Module 14

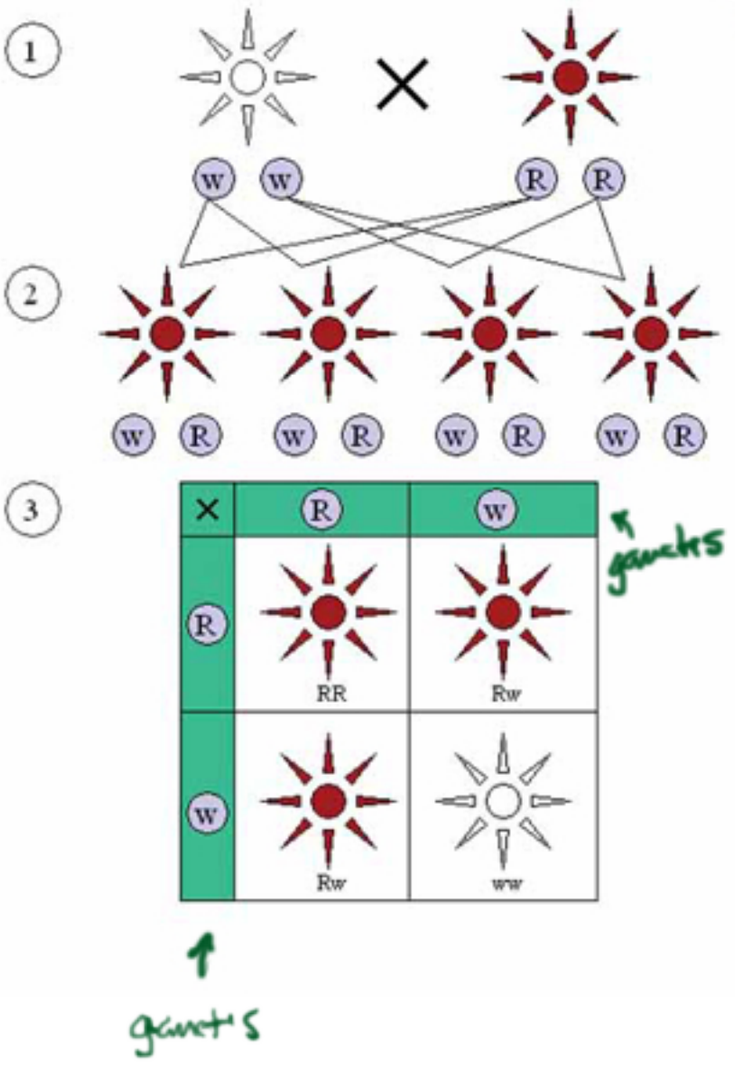
Transmission Genetics & Molecular Cell Biology

Session Slides with Notes

Mendel

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

- Law of segregation
- Law of Independent assortment



parental
- true breeding
- homozygous

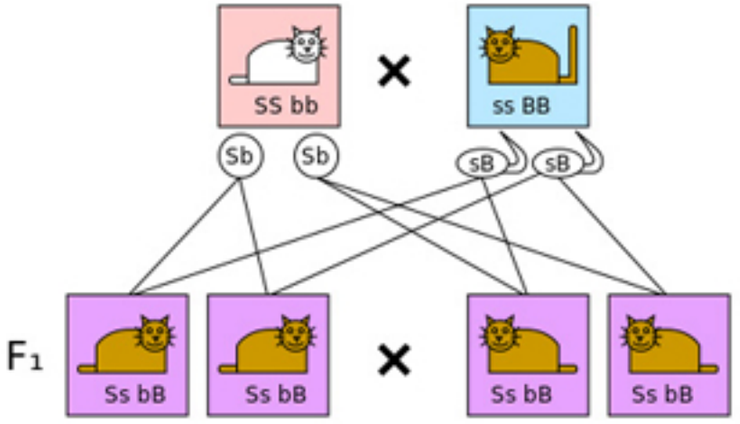
F1 - first filial

F2 -

3:1 phenotypic ratio

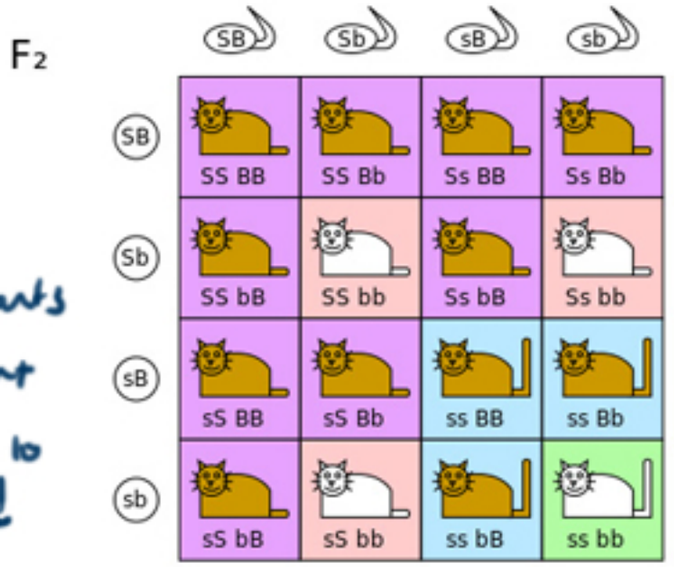
demonstrating independent assortment

- exception
- linkage



Dihybrid cross.

← dihybrids

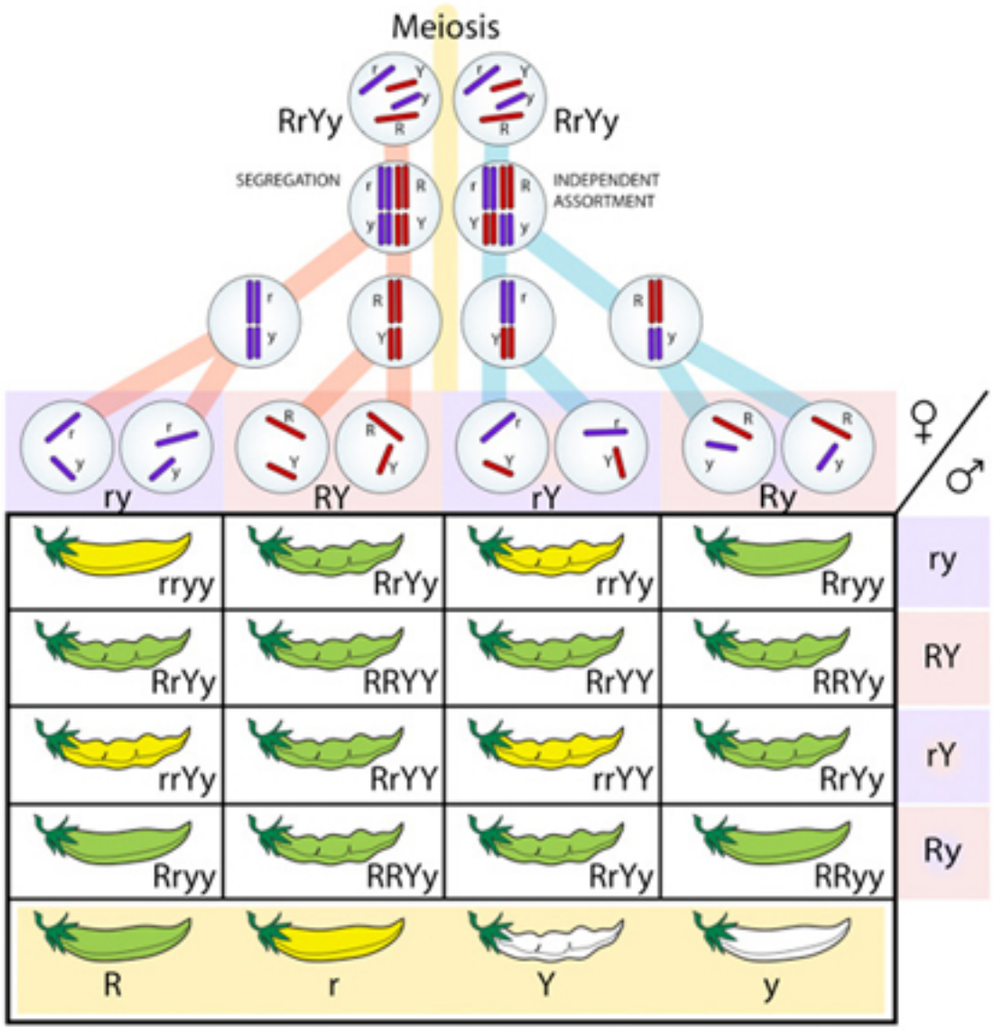


9:3:3:1

phenotypic ratio

NEAT!

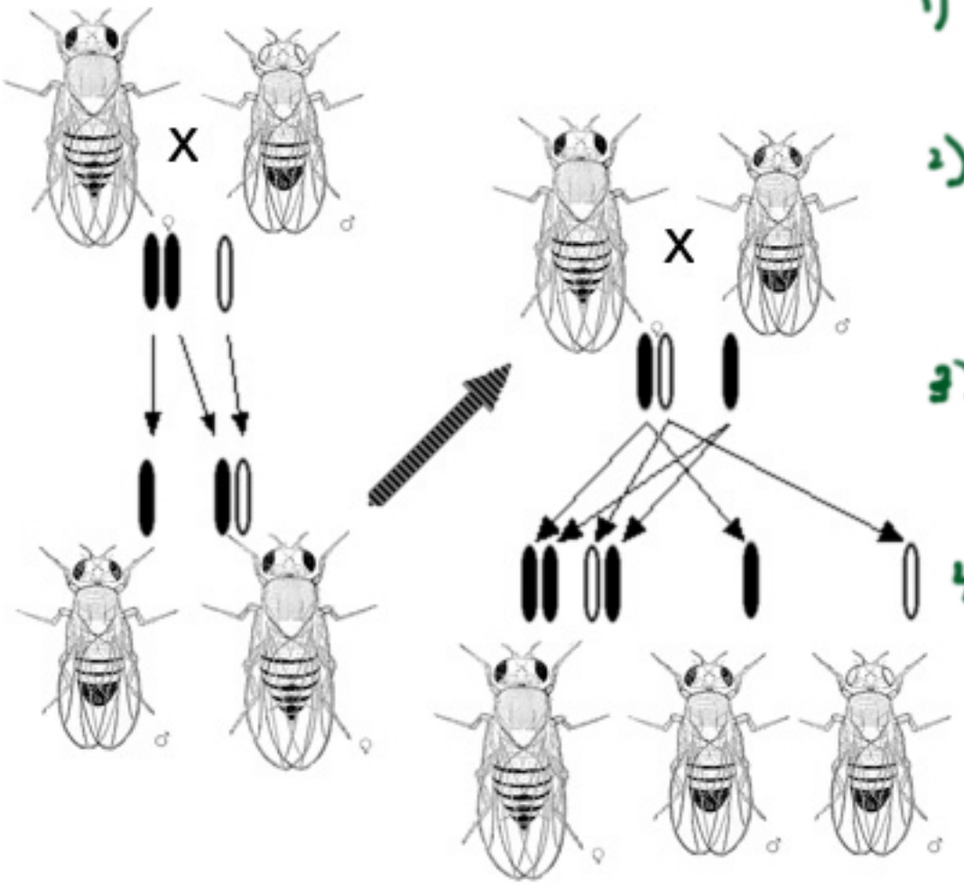
- genotype of parents will be different
- often don't have to fill them all in!


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



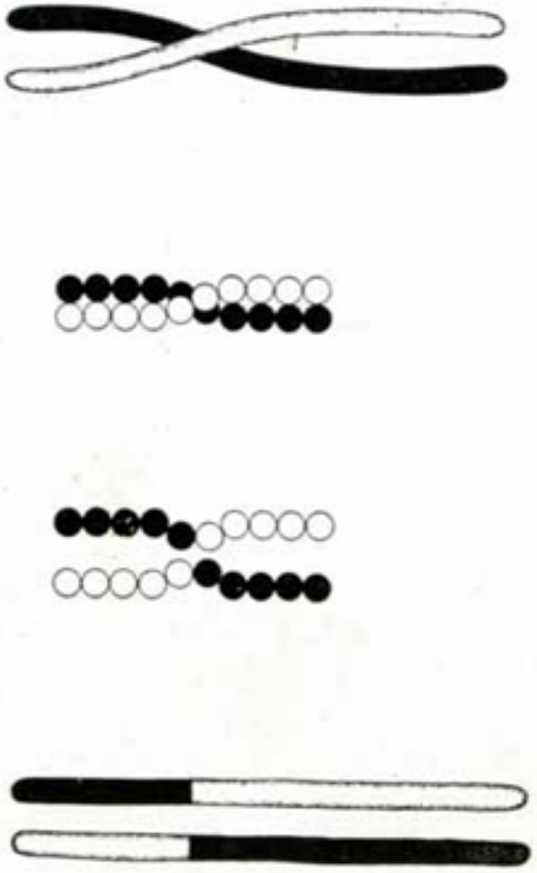
1) Noted white eyed mutant males

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

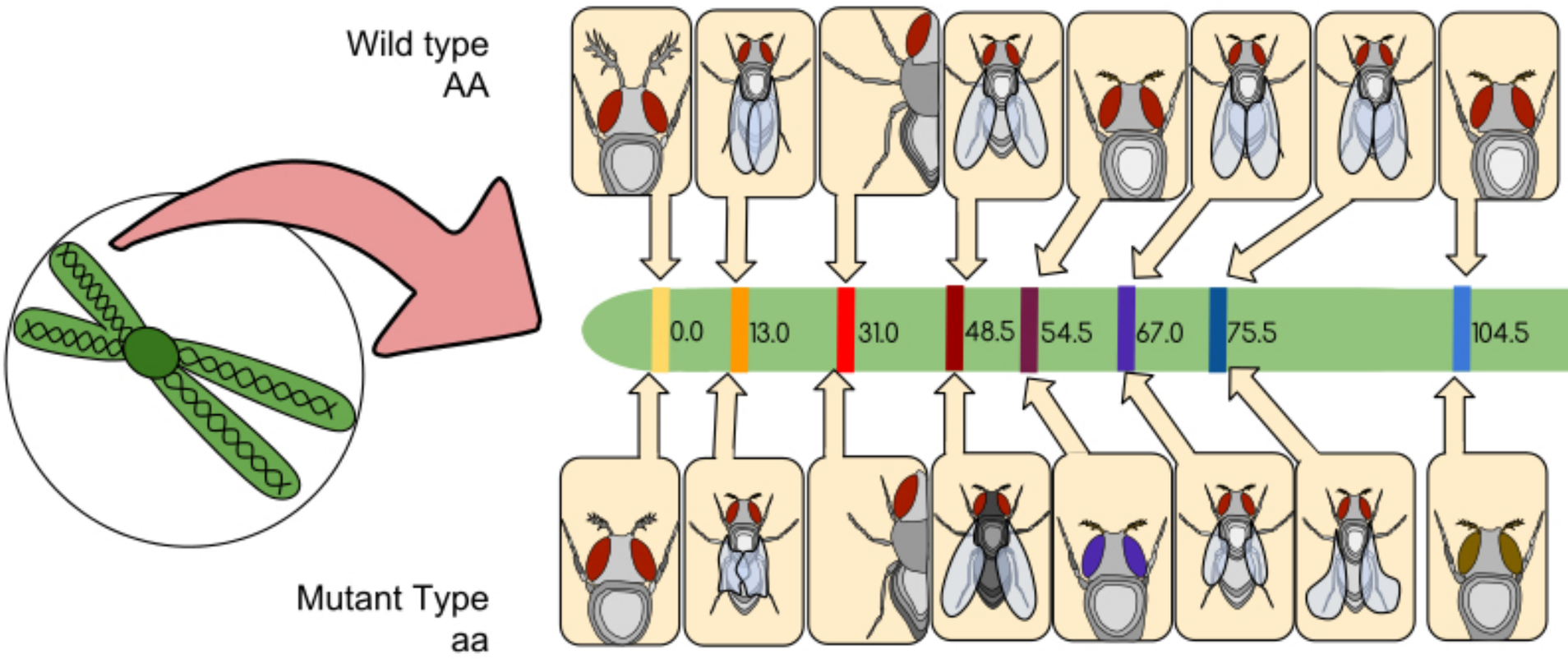
- Miniature winged mutant - also sex linked
- isolated miniature winged whited eyed male
- repeated original experiment.
- expected 25% miniature winged white eyed males

However - the two traits often assorted independently

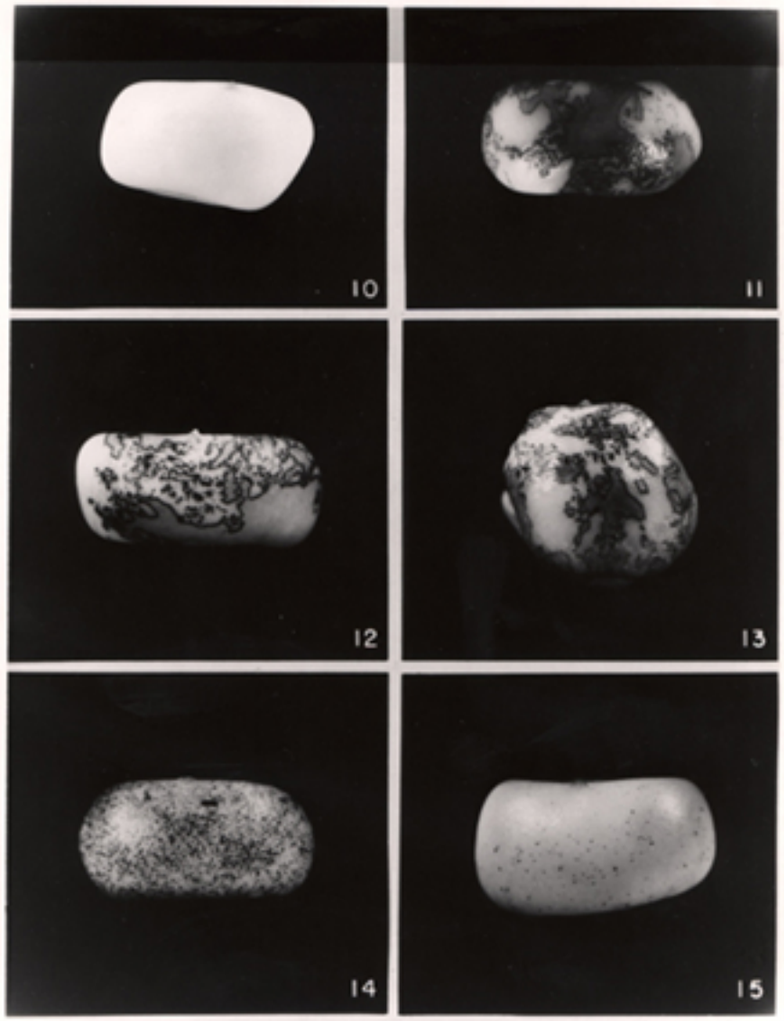
FIG. 64. Scheme to illustrate a method of crossing over of the chromosomes.

Stuyvesant

gene mapping



recombination frequency
of 1% = 1 centiMorgan



Barbara McClintock

Activator (Ac) - gene for synthesis of anthocyanin pigment

Dissociator (Ds) - disrupts activator

↑

transposon

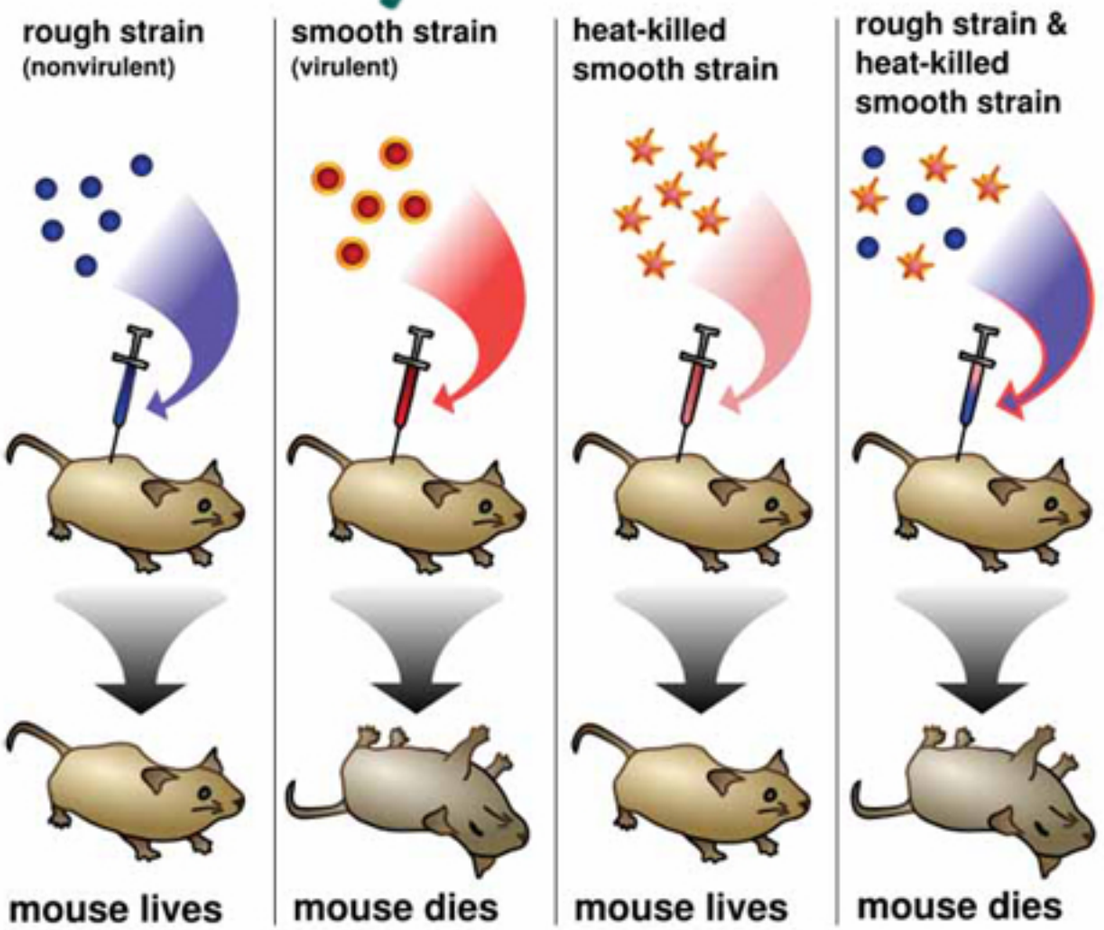
Mobile genetic elements

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



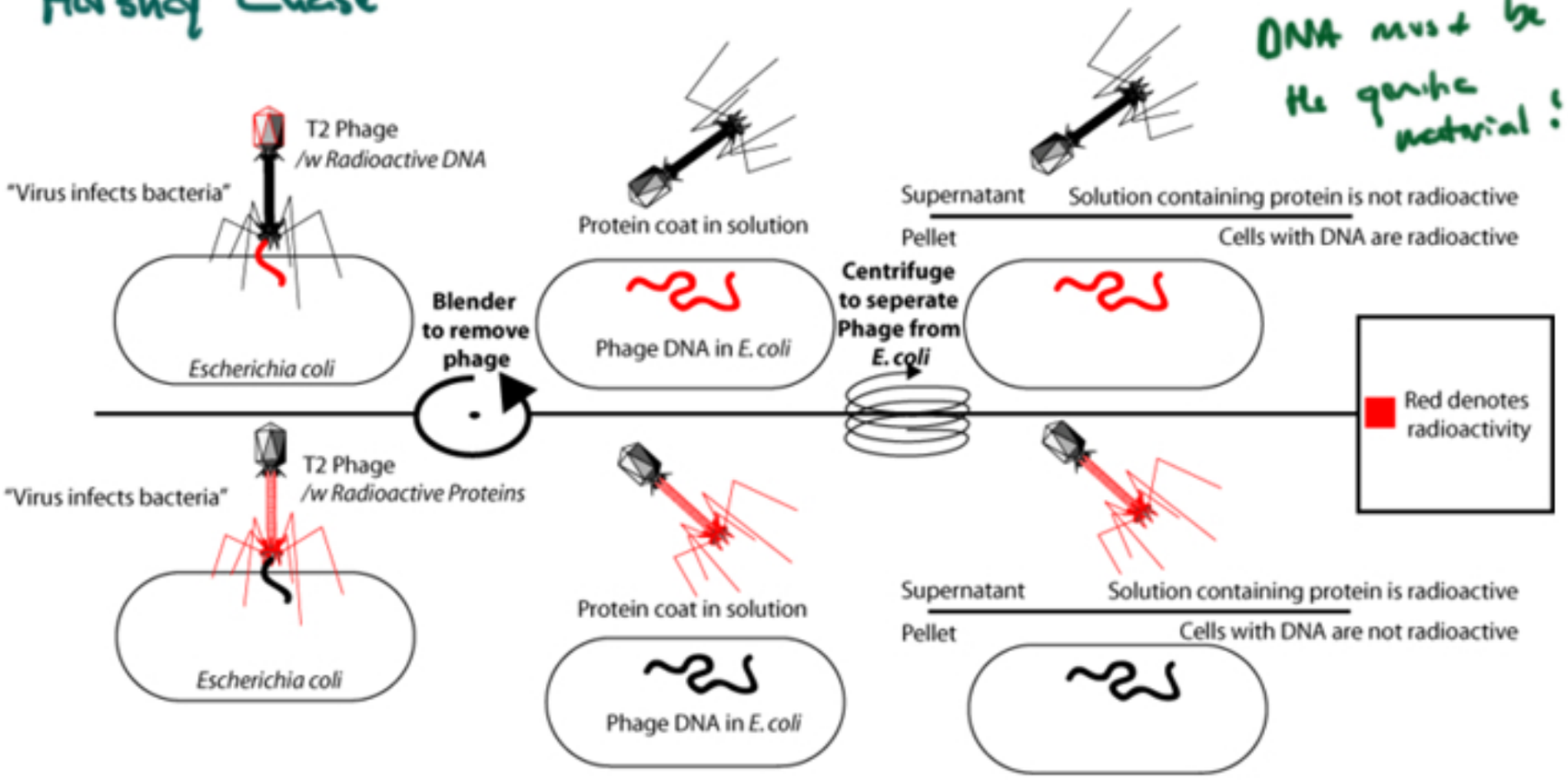
Griffiths

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

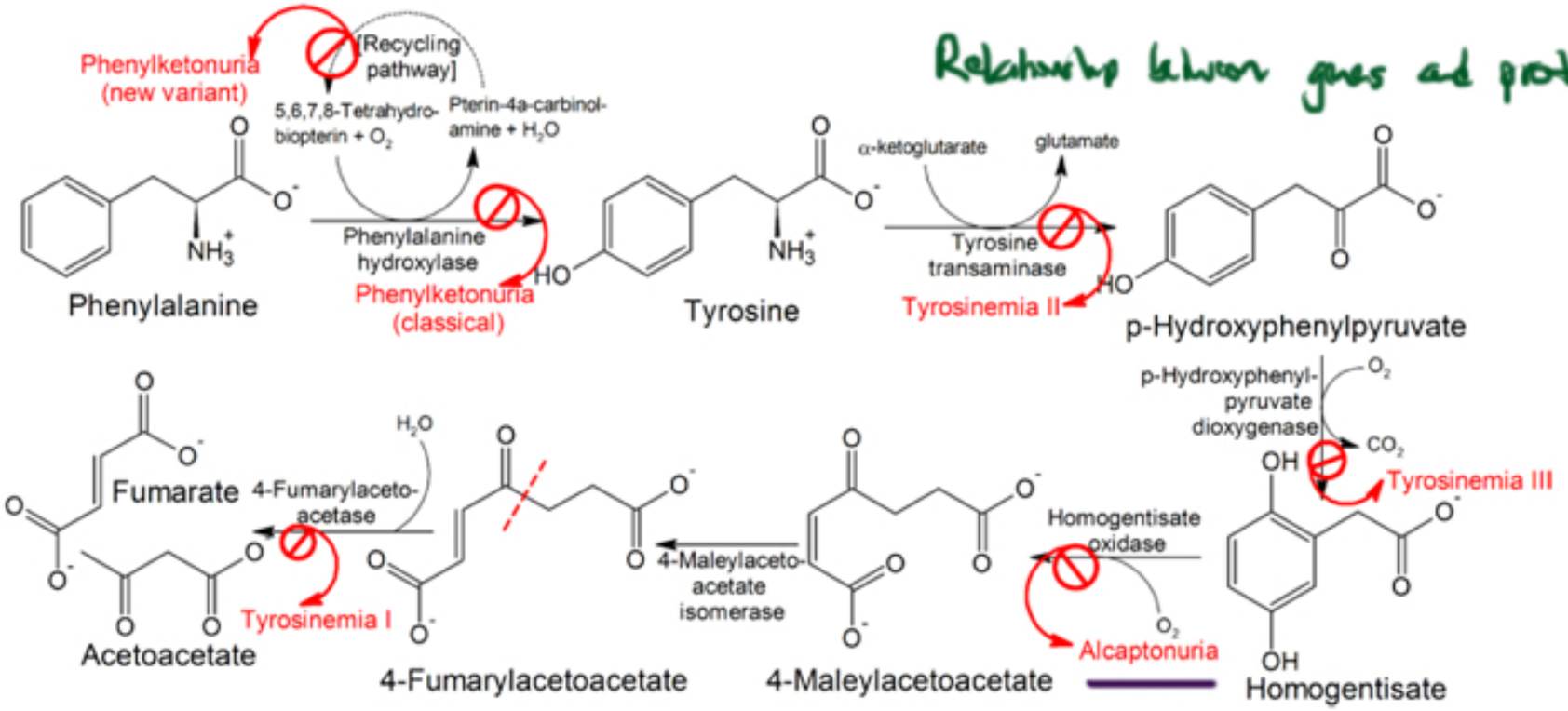
IV ssRNA (+) → ss (−)

V (−) ssRNA → viral polymerase

VI ssRNA-RT → dsDNA

VII dsDNA-RT

Relationship between genes and proteins

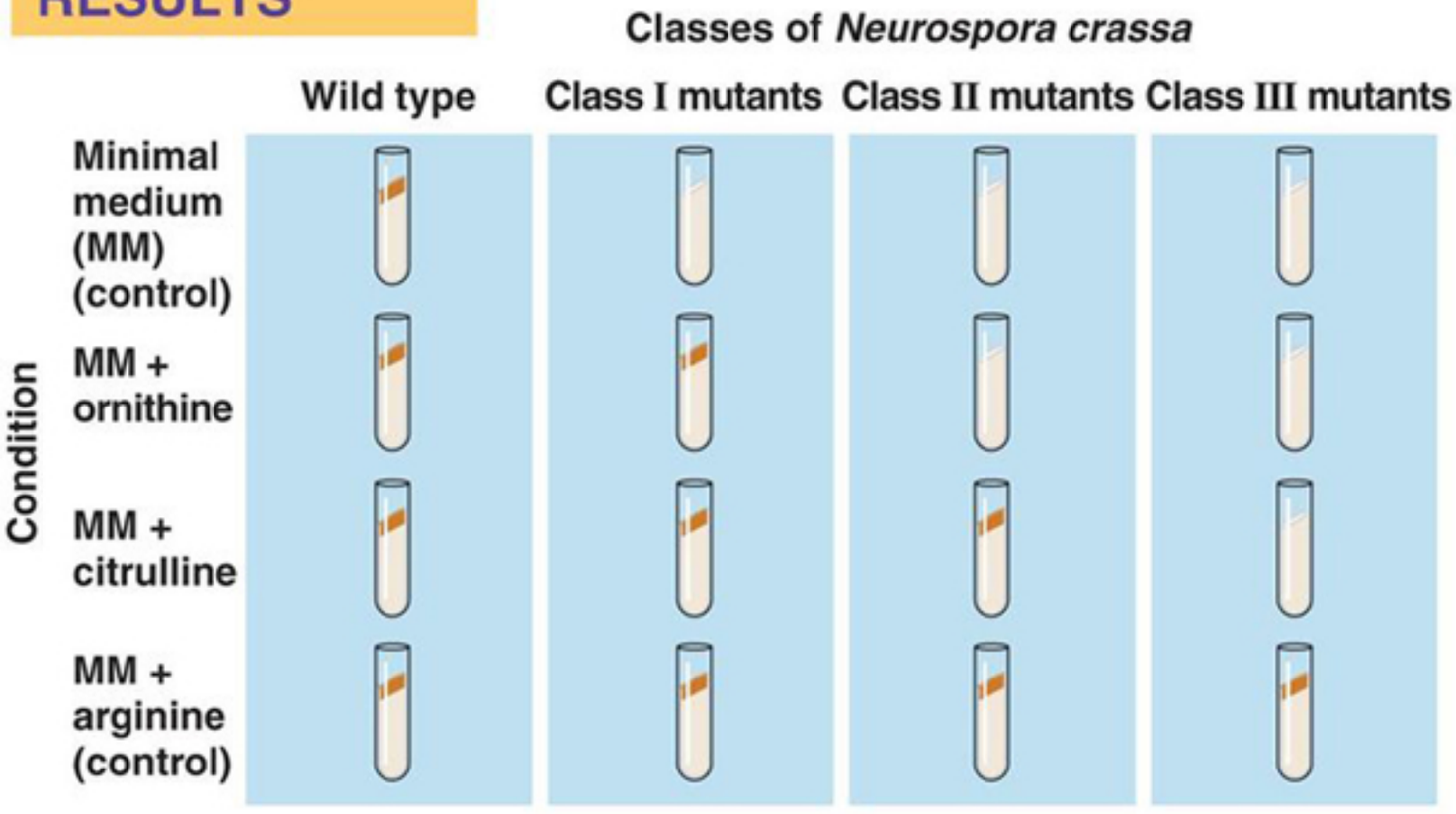


- Alcaptonuria
  - trait that obeyed Mendelian inheritance

Archibald Garrod

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

RESULTS

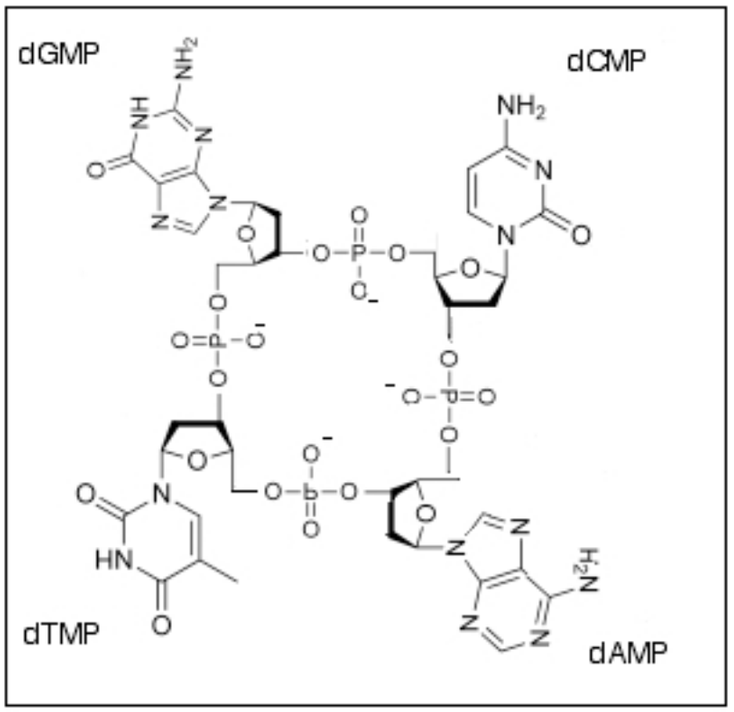


Beadle and Tatum

- 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

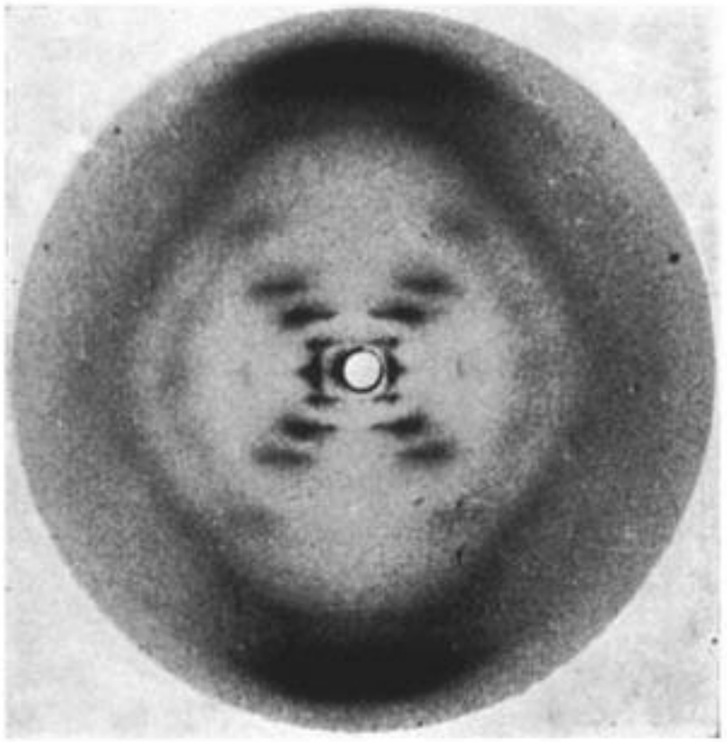
Through careful experimentation, Chargaff discovered two rules that helped lead to the discovery of the double helix structure of DNA.

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.

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

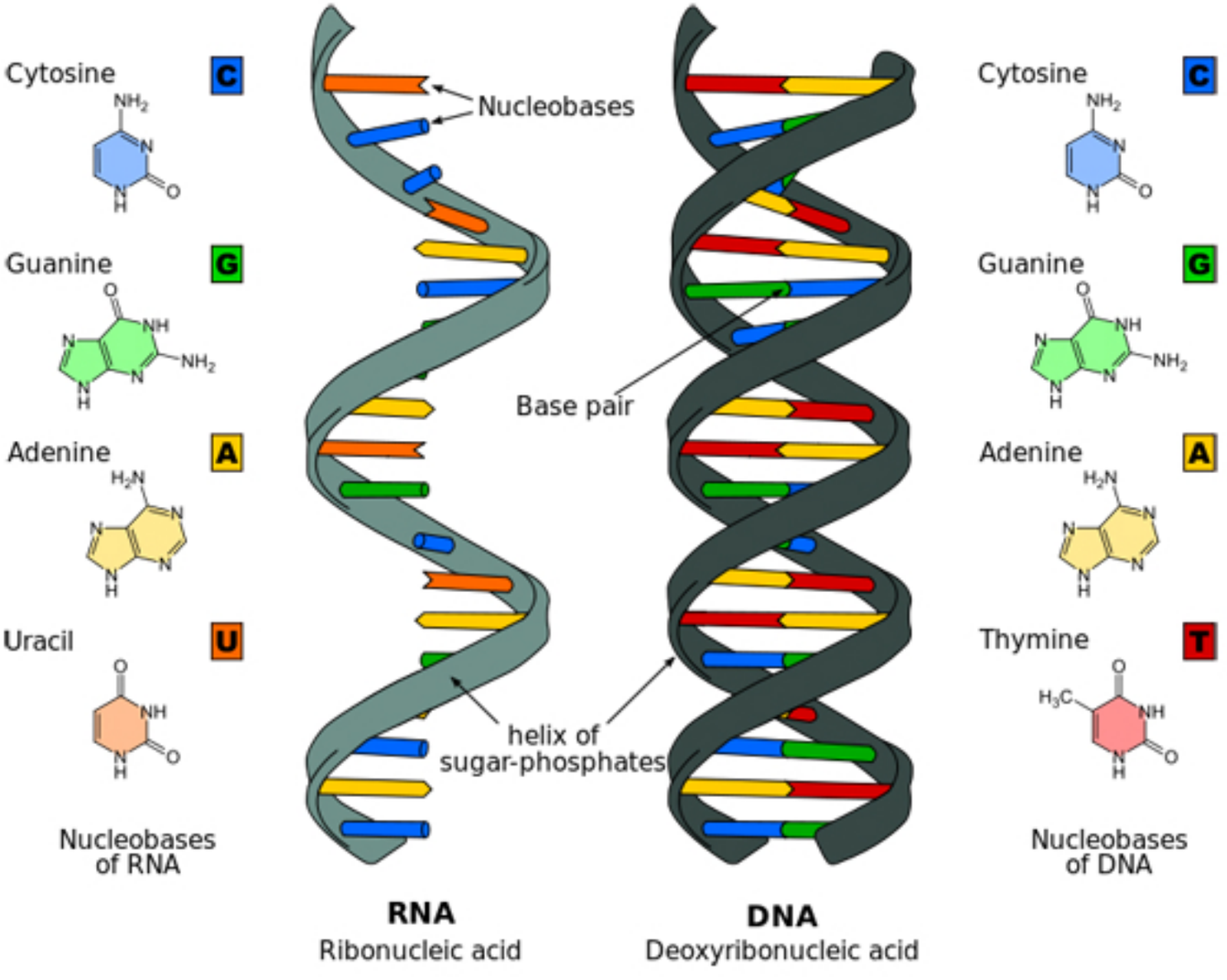
Rosalind Franklin

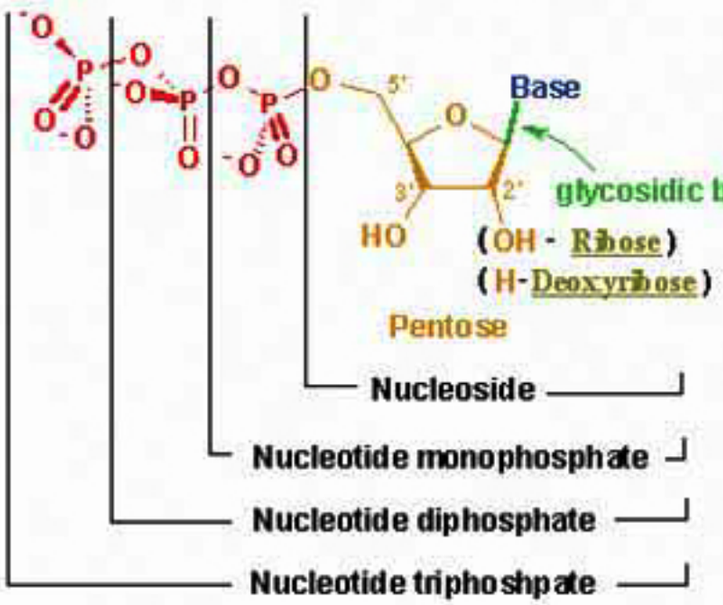


$2d\sin\theta = n\lambda$

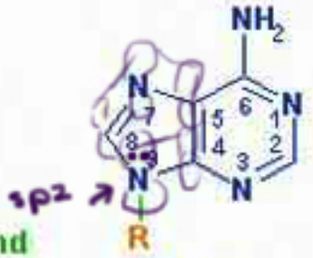
Bragg's Law

Watson & Crick





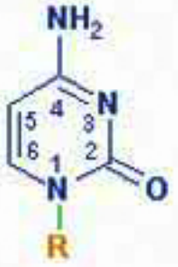
Purines



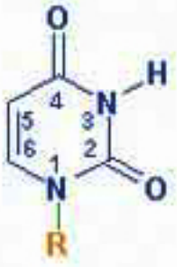
Adenine

Guanine

Pyrimidines



Cytosine

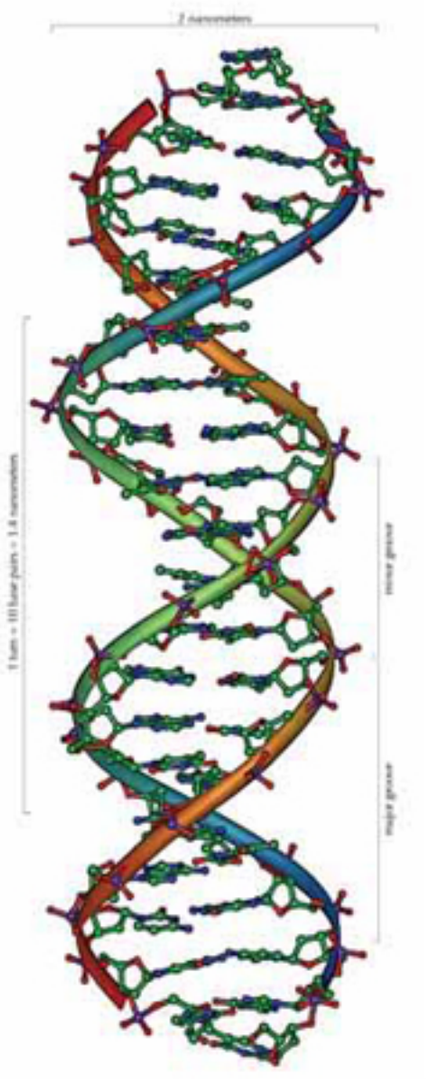


Uracil

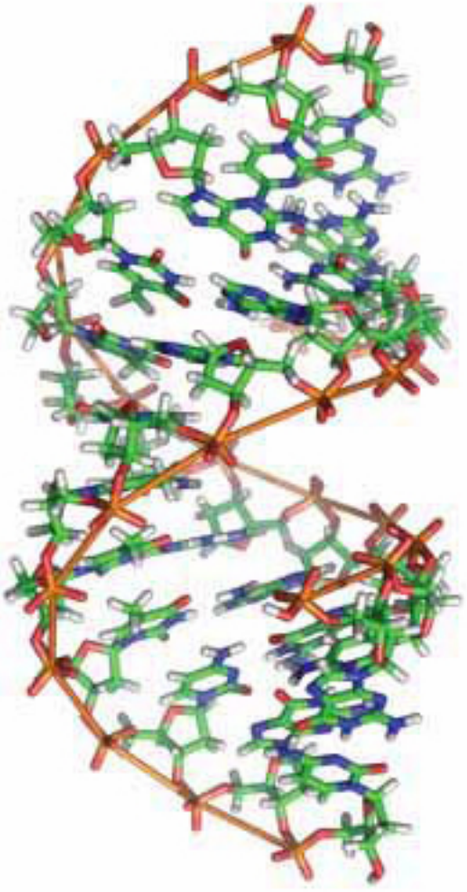
Thymine

(methylated uracil)

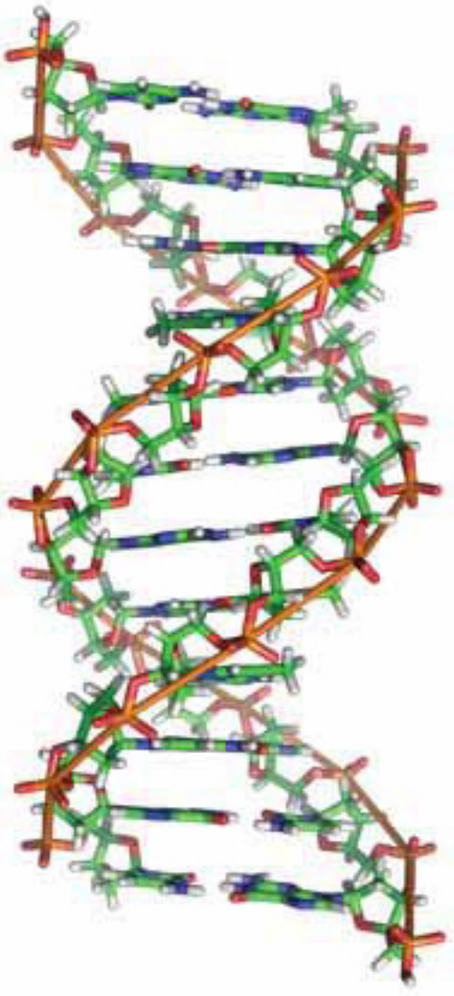
All rings are aromatic


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

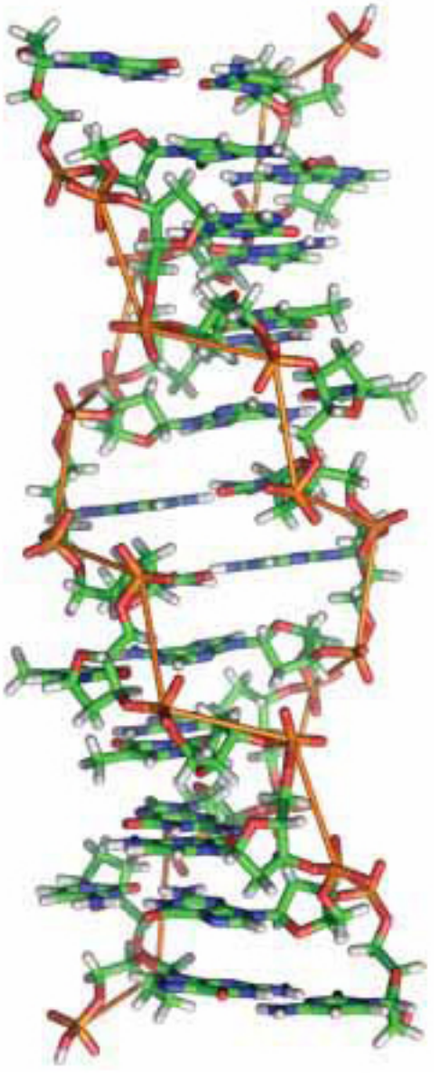
B Form



A Form

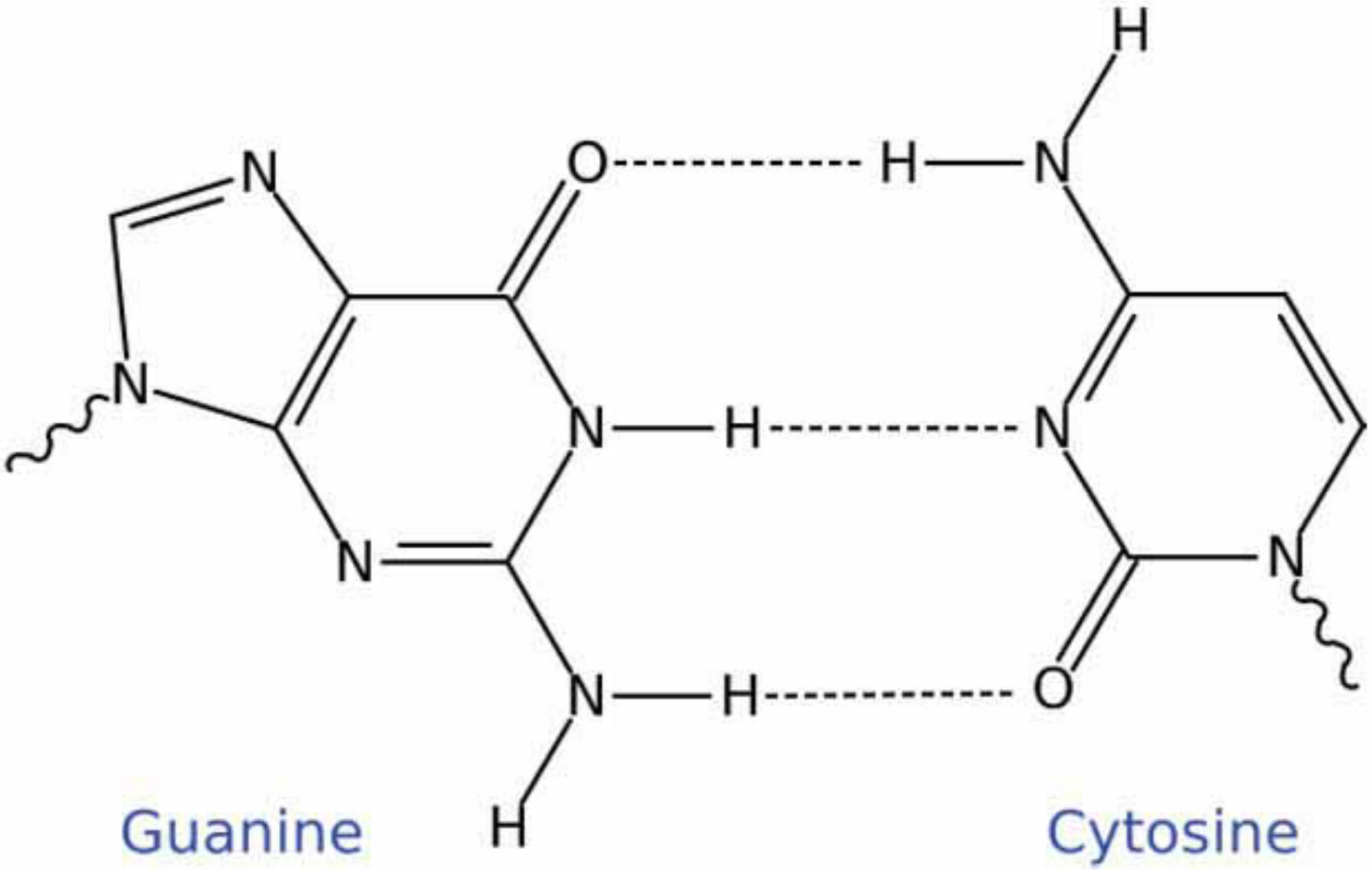


B Form



Z Form

(left handed)

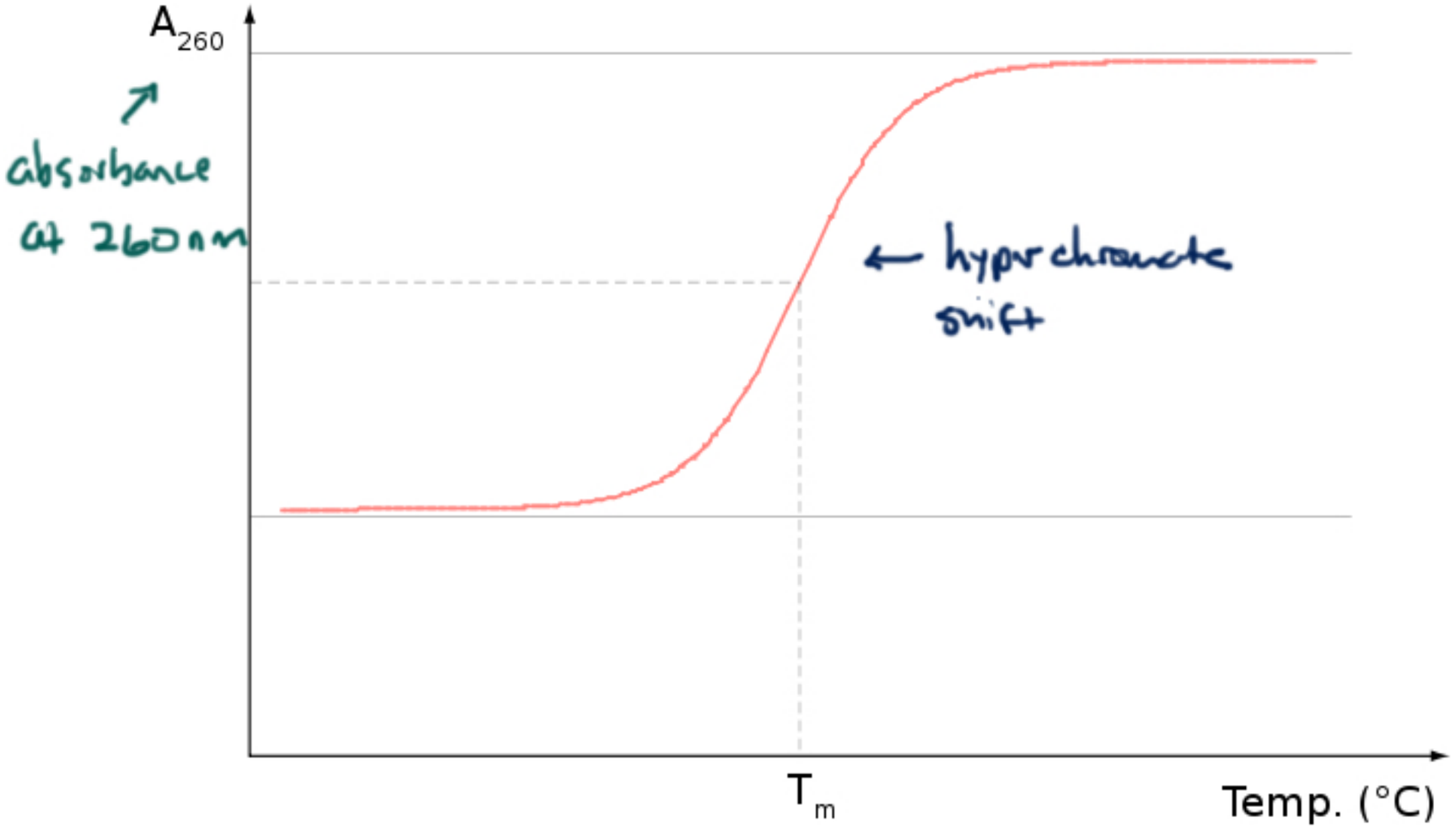


Guanine

Cytosine

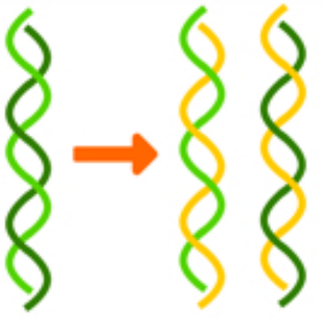
G ≡ C

A = T

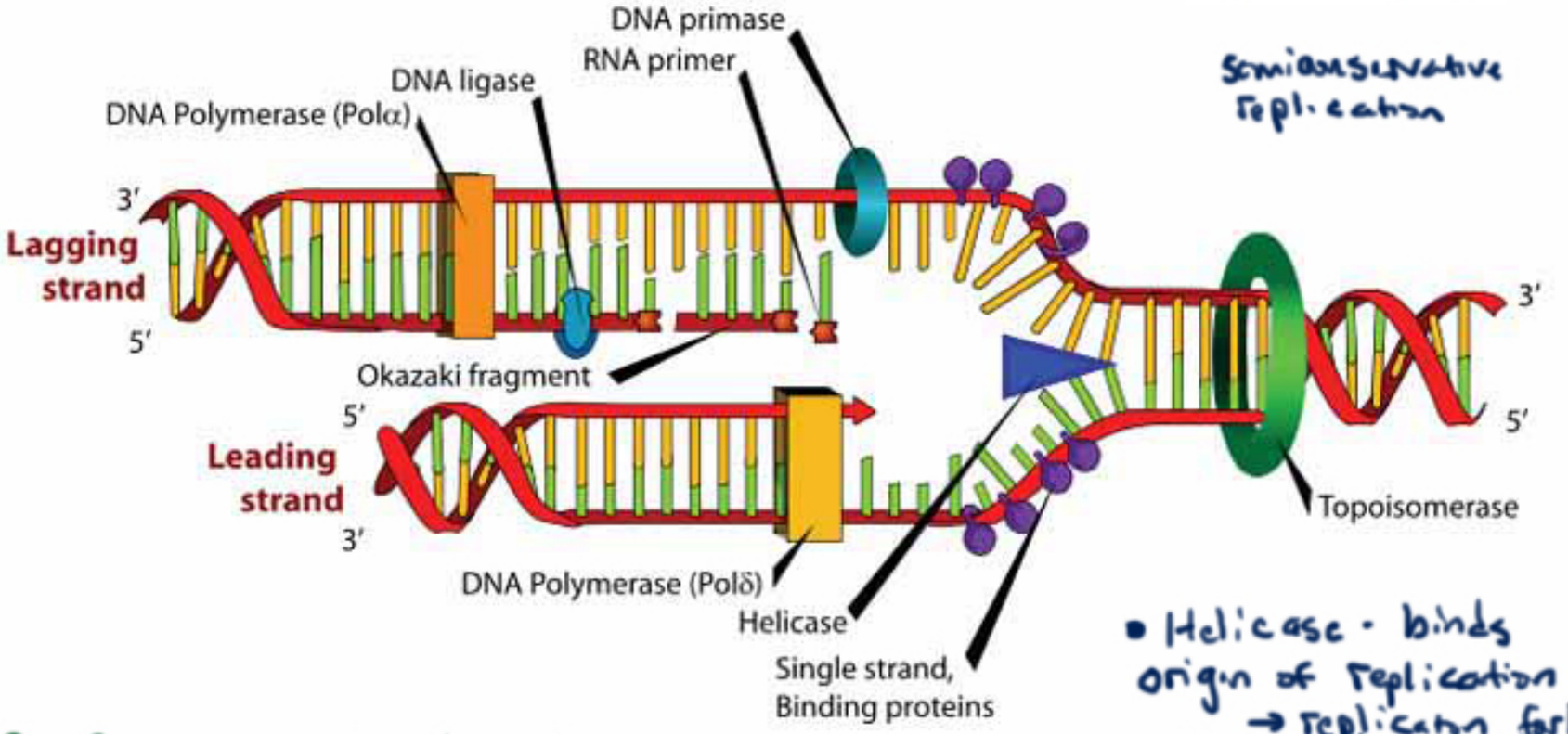


Melting DNA is synonymous with denaturation (unzipping)

DNA Replication



Semiconservative replication



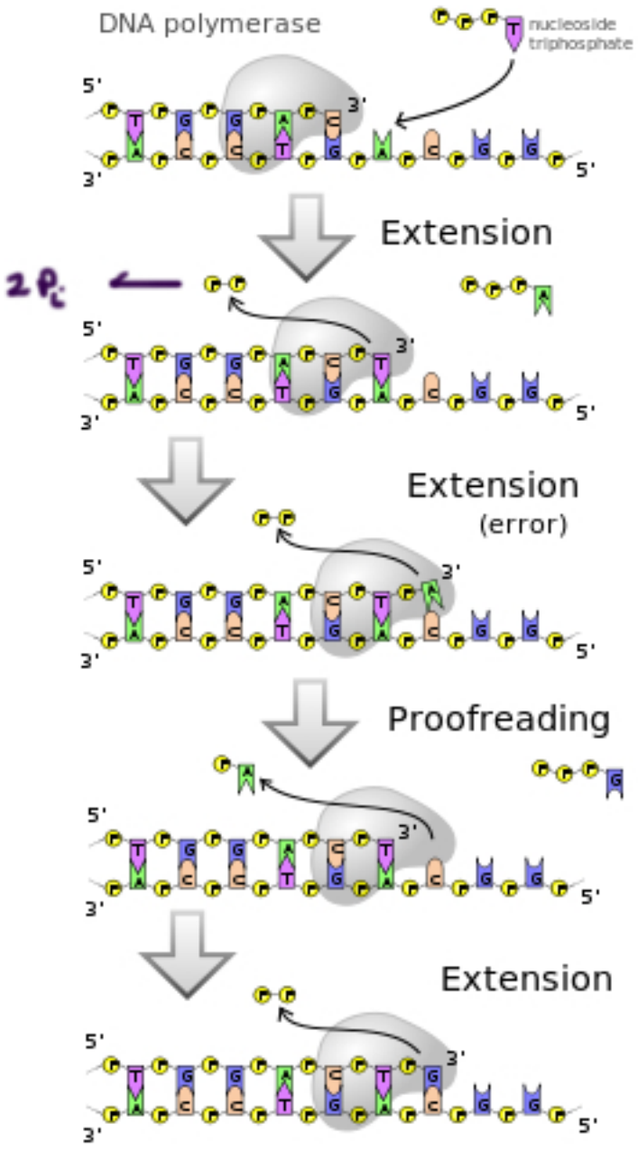
- Helicase - binds origin of replication → replicaton fork (make binding proteins)
- Primase - short complimentary RNA

- 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.

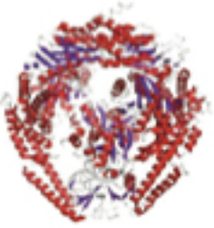
- DNA polymerase reads 3′ → 5′ writes 5′ → 3′
- leading strand and lagging strand (multiple primers and Okizaki fragments - DNA ligase

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



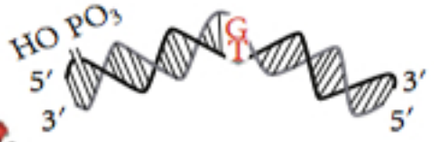
Proofreading

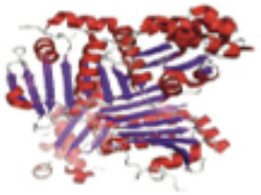
Mismatch Repair



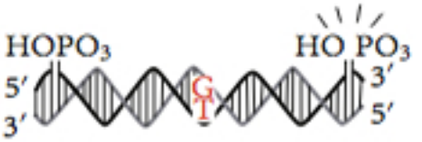
MutSα ↓ Recognition

DNA clamp binds





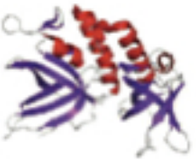
MutLα ↓ Incision



EXO1

↓ Removal

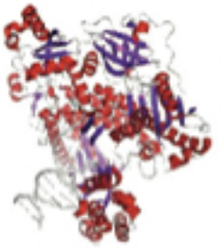
Exonuclease



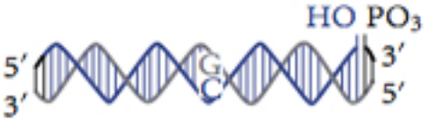
RPA

and

- homologous and nonhomologous end joining

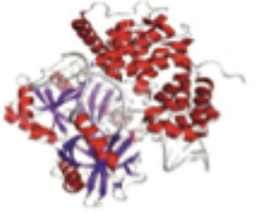


DNA polymerase δ ↓ Resynthesis



also:

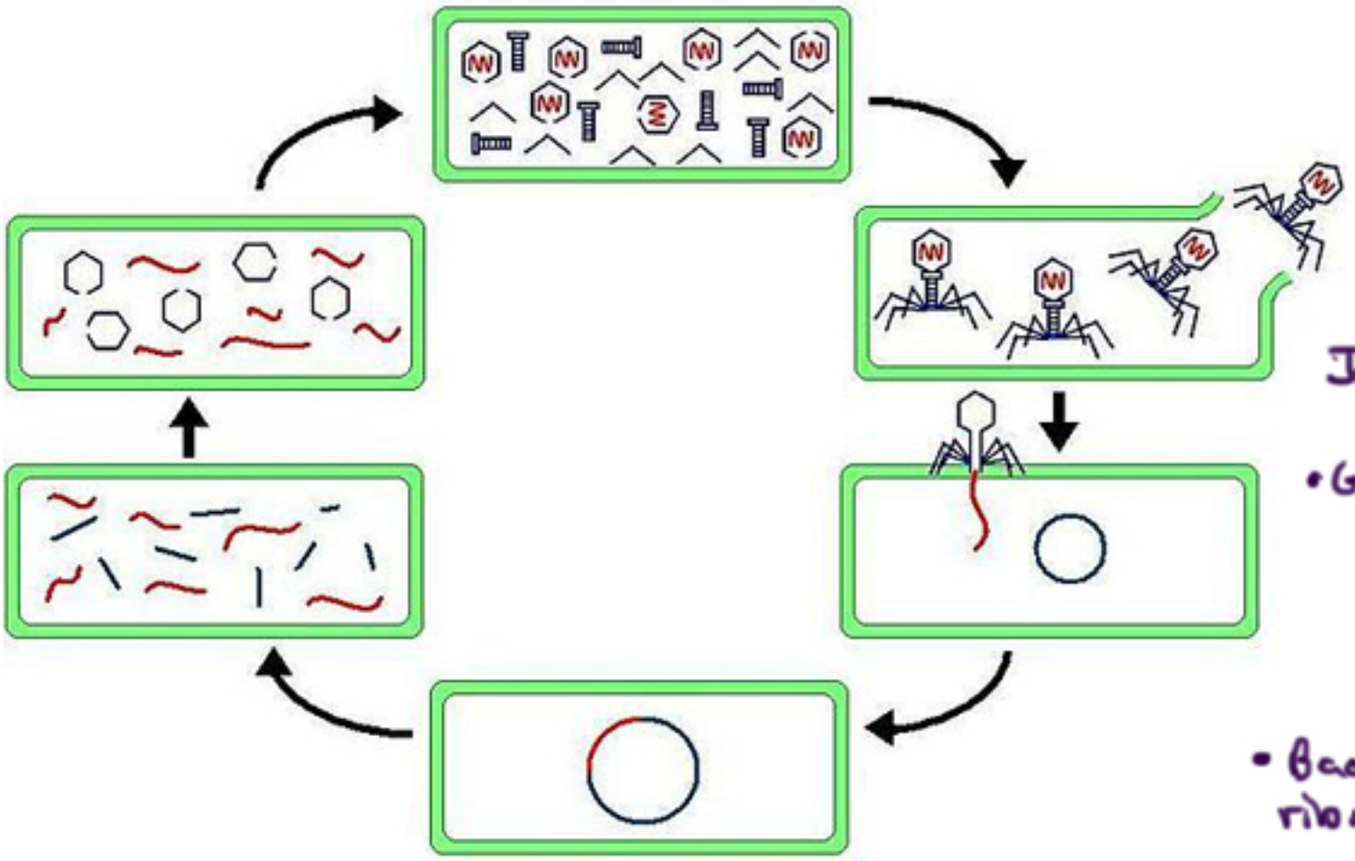
- nucleotide excision repair - for pyrimidine dimers from UV



DNA ligase ↓ Ligation

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

←



Discovery of mRNA by disproving the ribosome hypothesis.

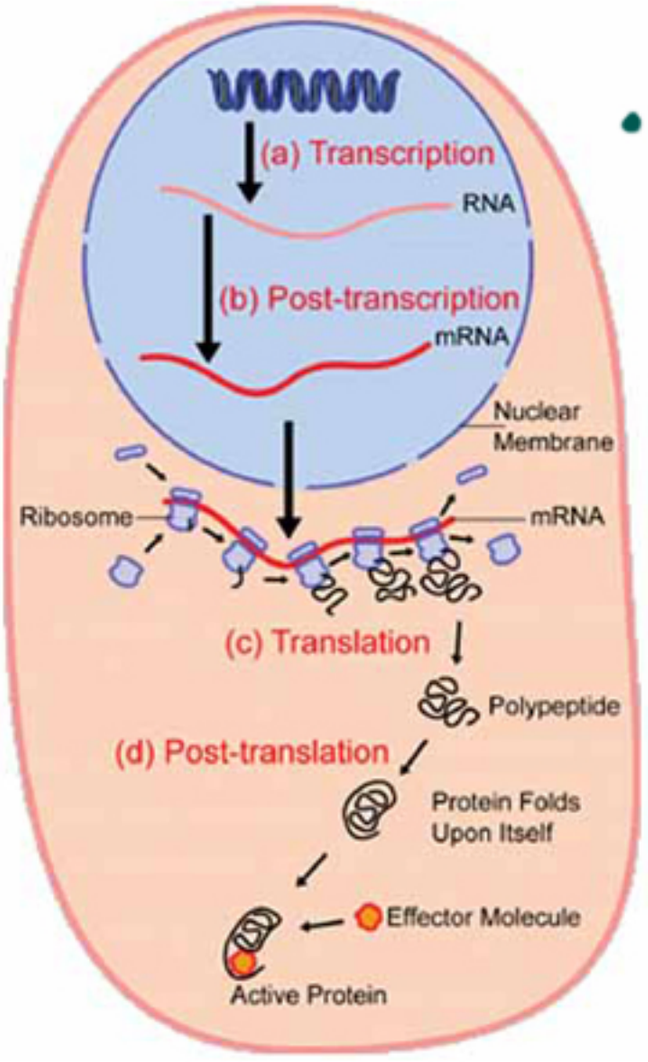
Jacob, Brenner, et al

- 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

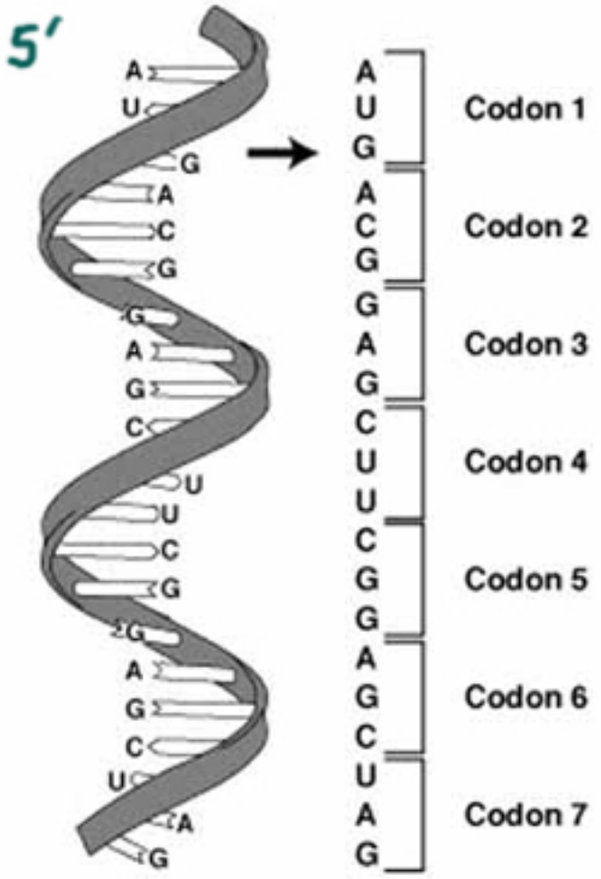
Conclusion - Another form of RNA was carrying the information for peptide sequence - mRNA

Central Dogma

Transcription & Translation



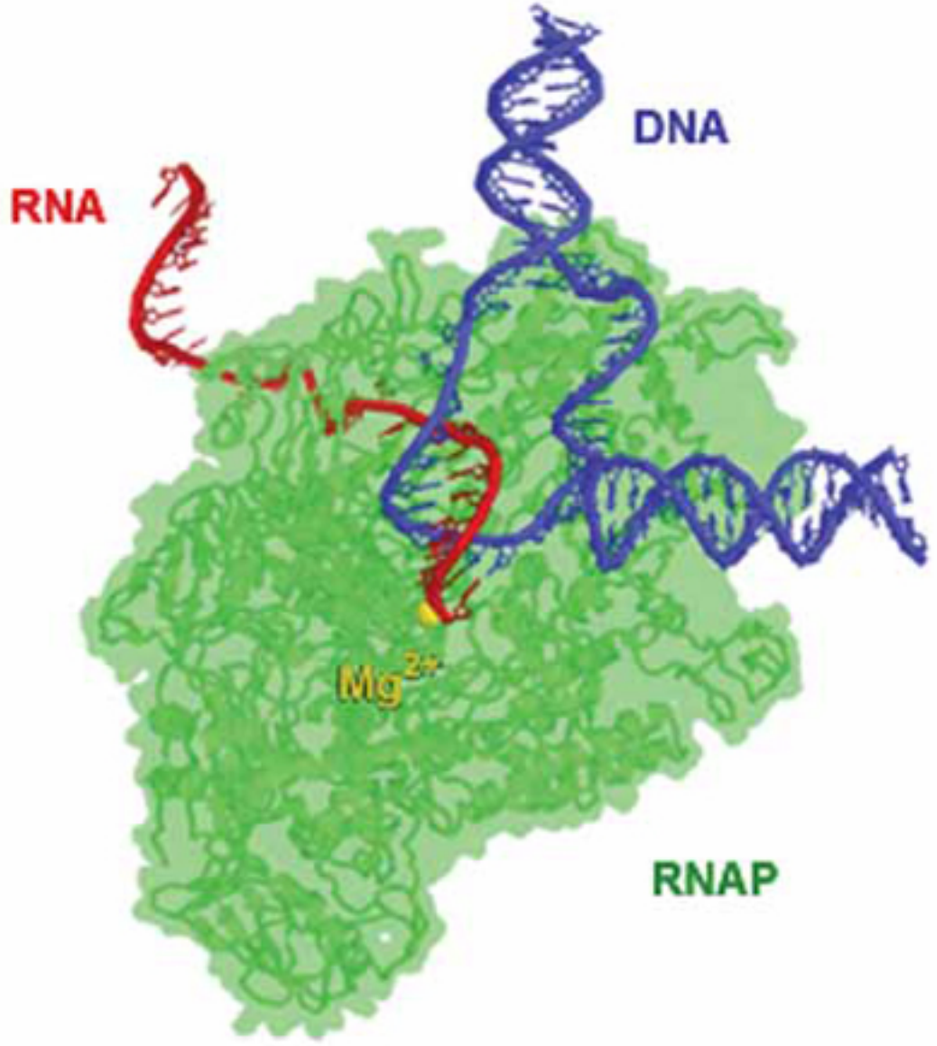
- Let's overview regulation of gene expression
  - Transcriptional control
    - Chromatin modeling
    - DNA methylation
    - Transcription factors
  - Post transcriptional
    - Alternative splicing
    - polyA tail - RNA half life - 3' untranslated region sequences
    - 5' cap
  - RNA interference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



RNA

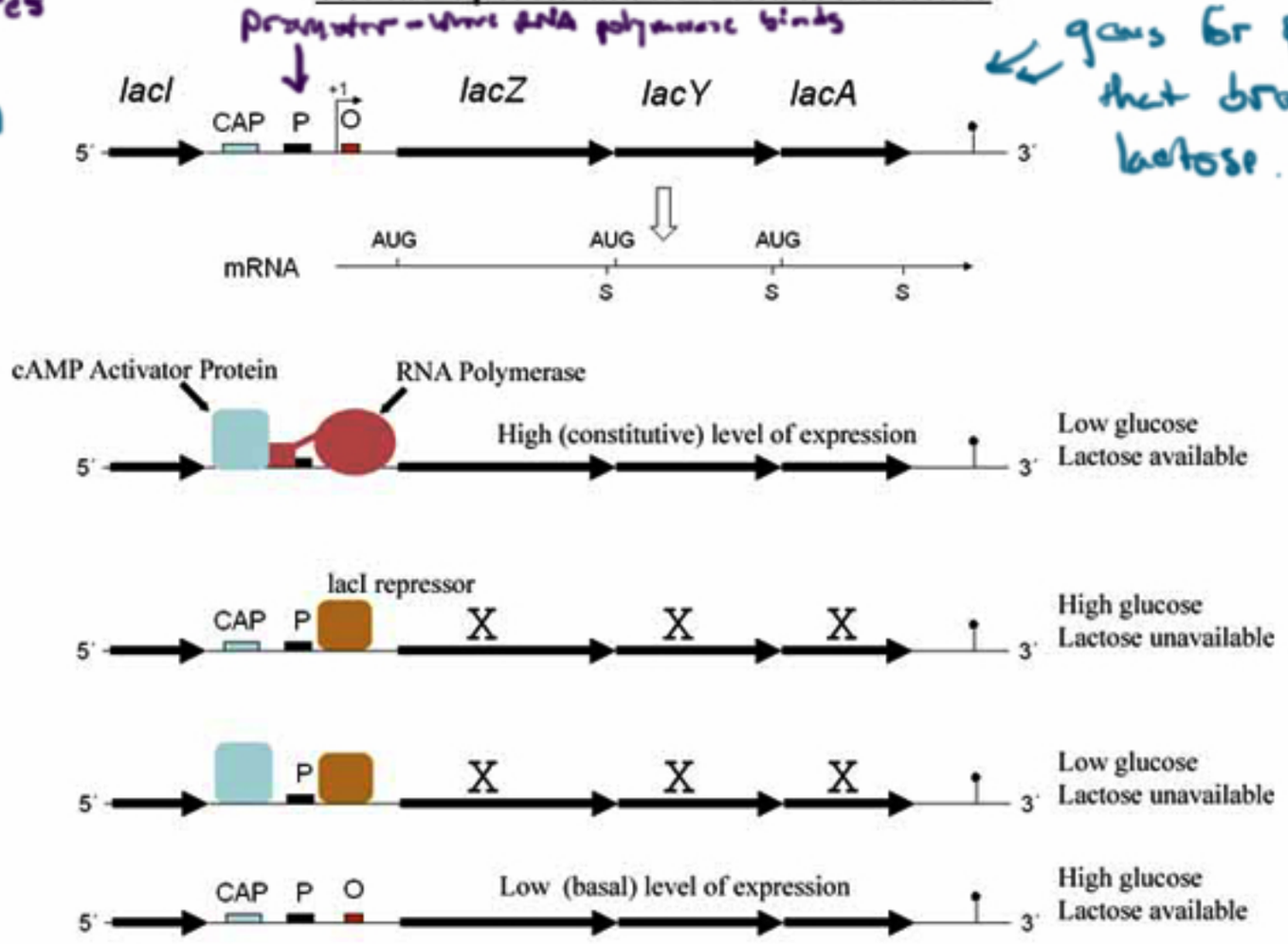
Ribonucleic acid

More in translation


DNA
RNA
Mg^{2+}
RNAP

Prokaryotes

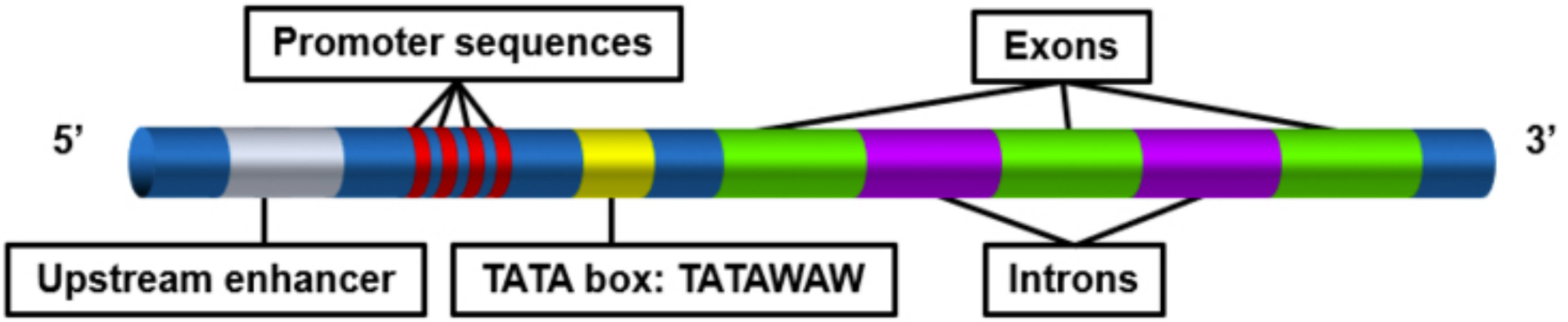
Lac operon
- an inducible operon



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

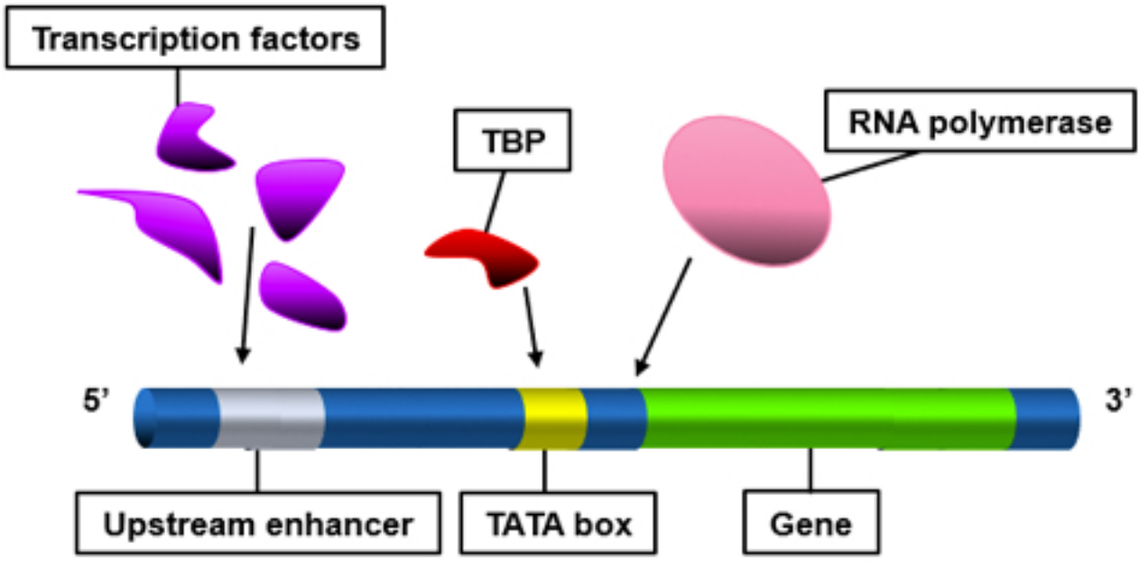
eukaryote gene

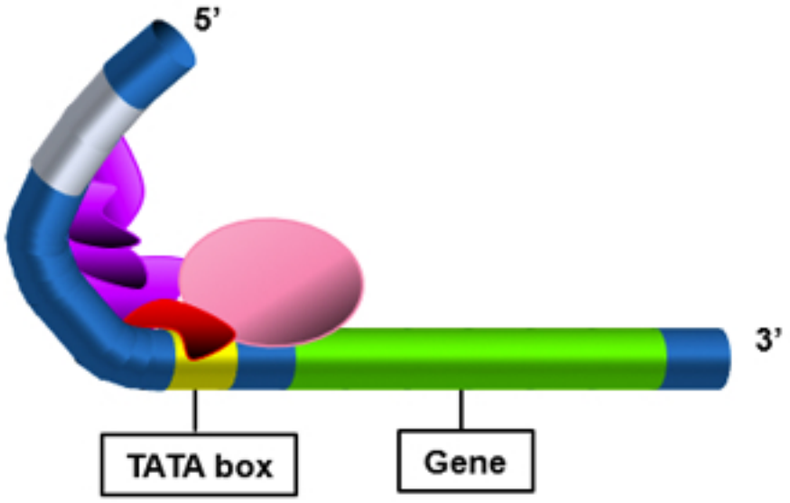


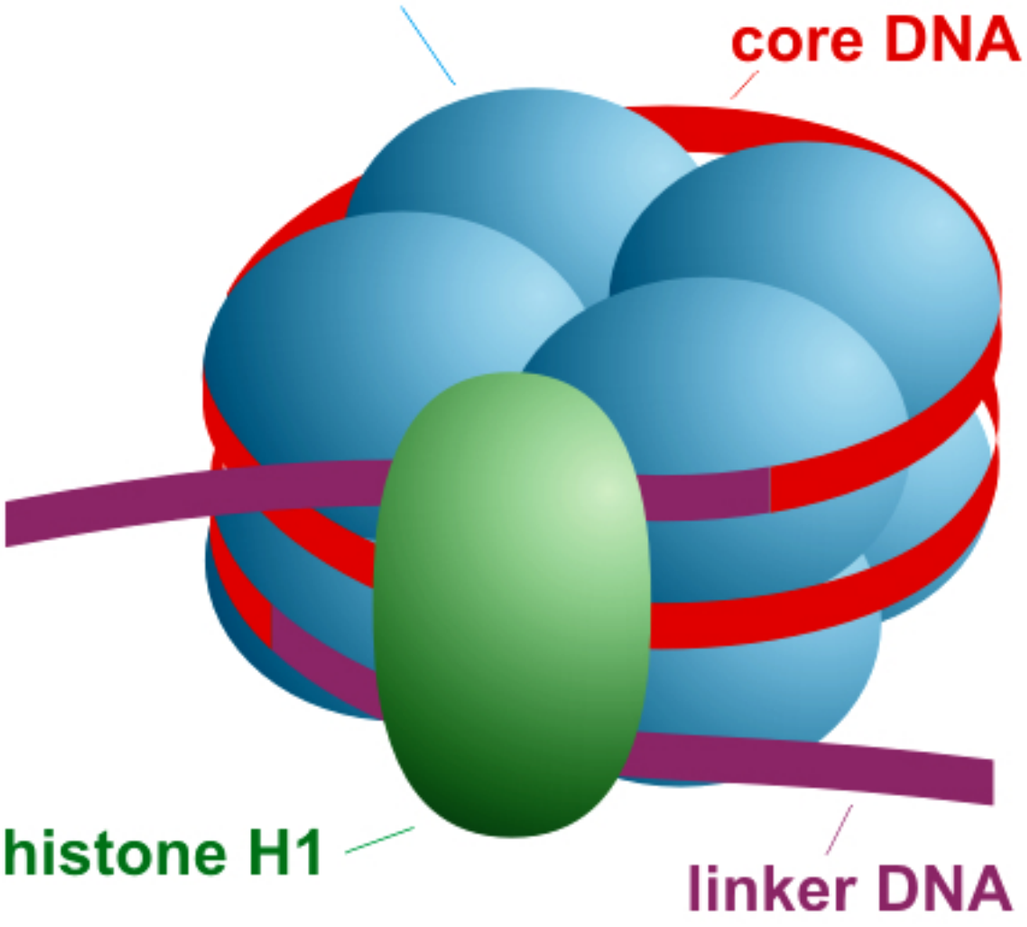
binding of specific transcription factors

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

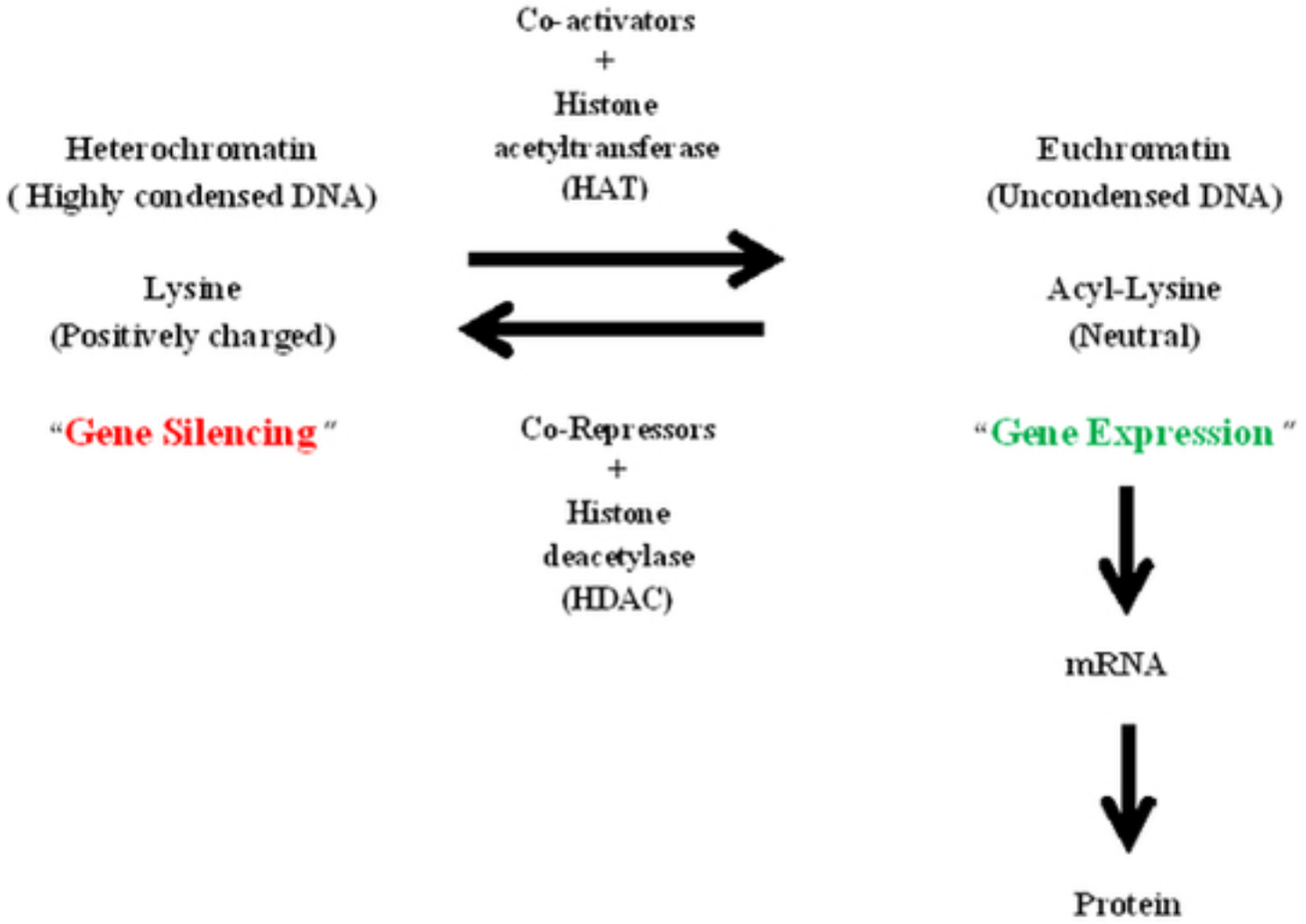


high pI · lots of lysine

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

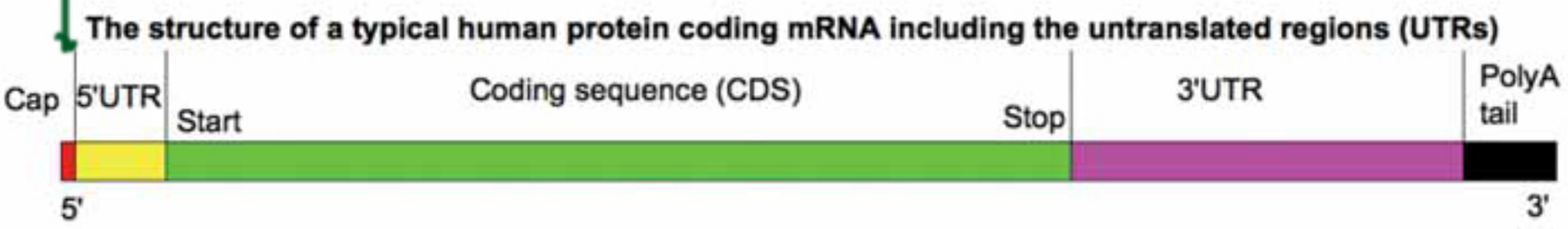
heterochromatin

euchromatin

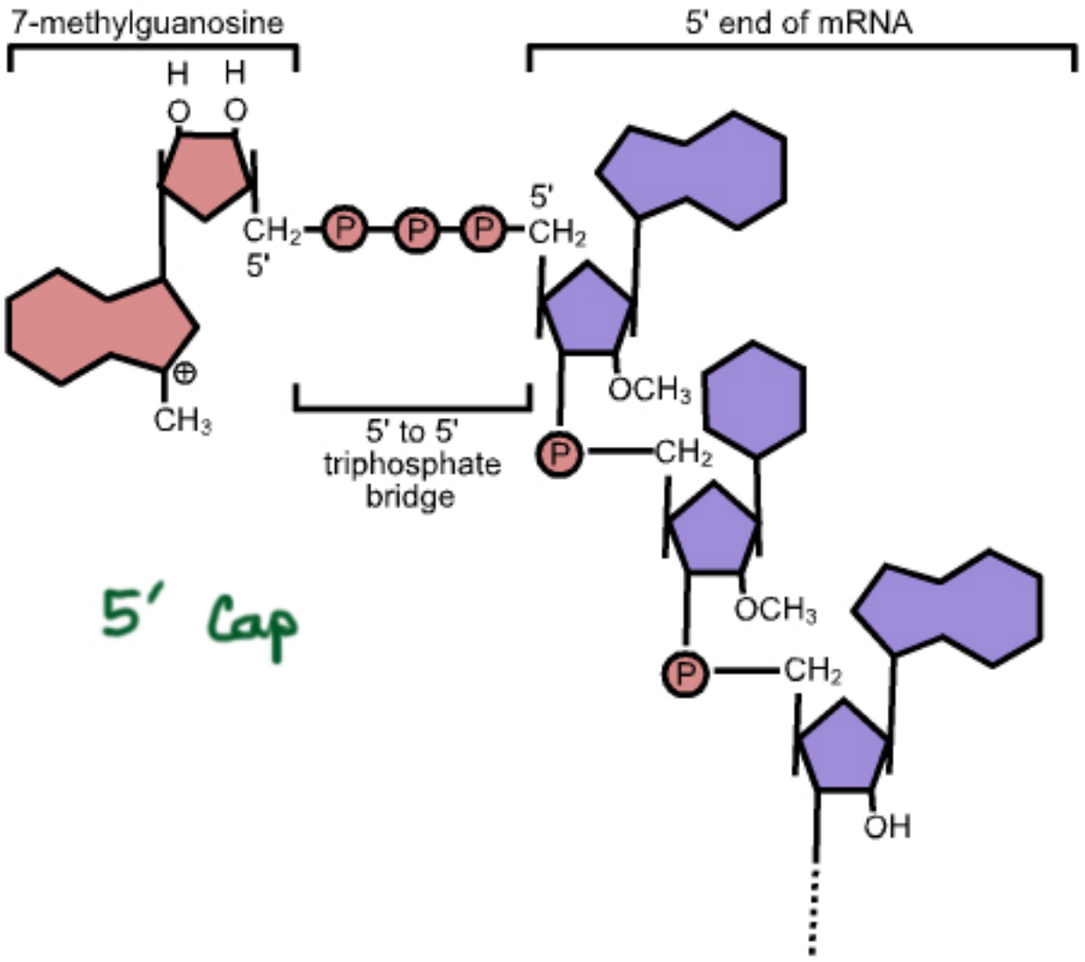

Co-activators
+
Histone
acetyltransferase
(HAT)
Heterochromatin
(Highly condensed DNA)
Euchromatin
(Uncondensed DNA)
Lysine
(Positively charged)
Acyl-Lysine
(Neutral)
"Gene Silencing"
Co-Repressors
+
Histone
deacetylase
(HDAC)
"Gene Expression"
mRNA
Protein

triphosphate bridge + methylguanosine

mRNA

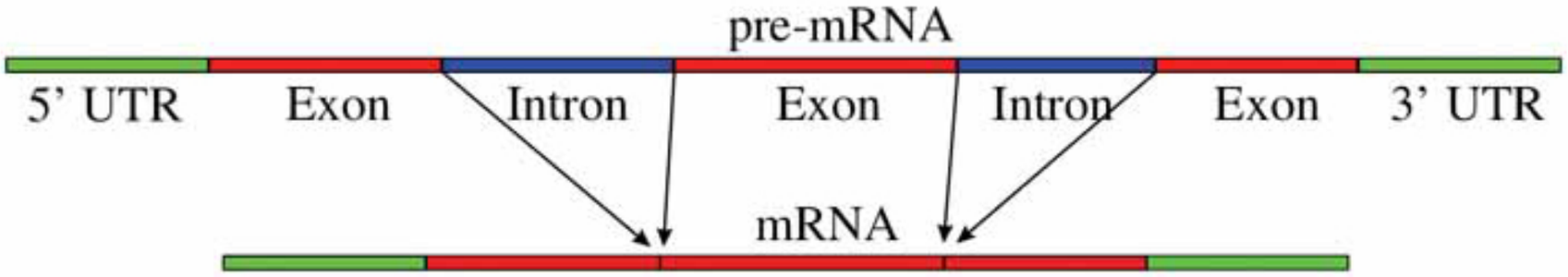


primary RNA transcript → 5' cap, poly A tail, splicing → mRNA

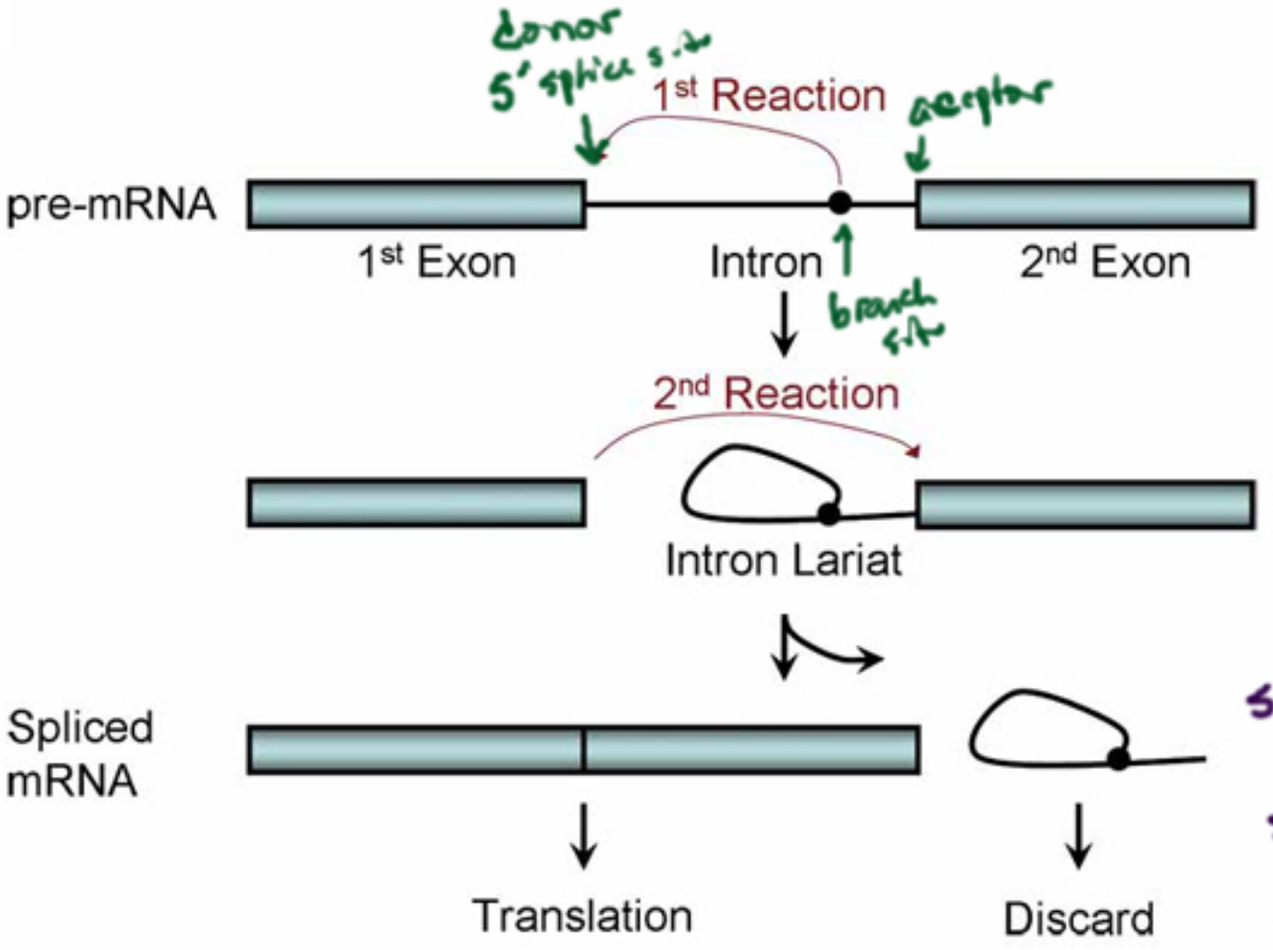

7-methylguanosine
5' end of mRNA
H H
O O
CH2
5'
P P P
5'
CH2
CH3
5' to 5'
triphosphate
bridge
OCH3
P
CH2
OCH3
P
CH2
OH

5′ Cap

Splicing



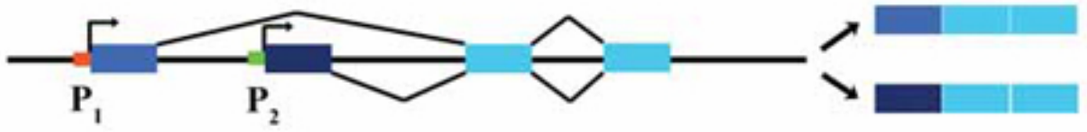
introns are excised



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

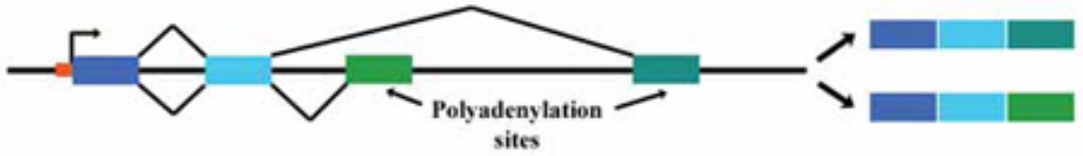
self splicing introns also exist

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



Alternative Splicing

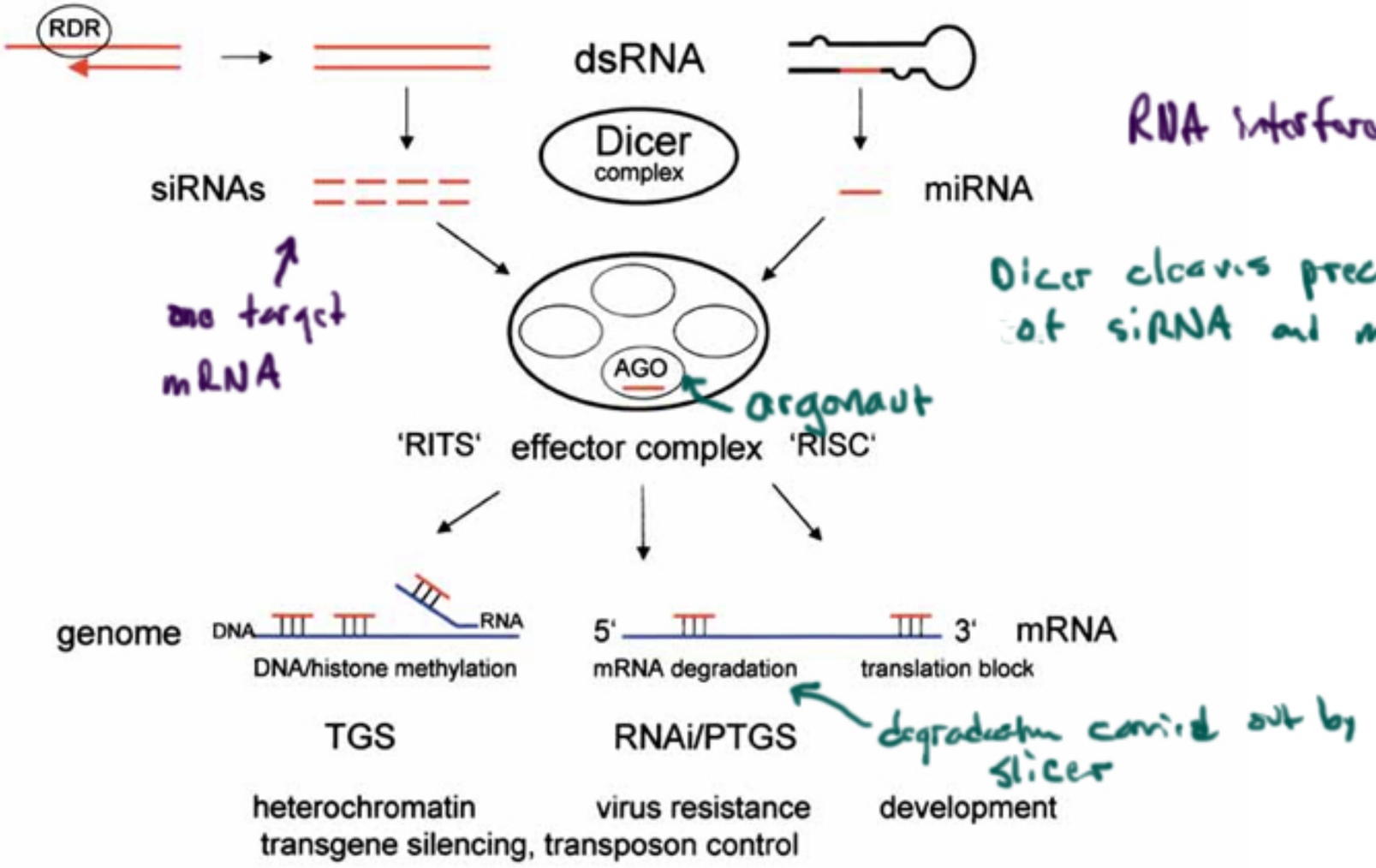
(b) Alternative selection of cleavage/polyadenylation sites (e.g., tropo*myosin* transcript)



(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

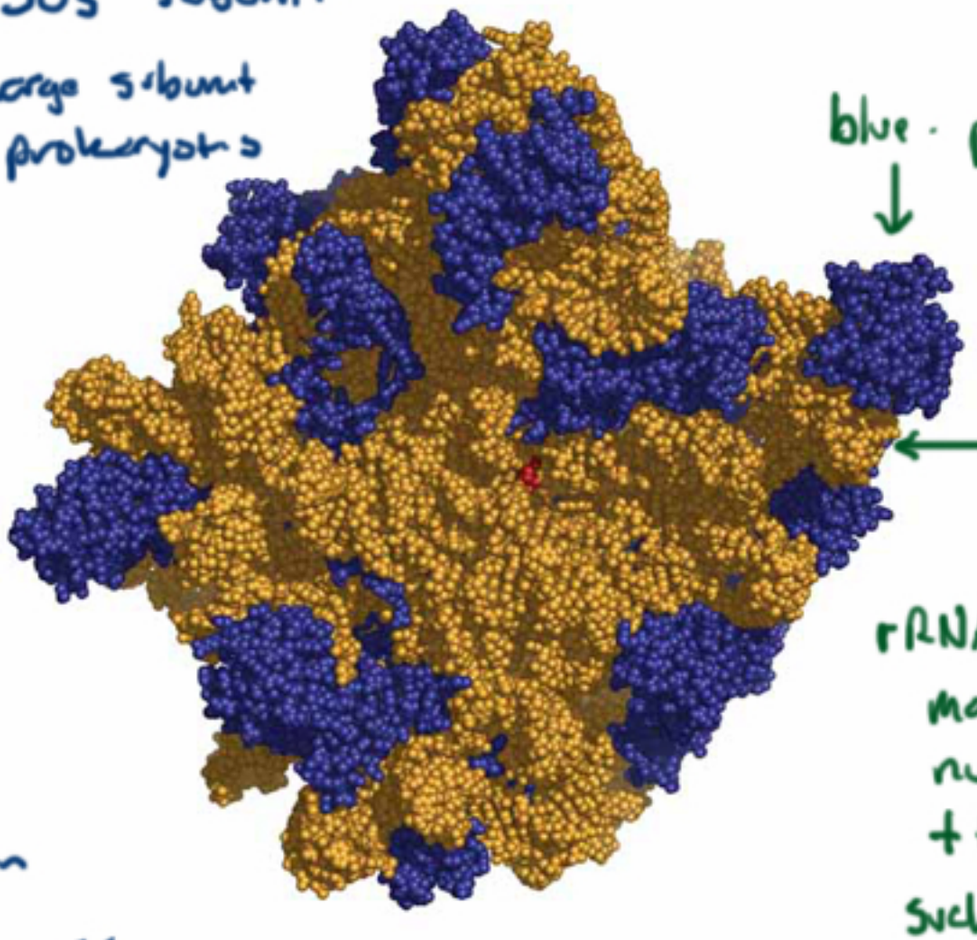
degradation carried out by slicer

50s
30s
70s
↑

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

eukaryotes 60s
40s

50s subunit
Large subunit prokaryotes



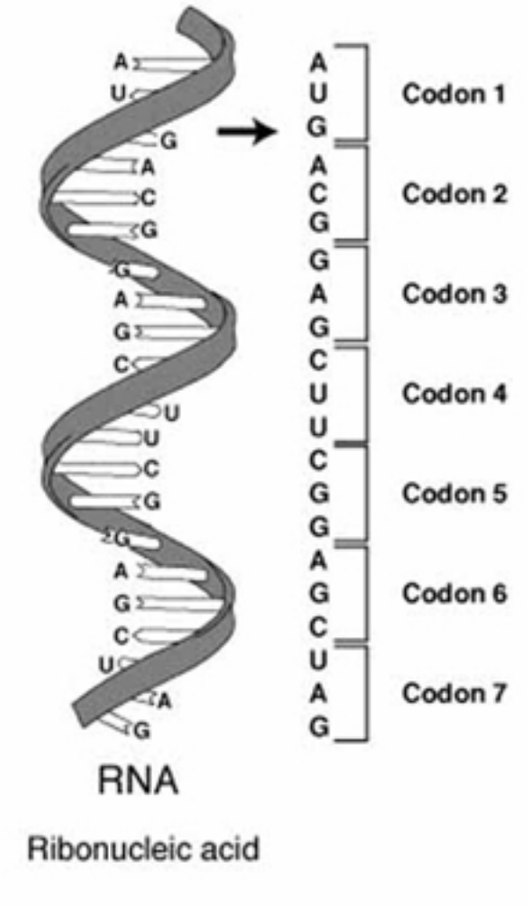
blue - protein
↓
← orange rRNA

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

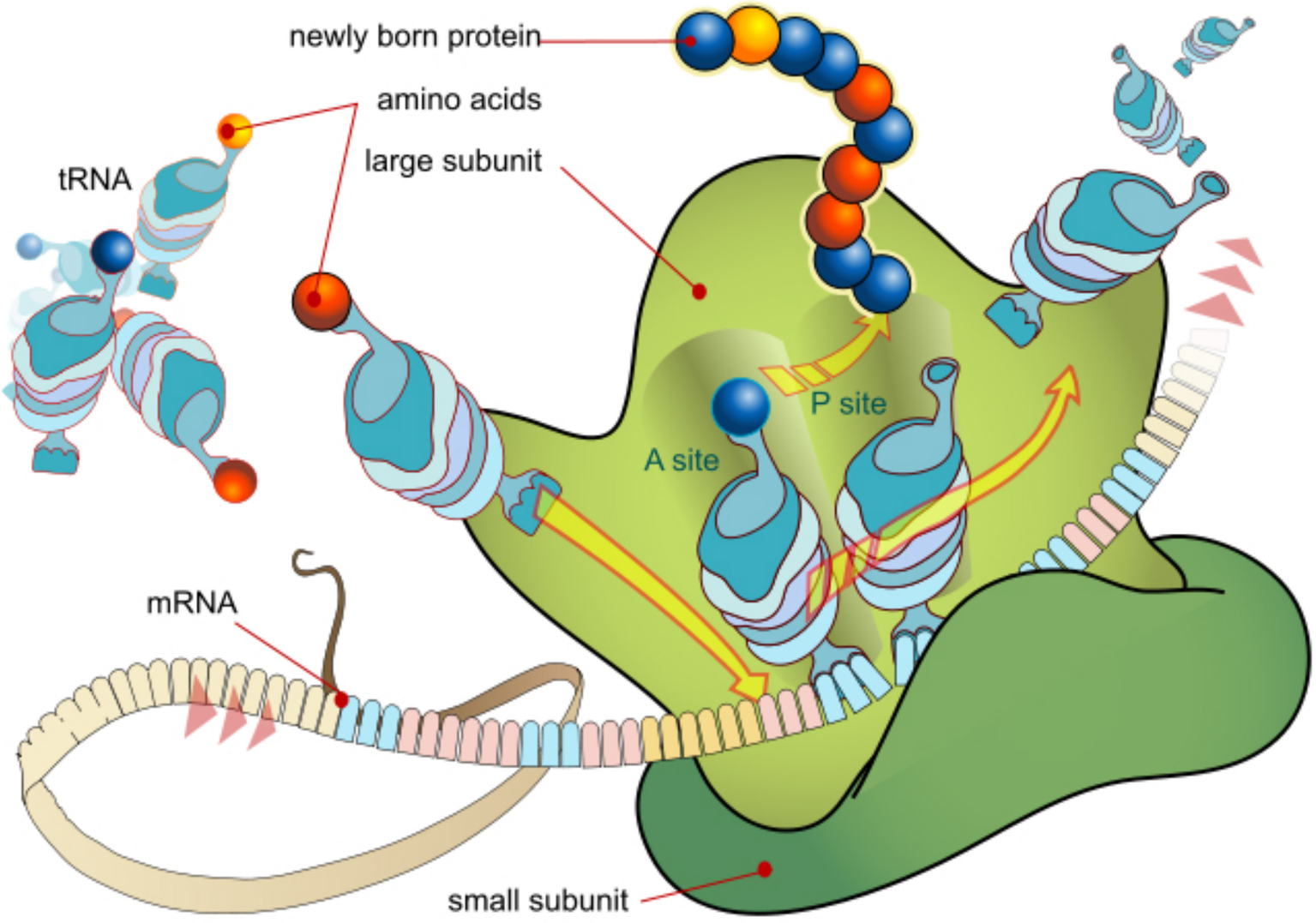
aminoacyl tRNA synthetase

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

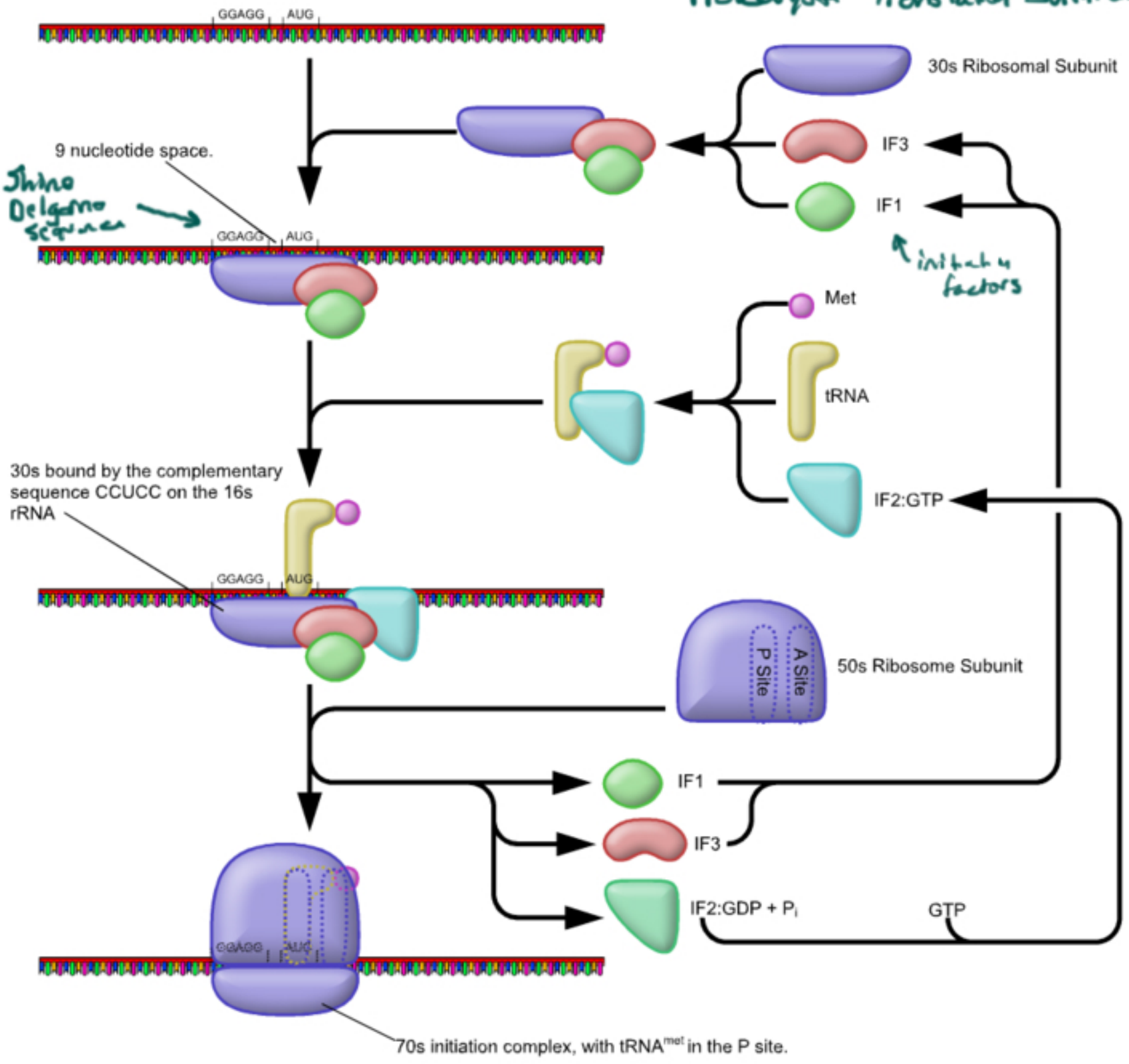
anticodon

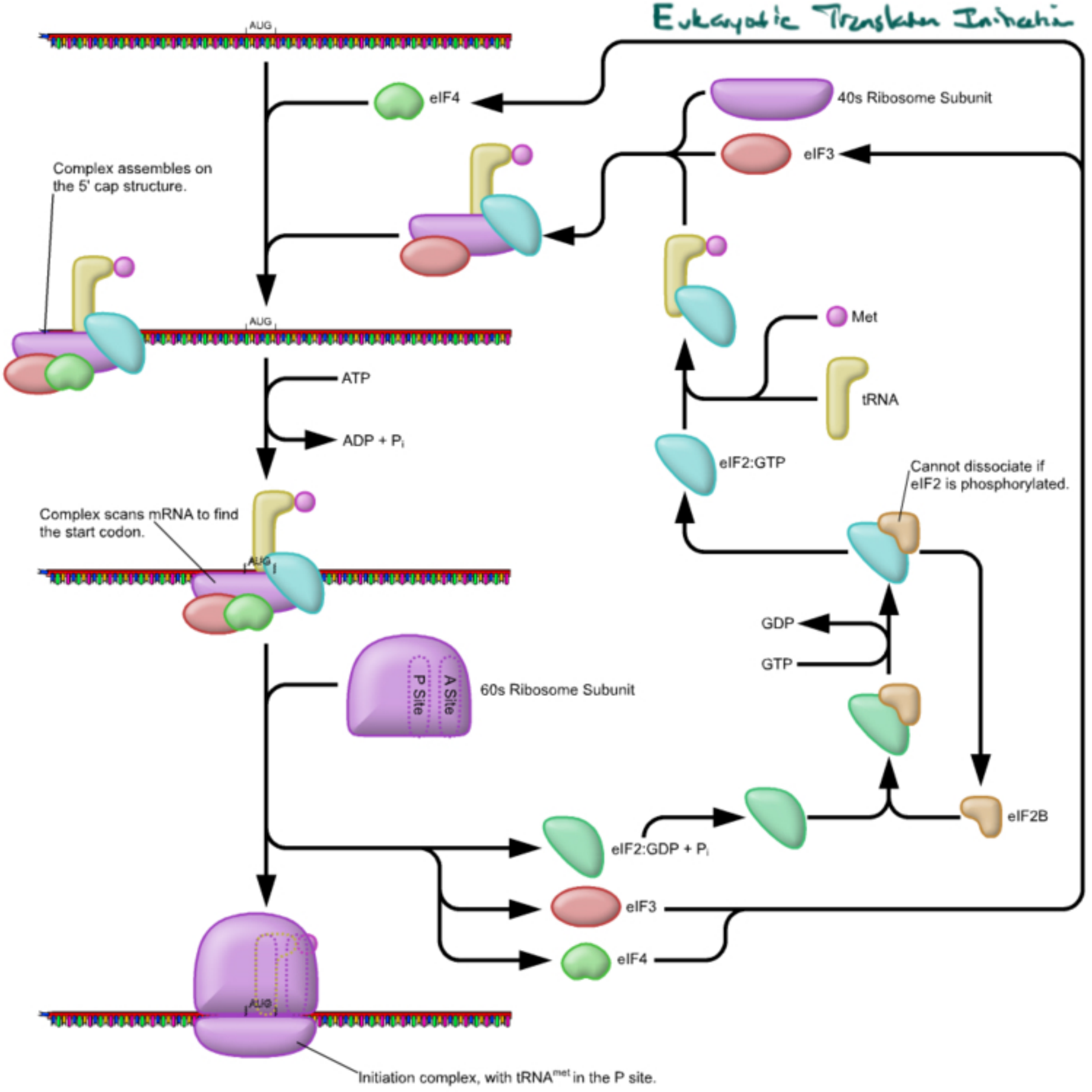
antiparallel binding

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.