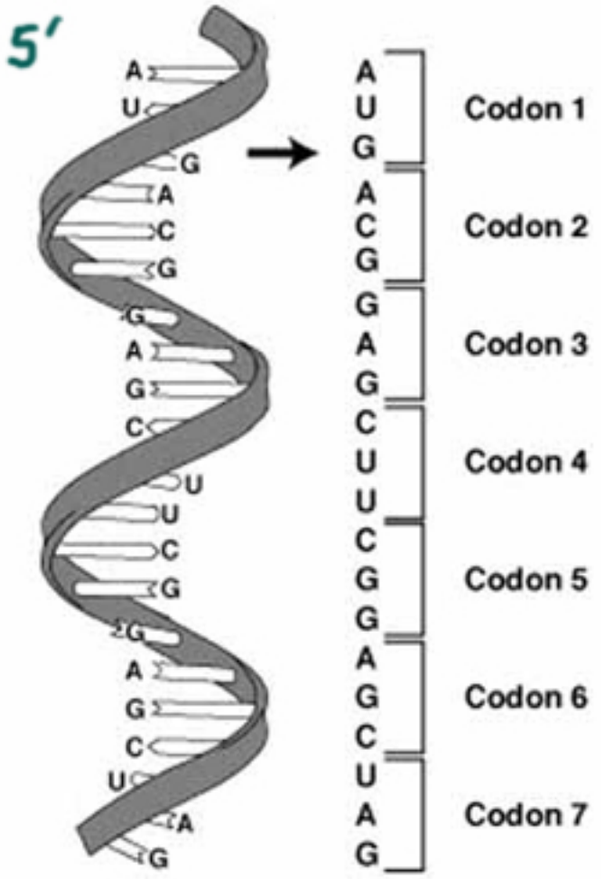
Conclusion - Another form of RNA was carrying the information for peptide sequence - mRNA

Central Dogma

Transcription & Translation



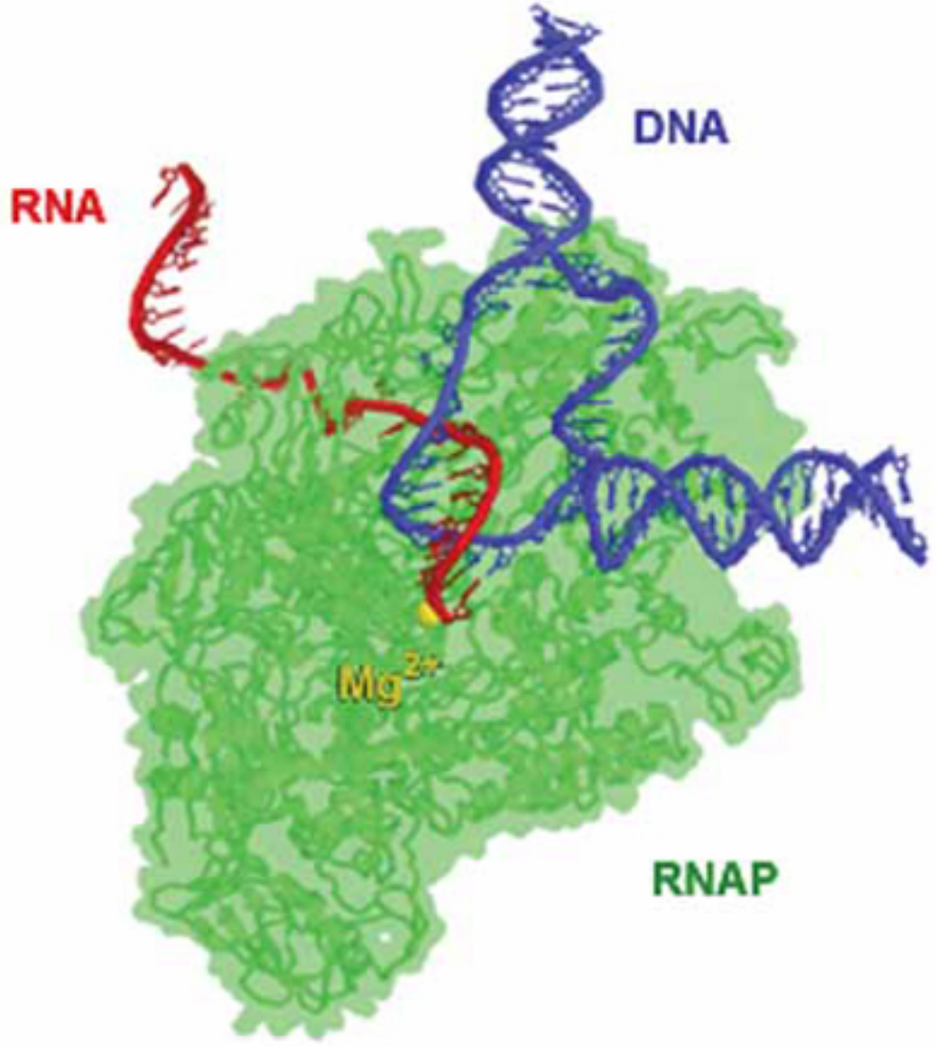
- Let's overview regulation of gene expression
  - Transcriptional control
    - Chromatin modeling
    - DNA methylation
    - Transcription factors
  - Post transcriptional
    - Alternative splicing
    - polyA tail - RNA half life - 3' untranslated region sequences
    - 5' cap
  - RNA interference
  - Translational Regulation
  - Post translational modification, activation, inhibition etc



RNA

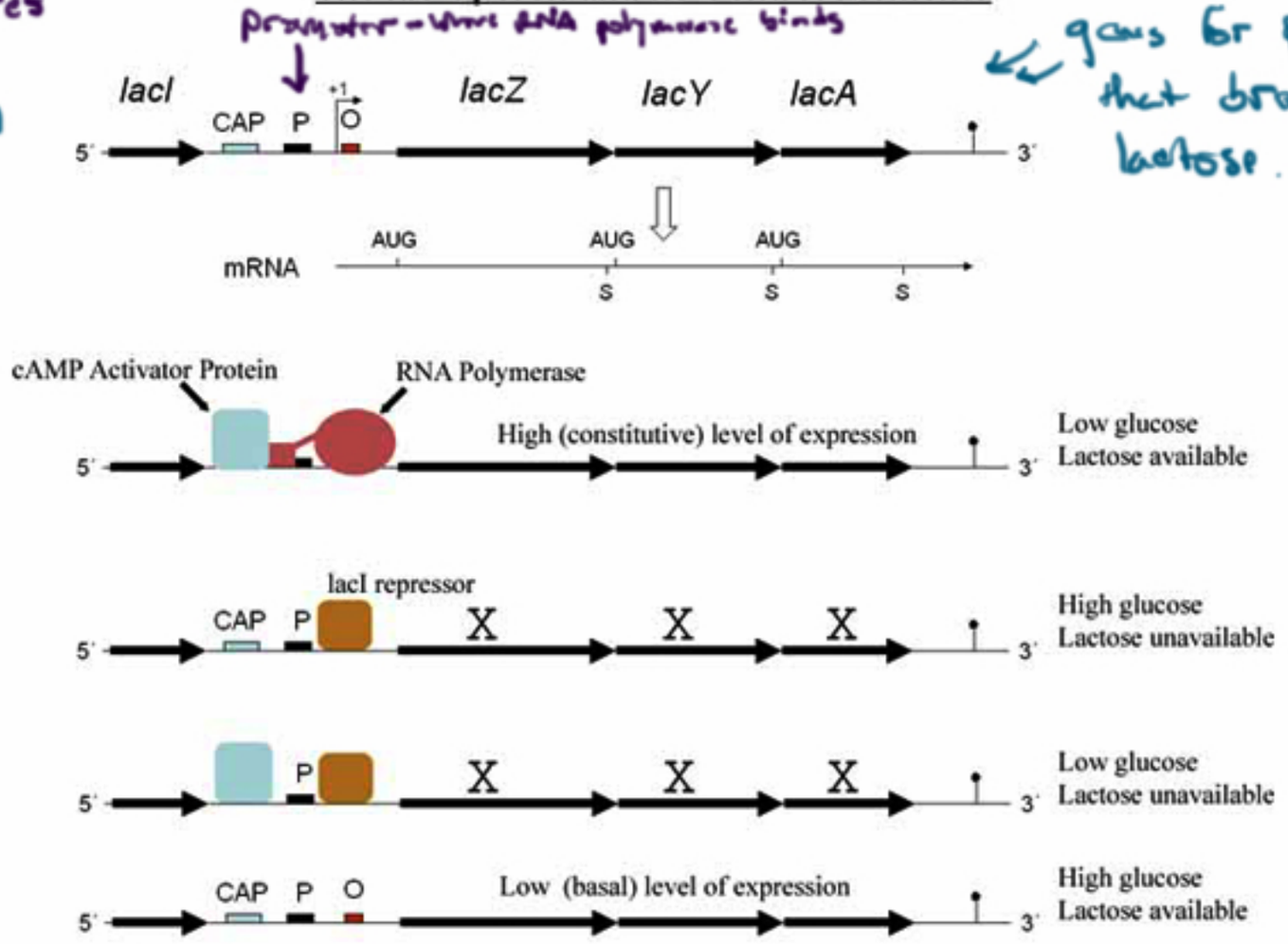
Ribonucleic acid

More in translation


DNA
RNA
Mg^{2+}
RNAP

Prokaryotes

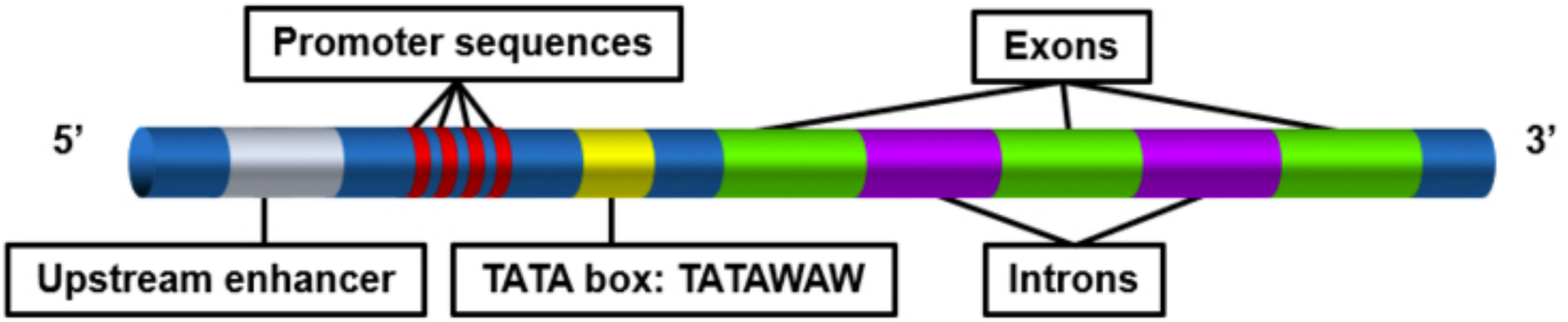
Lac operon
- an inducible operon



when glucose is low cAMP is high in E. coli:

Polycistronic
- Single promotor - a single reading frame with multiple genes
- Doesn't happen with eukaryotes

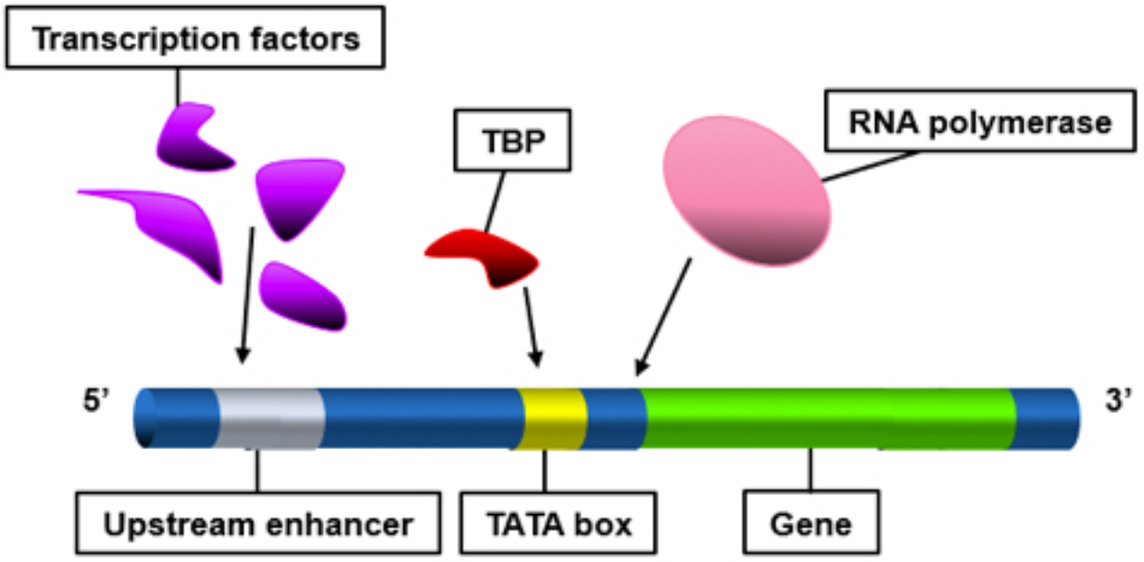
eukaryote gene

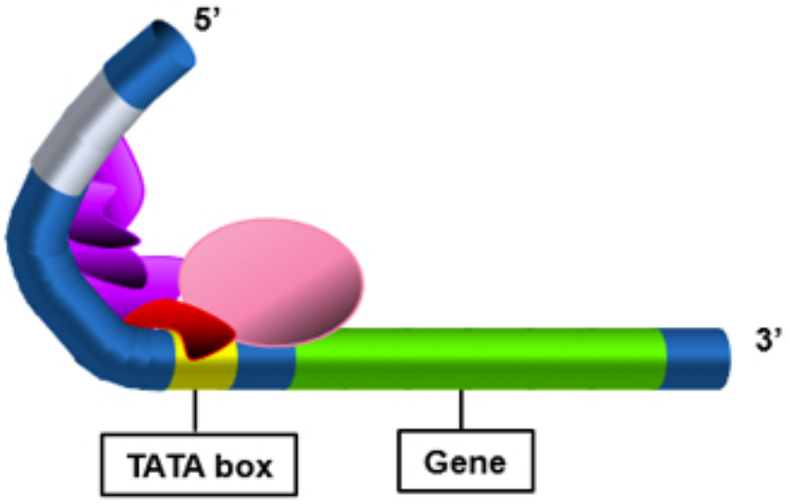


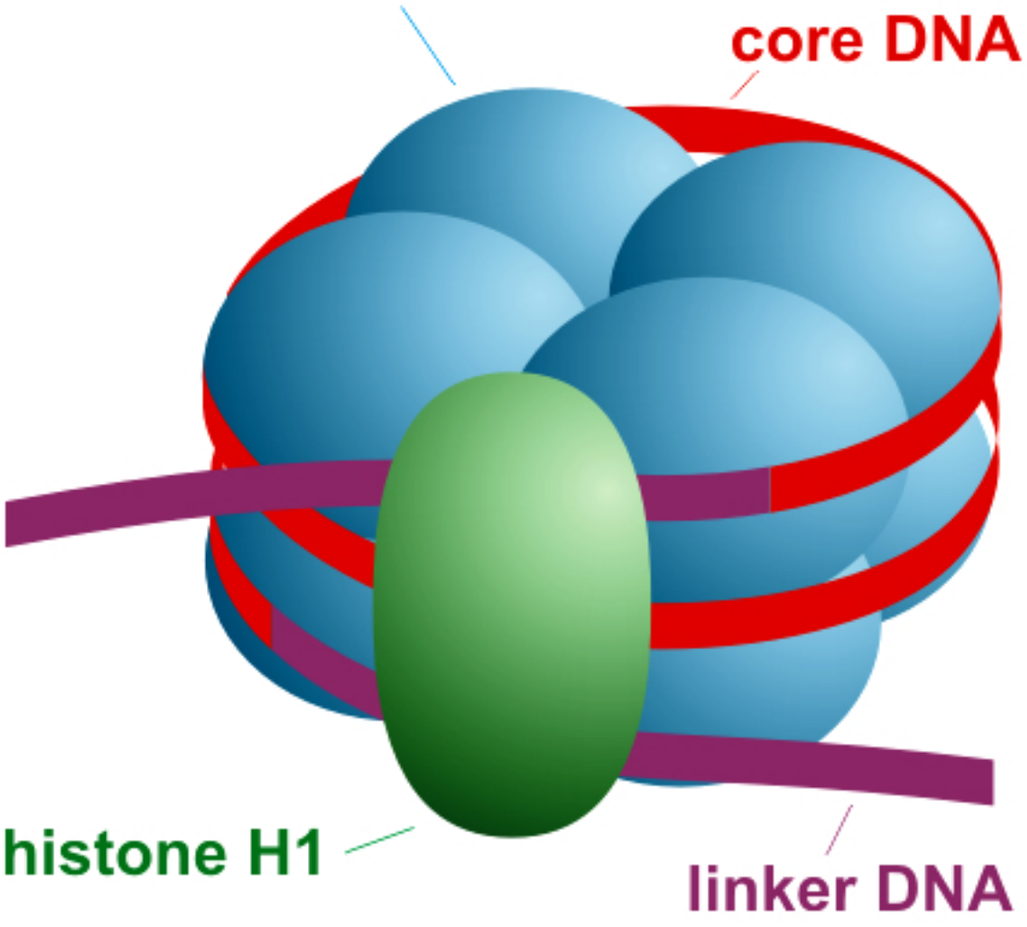
binding of specific transcription factors

Region of core promoter. Sites for binding of general transcription factors
- TATA box - bind TBP
- B recognition element
- Inr ('initiator element)

- DNA to which transcription factors bind often includes inverted repeats.


Transcription factors
TBP
RNA polymerase
5’
3’
Upstream enhancer
TATA box
Gene


5’
3’
TATA box
Gene

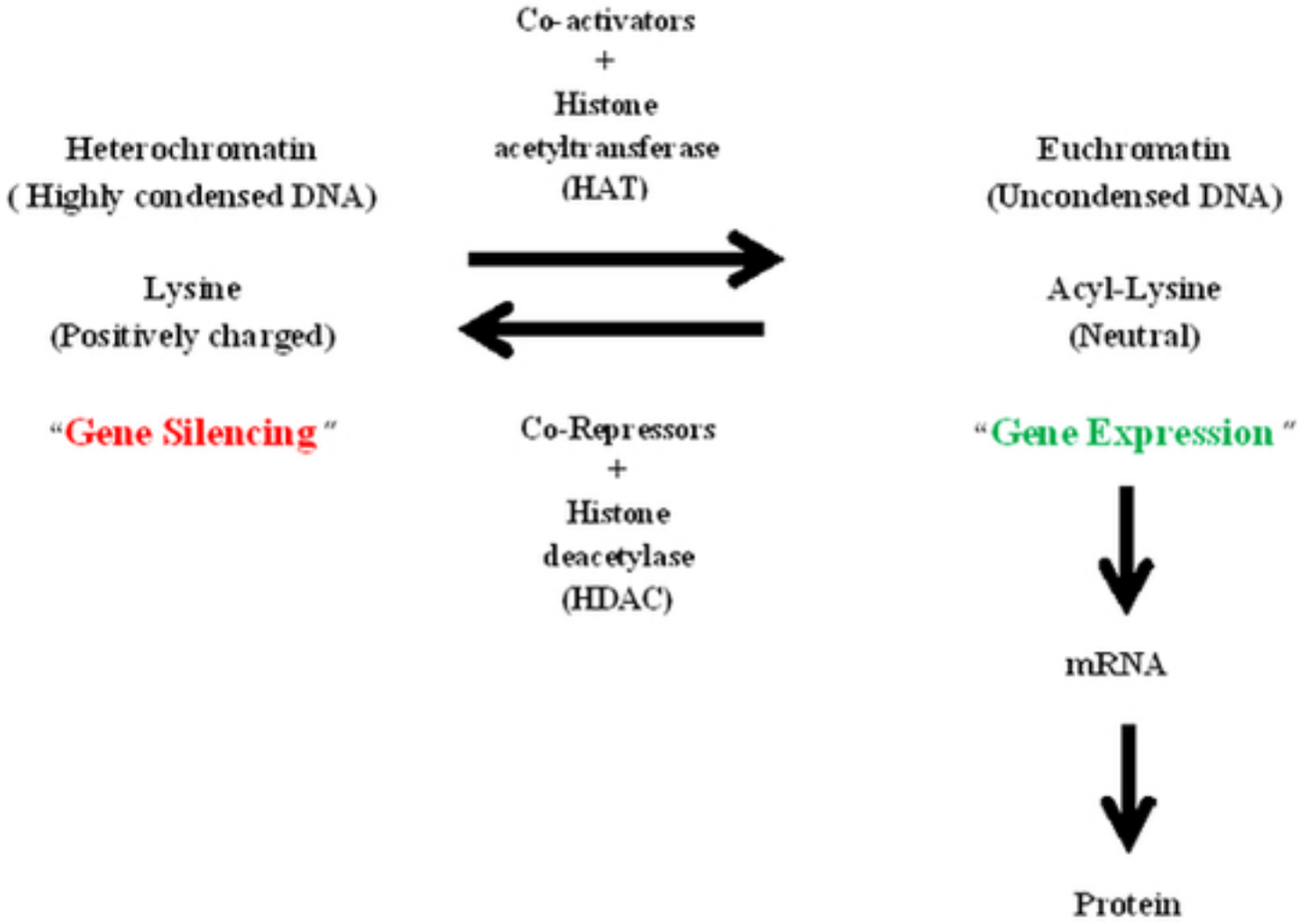


high pI · lots of lysine

$Lys-NH_3^+$ ⟵ histone deacetylase / ⟶ histone acetyl transferase ⟶ $Lys-NH-\overset{O}{\overset{\|}{C}}-CH_3$

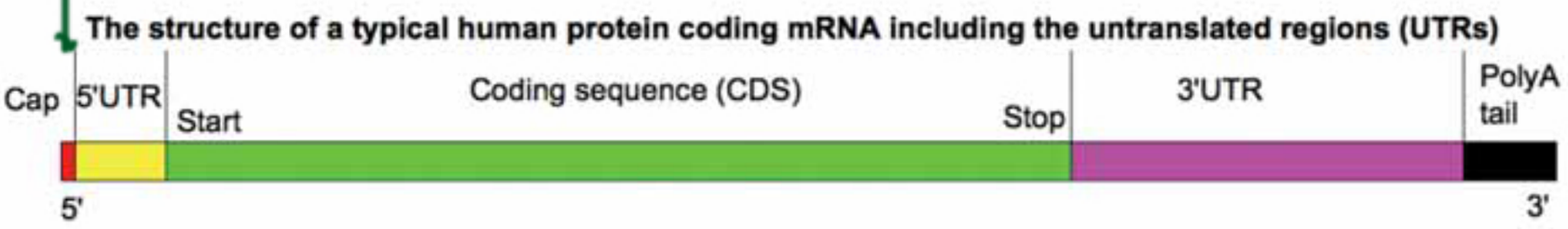
heterochromatin

euchromatin

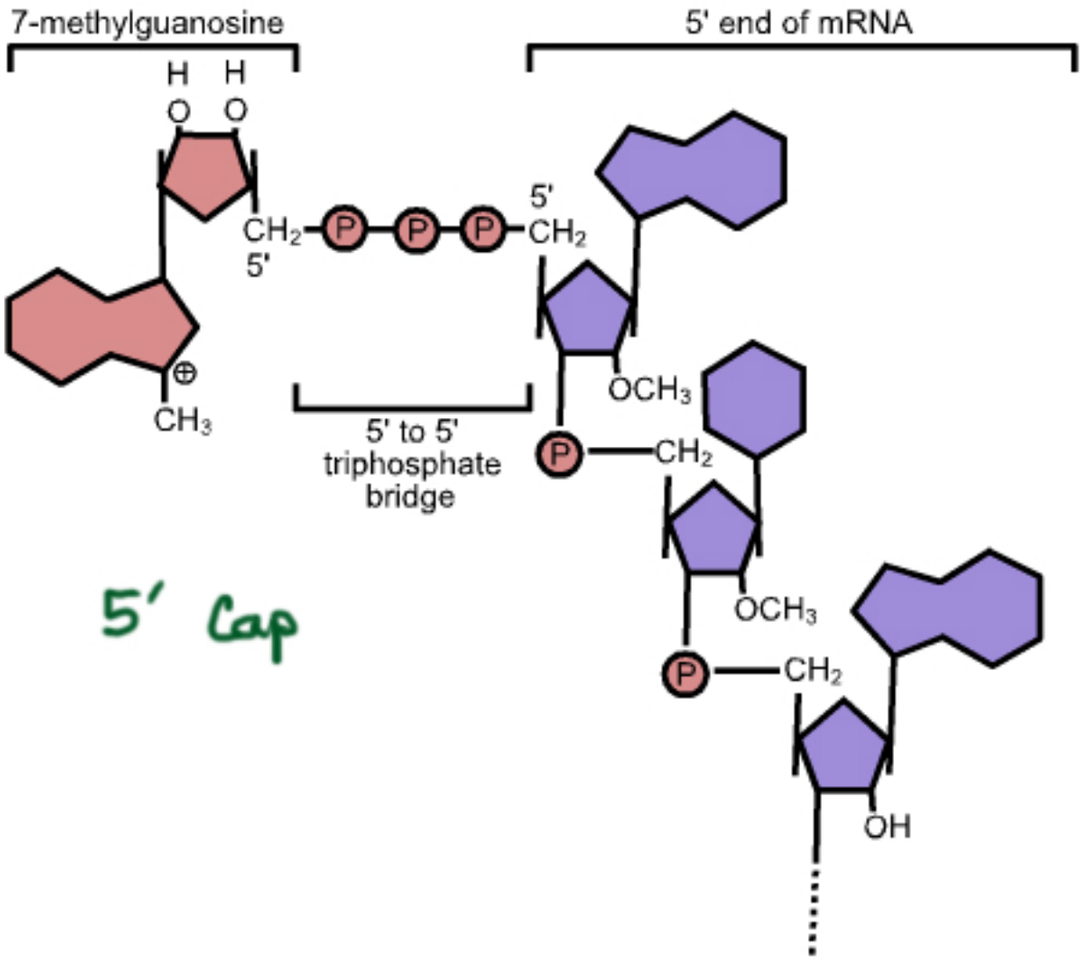

Co-activators
+
Histone
acetyltransferase
(HAT)
Heterochromatin
(Highly condensed DNA)
Euchromatin
(Uncondensed DNA)
Lysine
(Positively charged)
Acyl-Lysine
(Neutral)
"Gene Silencing"
Co-Repressors
+
Histone
deacetylase
(HDAC)
"Gene Expression"
mRNA
Protein

triphosphate bridge + methylguanosine

mRNA

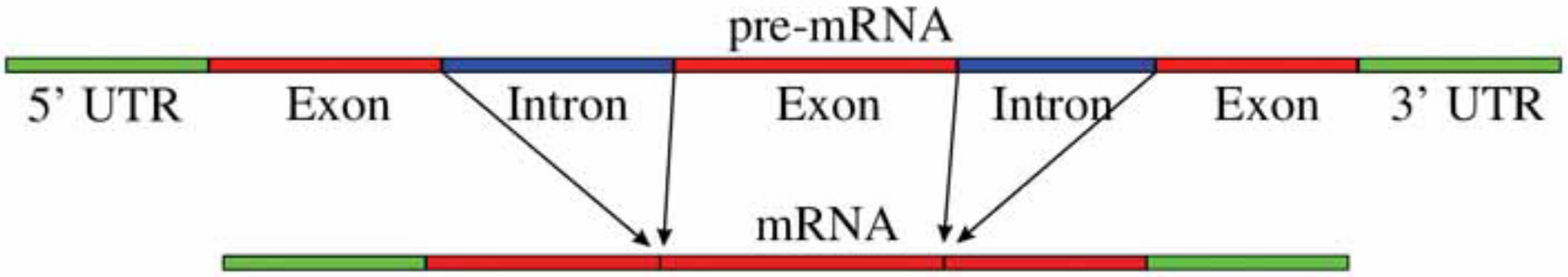


primary RNA transcript → 5' cap, poly A tail, splicing → mRNA

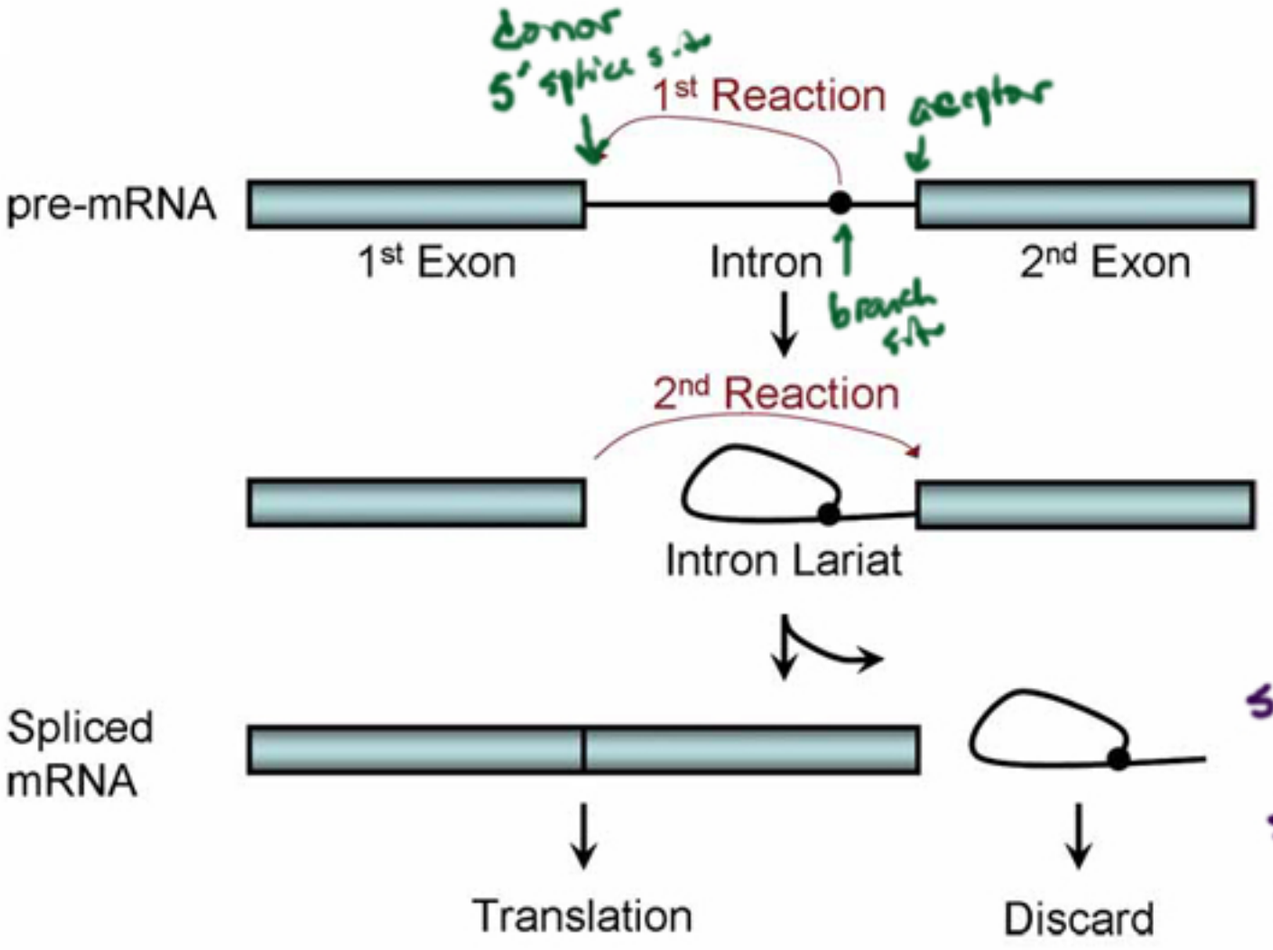

7-methylguanosine
5' end of mRNA
H H
O O
CH2
5'
P P P
5'
CH2
CH3
5' to 5'
triphosphate
bridge
OCH3
P
CH2
OCH3
P
CH2
OH

5′ Cap

Splicing



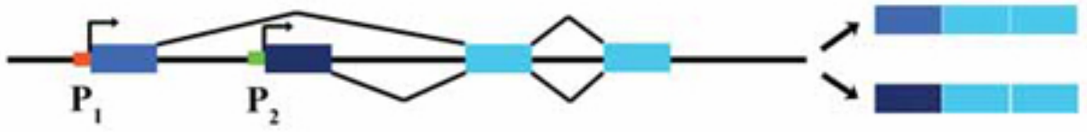
introns are excised



Transesterification reactions catalyzed by spliceosome
- complex of snRNPs
snRNA + protein

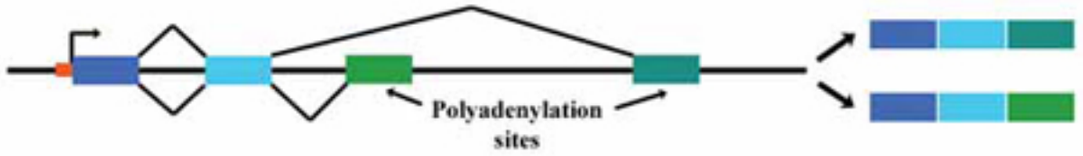
self splicing introns also exist

(a) Alternative selection of promoters (e.g., *myosin* primary transcript)



Alternative Splicing

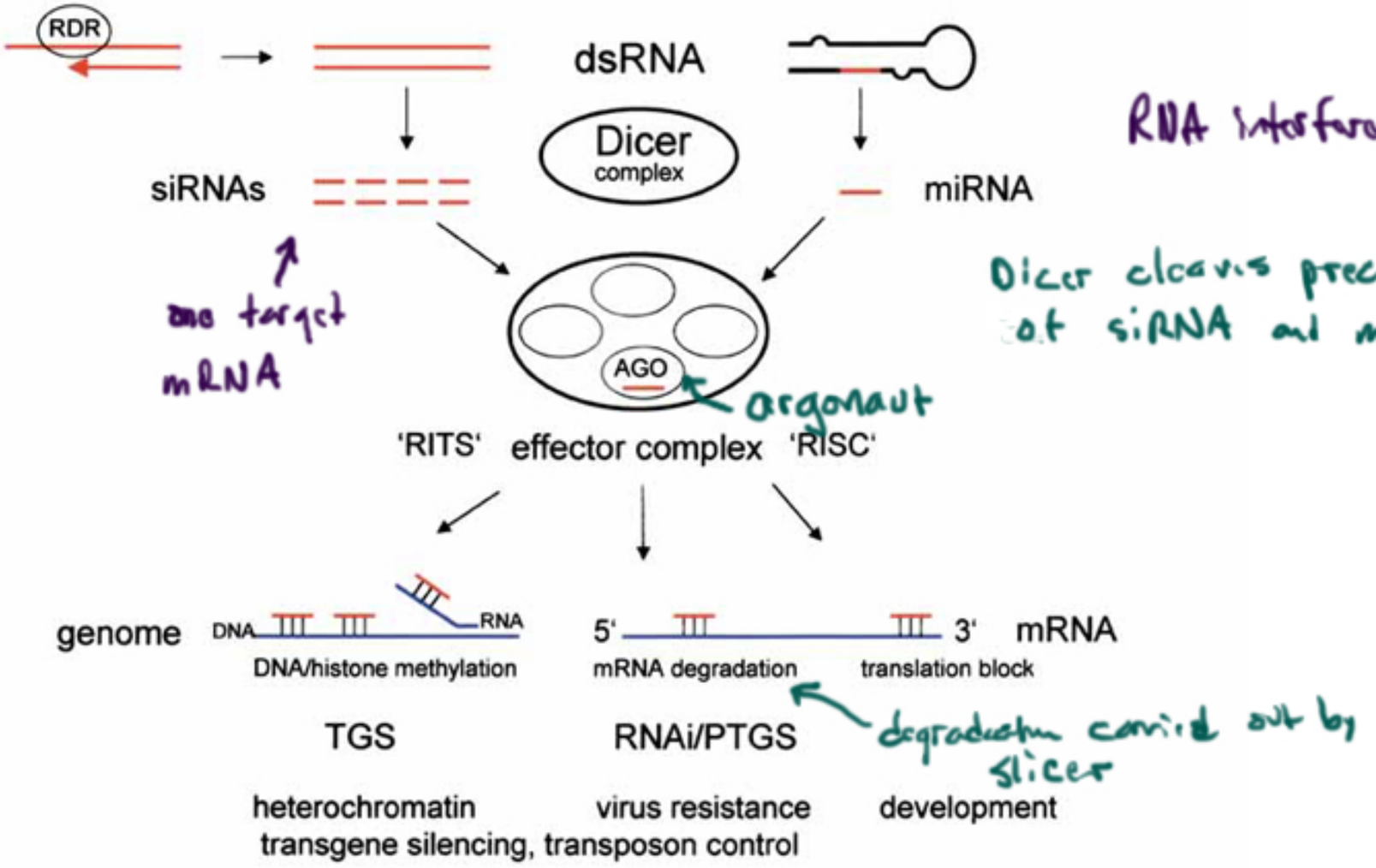
(b) Alternative selection of cleavage/polyadenylation sites (e.g., tropo*myosin* transcript)



(c) Intron retaining mode (e.g., *transposase* primary transcript)

(d) Exon cassette mode (e.g., *troponin* primary transcript)

different modes are supplement



RNA interference

Dicer cleaves precursor of siRNA and miRNA

one target mRNA

argonaut

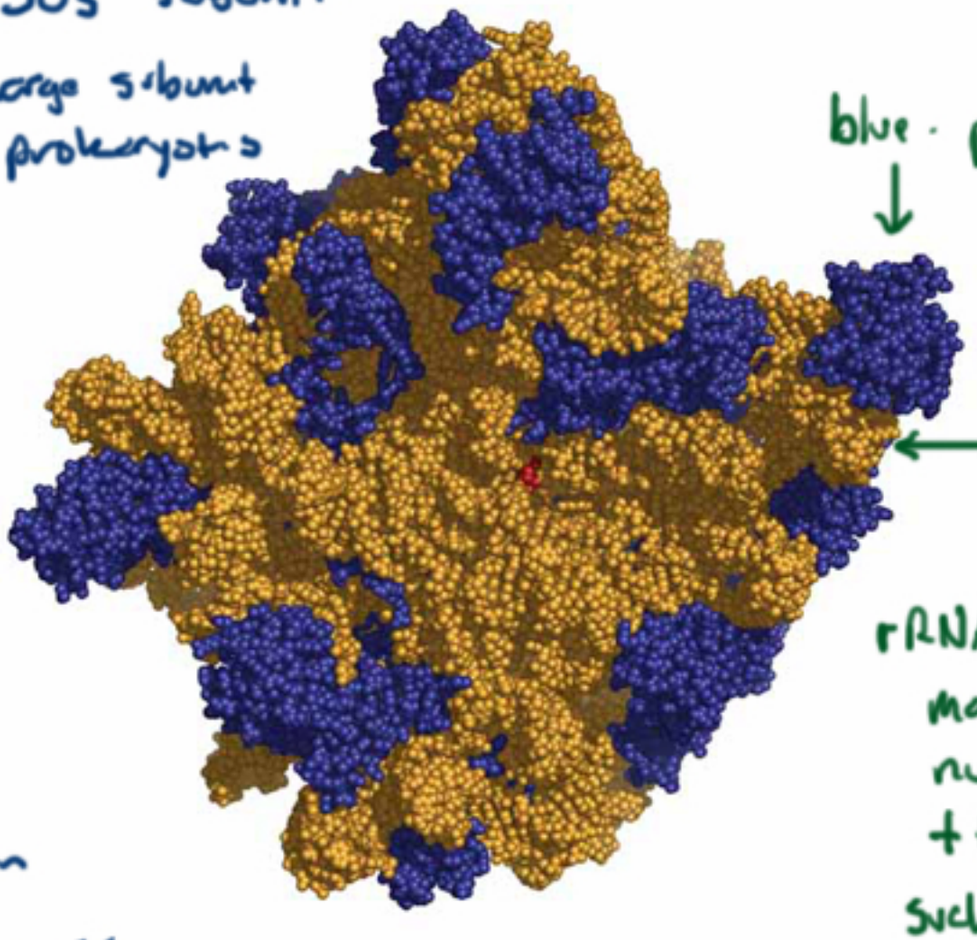
degradation carried out by slicer

50s
30s
70s
↑

Svedberg coefficient
- "sedimentation coefficient"
speed of migration under centrifugation
- logarithmic with mass.

eukaryotes 60s
40s

50s subunit
Large subunit prokaryotes



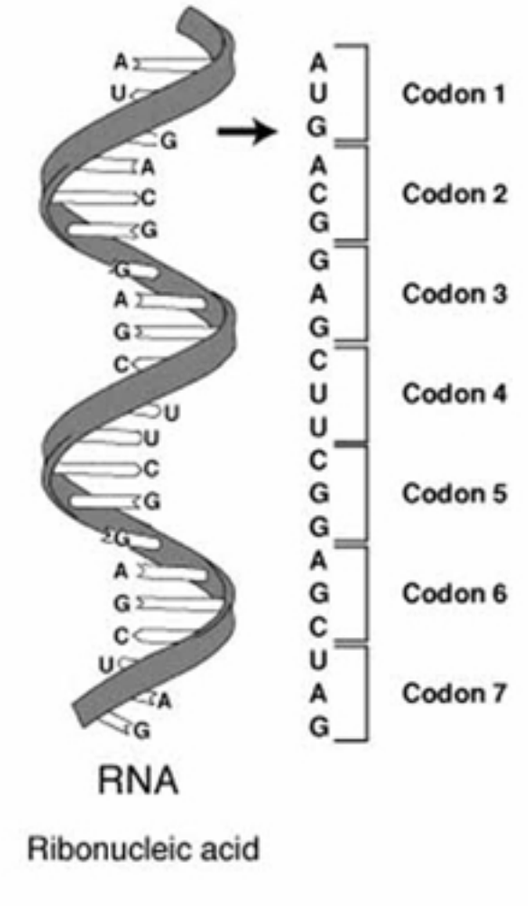
blue - protein
↓
← orange rRNA

rRNA -
may modified nucleobases (like +RNA)
such as pseudouridine (N & C switched)

ribozyme - RNA enzyme
peptidyl transferase is a ribozyme

First Base	Second Base: U	Second Base: C	Second Base: A	Second Base: G	Third Base
U	UUU, UUC — Phe	UCU, UCC, UCA, UCG — Ser	UAU, UAC — Tyr	UGU, UGC — Cys	U
	UUA, UUG — Leu		UAA, UAG — STOP	UGA — STOP; UGG — Trp	C, A, G
C	CUU, CUC, CUA, CUG — Leu	CCU, CCC, CCA, CCG — Pro	CAU, CAC — His; CAA, CAG — Gln	CGU, CGC, CGA, CGG — Arg	U, C, A, G
A	AUU, AUC, AUA — Ile	ACU, ACC, ACA, ACG — Thr	AAU, AAC — Asn; AAA, AAG — Lys	AGU, AGC — Ser; AGA, AGG — Arg	U, C, A, G
	AUG — Met or Start				
G	GUU, GUC, GUA, GUG — Val	GCU, GCC, GCA, GCG — Ala	GAU, GAC — Asp; GAA, GAG — Glu	GGU, GGC, GGA, GGG — Gly	U, C, A, G

wobble base



64 possible codons

61 code amino acids

3 stop - UAA, UGA, UAG

There are only 20 amino acids

- no punctuation
- degenerate - not specific from protein sequence → RNA
- universal

- Consequences of alteration
  - silent mutation
  - missense mutation
  - nonsense mutation
    - stop
  - Others - trinucleotide repeat
    - loop out structure
    - also in repair
  - splice site mutation
  - frameshift

tRNA

- modified nucleobases

aminoacyl attachment site

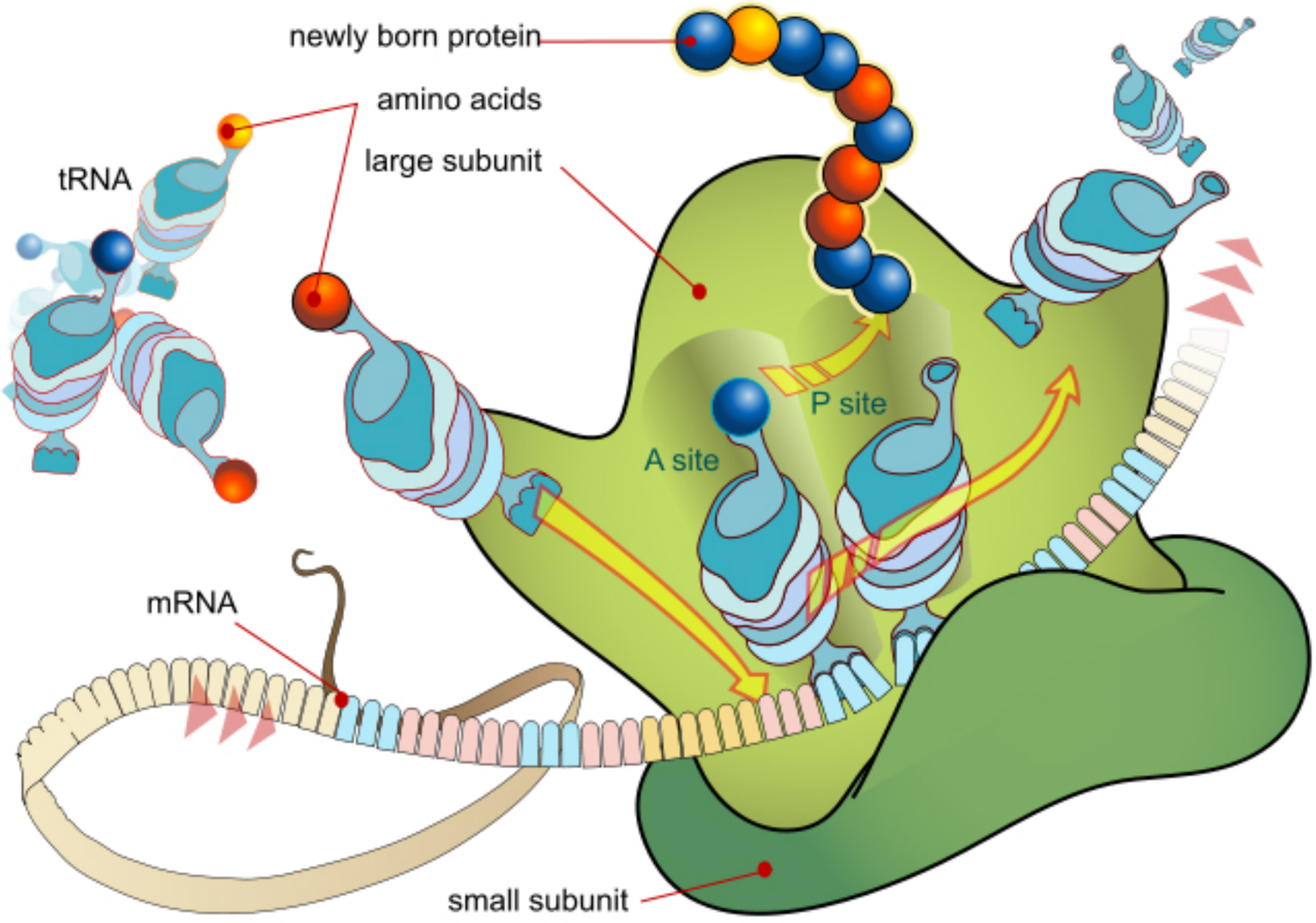
aminoacyl tRNA synthetase

there are a bunch of these - also has proofreading and editing

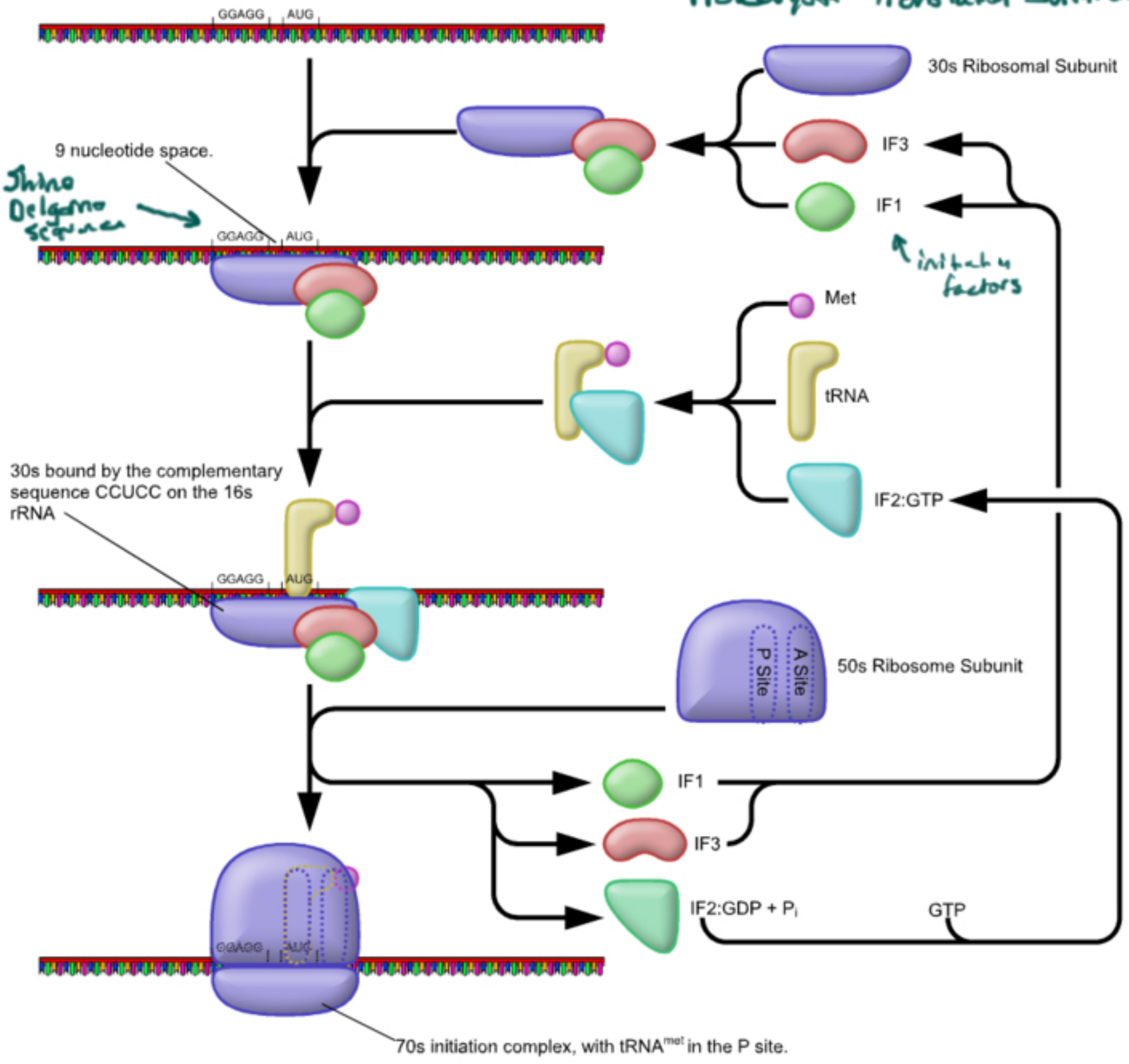
anticodon

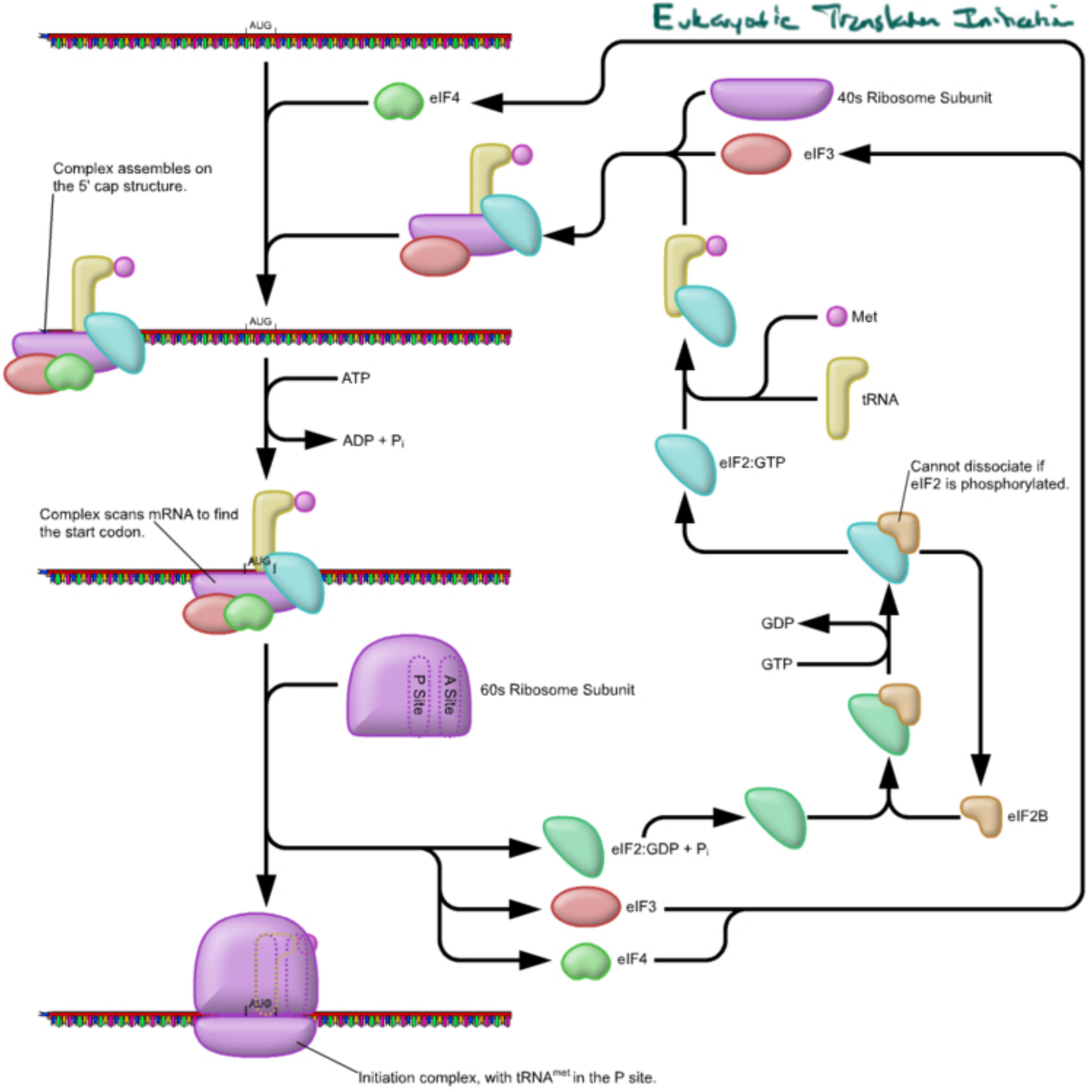
antiparallel binding

You still express tRNA sequence 5' → 3'. It will read in reverse of the mRNA sequence.

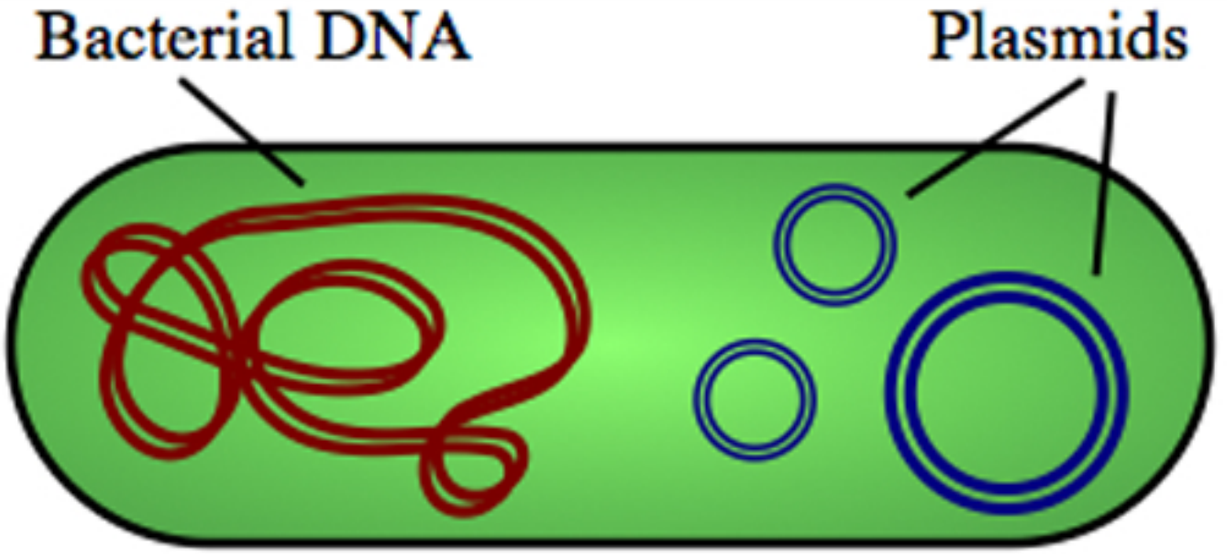

newly born protein
amino acids
large subunit
tRNA
P site
A site
mRNA
small subunit

Prokaryotic Translation Initiation




Eukaryotic Translation Initiation
AUG
eIF4
40s Ribosome Subunit
eIF3
Complex assembles on the 5' cap structure.
Met
tRNA
ATP
ADP + Pi
eIF2:GTP
Cannot dissociate if eIF2 is phosphorylated.
Complex scans mRNA to find the start codon.
GDP
GTP
A Site
P Site
60s Ribosome Subunit
eIF2B
eIF2:GDP + Pi
eIF3
eIF4
Initiation complex, with tRNA^met in the P site.

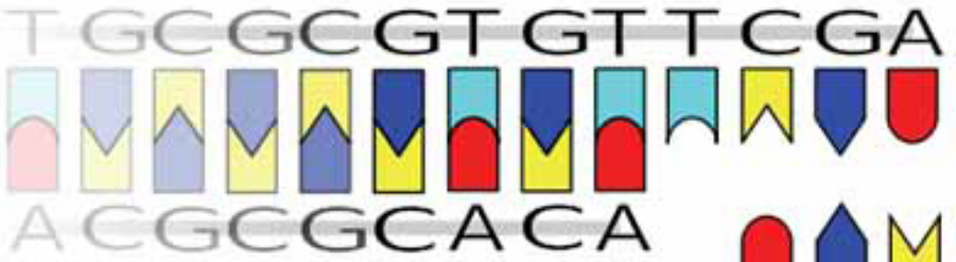
DNA Lab



Cohen ad Boyer

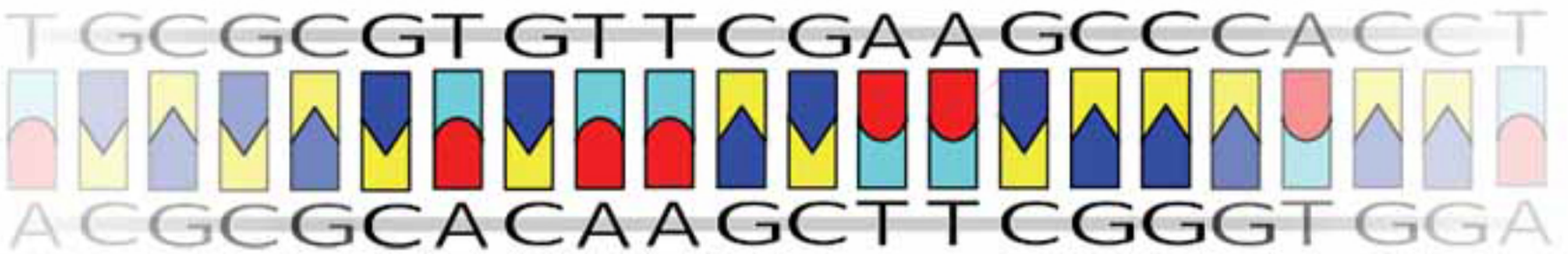
Transformed bacteria with recombinant plasmid.

Using restriction enzyme - (restriction endonuclease)

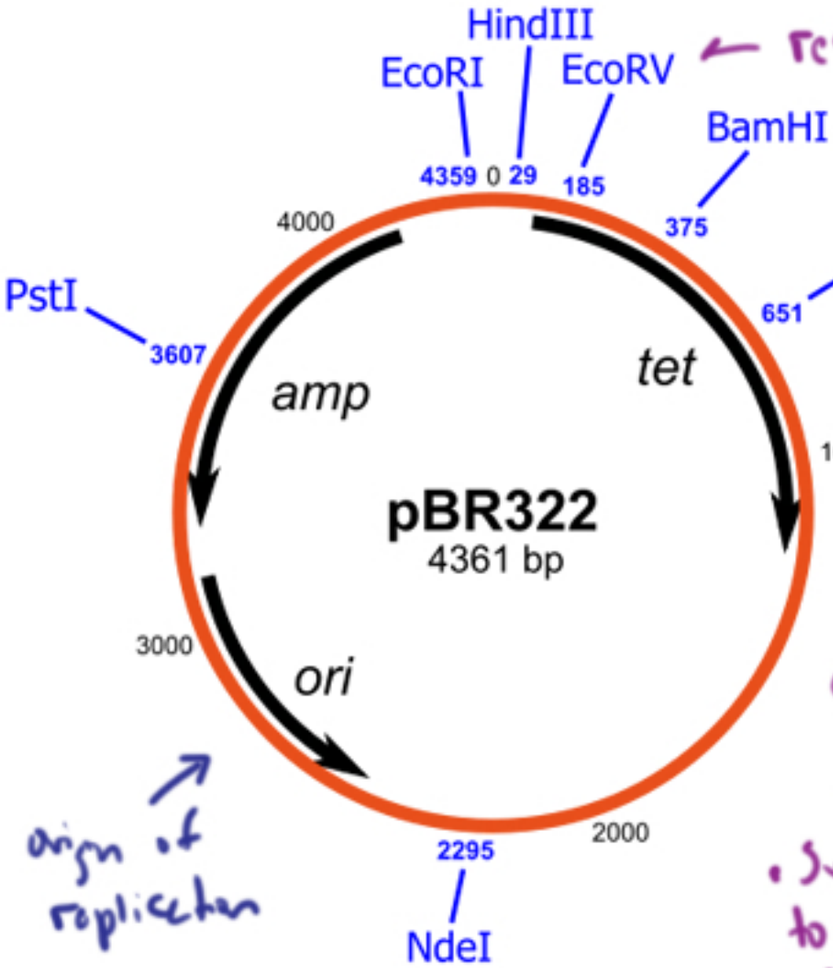


- 2 fold palindromic symmetry
- sticky ends are identical

sticky end



Any DNA cleaved by same restriction enzyme may ligated.

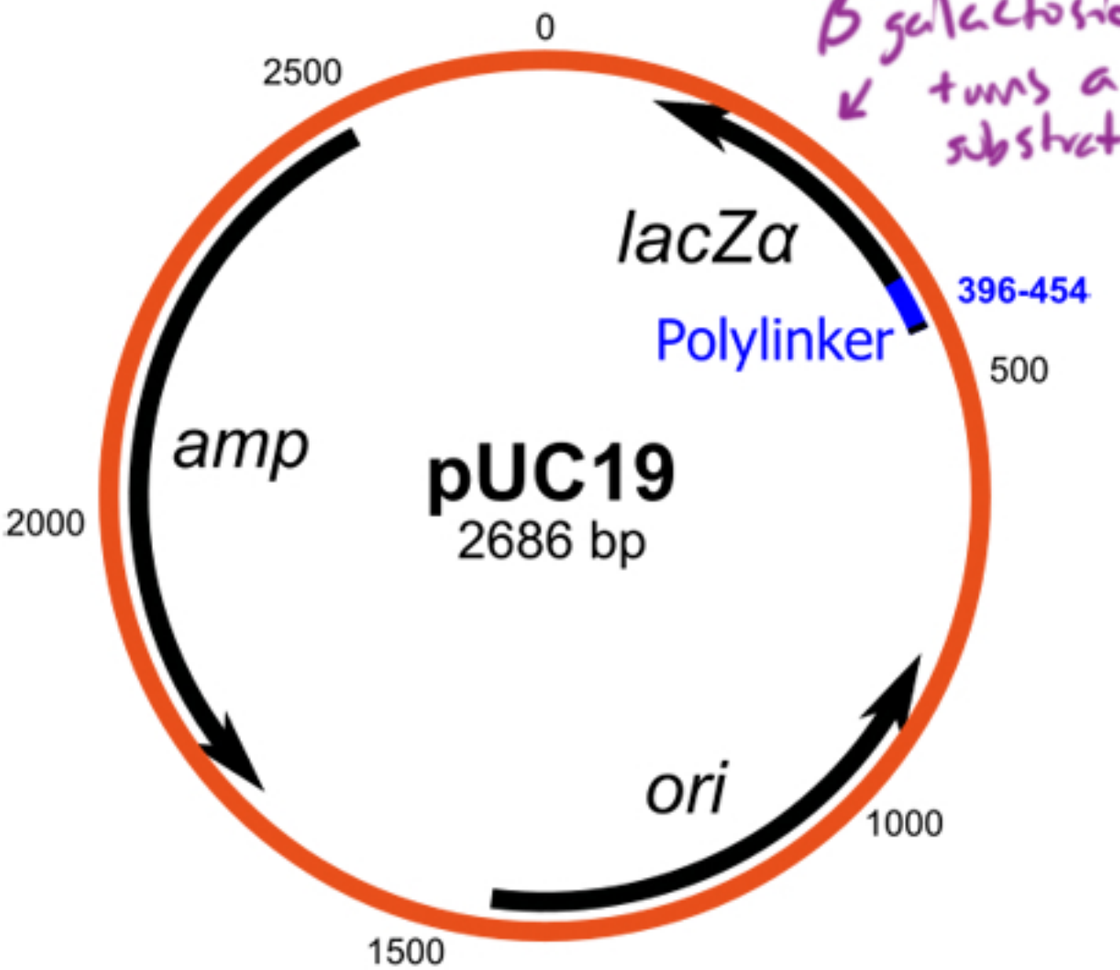


← restriction sites

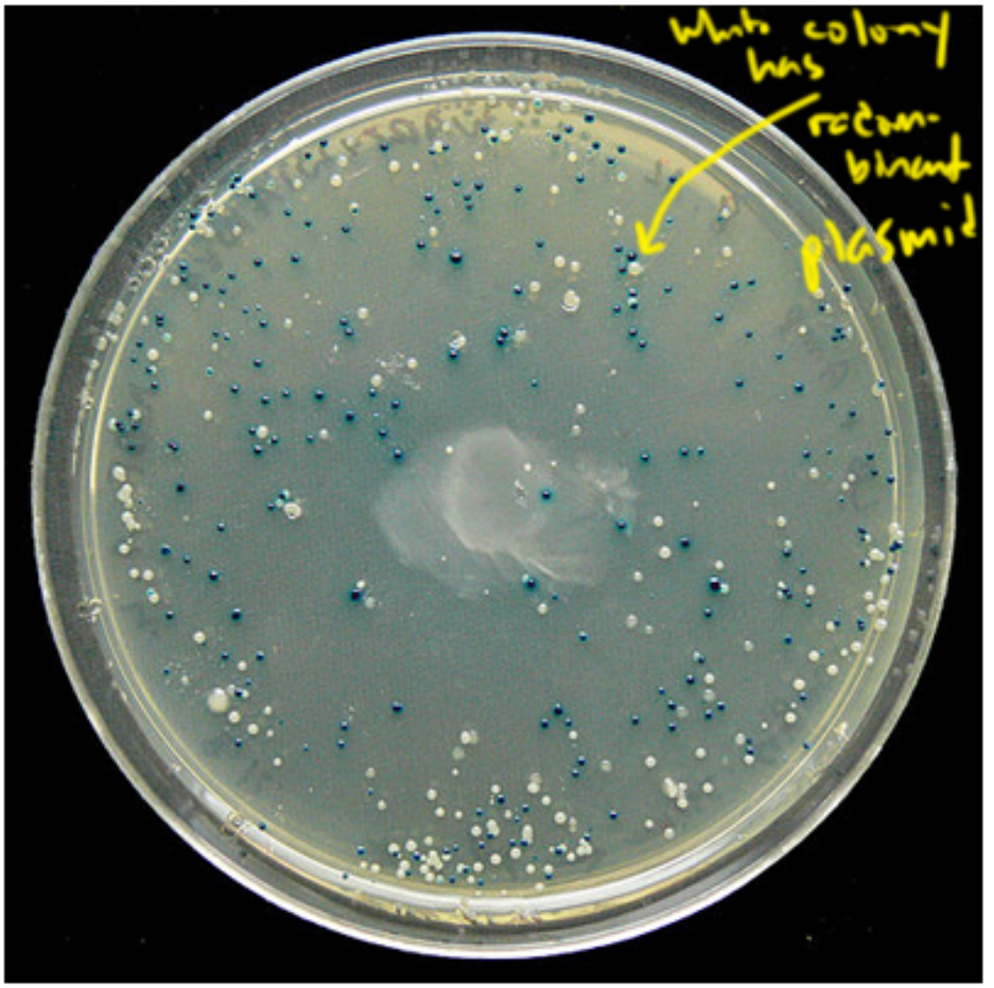
origin of replication

amp & tet antibiotic resistance genes for screening

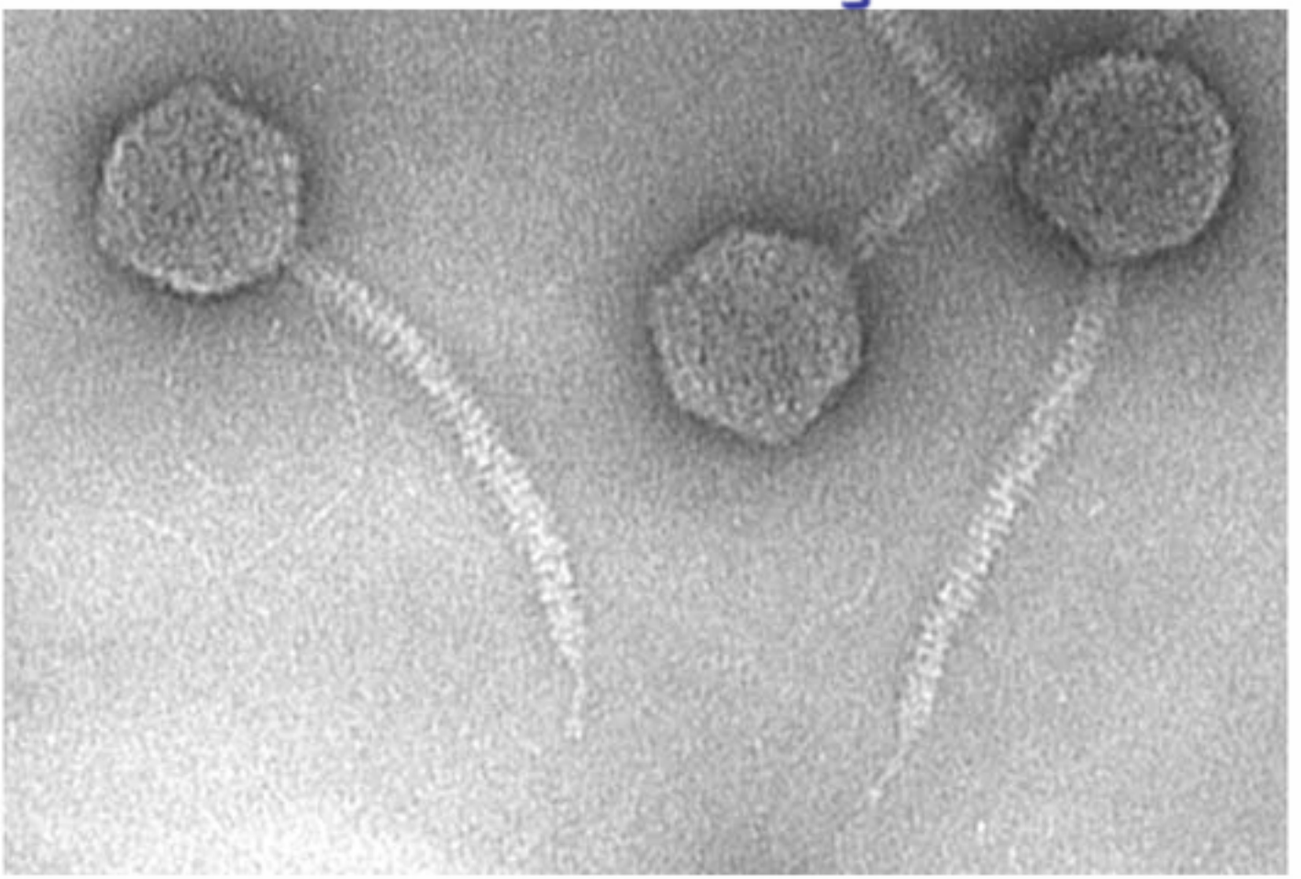
- Suppose we used BamHI to form recombinant plasmids and transform bacteria.
- Is a colony ampicillin resistant? It received plasmid
- Is tetracycline resistance missing? The plasmid was recombinant.



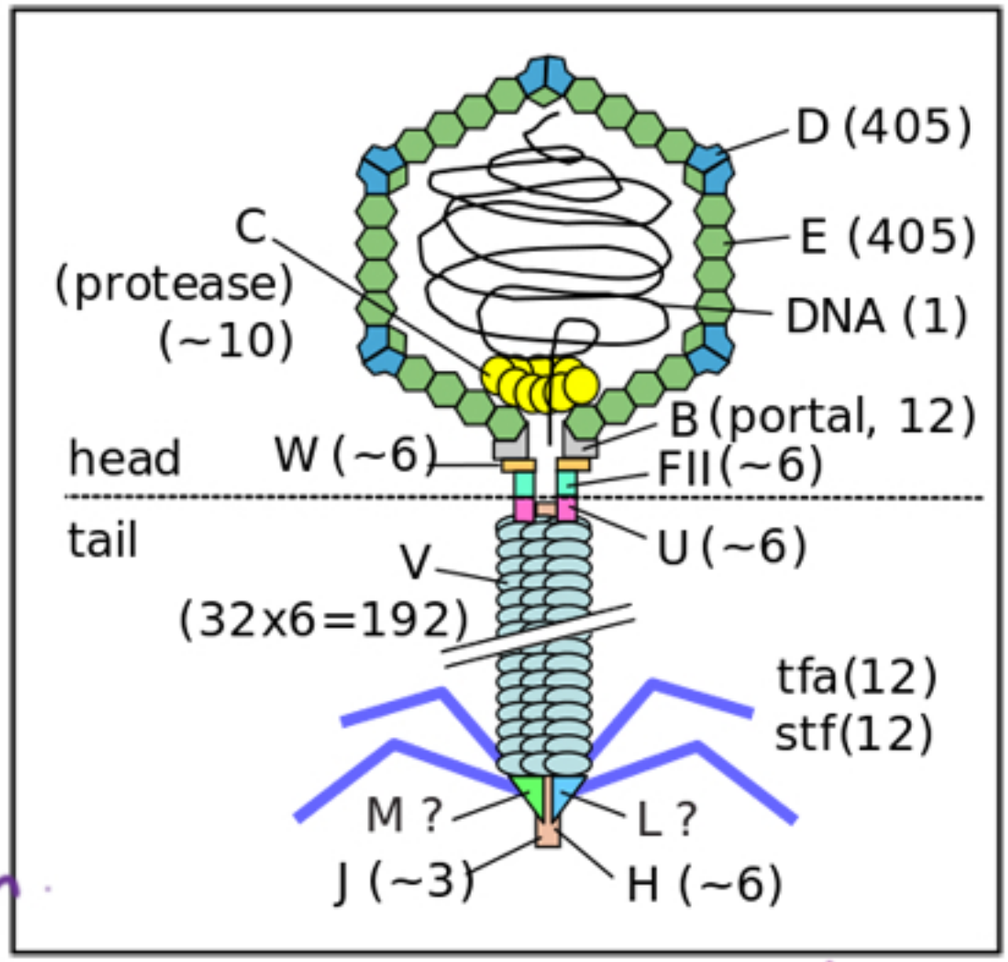
β galactosidase
↙ turns a special substrate blue


white colony has recombinant plasmid

Lambda Phage



λ Phage



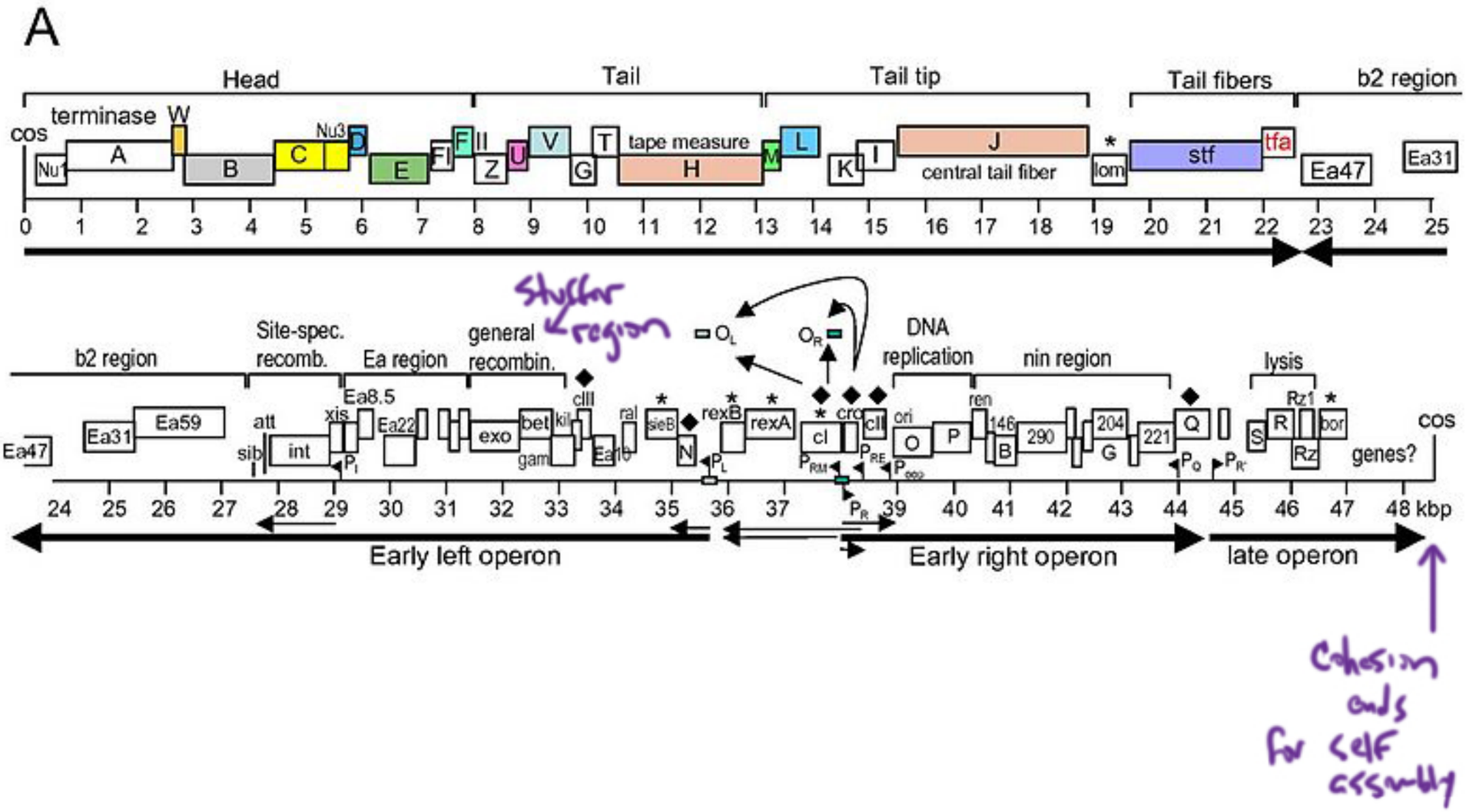
λ phage can accomodate several tens of thousands of BP DNA in its stuffer region.

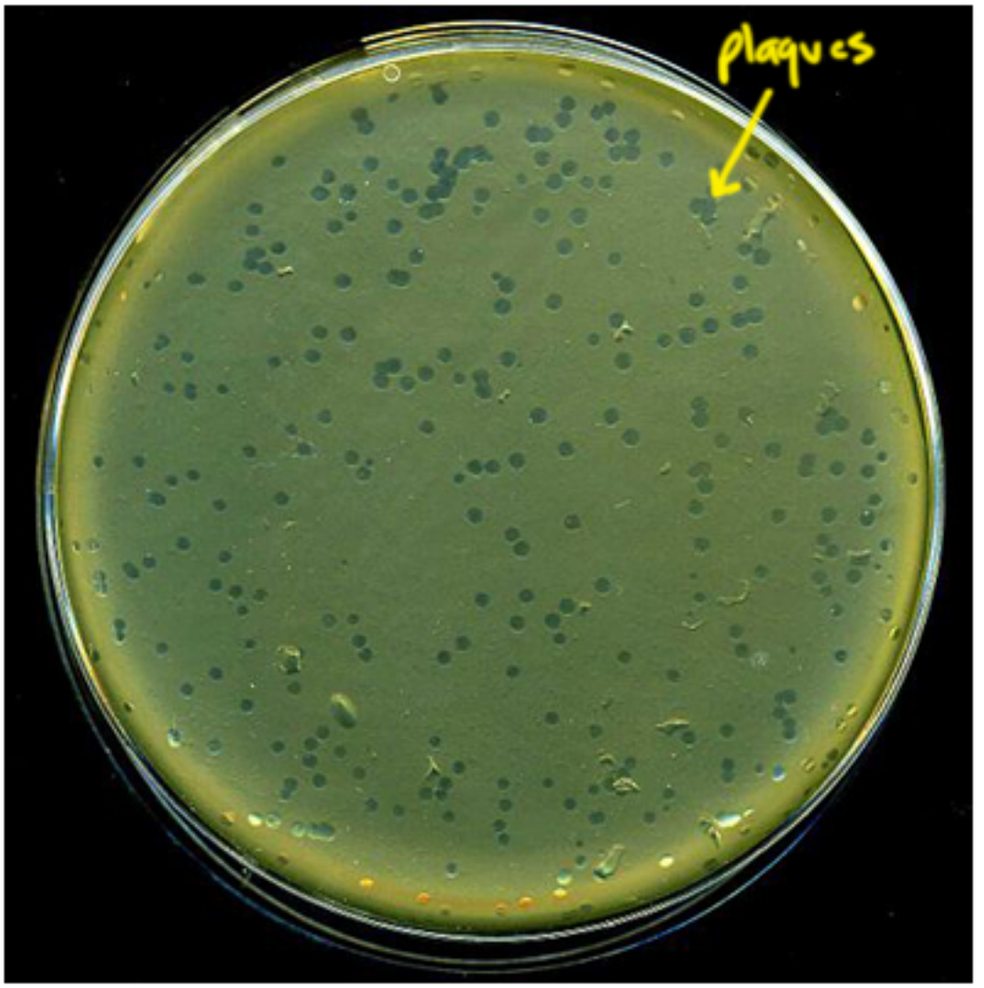
The virus will self assemble as a recombinant

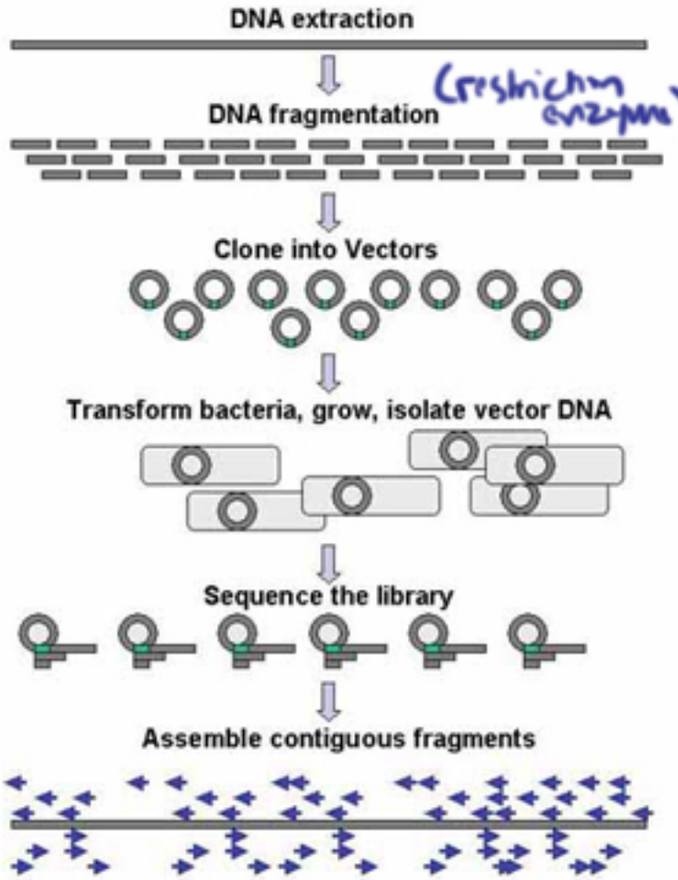
Important note - viral self-assembly with a phage sections of DNA called cohesion ends (cos)

Certain capsid proteins recognize and bind cohesion ends and nucleate self-assembly

Figure 1.


A
Head
Tail
Tail tip
Tail fibers
b2 region
cos
terminase
W
Nu1
A
B
Nu3
C
D
E
FI
FII
Z
U
V
G
T
tape measure
H
M
L
K
I
J
central tail fiber
*
lom
stf
tfa
Ea47
Ea31
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
b2 region
Site-spec. recomb.
Ea region
general recombin.
O_L
O_R
DNA replication
nin region
lysis
Ea8.5
cIII
xis
att
sib
int
P_I
Ea22
exo
bet
gam
kil
ral
Ea10
sieB
N
P_L
rexB
rexA
cro
cI
cII
P_{RM}
P_{RE}
P_{aQ2}
ori
O
P
ren
146
B
290
204
G
221
Q
P_Q
$P_{R'}$
S
R
Rz1
Rz
bor
genes?
cos
24 25 26 27 28 29 30 31 32 33 34 35 36 37 39 40 41 42 43 44 45 46 47 48 kbp
P_R
Early left operon
Early right operon
late operon
stuffer region
Cohesive ends for cell assembly


plaques

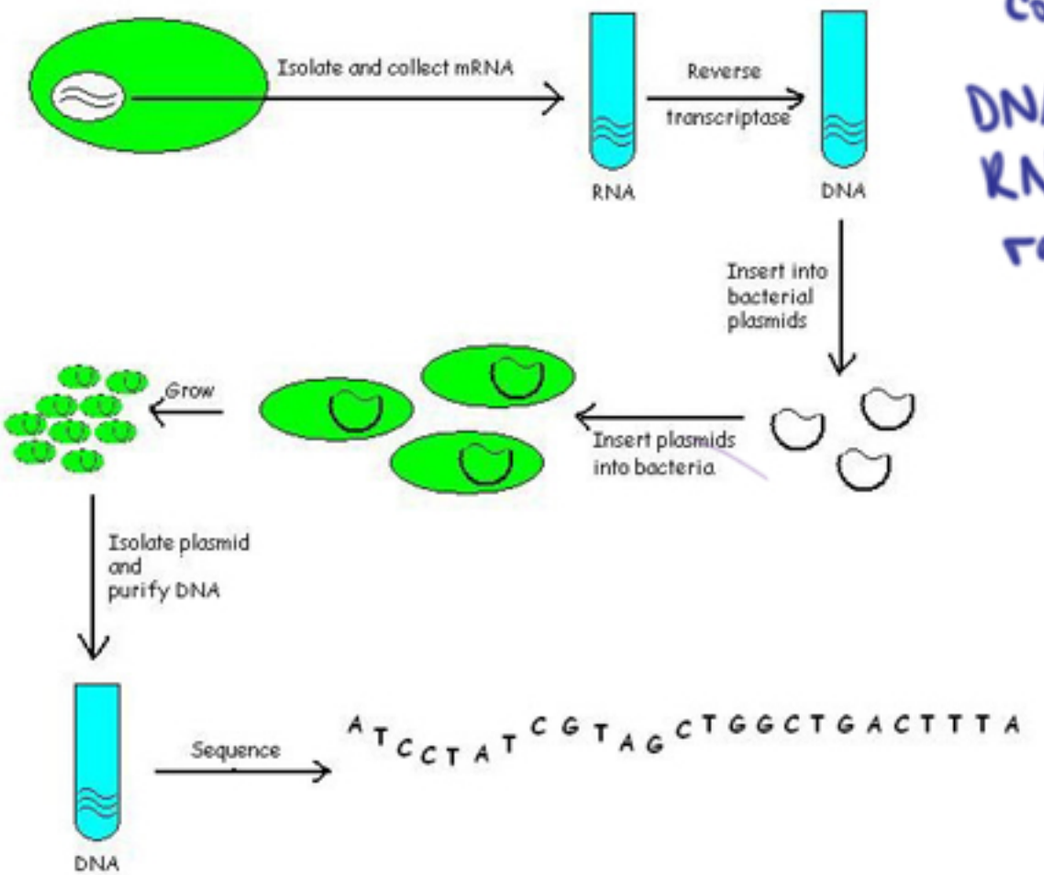


(restriction enzyme)

DNA Library

shotgun sequencing

Formation of a cDNA Library

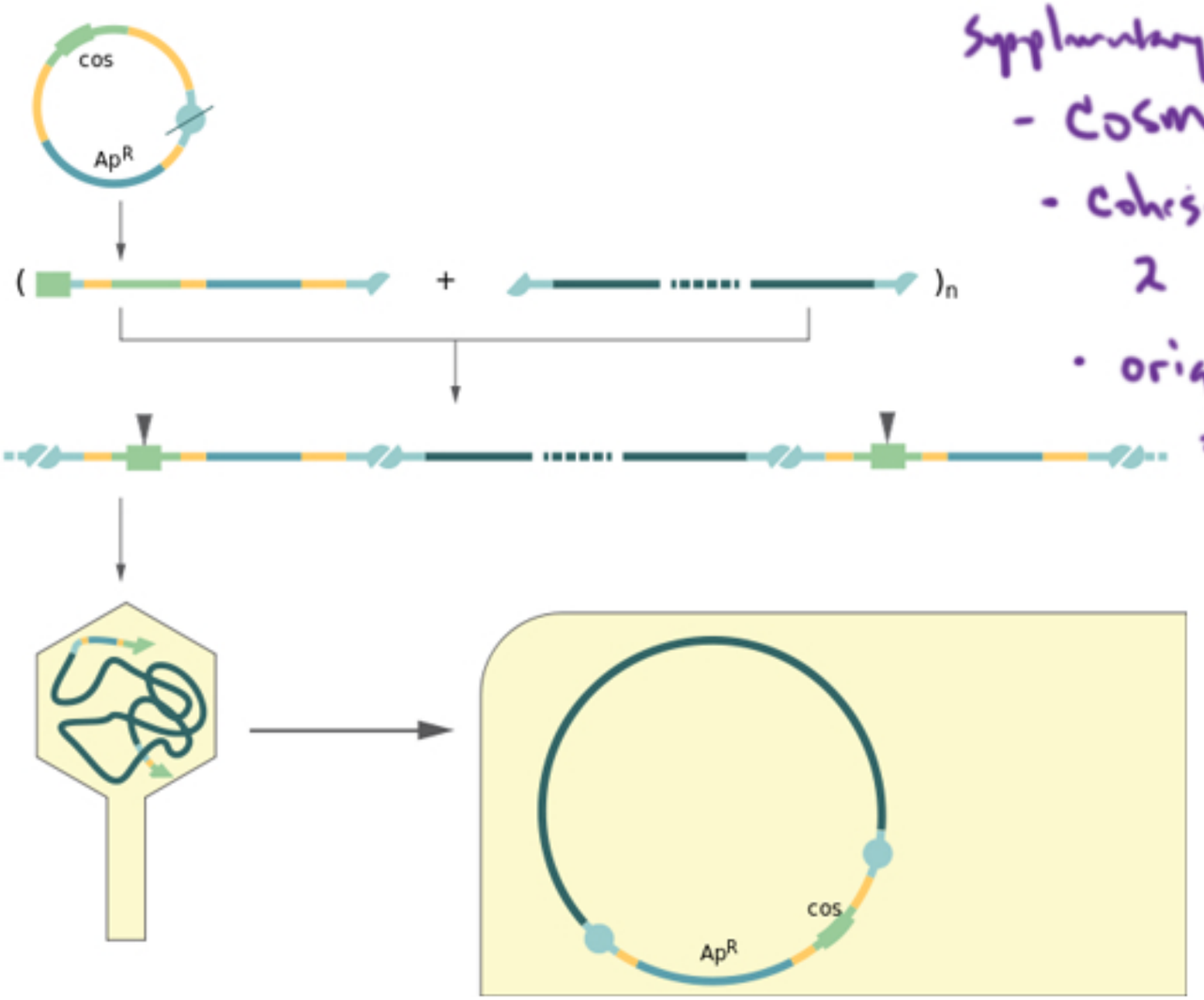


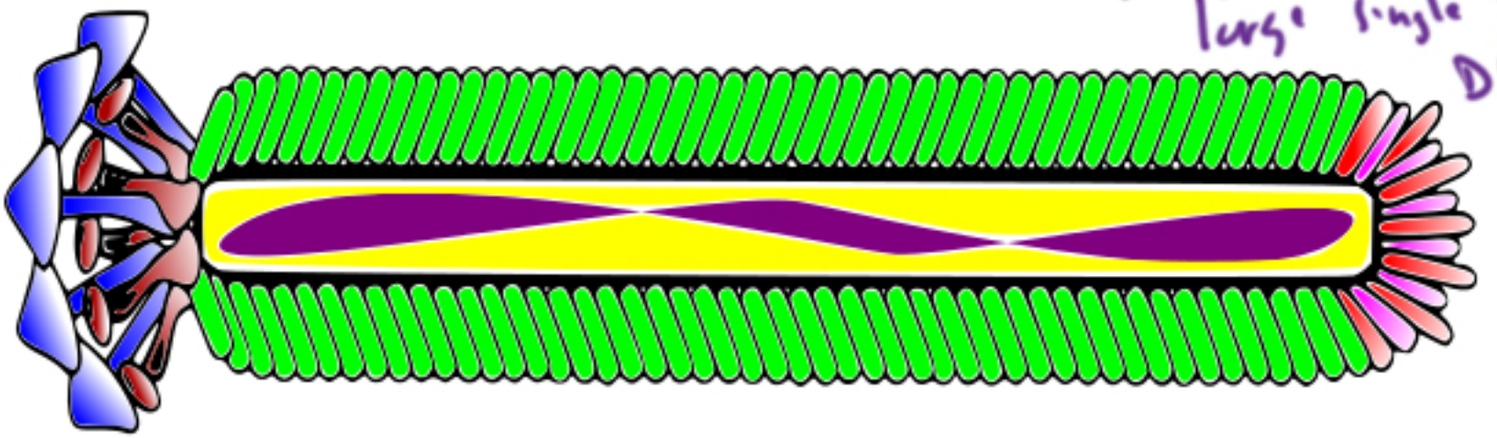
cDNA - Complimentary DNA

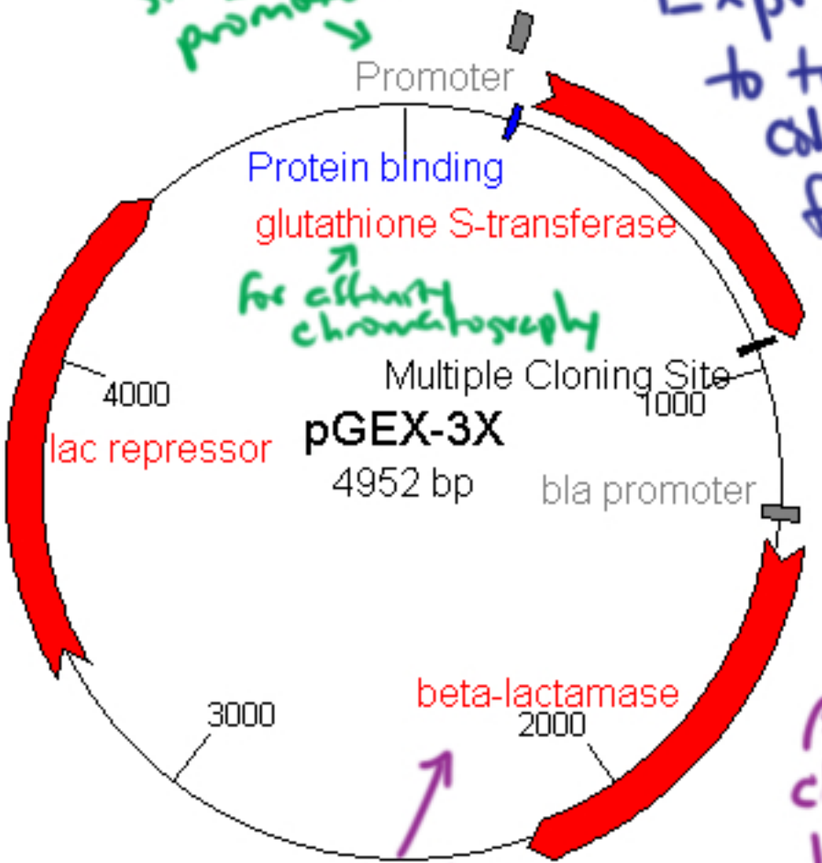
DNA found from RNA using reverse transcriptase

Supplementary

- Cosmid
  - Cohesion ends of λ phage
  - origin of replication of a plasmid.




phagemid -
large single stranded
DNA



strong promoter

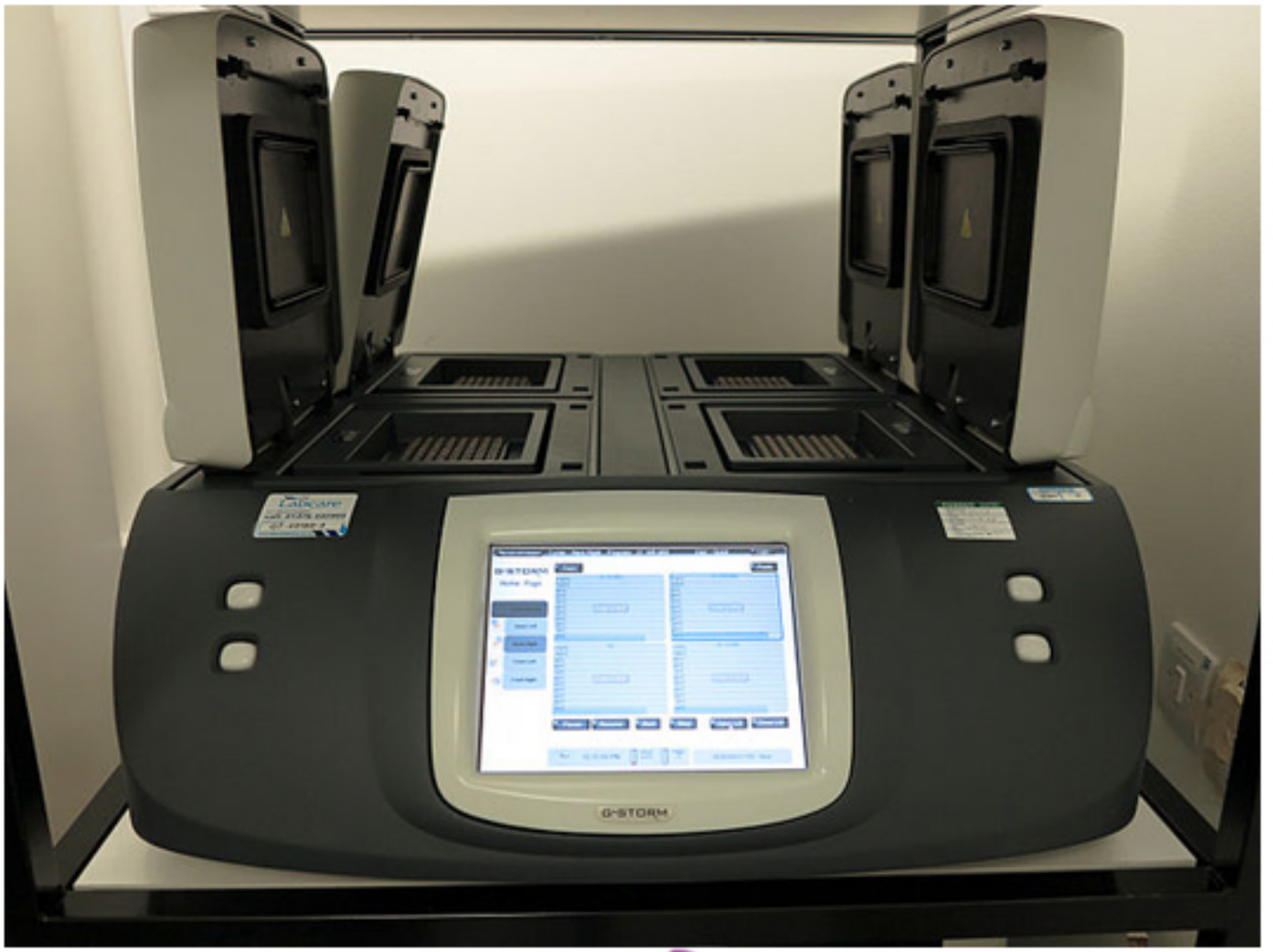
Expression vector to turn a bacterial colony into a protein factory

for affinity chromatography

can control rate with lactose levels

β lactams - class of antibiotics like penicillin

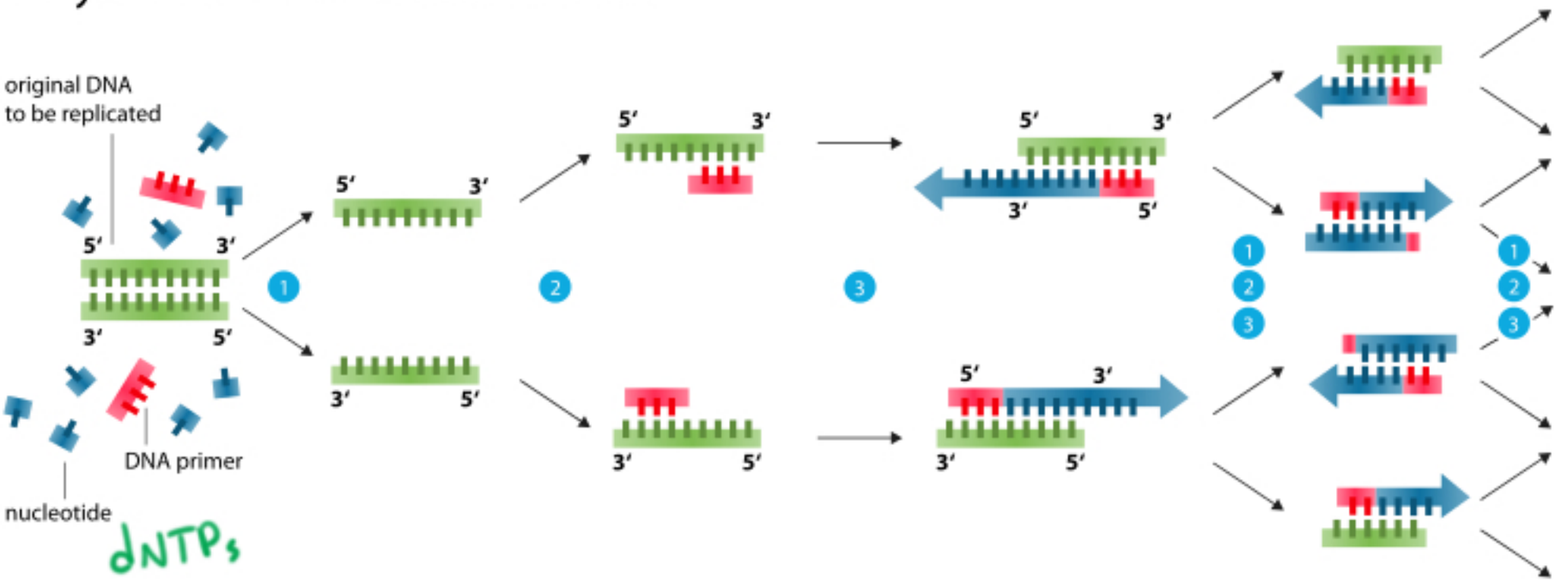
antibiotic resistance for screening



- denature
- anneal primers
- elongate

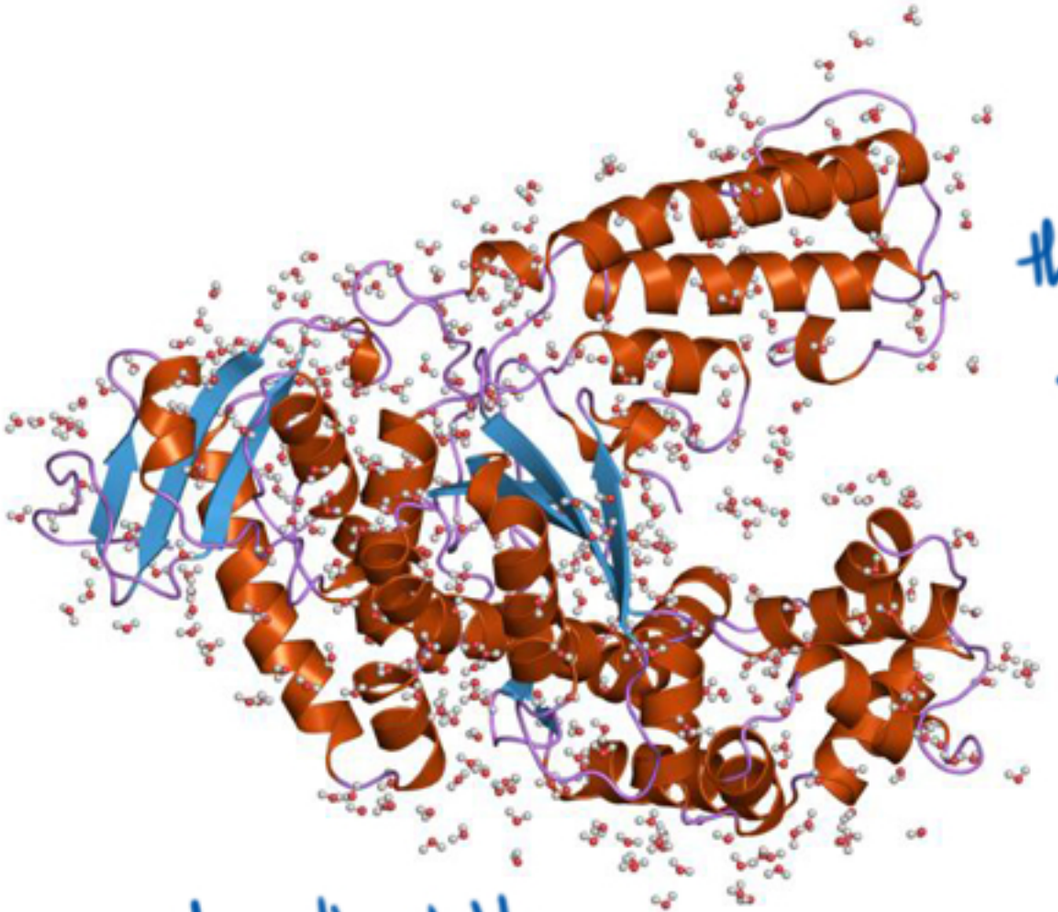
thermal cycler for PCR
(polymerase chain reaction)
to turn a small amount of DNA
into a large amount

Polymerase chain reaction - PCR



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C

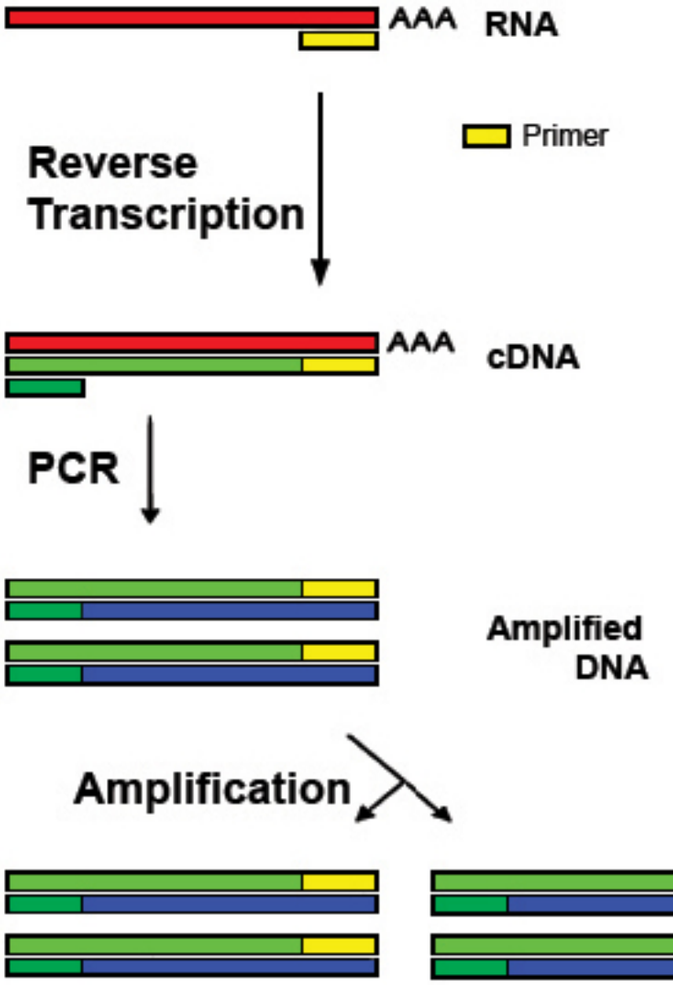
Taq polymerase



Taq thermoaquaticus

- bacteria discovered in oceanic hydrothermal vents

thermally stable DNA polymerase



RT PCR

↑

reverse transcriptase

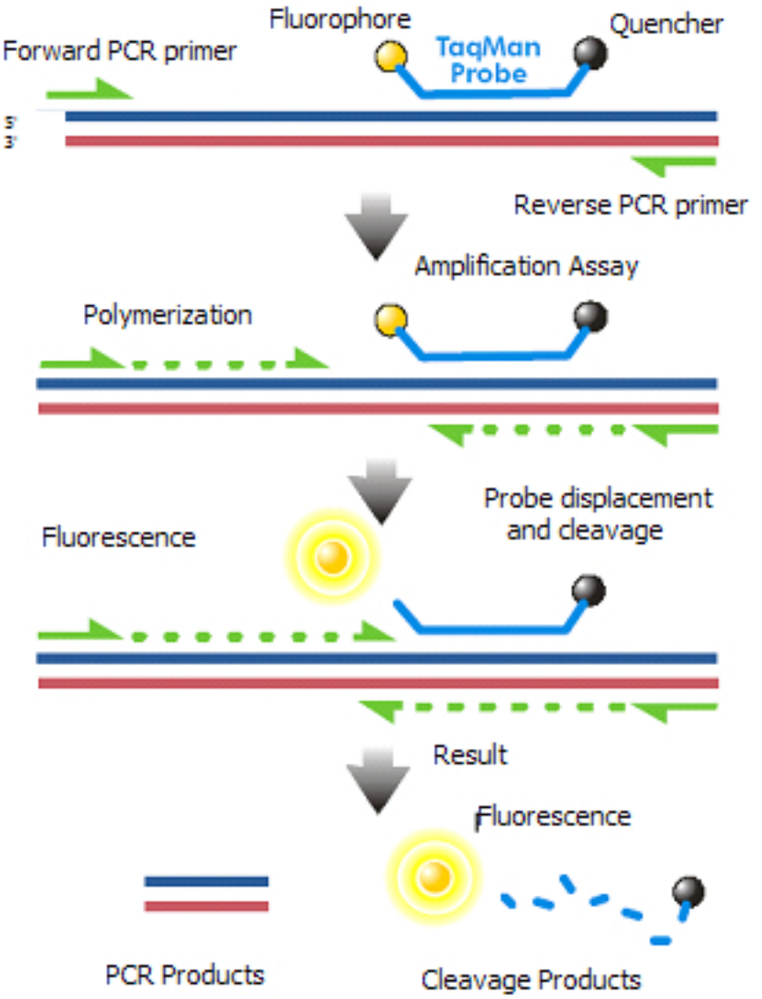
(RT is not 'real time')

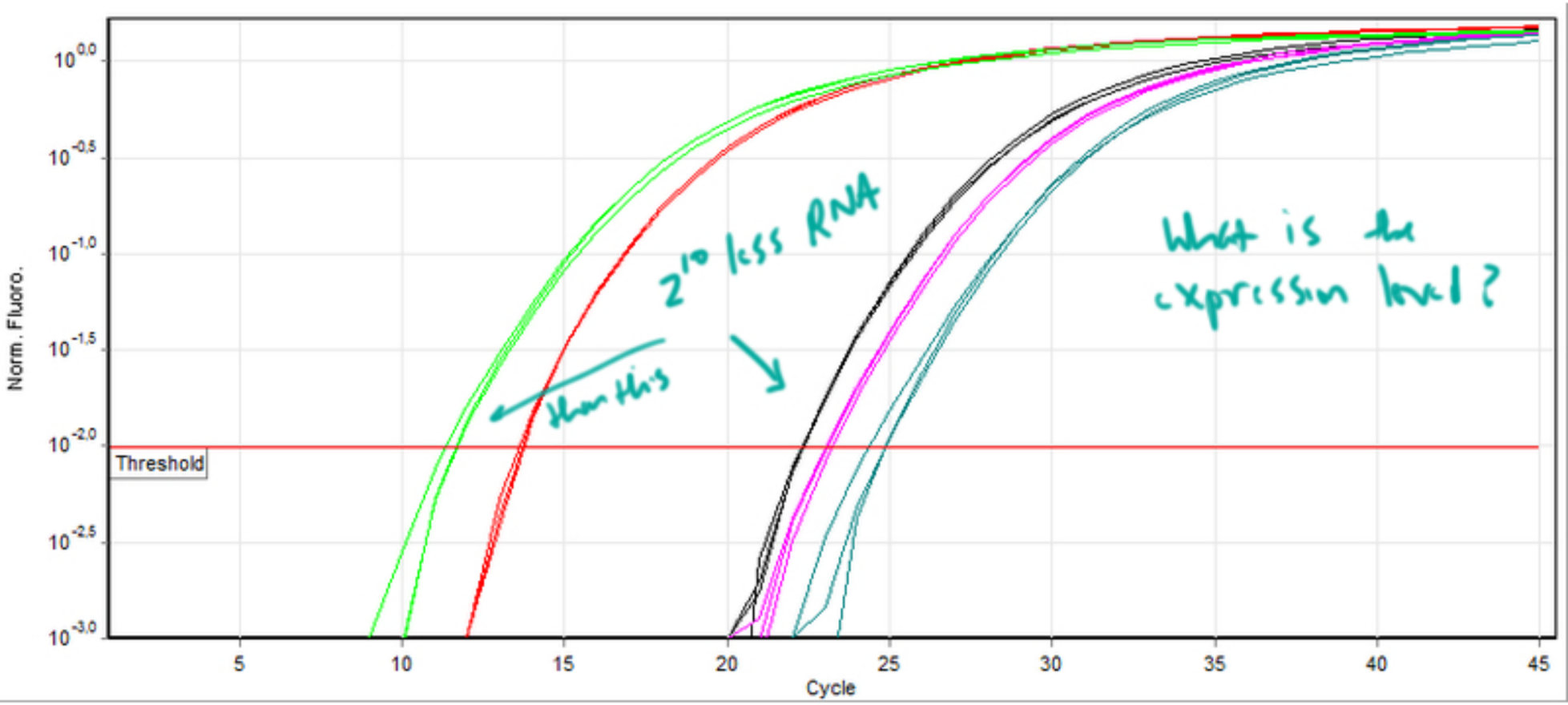
Real Time PCR

"Quantitative PCR"

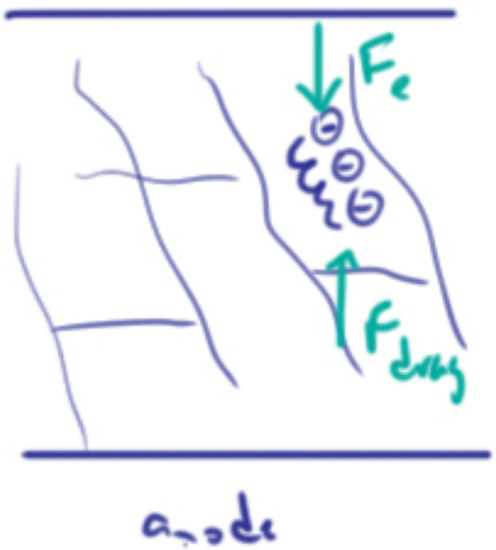
How many cycles does it take to achieve threshold of detection?

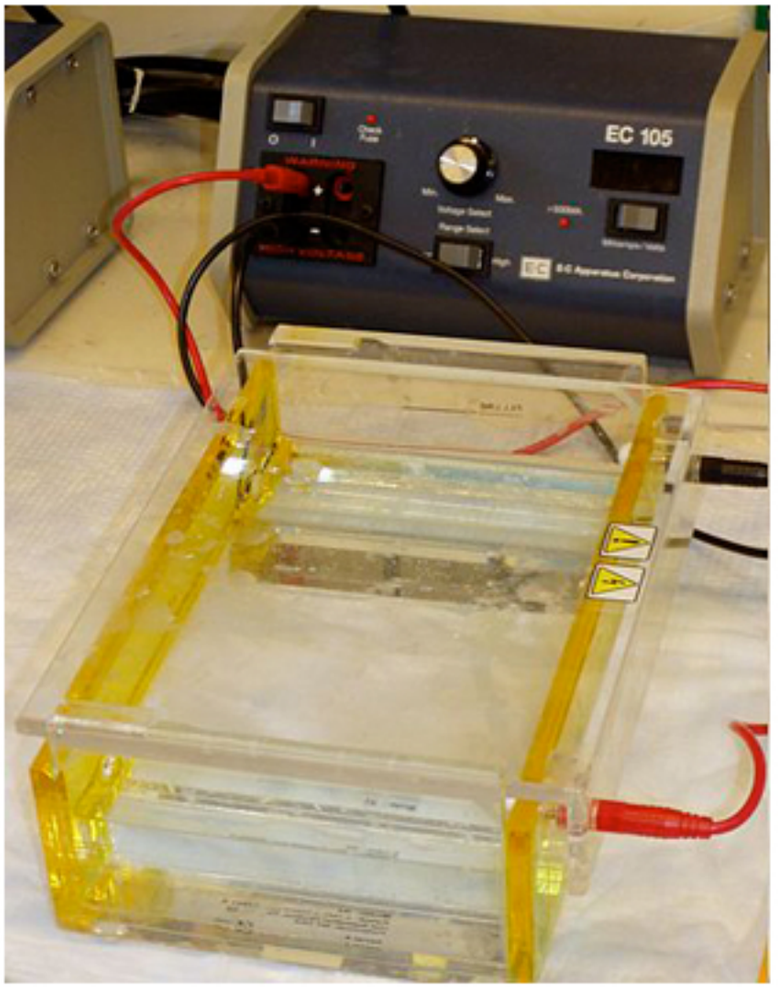
This depends on how much you started with.





Data from Real Time RT PCR





DNA ad RNA electrophoresis.

PAGE - polyacrylamide gel electrophoresis

or

agarose (for bigger stands)

Denaturing (PAGE) urea - H_2NCNH_2 (O)

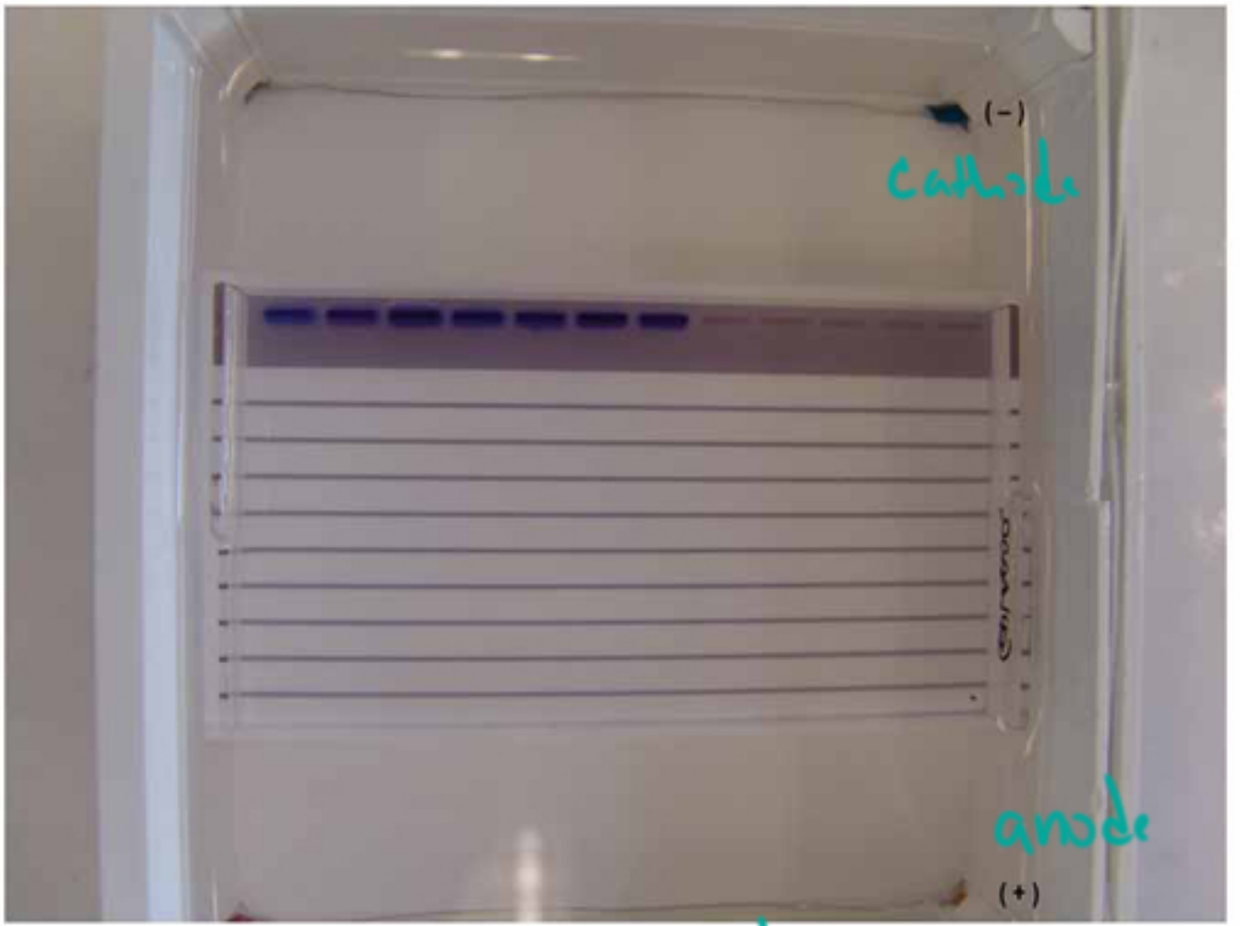
or

Native (double stand)

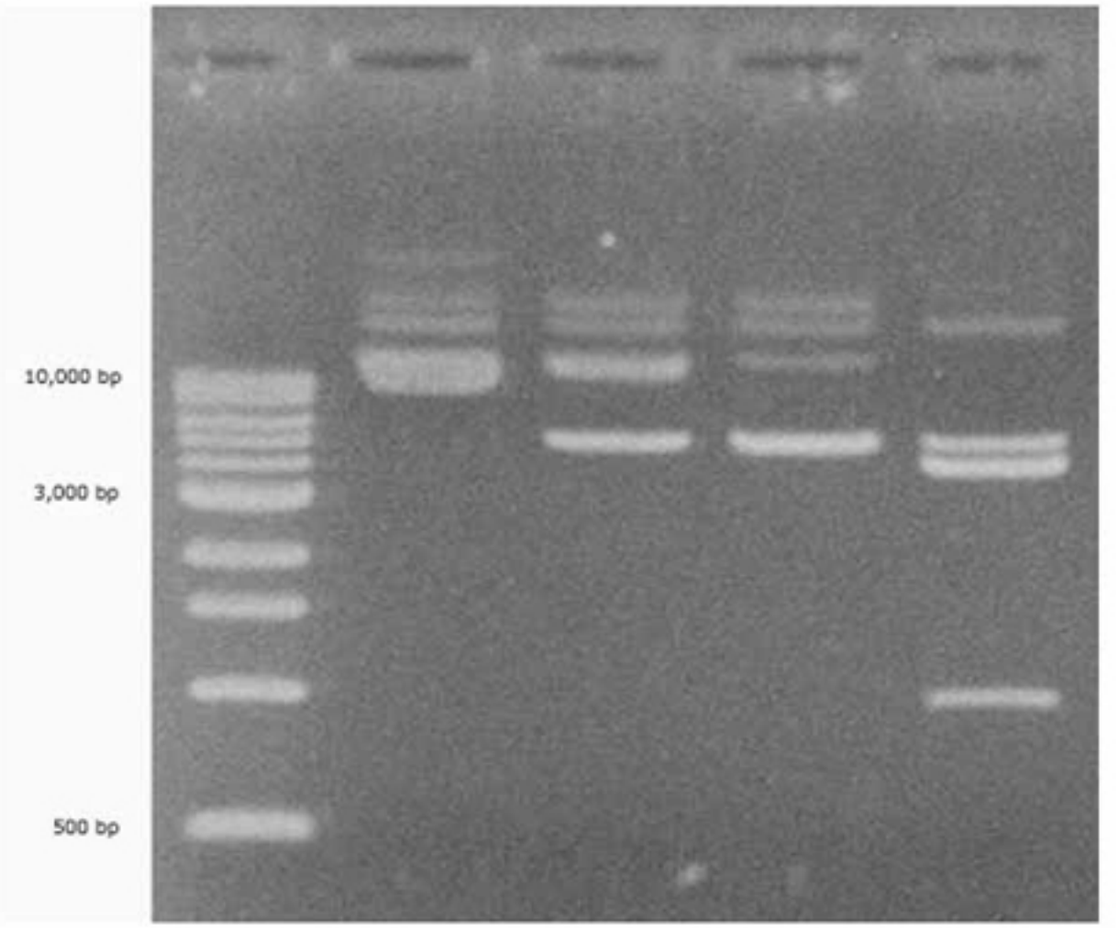
Post Electrophoresis - staining or autoradiography (^{32}P labeled DNA) photographic visualizing of radionuclids

-Blotting - Southern DNA

Northern RNA



agarose gel



agarose gel stained with ethidium bromide

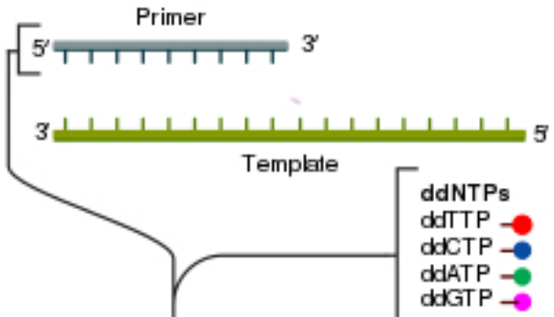
Sanger Sequencing

① **Reaction mixture**

- **Primer and DNA template**
- **DNA polymerase**
- **ddNTPs with flourochromes**
- **dNTPs (dATP, dCTP, dGTP, and dTTP)**

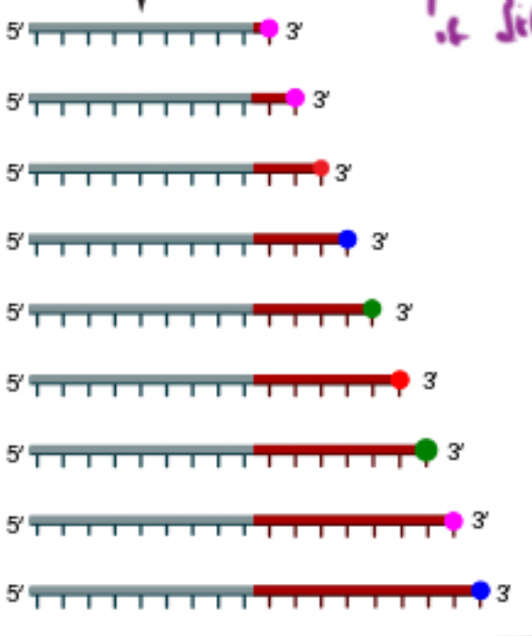
the regular nucleoside triphosphates

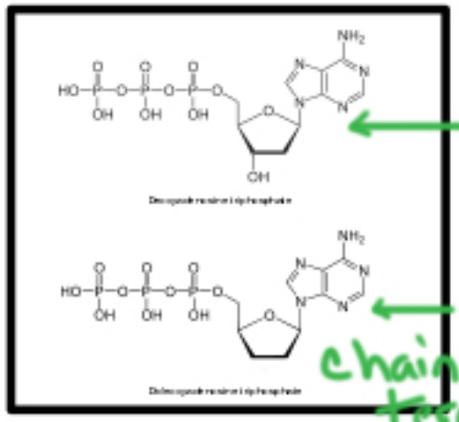
plus a small amount of these dye terminators



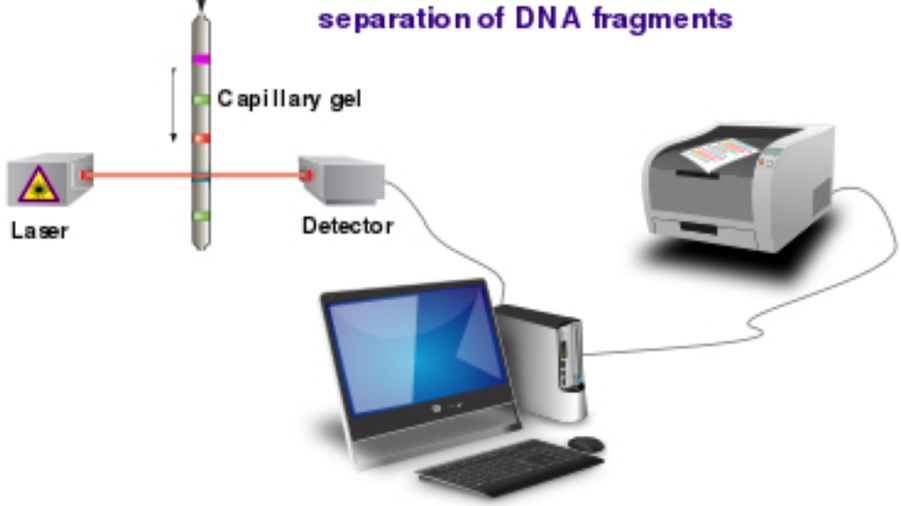
② **Primer elongation and chain termination**

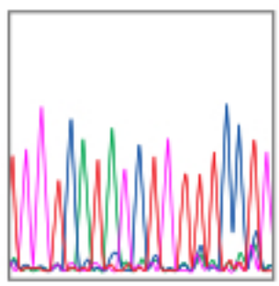
you get fragments of different length





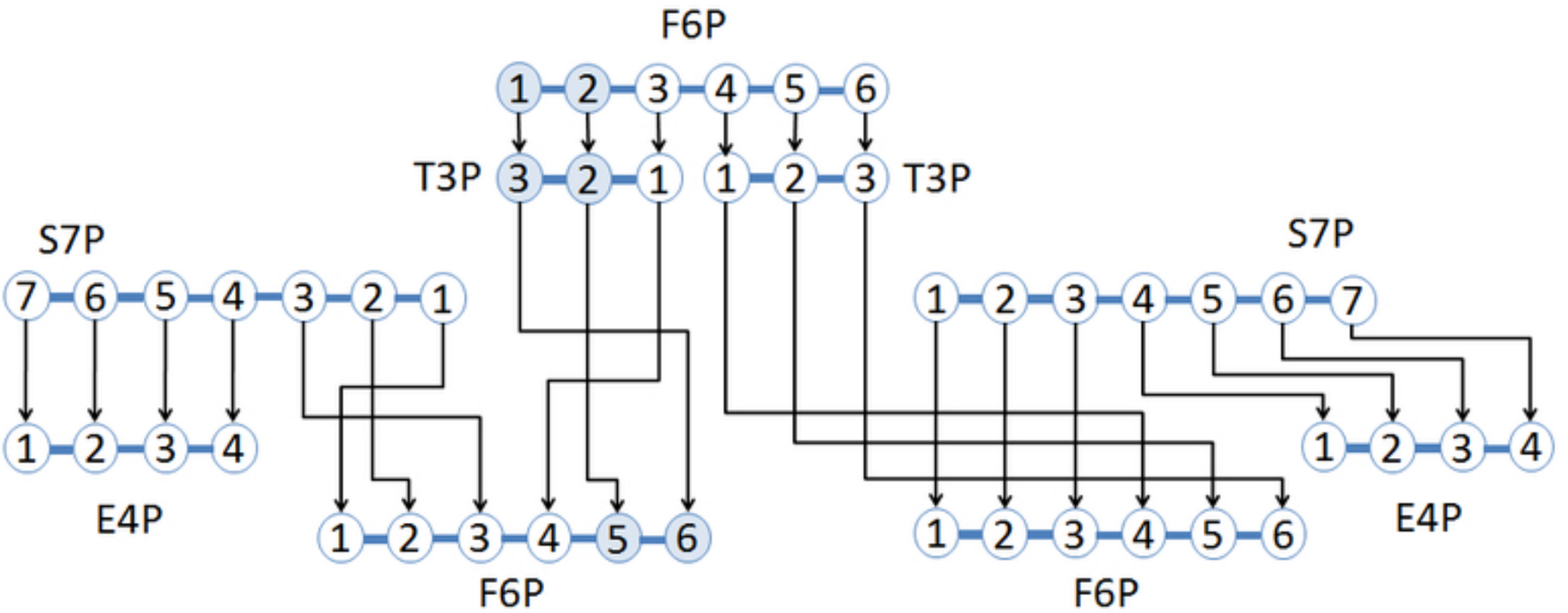
③ **Capillary gel electrophoresis separation of DNA fragments**





Chromatograph

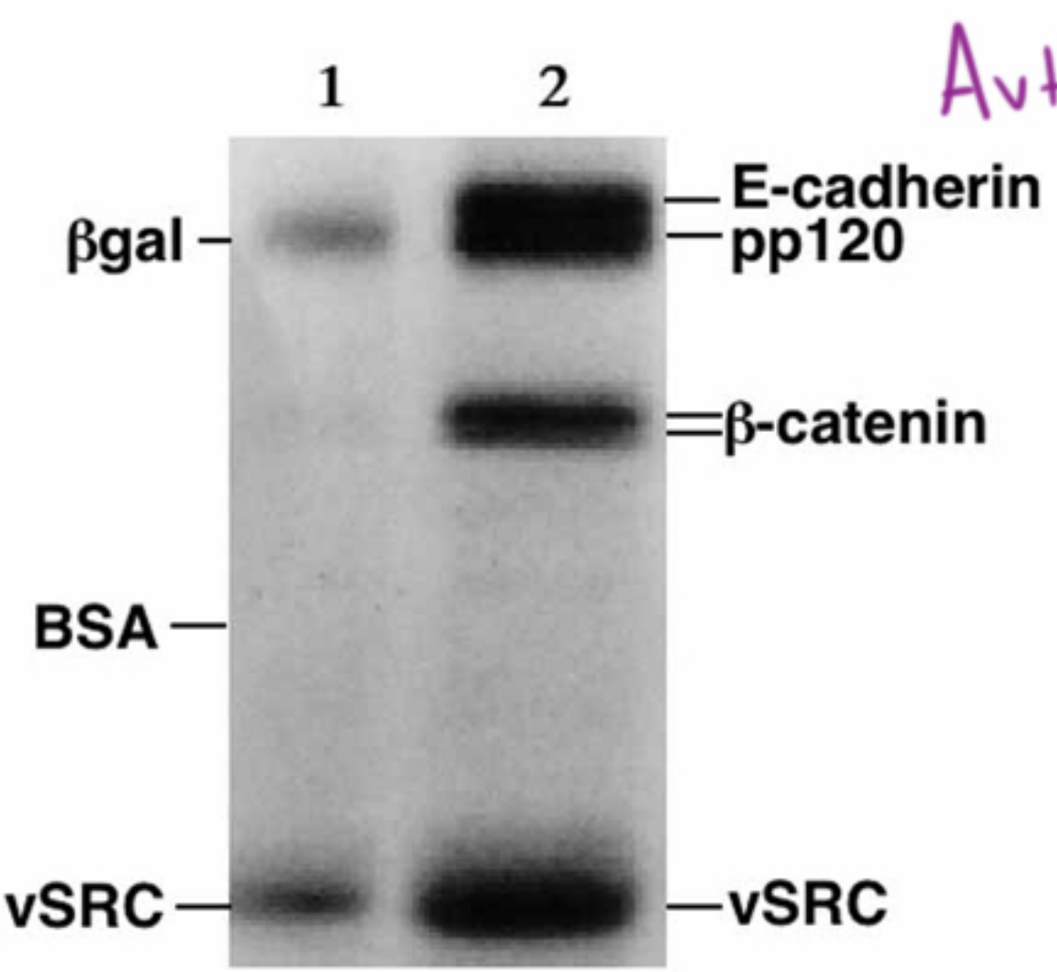
④ **Laser detection of flourochromes and computational sequence analysis**



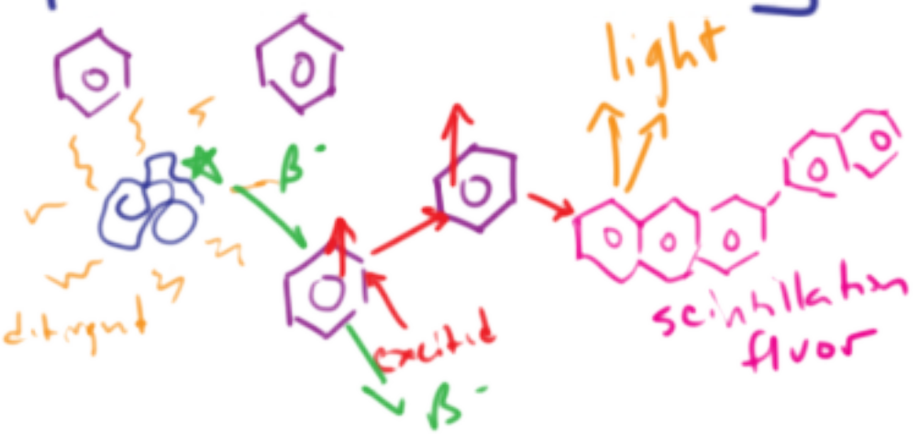
Radiolabeling

- β^- emitters
  ^3H, ^{14}C, ^{32}P, ^{35}S
- β^+ emitter ^{18}F
- Heavy
  ^2H, ^{13}C, ^{15}N, ^{18}O

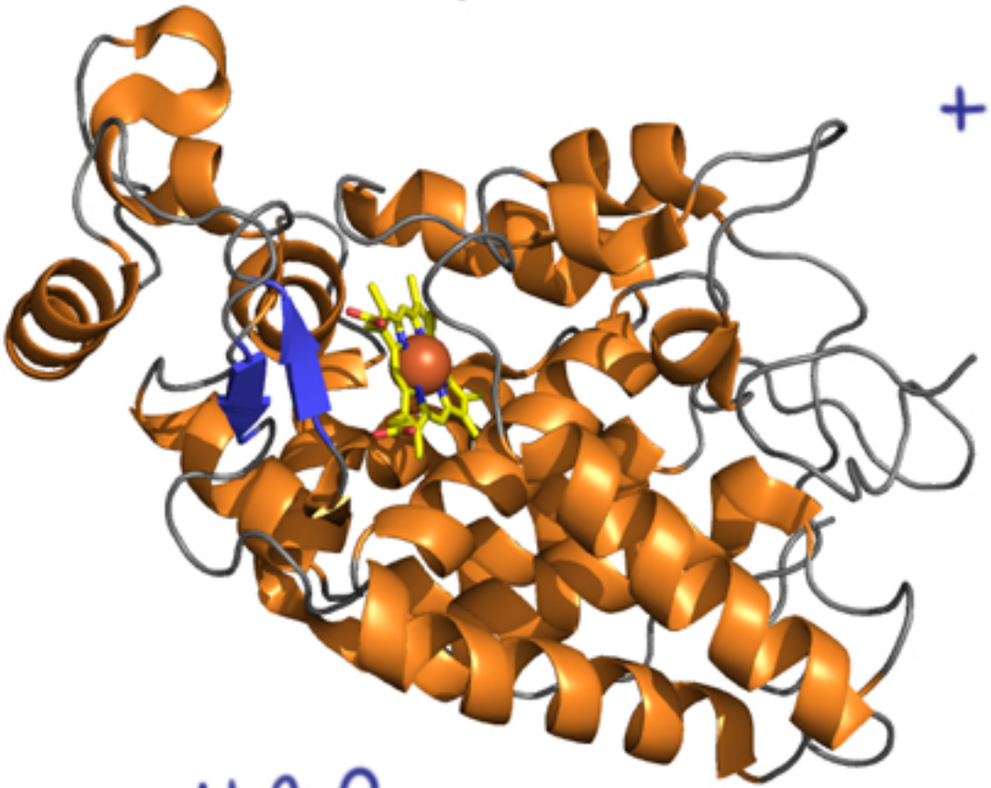
Autoradiography



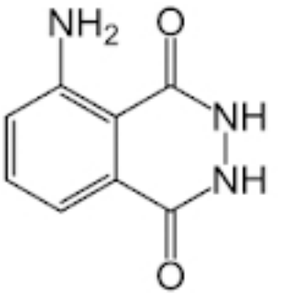
Liquid Scintillation Counting



Enzyme Label



+



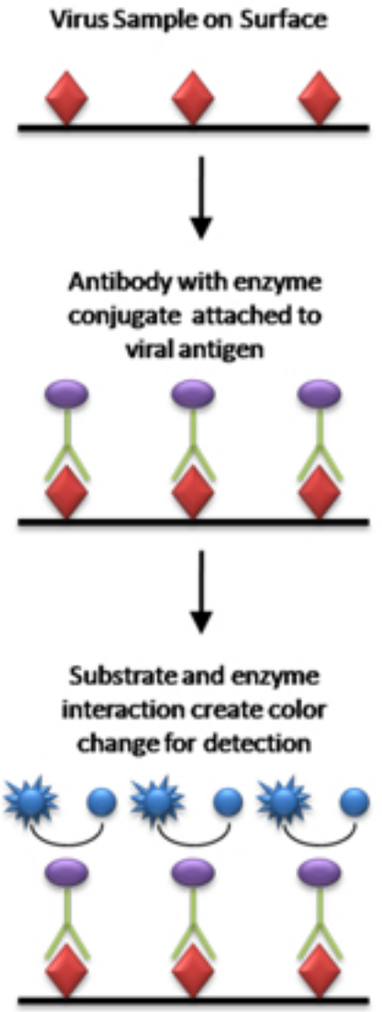
→ light

luminol

often on secondary antibody in Western blotting

HRP

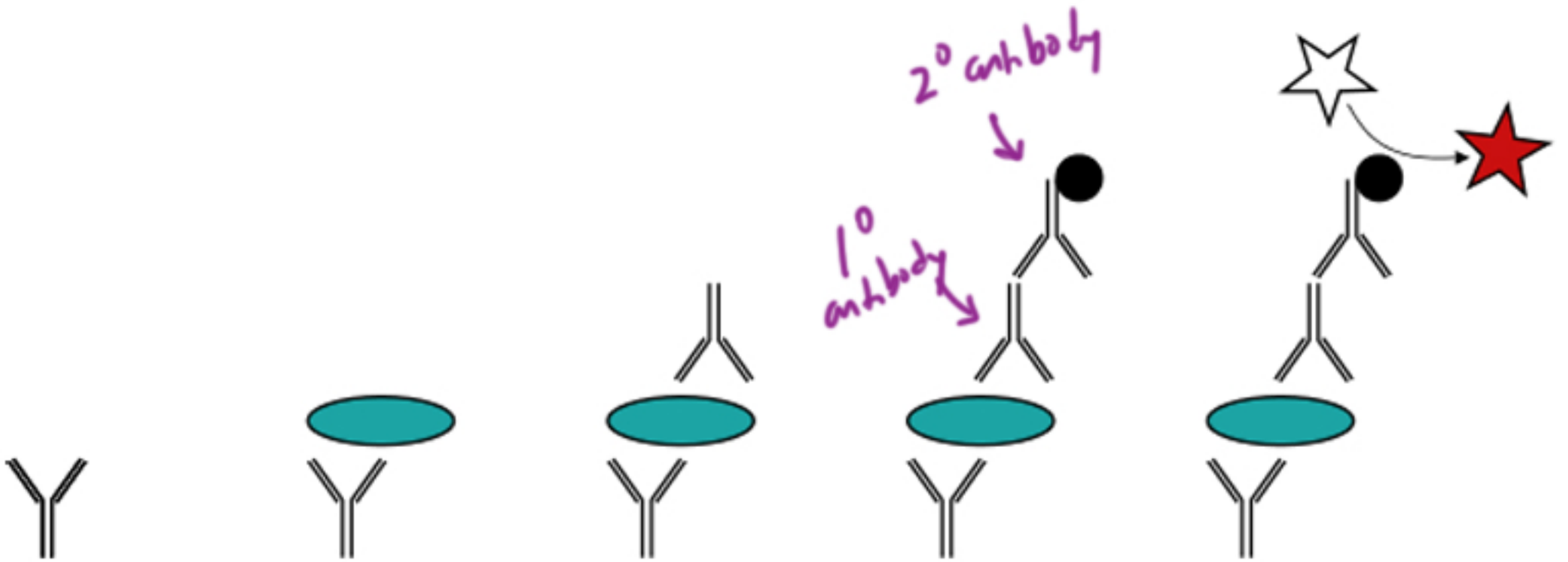
Horseradish Peroxidase



ELISA

enzyme linked immunosorbent assay

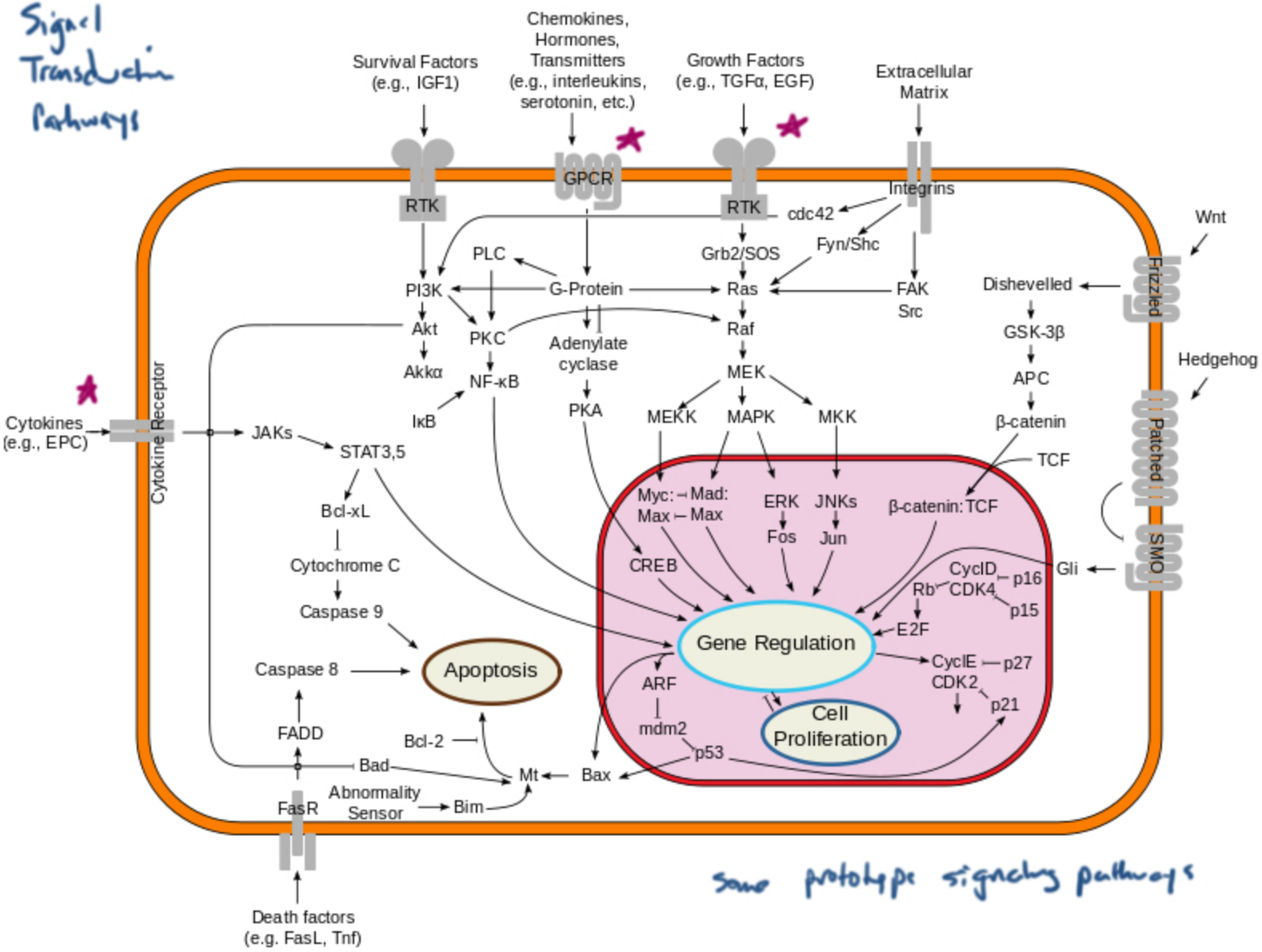
(1) (2) (3) (4) (5)



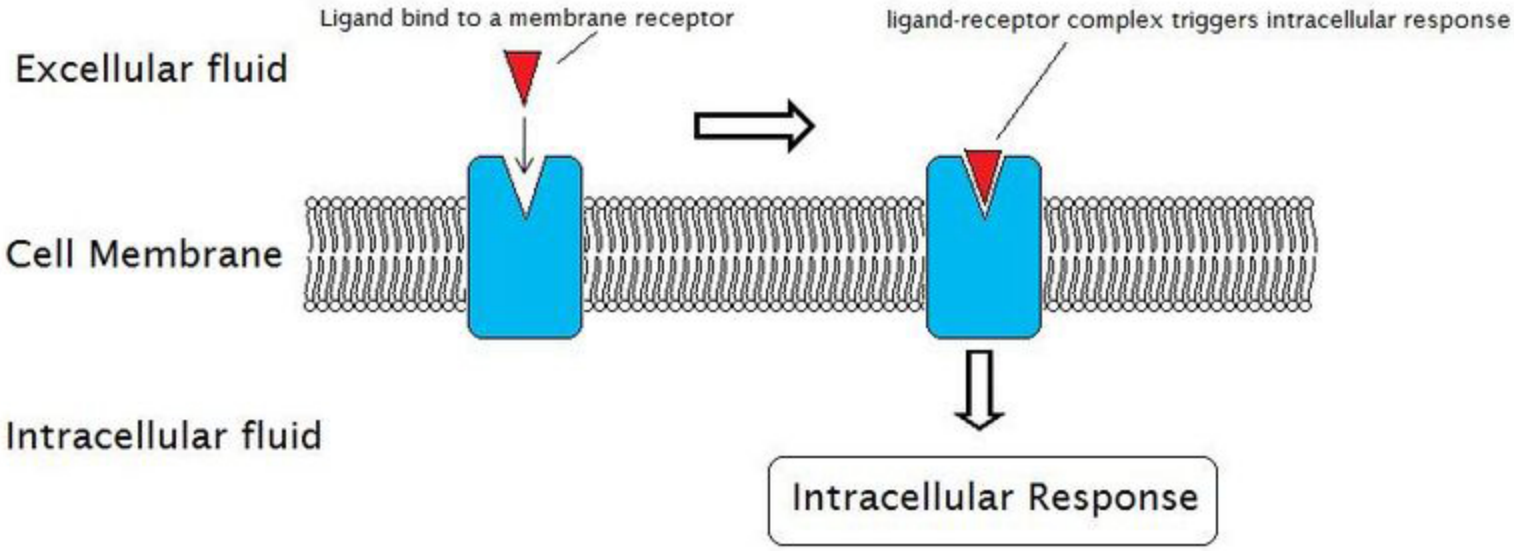
making monoclonal antibodies.

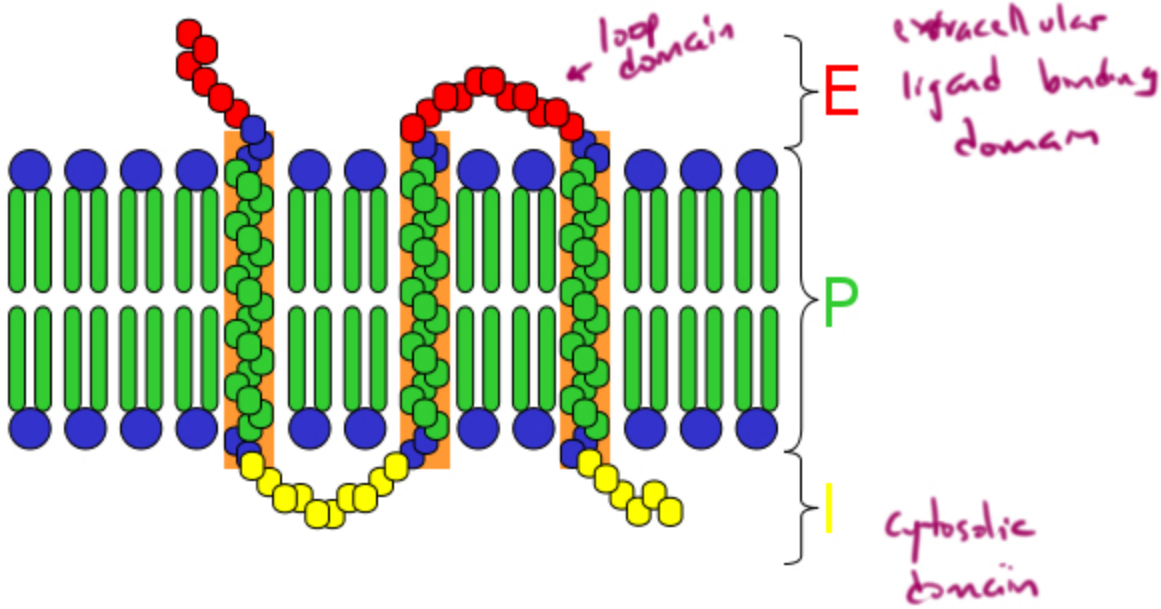
- harvest a B cell from rat thymus
- fuse this with multiple myeloma cell such as HLA (Henrietta Lacks)
- creates a hybridoma - leads to an antibody producing cell culture

Signal Transduction Pathways

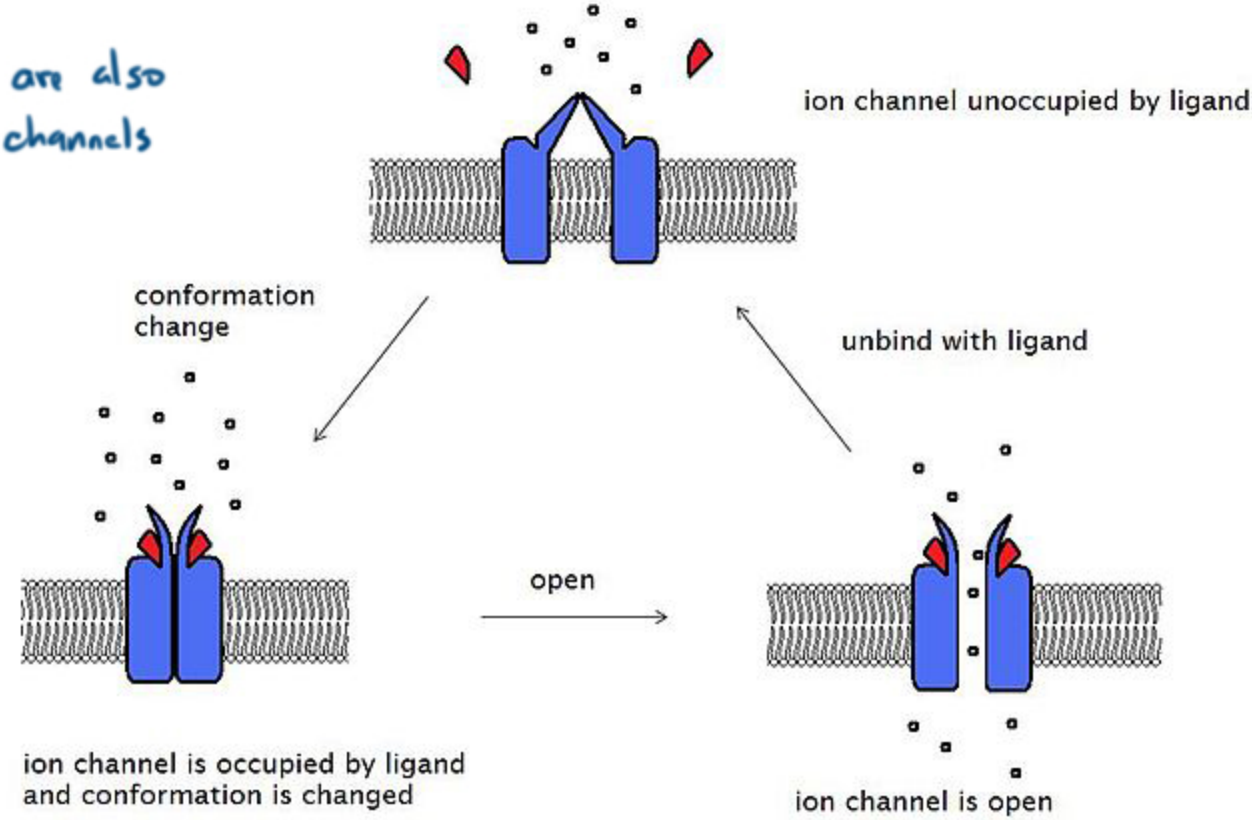


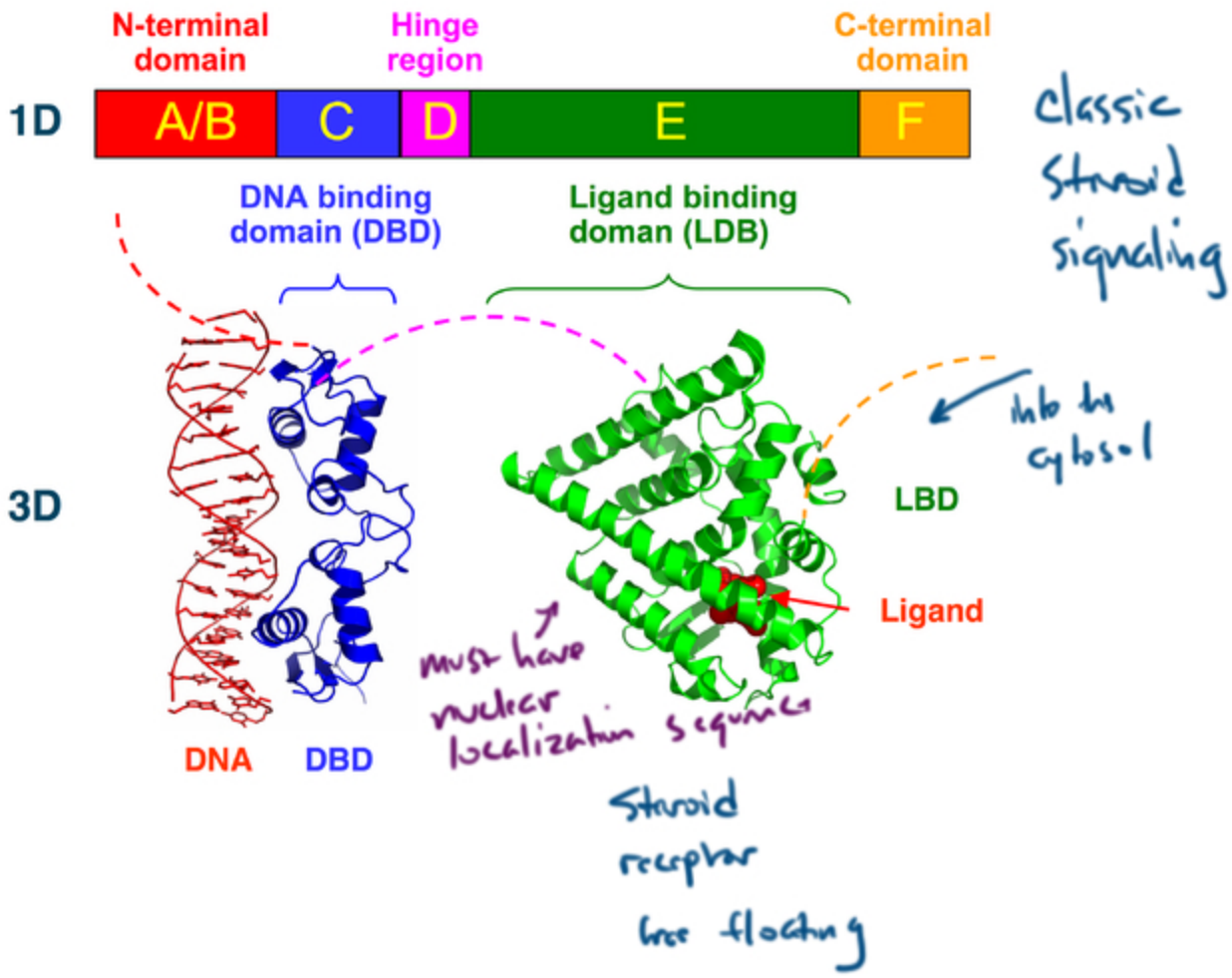
some prototype signaling pathways


Ligand bind to a membrane receptor
ligand-receptor complex triggers intracellular response
Excellular fluid
Cell Membrane
Intracellular fluid
Intracellular Response

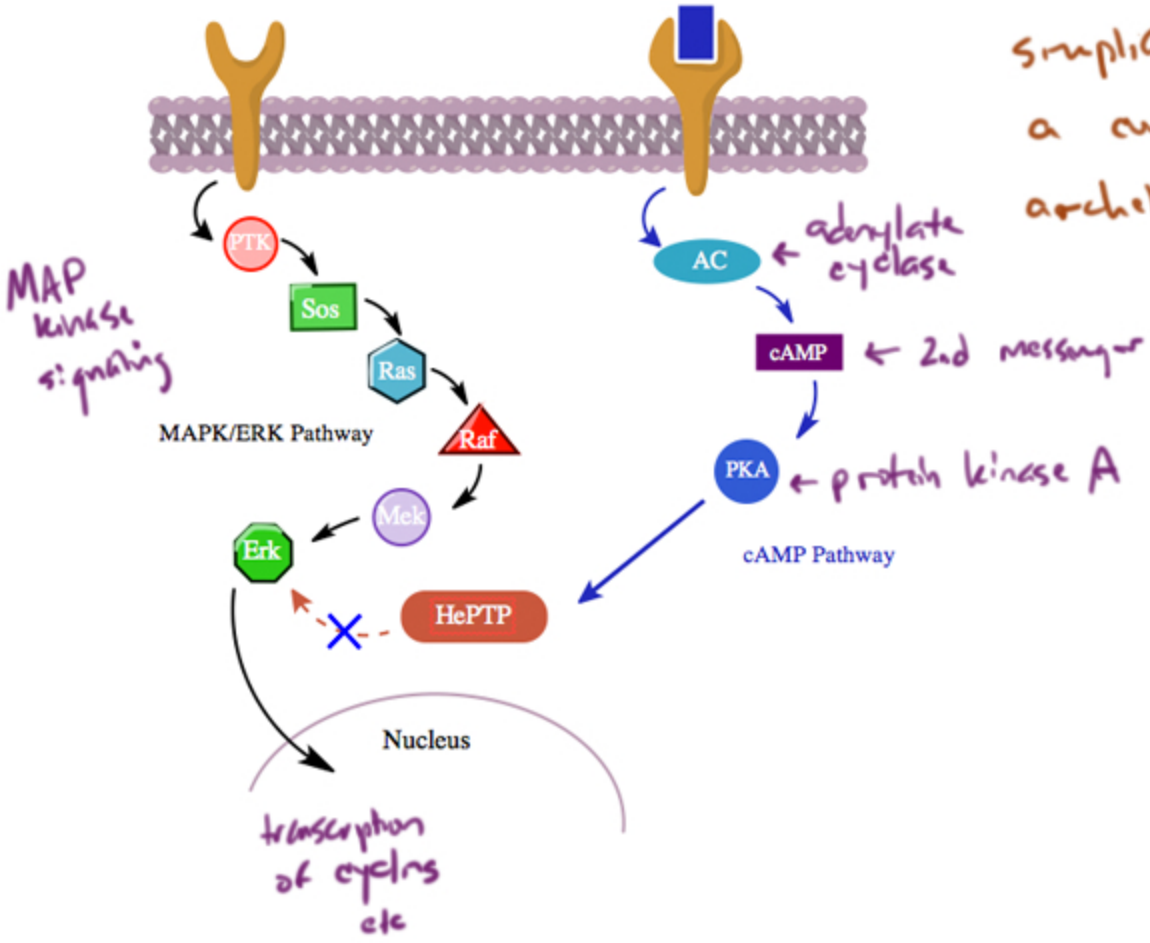

loop domain
extracellular ligand binding domain
E
P
I
cytosolic domain

There are also ion channels



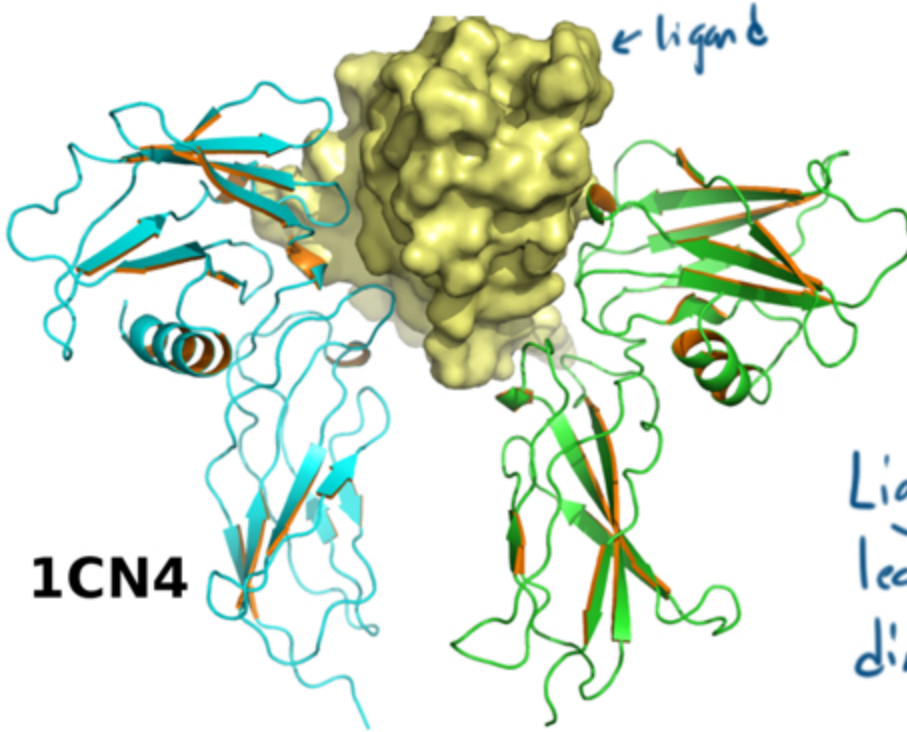

N-terminal domain
Hinge region
C-terminal domain
1D
A/B
C
D
E
F
DNA binding domain (DBD)
Ligand binding doman (LDB)
3D
LBD
Ligand
DNA
DBD
must have nuclear localization sequence
classic steroid signaling
into the cytosol
steroid receptor free floating

simplified pictures of a couple of archetypal pathways



Catalytic receptors

(growth factor receptors ; cytokine receptors)

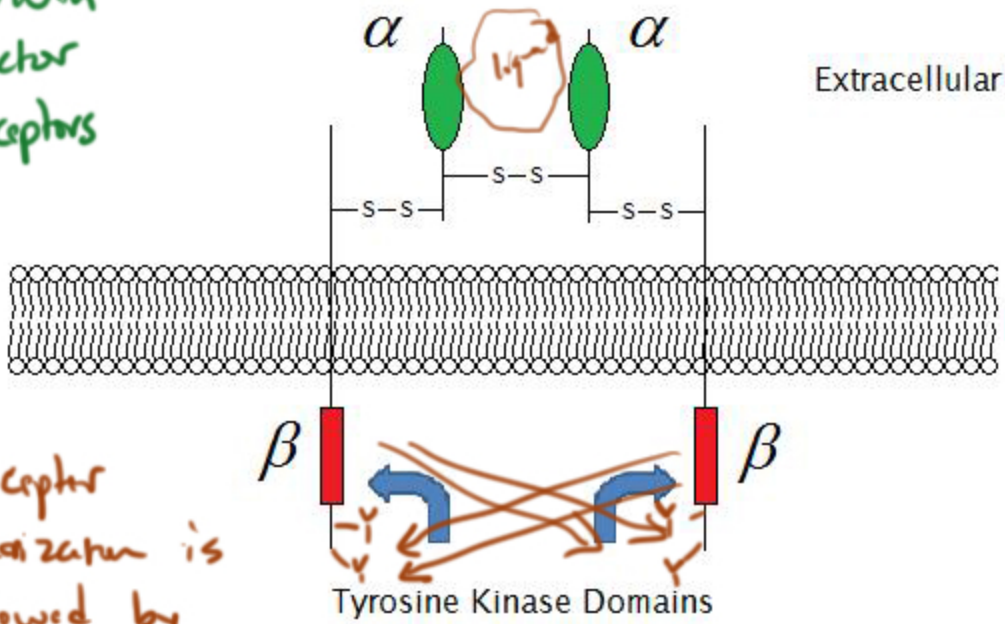


Ligand binding leads to dimerization

EPOR-EPO

erythropoetin receptor
- a cytokine receptor

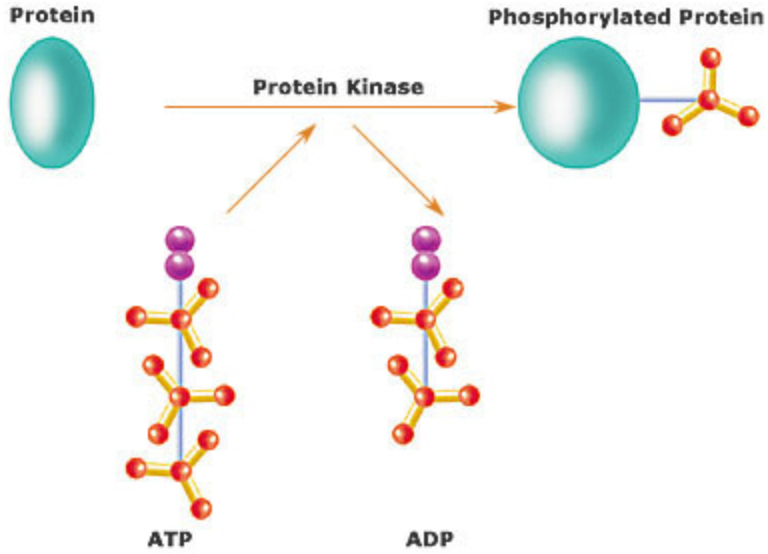
Growth Factor Receptors



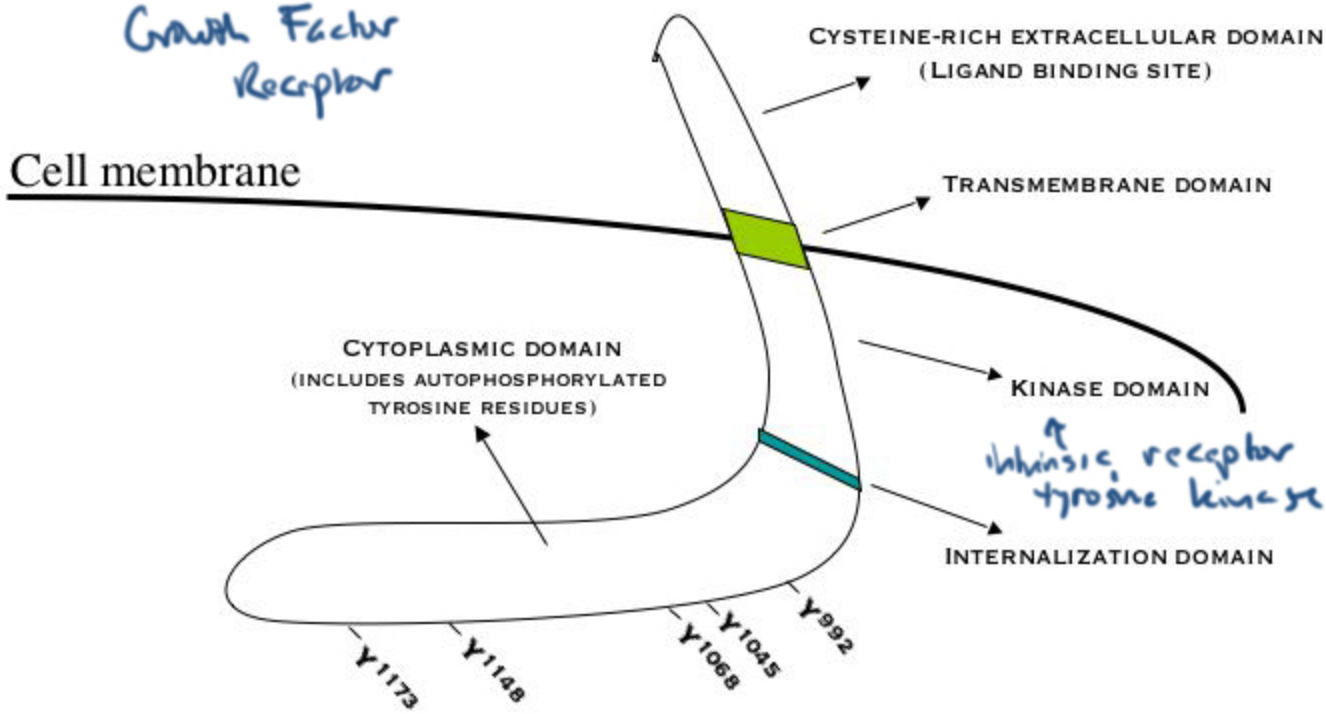
- Receptor dimerization is followed by transautophosphorylation
- Phosphorylated tyrosines serve as docking sites for signaling proteins (with SH2 domains)

Growth factor receptors possess intrinsic receptor tyrosine kinase.

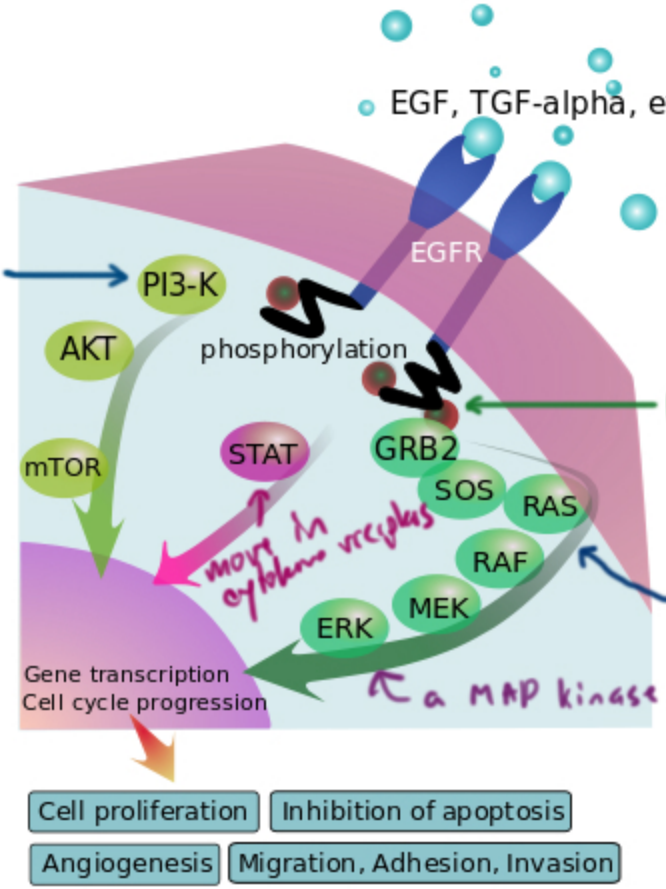
(cytokine receptors are just like this but they utilize a non receptor tyrosine kinase)


Protein
Phosphorylated Protein
Protein Kinase
ATP
ADP

Growth Factor Receptor



Some simplified archetypal pathways



phosphatidyl inositol 3-kinase (don't confuse with phospholipase C)

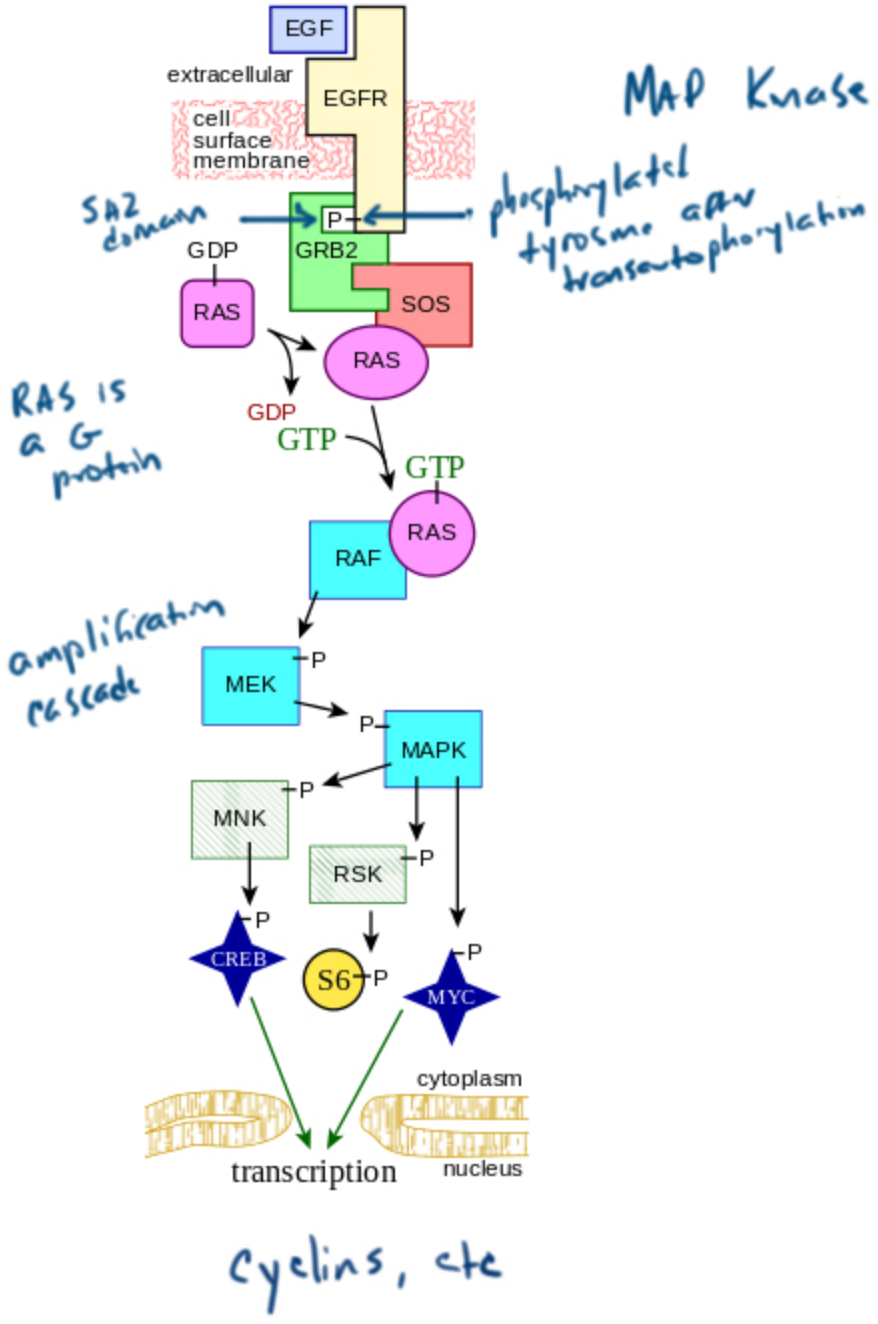
phosphorylated tyrosine

moves in cytokine receptors

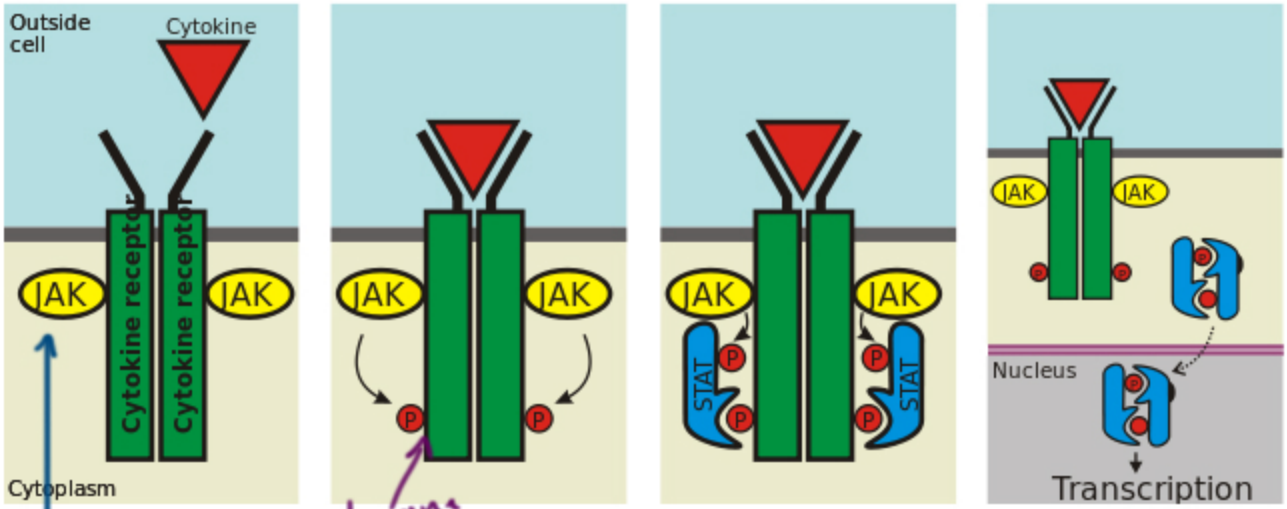
a MAP kinase

RAS is a G protein (don't confuse with GPCR) activated by exchange of GDP with GTP

deactivated by GTPase activity


MAP Kinase
EGF
extracellular
EGFR
cell surface membrane
SH2 domain
P
phosphorylated tyrosine after transautophosphorylation
GDP
GRB2
RAS
SOS
RAS
RAS is a G protein
GDP
GTP
GTP
RAS
RAF
amplification cascade
MEK
P
P
MAPK
MNK
P
RSK
P
P
CREB
S6
P
P
MYC
cytoplasm
transcription
nucleus
cyclins, etc

Cytokine Receptor



tyrosine

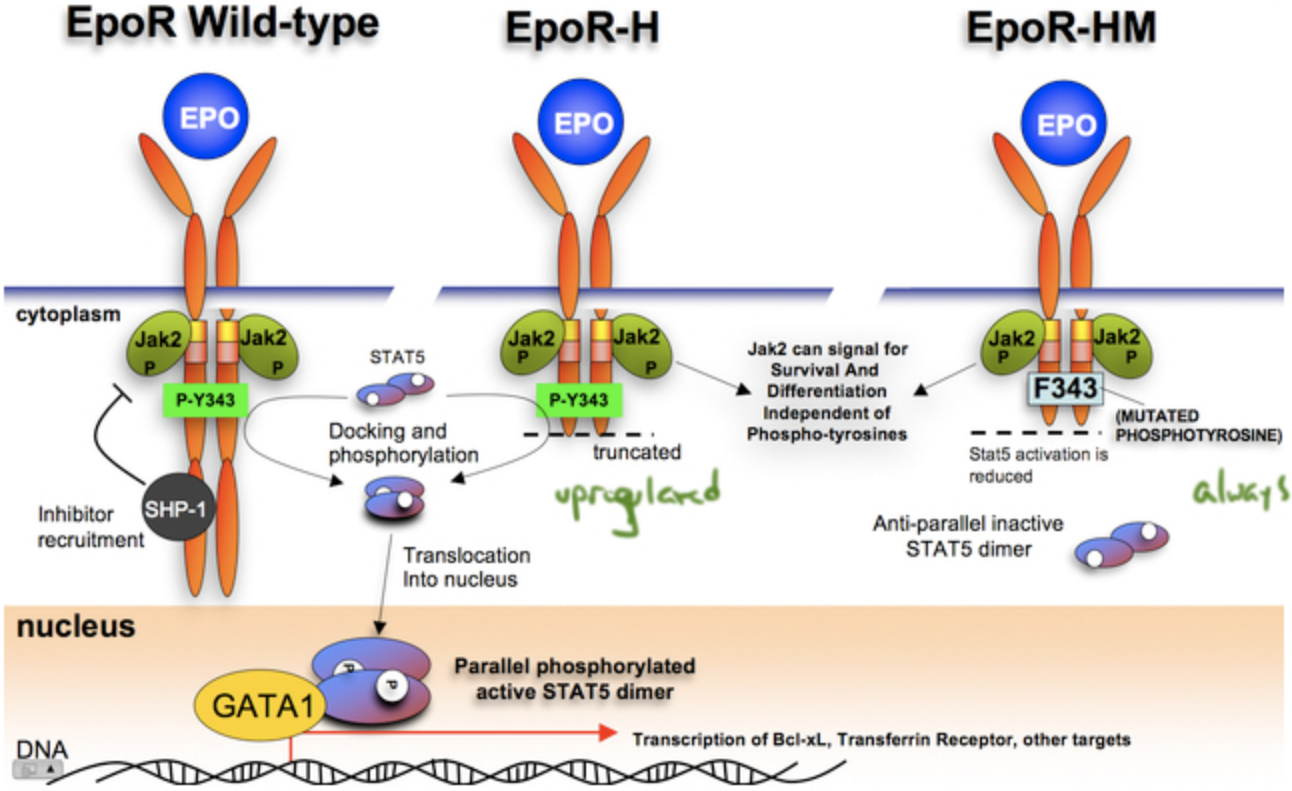
JAK STAT

non receptor tyrosine kinase

Janus Kinase

Truncated Epo Receptors

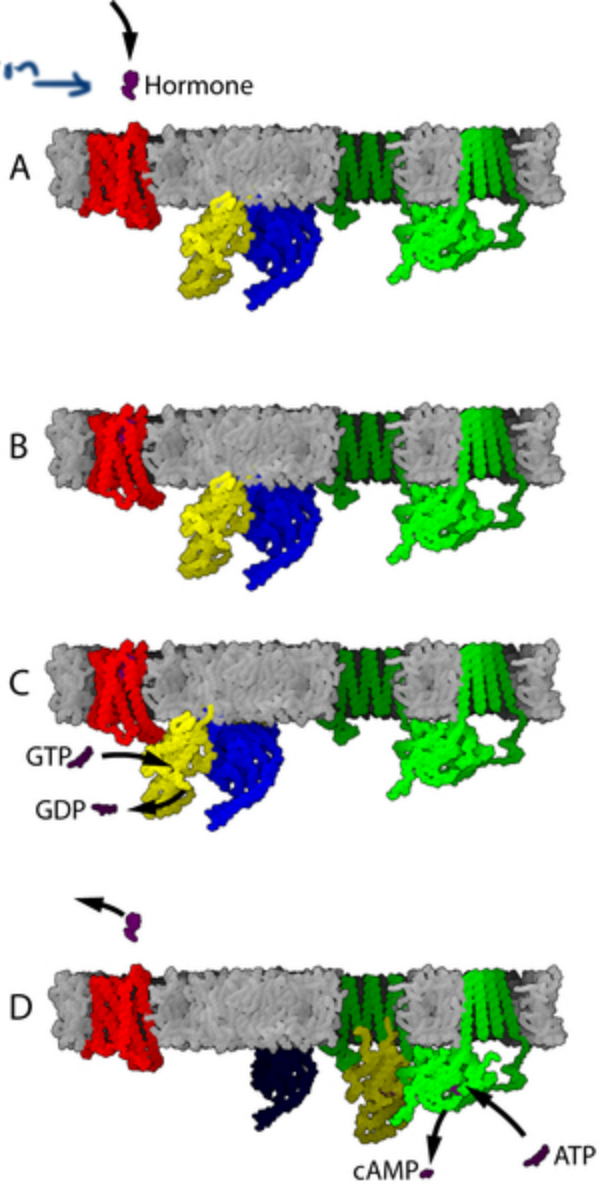
MCAT logic



example in liver

glucagon → Hormone

2nd Messengers



cAMP

↓

PKA → phosphorylase kinase (ON)

↓

glycogen phosphorylase

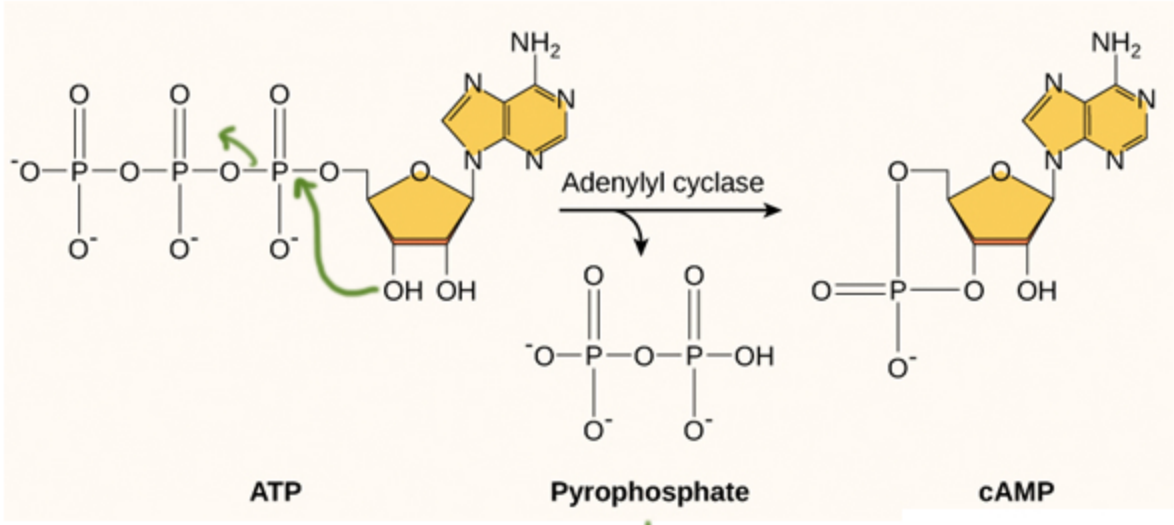
PKA → CREB

↓

PEP carboxykinase

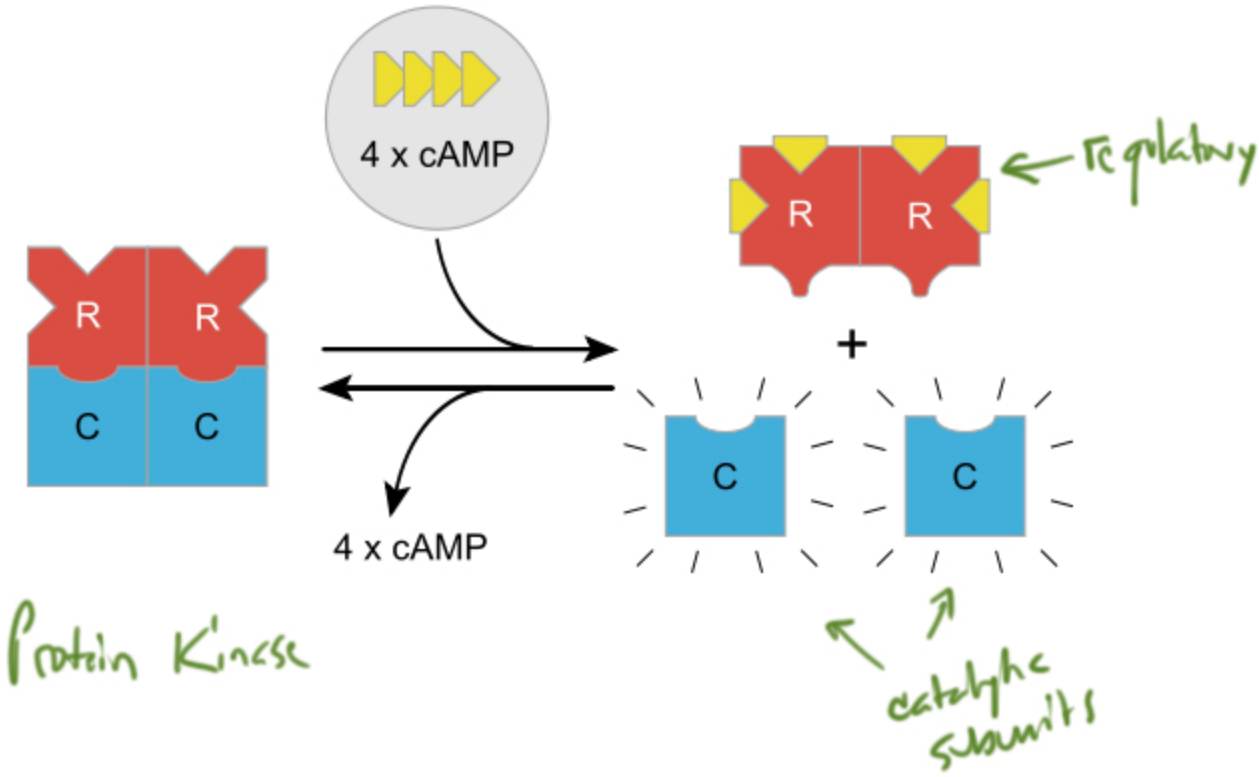
PKA → transcription factor

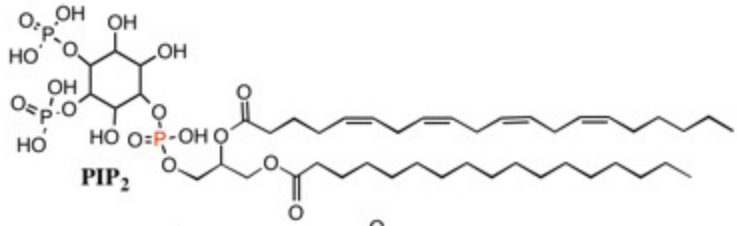
PFK II OFF



more $-\Delta G$

$2P_i$


4 x cAMP
R
R
C
C
4 x cAMP
R
R
+
C
C
Protein Kinase
regulatory
catalytic subunits

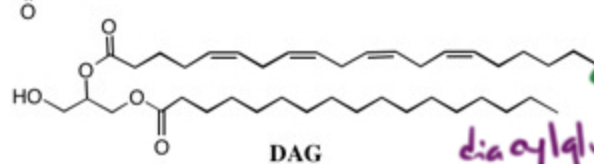


PIP$_2$

Phosphatidyl inositol diphosphate

Phospholipase C

PLC

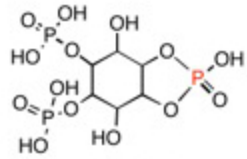


DAG

stays in membrane

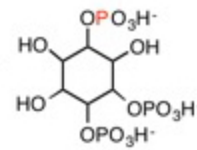
diacylglycerol

2nd messengers



Cyclic-IP$_3$

H_2O



IP$_3$

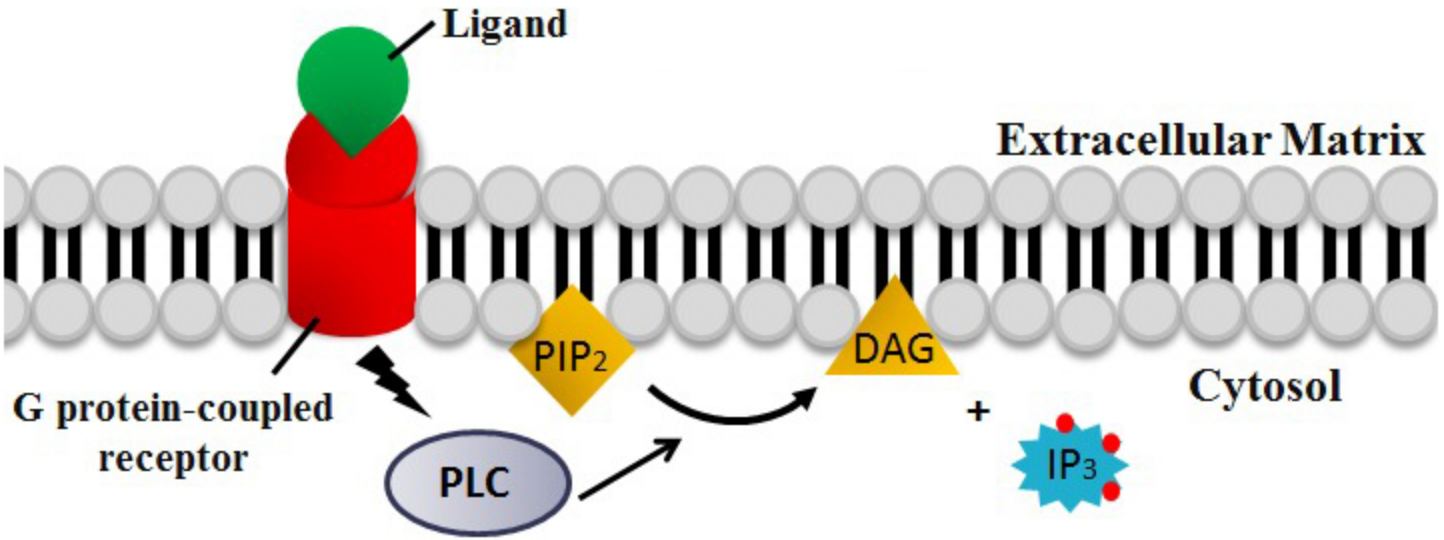
inositol triphosphate

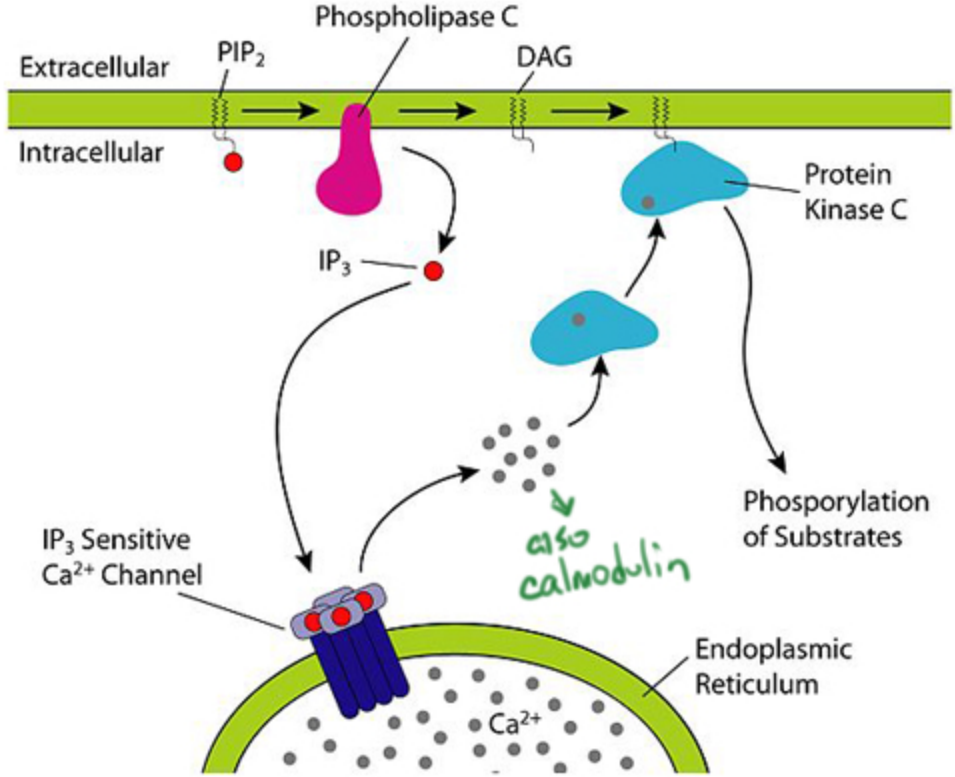
cytosol

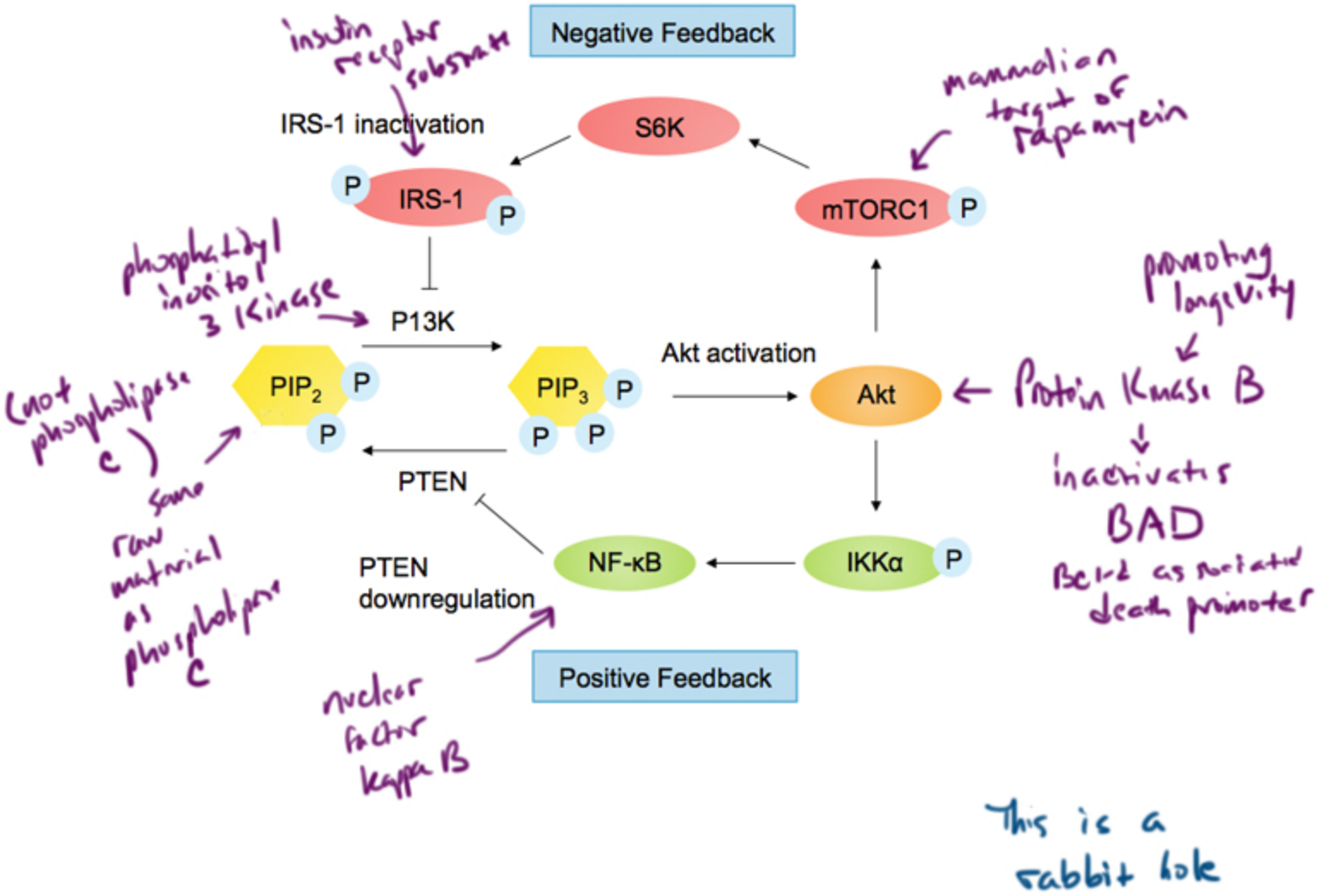
ER → Ca^{2+} + DAG → protein kinase C

↓

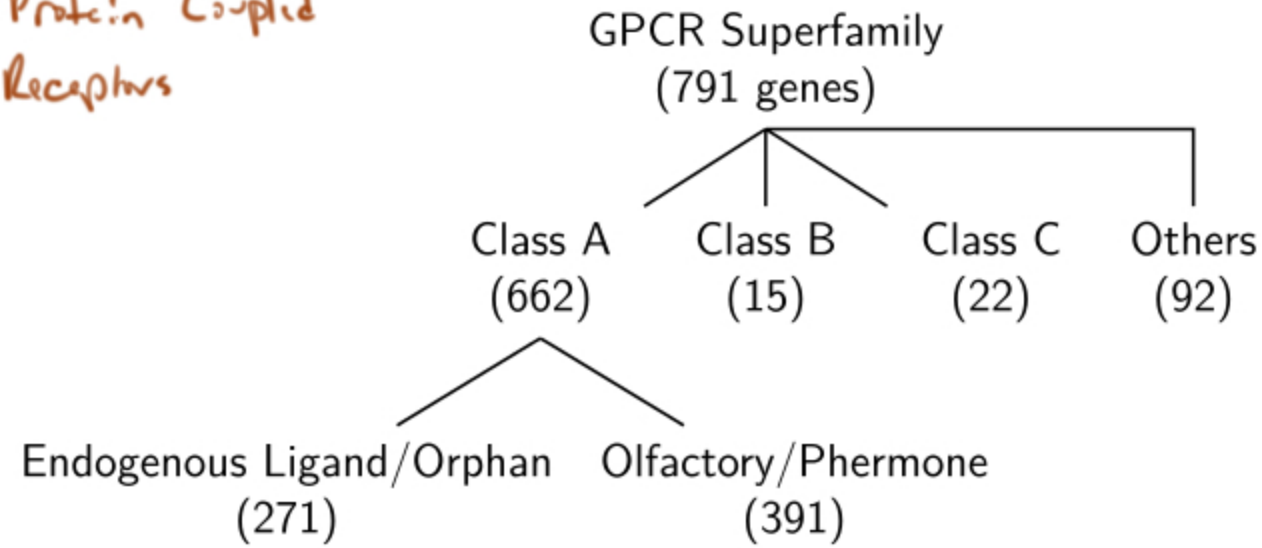
Calmodulin

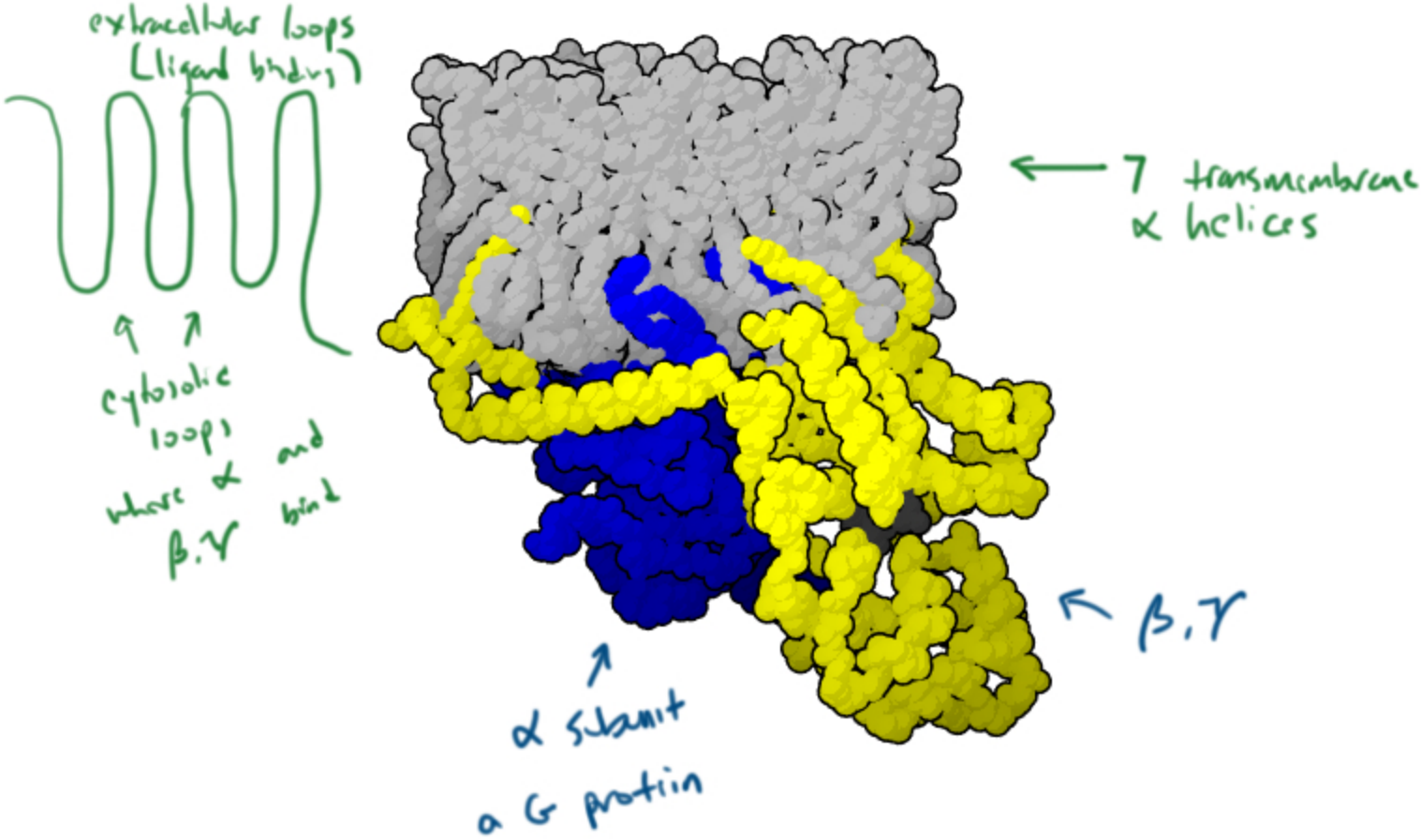

Ligand
Extracellular Matrix
PIP2
DAG
Cytosol
G protein-coupled
receptor
PLC
+
IP3

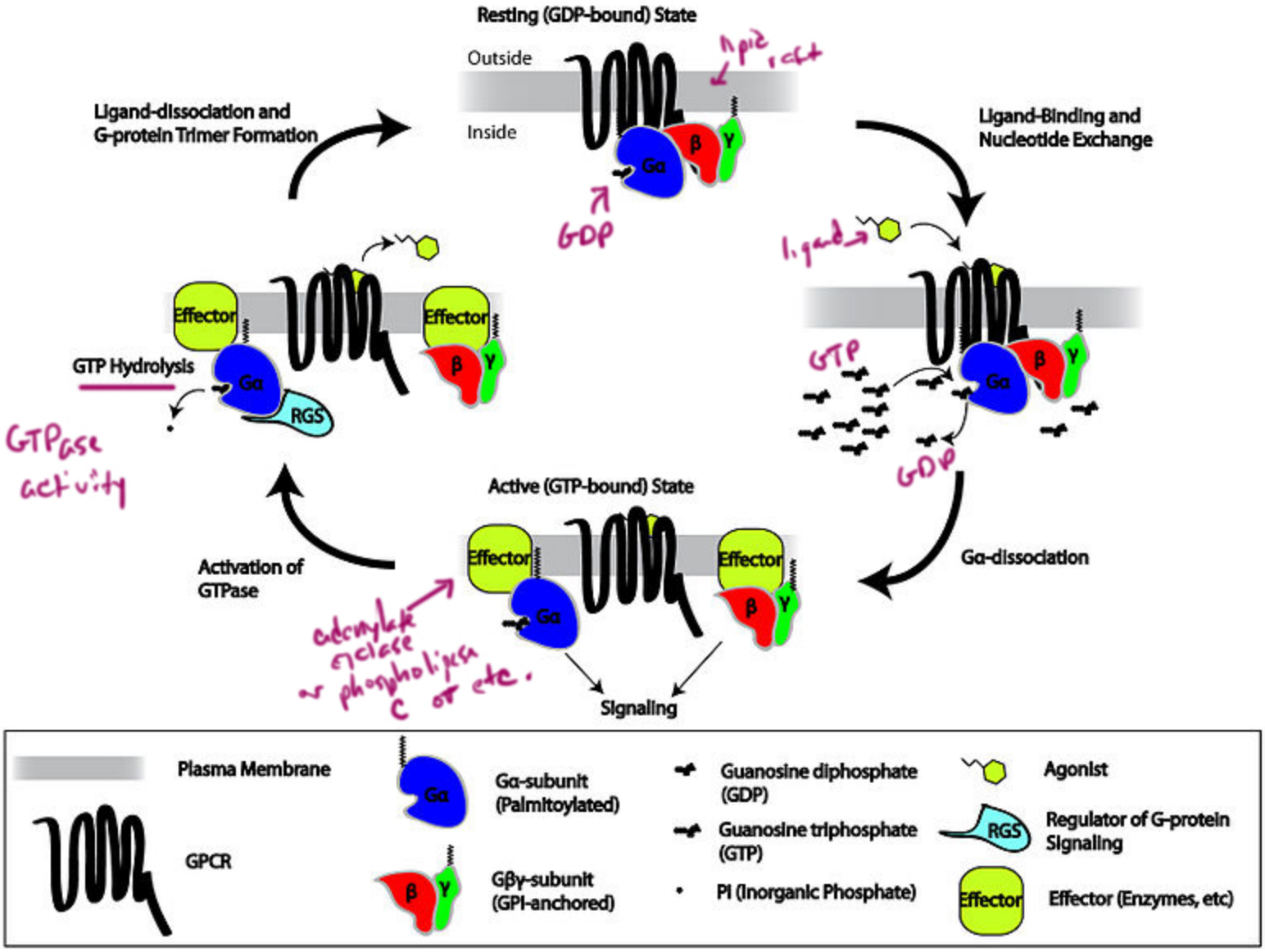

Phospholipase C
Extracellular
PIP2
DAG
Intracellular
Protein
Kinase C
IP3
IP3 Sensitive
Ca2+ Channel
also
calmodulin
Phosporylation
of Substrates
Endoplasmic
Reticulum
Ca2+

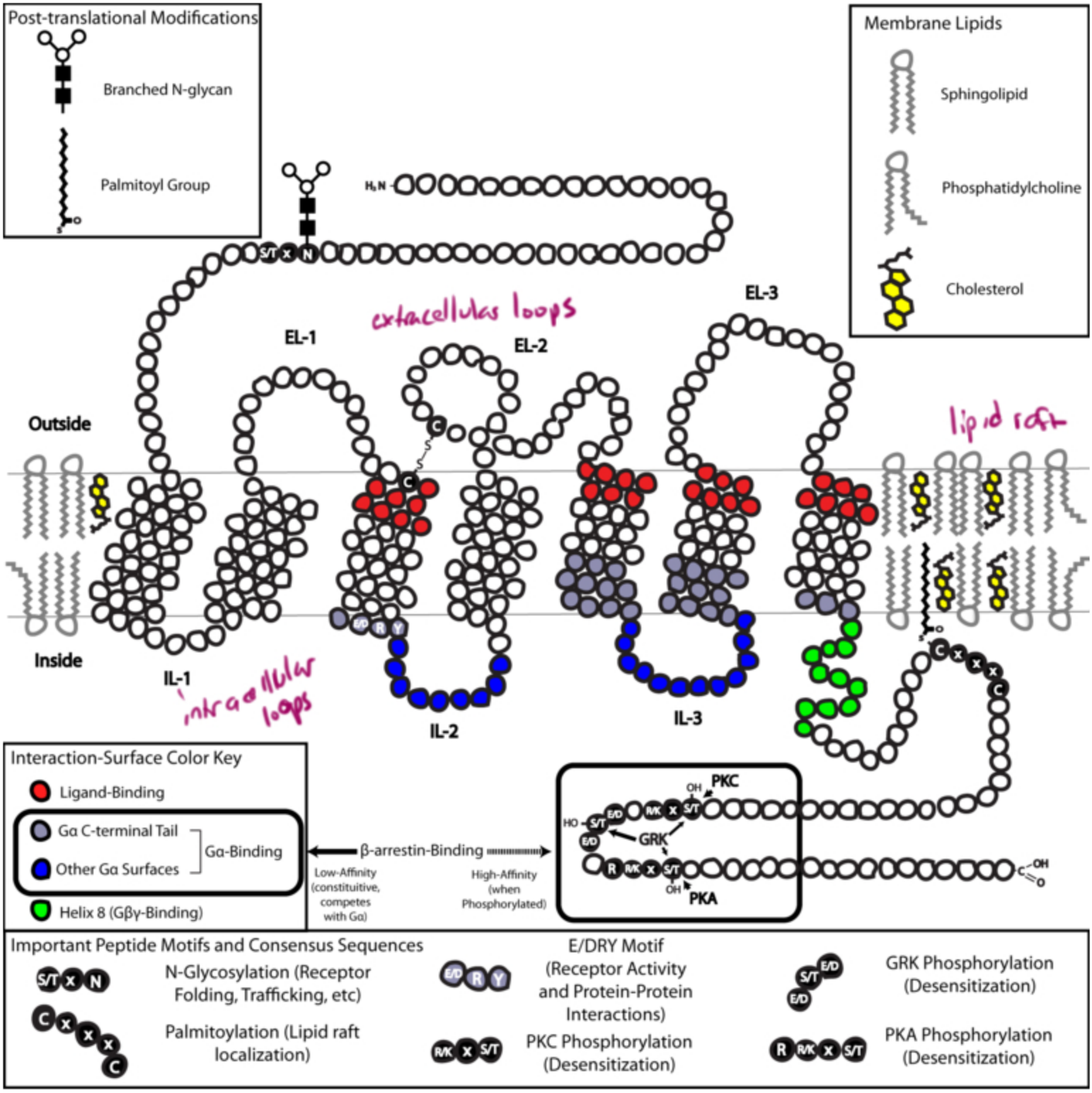

Negative Feedback
IRS-1 inactivation
S6K
IRS-1
P
mTORC1
P
P13K
PIP2
PIP3
Akt activation
Akt
PTEN
NF-κB
IKKα
PTEN downregulation
Positive Feedback

G Protein Coupled Receptors

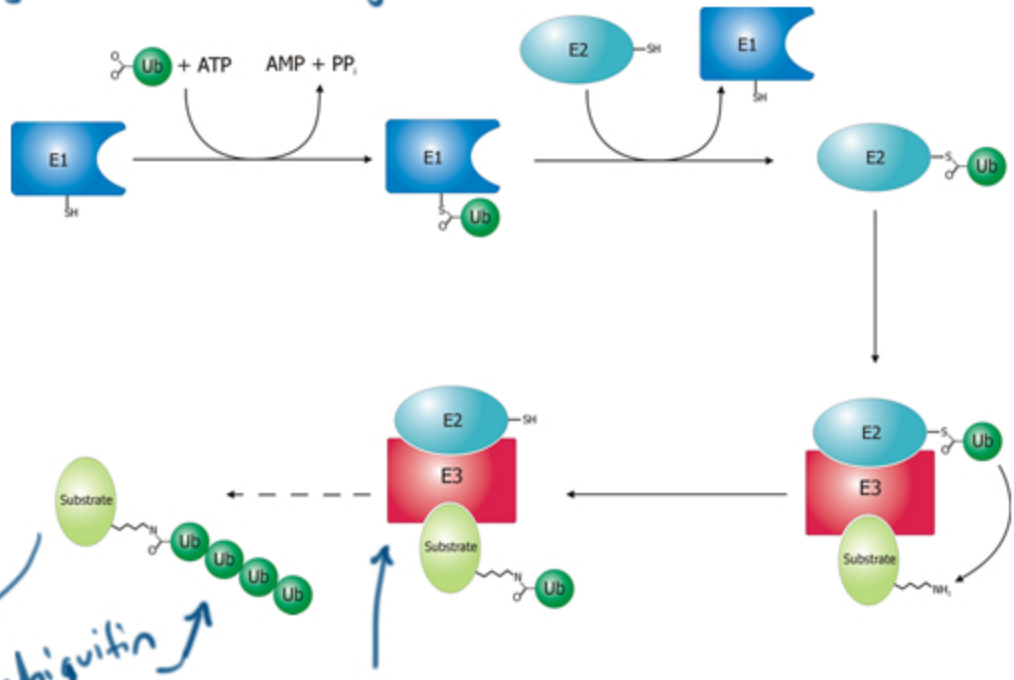



extracellular loops
(ligand binding)
cytosolic loops where α and β,γ bind
7 transmembrane α helices
β,γ
α subunit a G protein


Resting (GDP-bound) State
Outside
Inside
Gα
β
γ
GDP
Ligand-Binding and Nucleotide Exchange
Ligand
GTP
GDP
Gα-dissociation
Active (GTP-bound) State
Effector
Effector
Signaling
Activation of GTPase
adenylate cyclase or phospholipase C or etc.
GTP Hydrolysis
RGS
GTPase activity
Ligand-dissociation and G-protein Trimer Formation
Plasma Membrane
GPCR
Gα-subunit (Palmitoylated)
Gβγ-subunit (GPI-anchored)
Guanosine diphosphate (GDP)
Guanosine triphosphate (GTP)
PI (Inorganic Phosphate)
Agonist
Regulator of G-protein Signaling
Effector (Enzymes, etc)


Post-translational Modifications
Branched N-glycan
Palmitoyl Group
Membrane Lipids
Sphingolipid
Phosphatidylcholine
Cholesterol
H2N
S/T X N
extracellular loops
EL-1
EL-2
EL-3
Outside
Inside
lipid raft
C
C
E/D R Y
C X X X C
IL-1
intracellular loops
IL-2
IL-3
Interaction-Surface Color Key
Ligand-Binding
Gα C-terminal Tail
Other Gα Surfaces
Gα-Binding
Helix 8 (Gβγ-Binding)
β-arrestin-Binding
Low-Affinity (constituitive, competes with Gα)
High-Affinity (when Phosphorylated)
PKC
GRK
PKA
OH
HO
R/K X S/T
E/D
Important Peptide Motifs and Consensus Sequences
S/T X N
N-Glycosylation (Receptor Folding, Trafficking, etc)
C X X X C
Palmitoylation (Lipid raft localization)
E/D R Y
E/DRY Motif (Receptor Activity and Protein-Protein Interactions)
R/K X S/T
PKC Phosphorylation (Desensitization)
S/T E/D E/D
GRK Phosphorylation (Desensitization)
R R/K X S/T
PKA Phosphorylation (Desensitization)

Targeted Protein Degradation



Proteosome

ubiquitin

E3 ligase
(lots of these
for many specific
target proteins)

Gene Nomenclature

one gene in a pathway

leuA

bacterial gene

italics

all lower case

LeuA

bacterial protein

normal font

1st letter capitalized

α subunit

DNA polymerase III

rpoA

polC

RNA polymerase

leuA

leuA273

allele

wild type

$leuA^{+}$

mutant or knock-out

$leuA^{-}$

In diploid organisms these superscripts may reflect the entire genotype

+/-

temperature sensitive

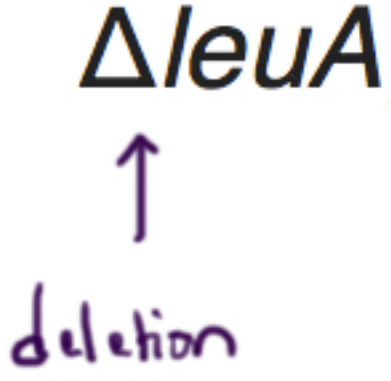
$leuA^{ts}$

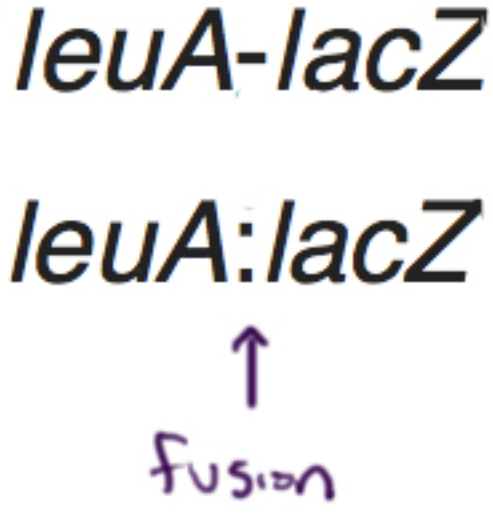
$leuA_{am}$

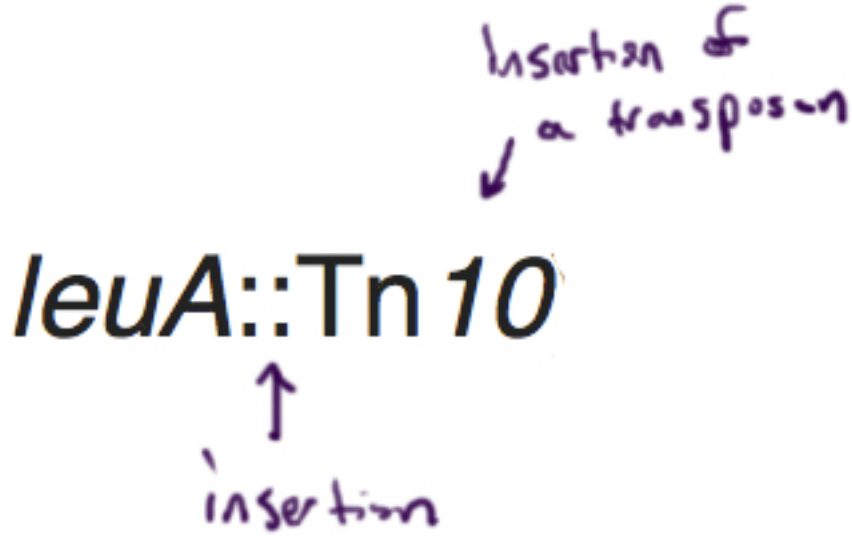
amber mutation

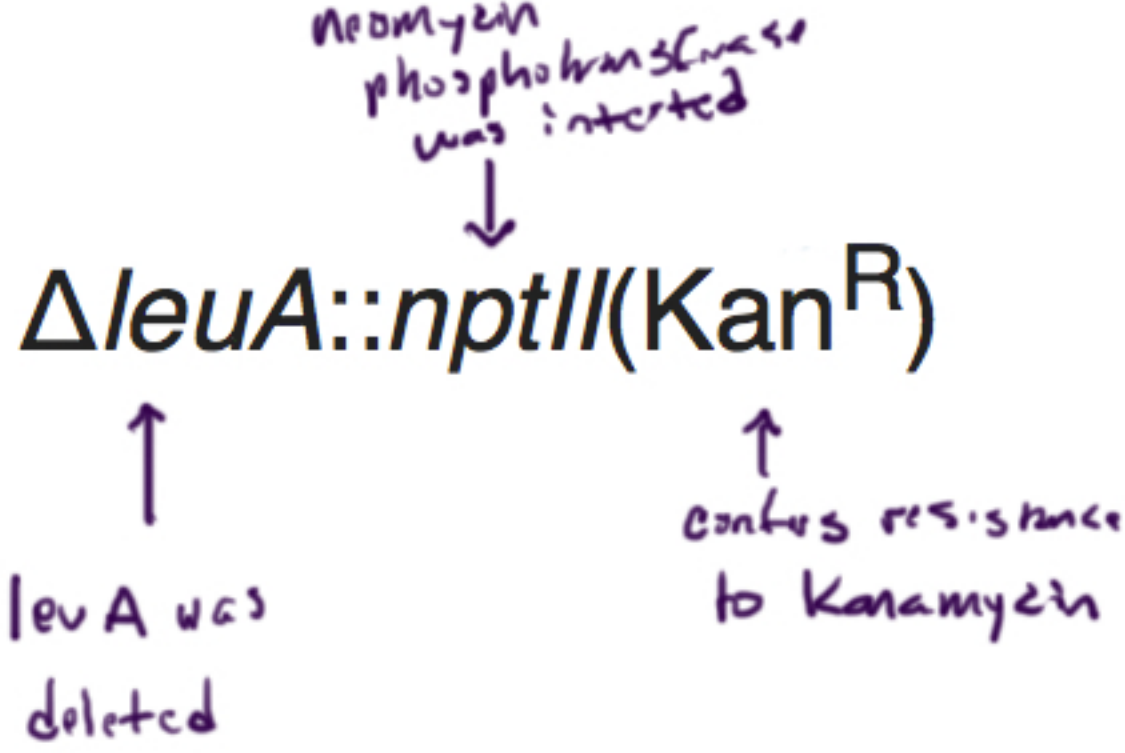
- nonsense mutation

stop.


ΔleuA
deletion


leuA-lacZ
leuA:lacZ
fusion


Insertion of a transposon
leuA::Tn10
insertion


neomycin phosphotransferase was inserted
ΔleuA::nptII(Kan^R)
leu A was deleted
confers resistance to Kanamycin

Bacterial Proteins or Phenotypes

DnaA LeuA^{-} Amp^{R}

- not italics
- 1st letter capitalized in bacteria

Human gene

CTLA4

all caps

italics

SHH Human

Shh Rat or Mouse

A Human Gene Family

SERPIN1 SERPIN2 SERPIN3

Human Gene

SHH

Human Protein or Phenotype

SHH

Rat Gene

Shh

Rat Protein or Phenotype

SHH