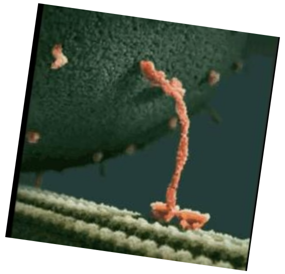
OSHER LIFELONG LEARNING INSTITUTE

NanoMachines:

The Tiny Biological Gadgets that Animate Life



I Love Lucy (1952)

Session 4
DNA and Proteins

OLLI at Illinois
Spring 2023

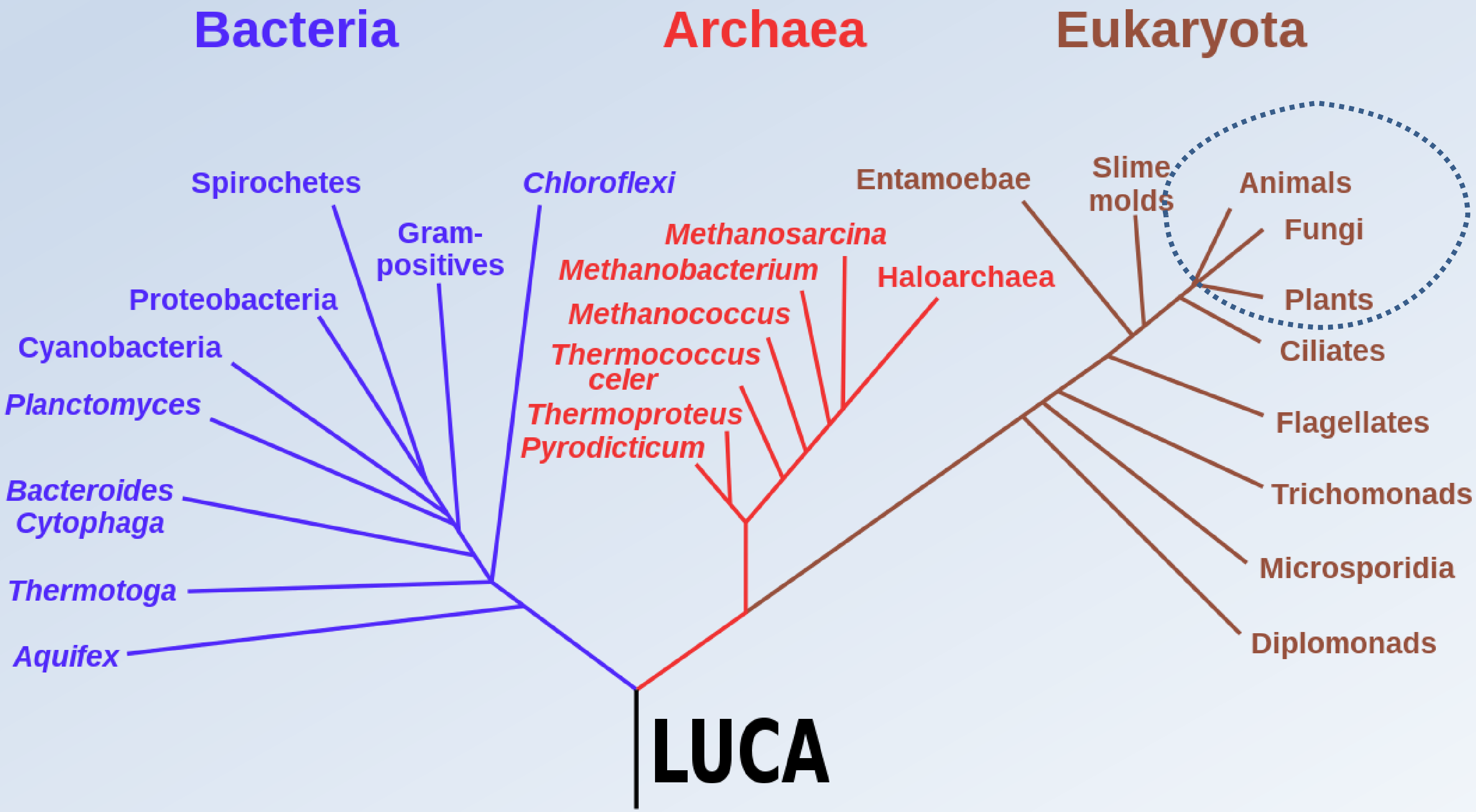
D. H. Tracy

Course Outline

1. The nanoscopic world: Building blocks, energy flow, how we observe it
2. Energy harvesting, storage and consumption
3. Movement, motors and locomotion
4. **Genetic information processing, protein manufacturing**

"LUCA"

Last **U**niversal **C**ommon **A**ncestor



Session 4 Outline

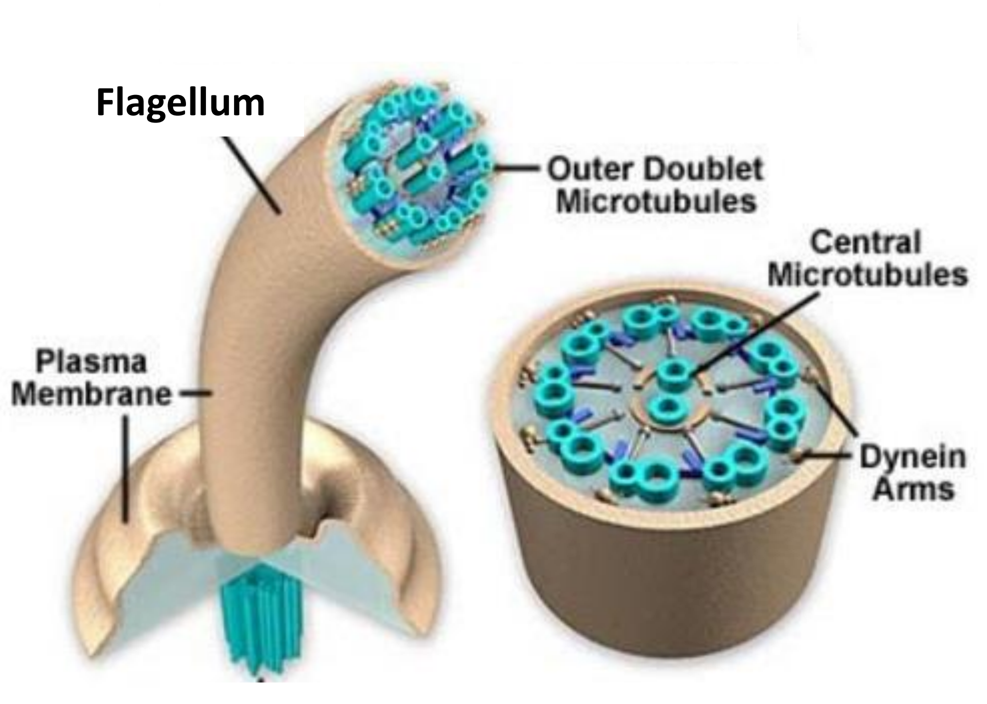
- Finish Molecular Motors
- DNA & RNA: The Central Dogma
- DNA Replication
- RNA Blueprints: Transcription
- Protein Factories: Ribosomes

Two very different ways to build a Flagellum

Eukaryotic Flagella

(*e.g.* Sperm or Algae)

Complex Flagellum **Bends**
using Dynein actuators



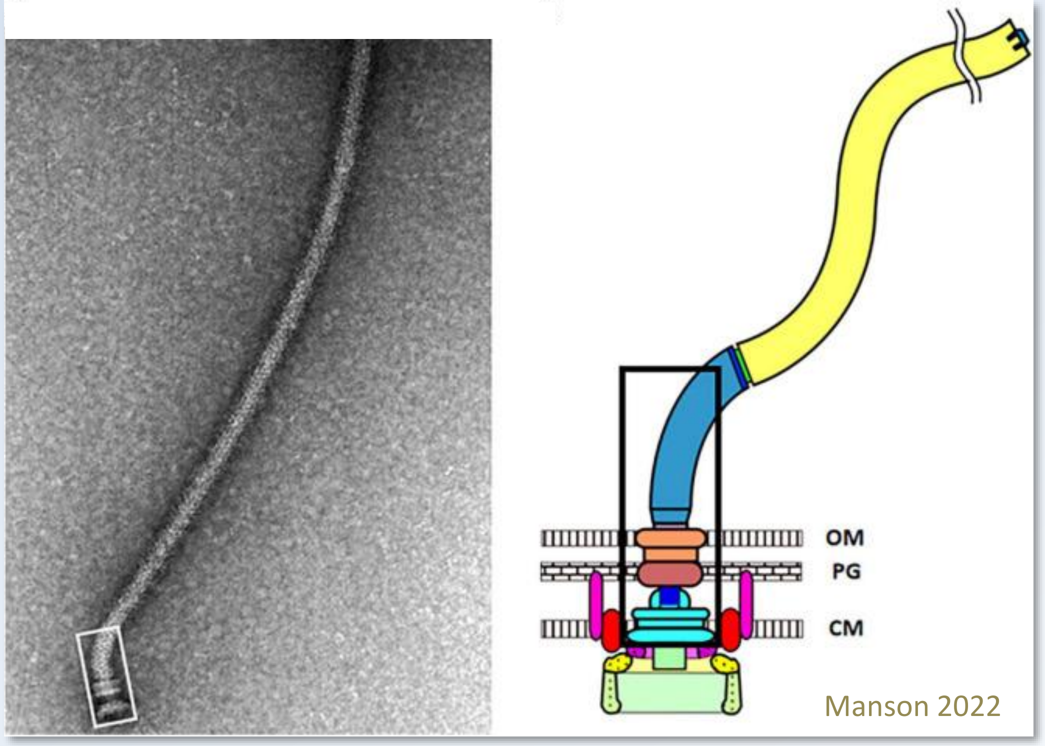
Eukaryotic "9+2" Flagellum

Quora

Prokaryotic Flagella

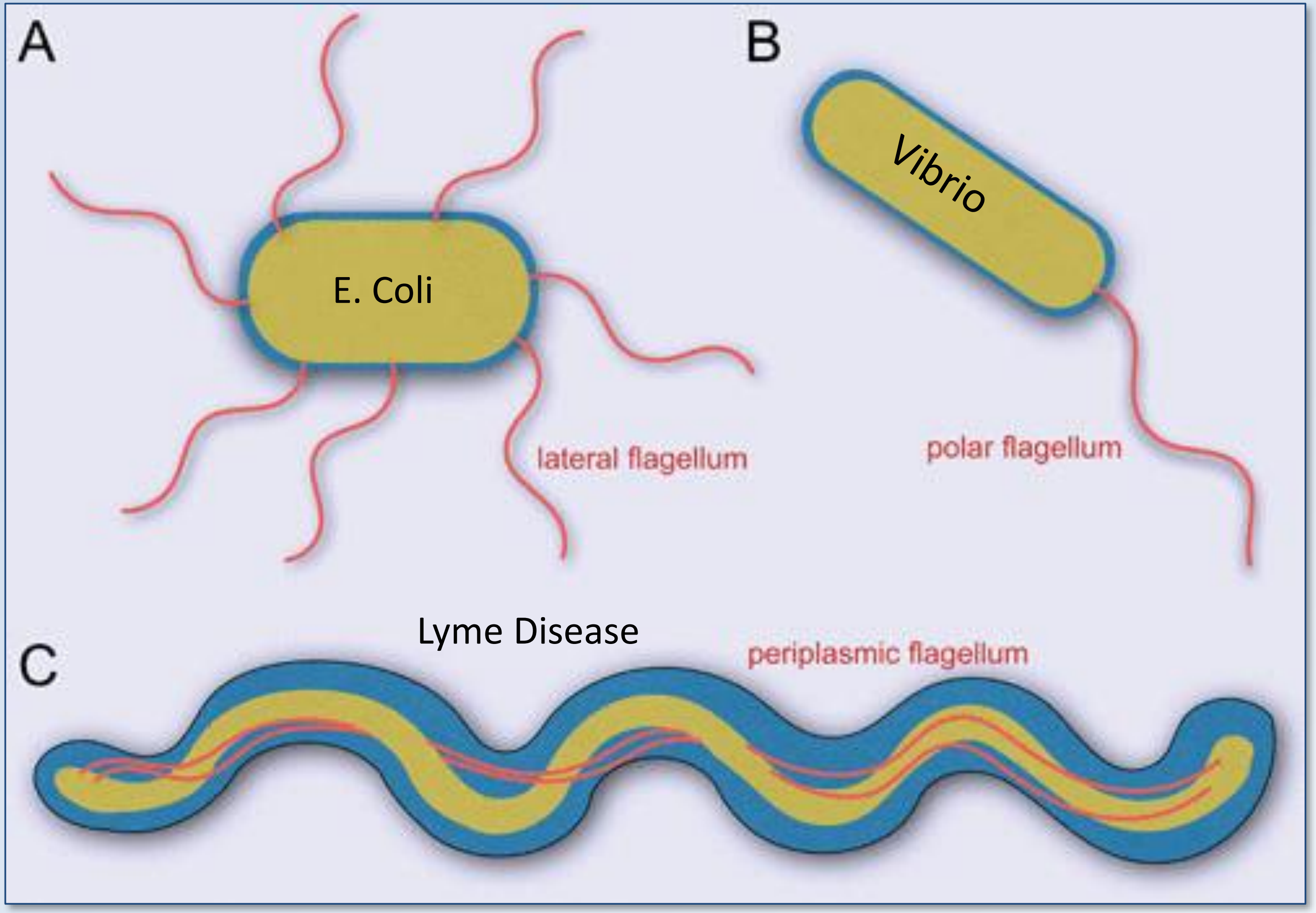
(*Bacteria & Archaea*)

Simple Flagellum **Spins**
using a Rotary Motor



Manson 2022

Bacterial Flagella take many forms



Chang *et al* Nature (2020)

The Bacterial Flagellar Motor

Movie

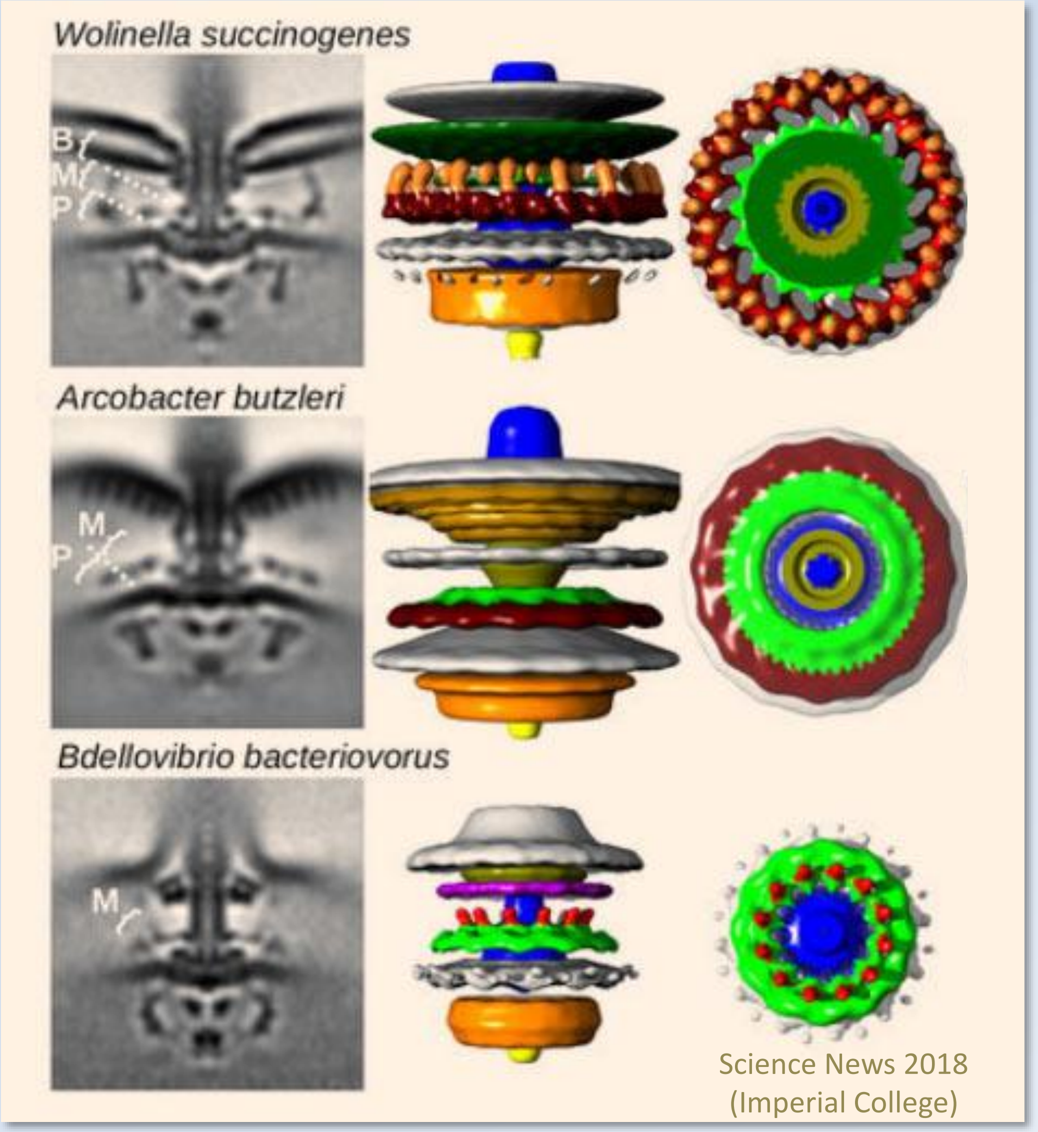
ATP Synthase

~ 50nm

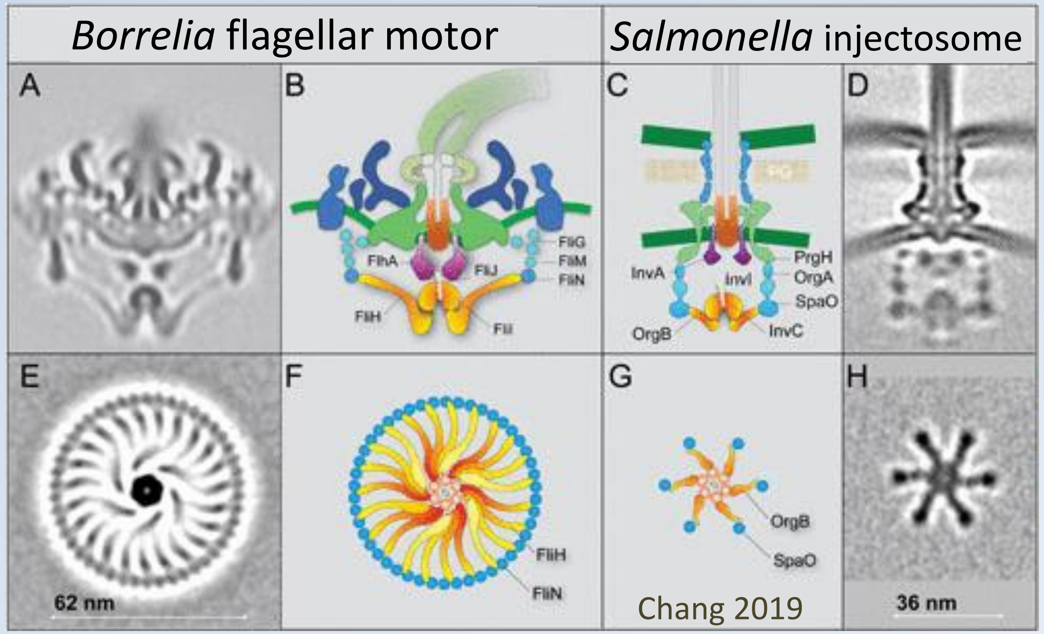
BFM

Stewart Lab
Callum Smits
ca 2018

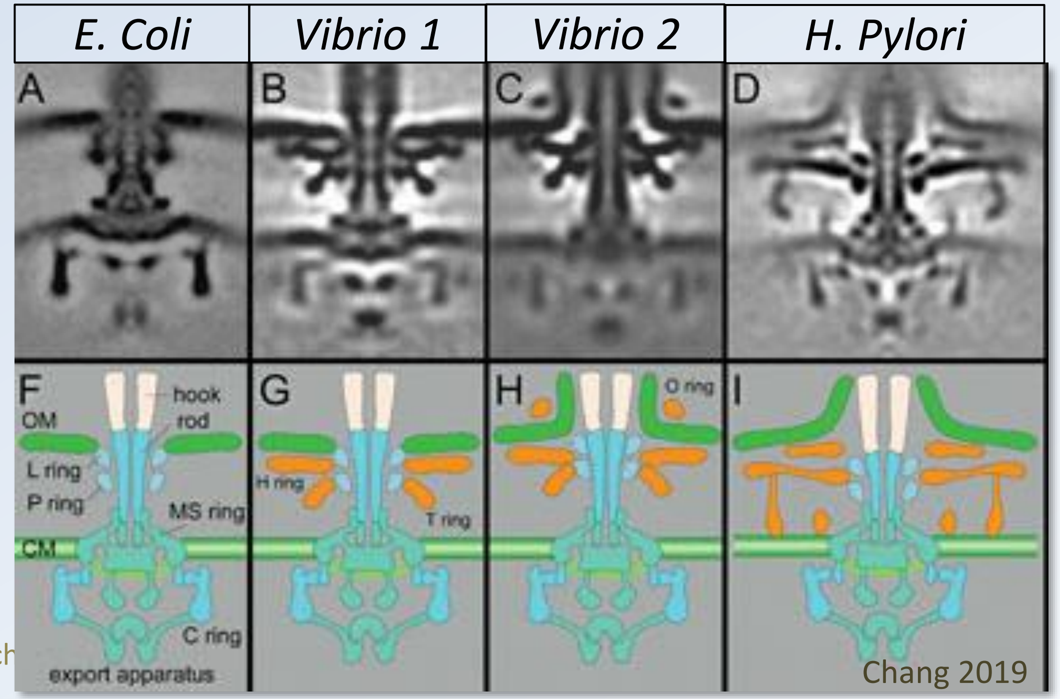
Bacterial Flagellar Motors come in many variations



Science News 2018 (Imperial College)



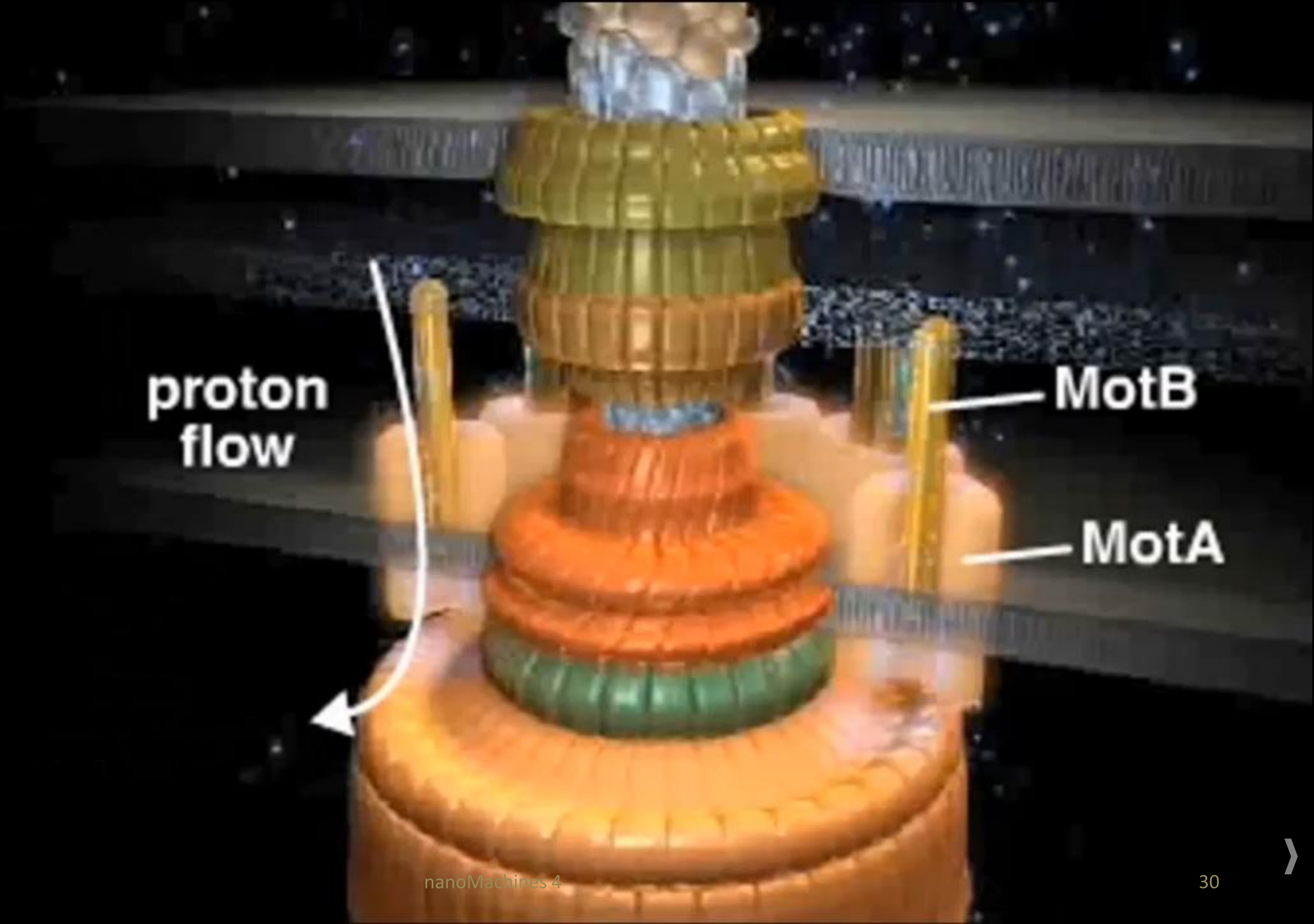
Chang 2019



Chang 2019

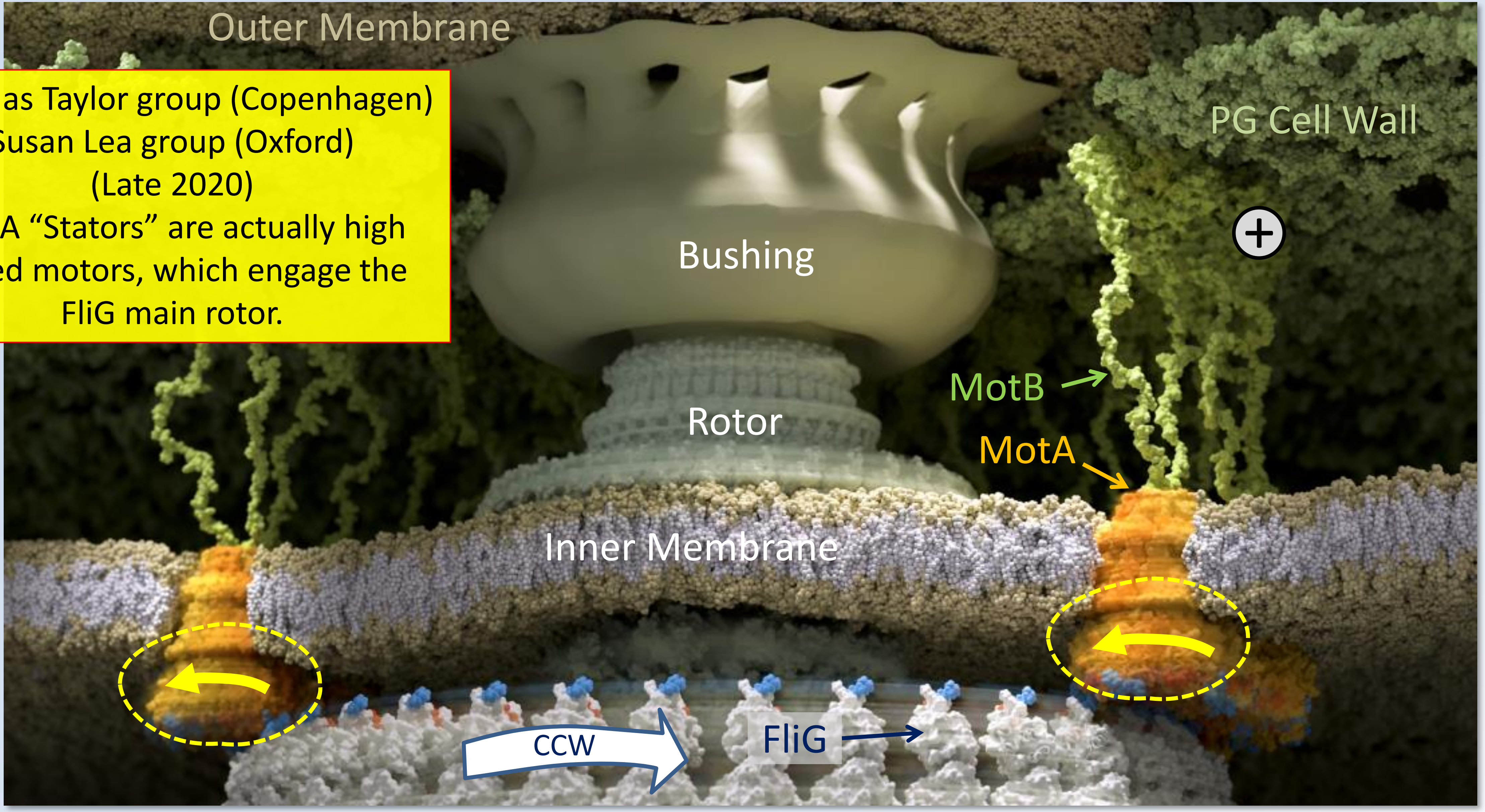
Bacterial Flagella

Movie



Biology Help
ca 2018

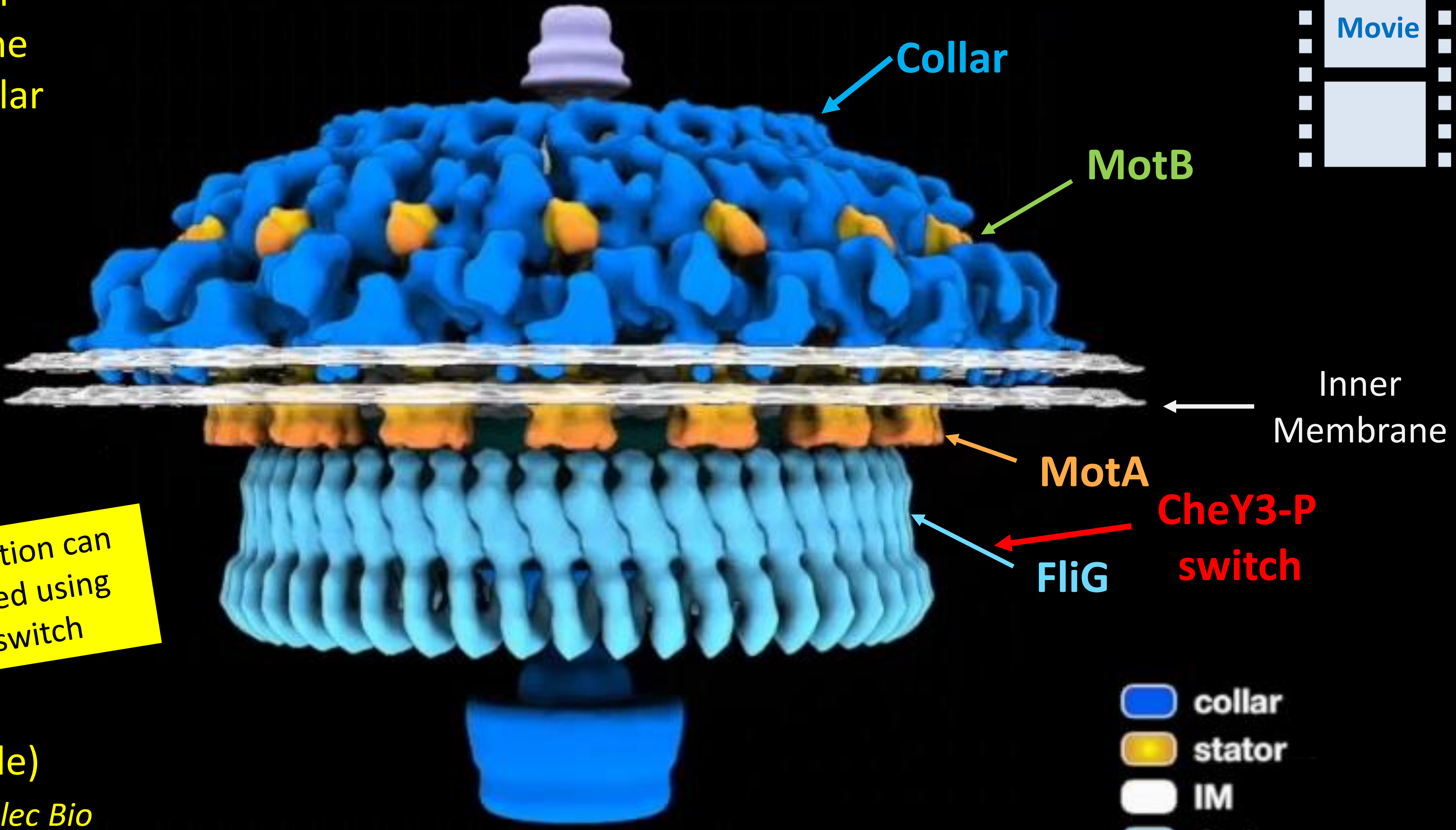
Nicholas Taylor group (Copenhagen)
Susan Lea group (Oxford)
(Late 2020)
MotA "Stators" are actually high speed motors, which engage the FliG main rotor.



U of Copenhagen

Molecular Mechanism for Rotational Switching in the Bacterial Flagellar Motor

Motor from *B. burgdorferi* (Lyme disease spirochete)

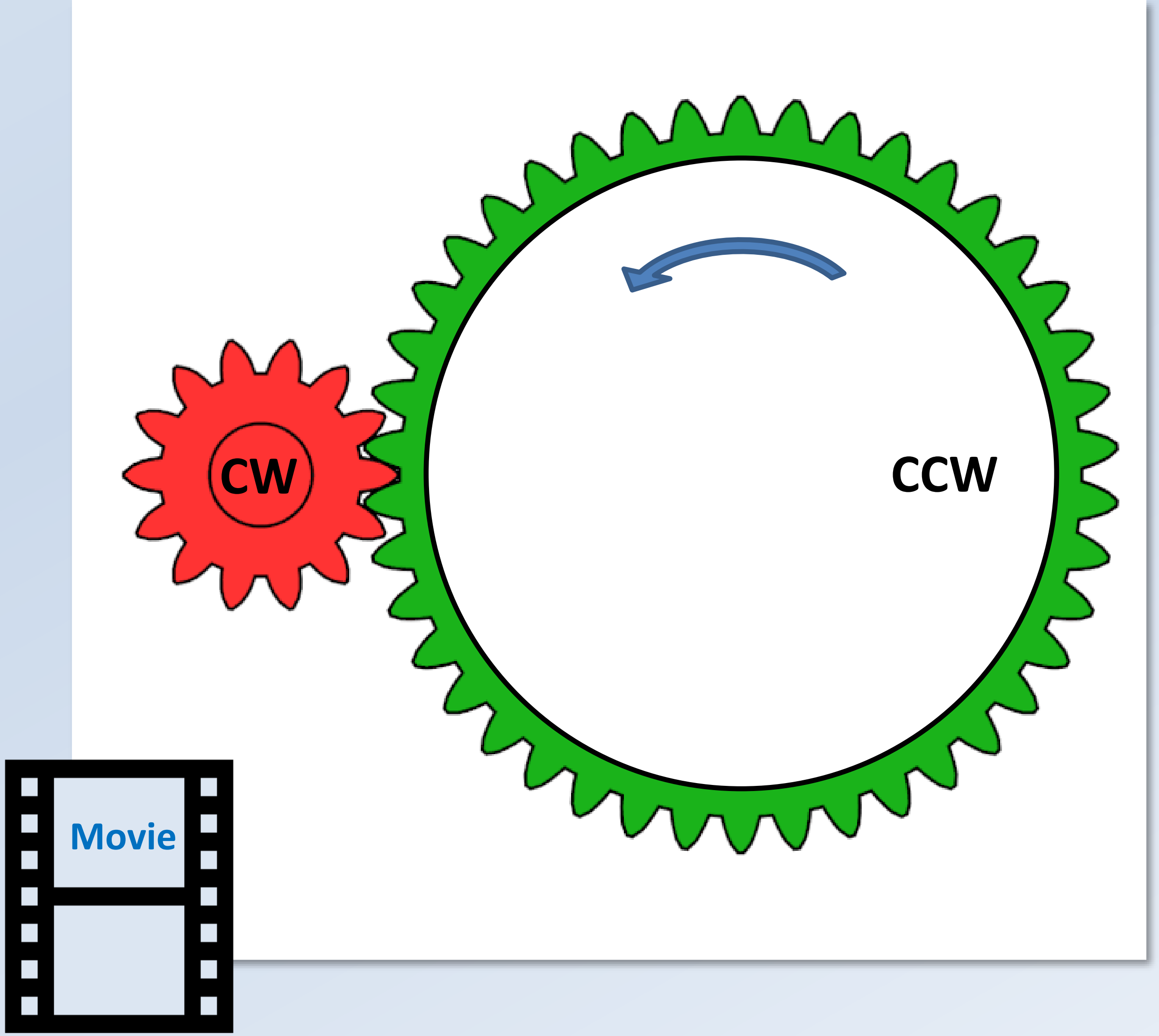


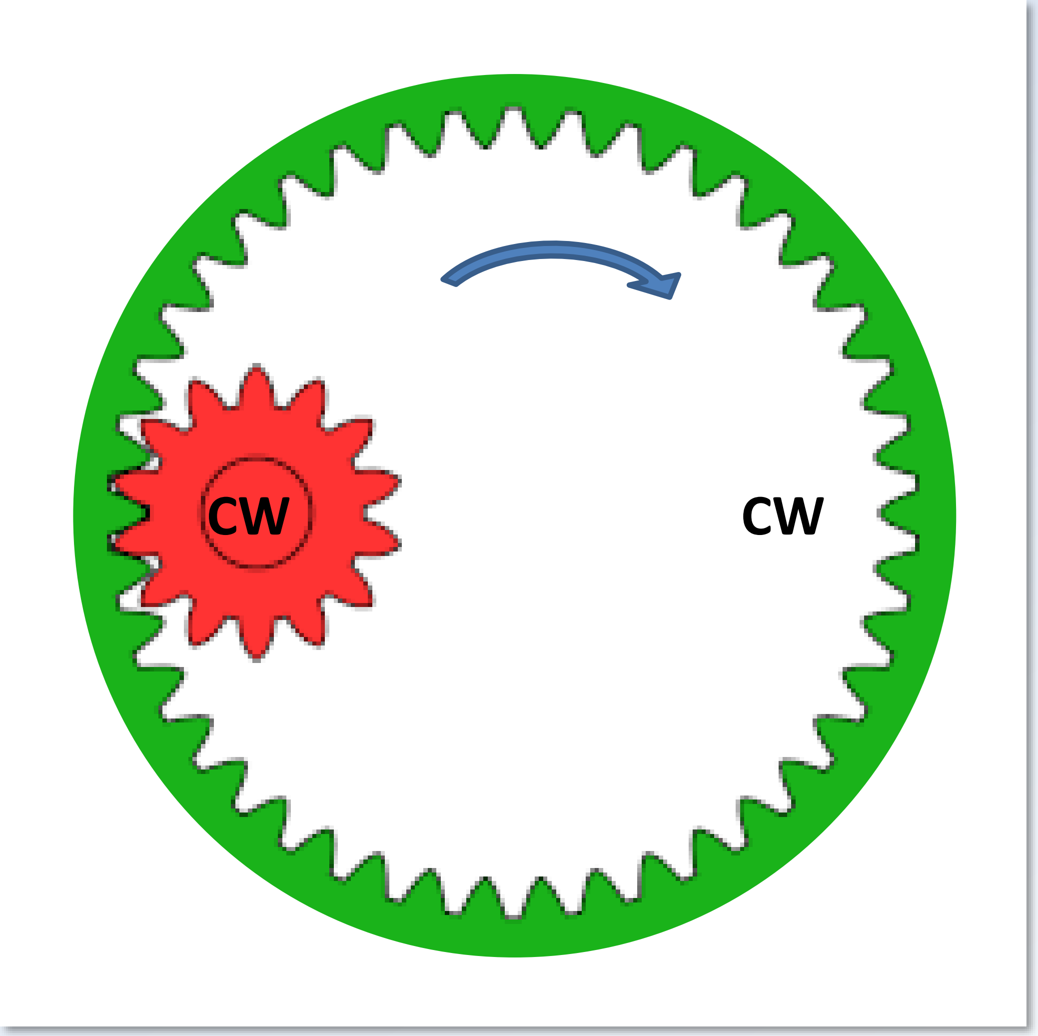
Rotor rotation can be reversed using CheY3-P switch

Movie

Chang *et al* (Yale)
Nature Structural Molec Bio
Nov 2020

Shifting into Reverse

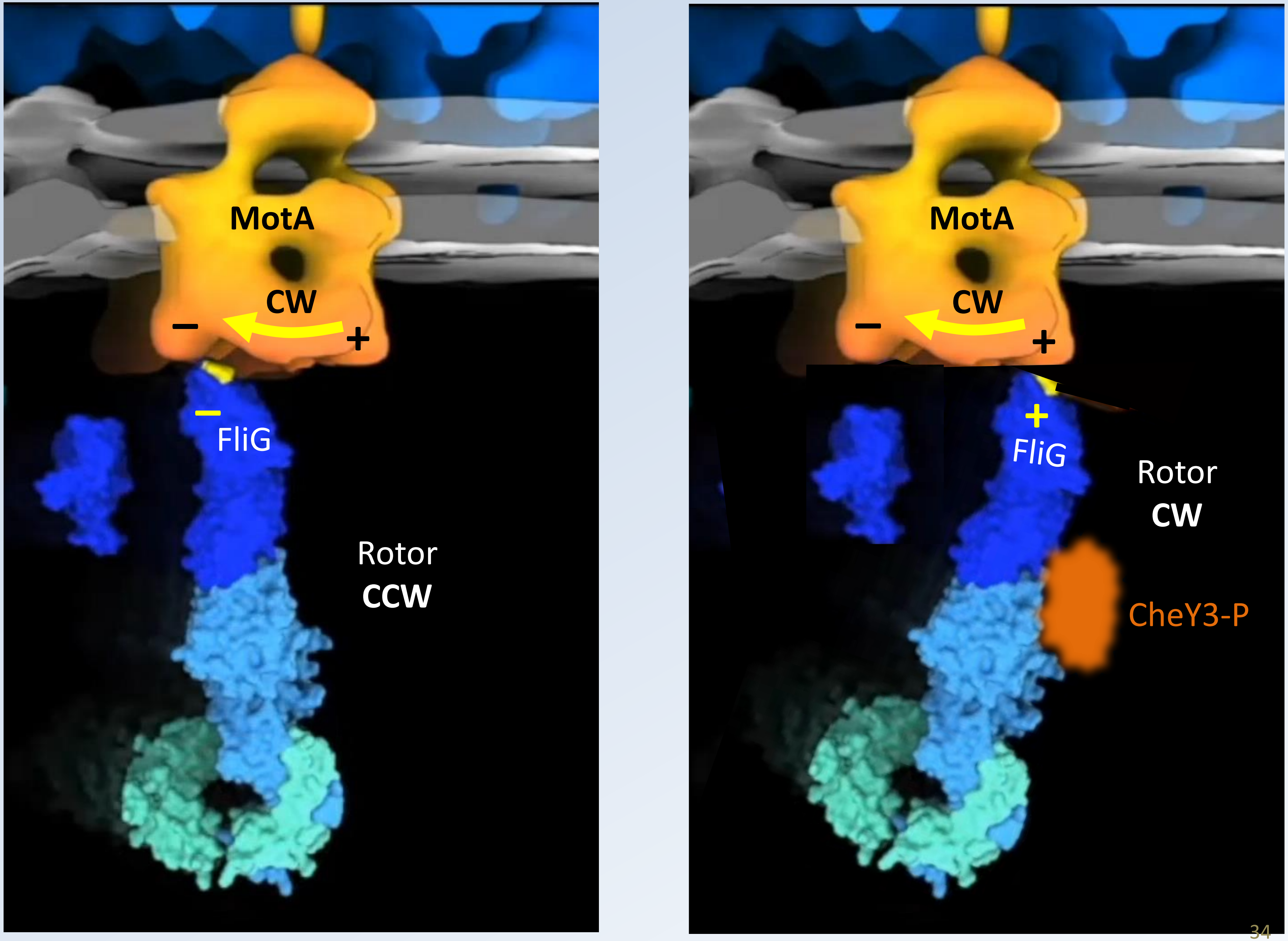




Jahobr (Wikimedia Commons)

Shifting Gears into Reverse

CheY3-P bends the FliG rotor units outward so they engage the outer sides of the MotA motors

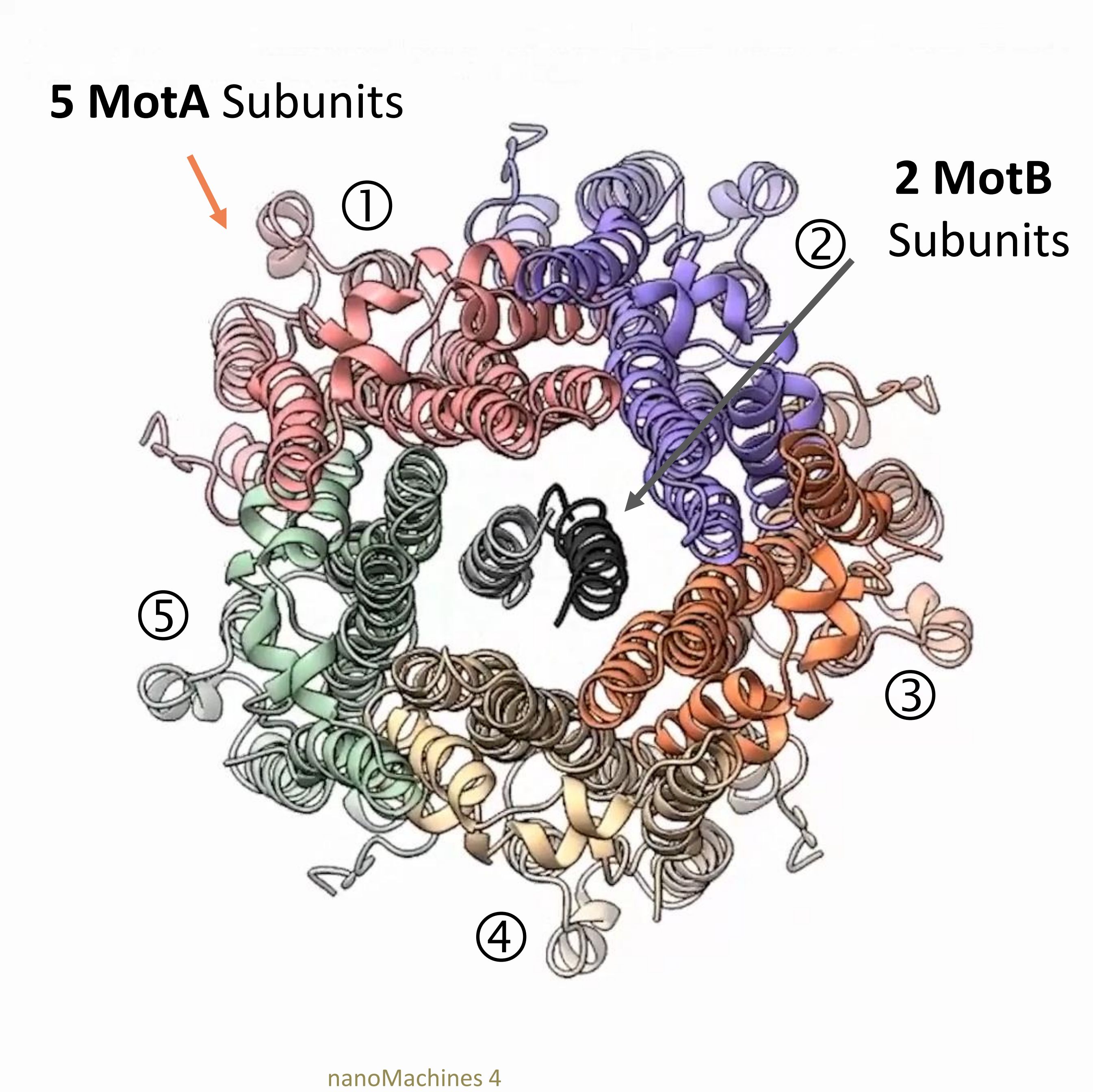


The Bacterial Flagella MotA/MotB Motor in Action

from numerical modeling

MotA Pentamer rotates 36º for each proton flowing through

Santiveri et al, Cell **183** (2020)
Structure and Function of Stator Units of the BFM
Video S5



Movie

Selected List of Molecular Machines

I. MOLECULAR MACHINES THAT SCIENTISTS HAVE ARGUED SHOW IRREDUCIBLE COMPLEXITY

1. BACTERIAL FLAGELLUM

The flagellum is a rotary motor in bacteria that drives a propeller to spin, much like an outboard motor, powered by ion flow to drive rotary motion. Capable of spinning up to 100,000 rpm,[13] one paper in *Trends in Microbiology* called the flagellum "an exquisitely engineered chemi-osmotic nanomachine; nature's most powerful rotary motor, harnessing a transmembrane ion-motive force to drive a filamentous propeller."[14] Due to its motor-like structure and internal parts, one molecular biologist wrote in the journal *Cell*, "[m]ore so than other motors, the flagellum resembles a machine designed by a human."[15] Genetic knockout experiments have shown that the *E. coli* flagellum is irreducibly complex with respect to its approximately 35 genes.[16] Despite the fact that this is one of the best studied molecular machines, a

Questions?

Building Cells from a DNA Blueprint

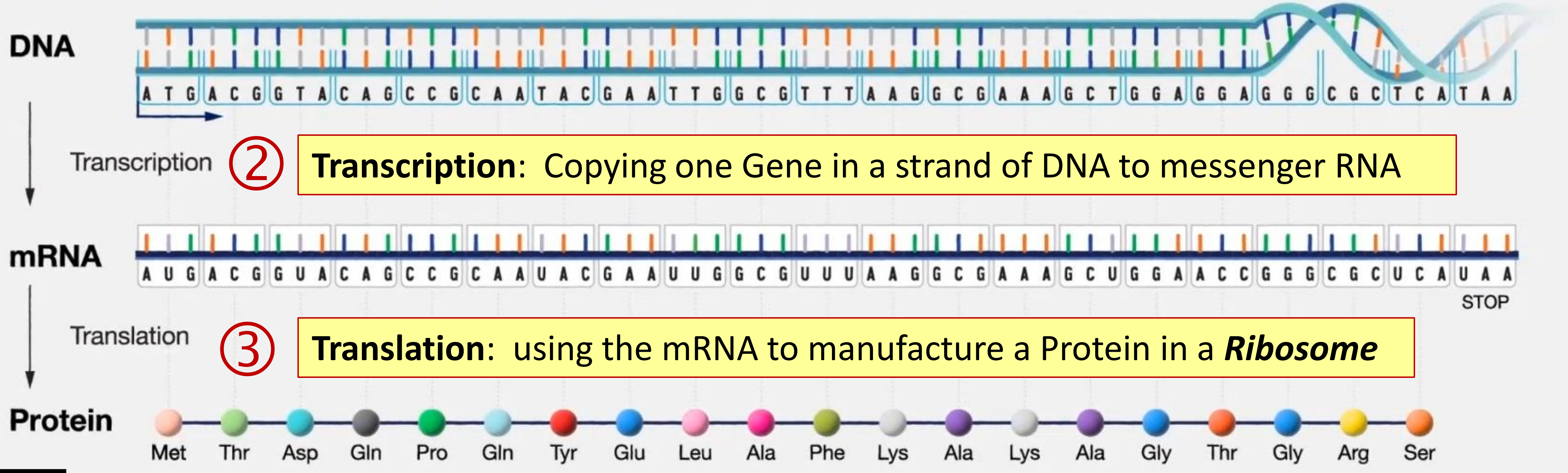

The Central Dogma
Ely cathedral .org

Central Dogma

① **Replication**: Duplicating DNA when cells divide.

② **Transcription**: Copying one Gene in a strand of DNA to messenger RNA

③ **Translation**: using the mRNA to manufacture a Protein in a ***Ribosome***

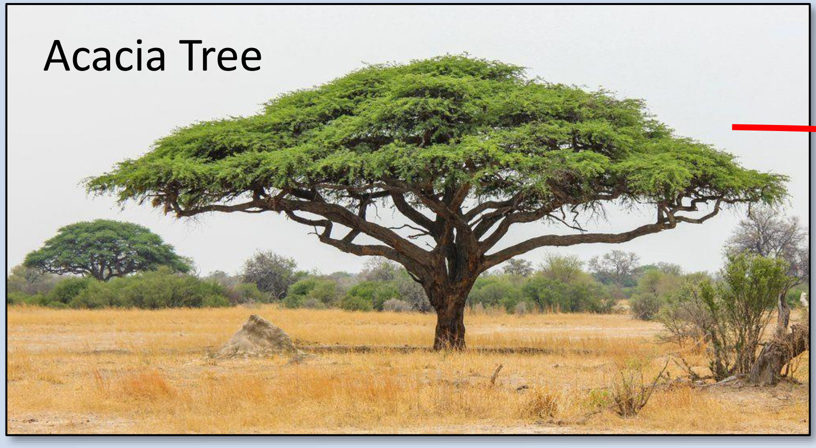


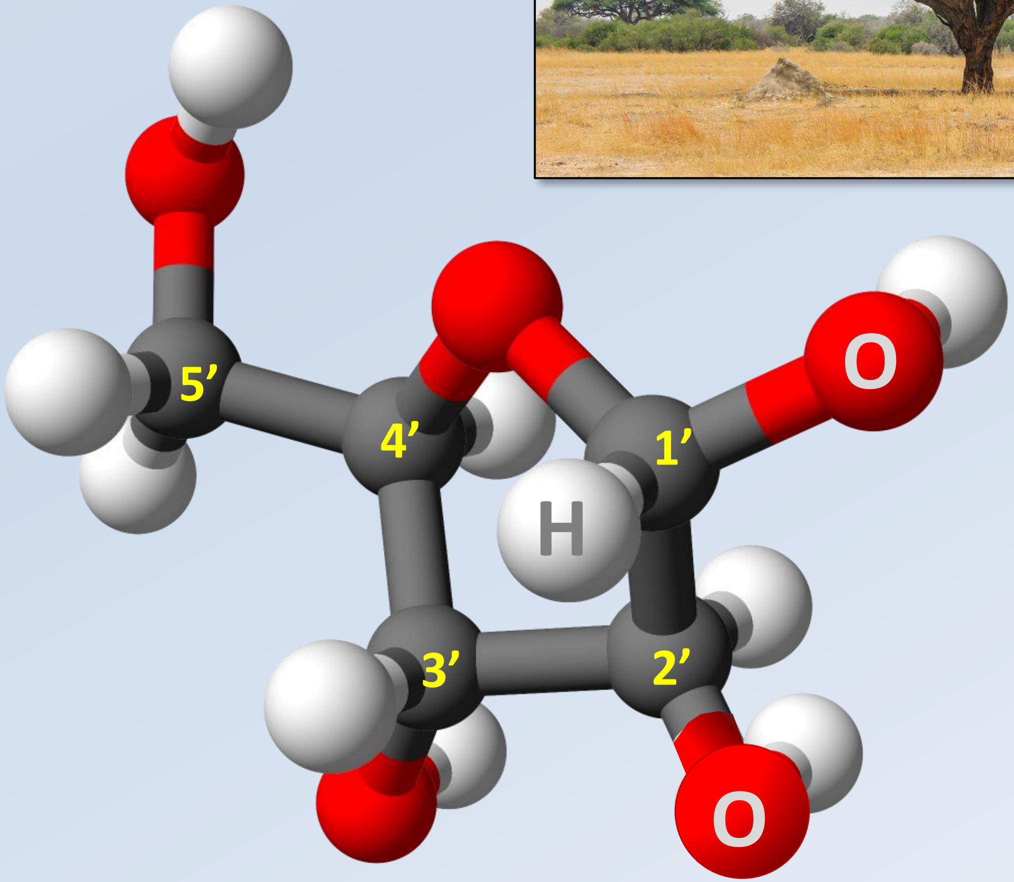
Movie

National Human Genome Research Institute (2022)

Structure of DNA and RNA

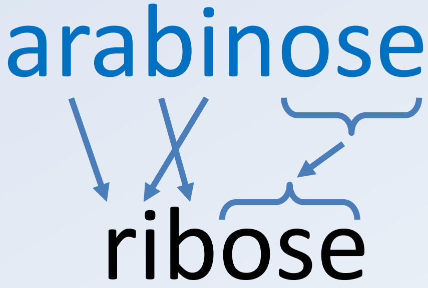
Nucleic Acids





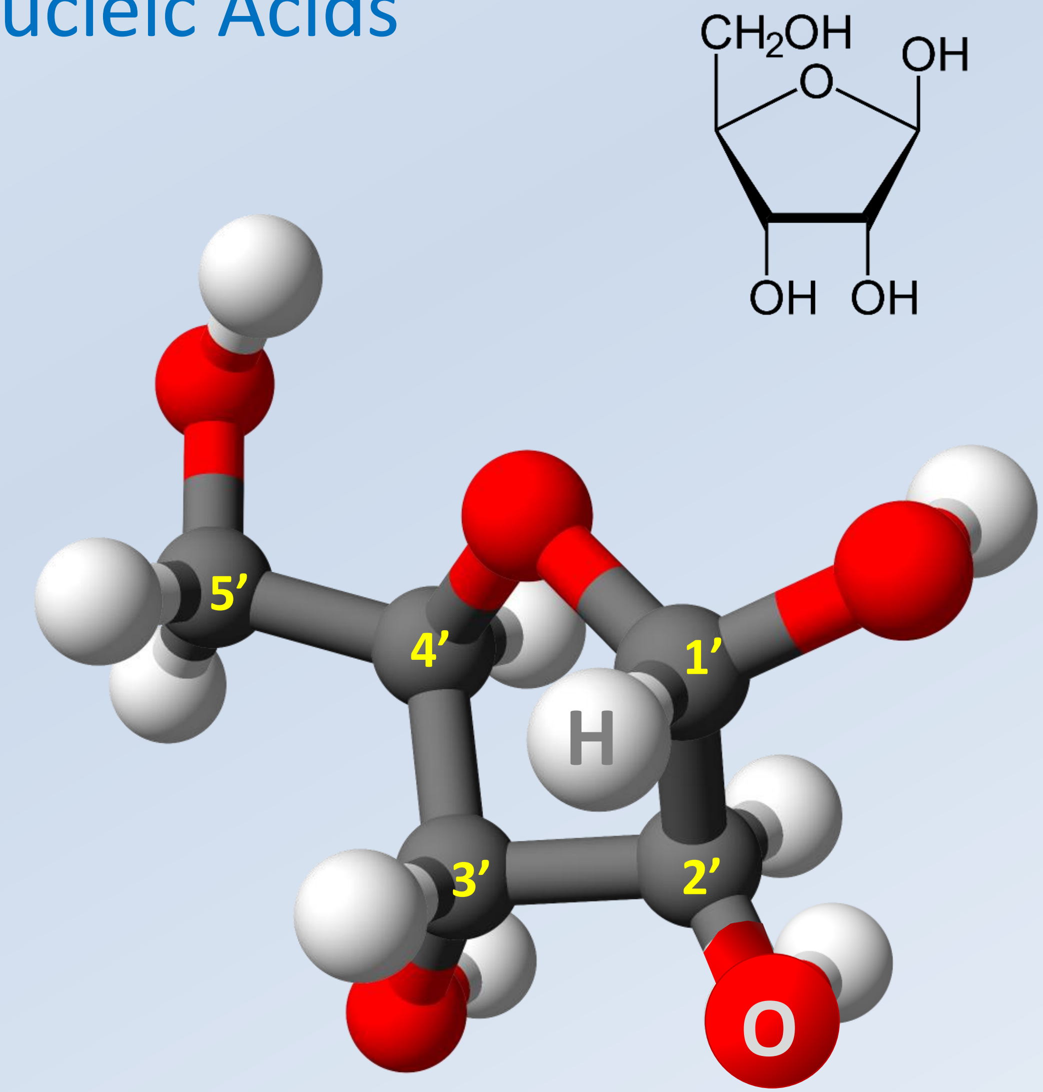
Ribose

A similar 5 carbon sugar:

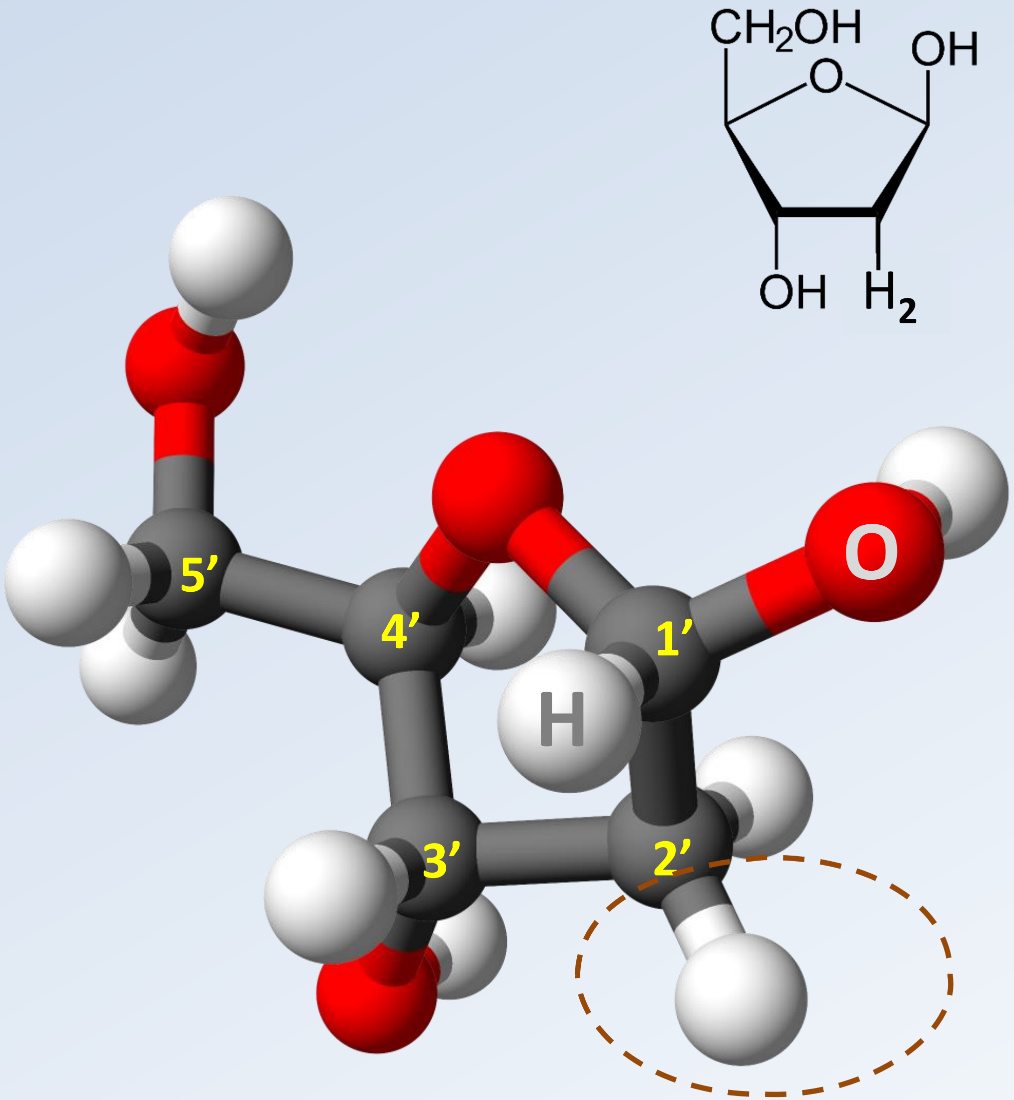


Named after arabinose, in turn named after the Gum Arabic in which it was found.

Nucleic Acids



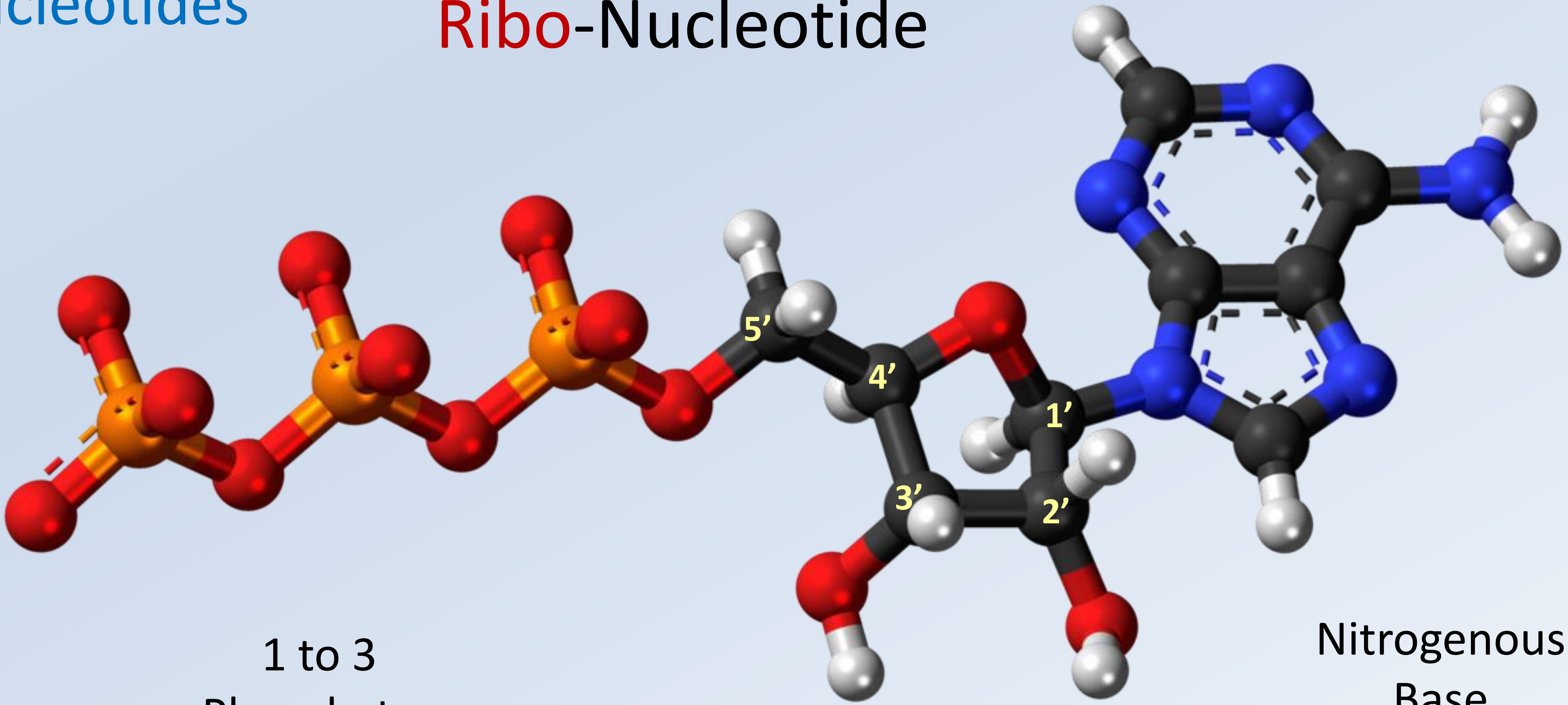
Ribose



DeOxy-Ribose

Nucleotides

Ribo-Nucleotide



1 to 3
Phosphates
PO_4

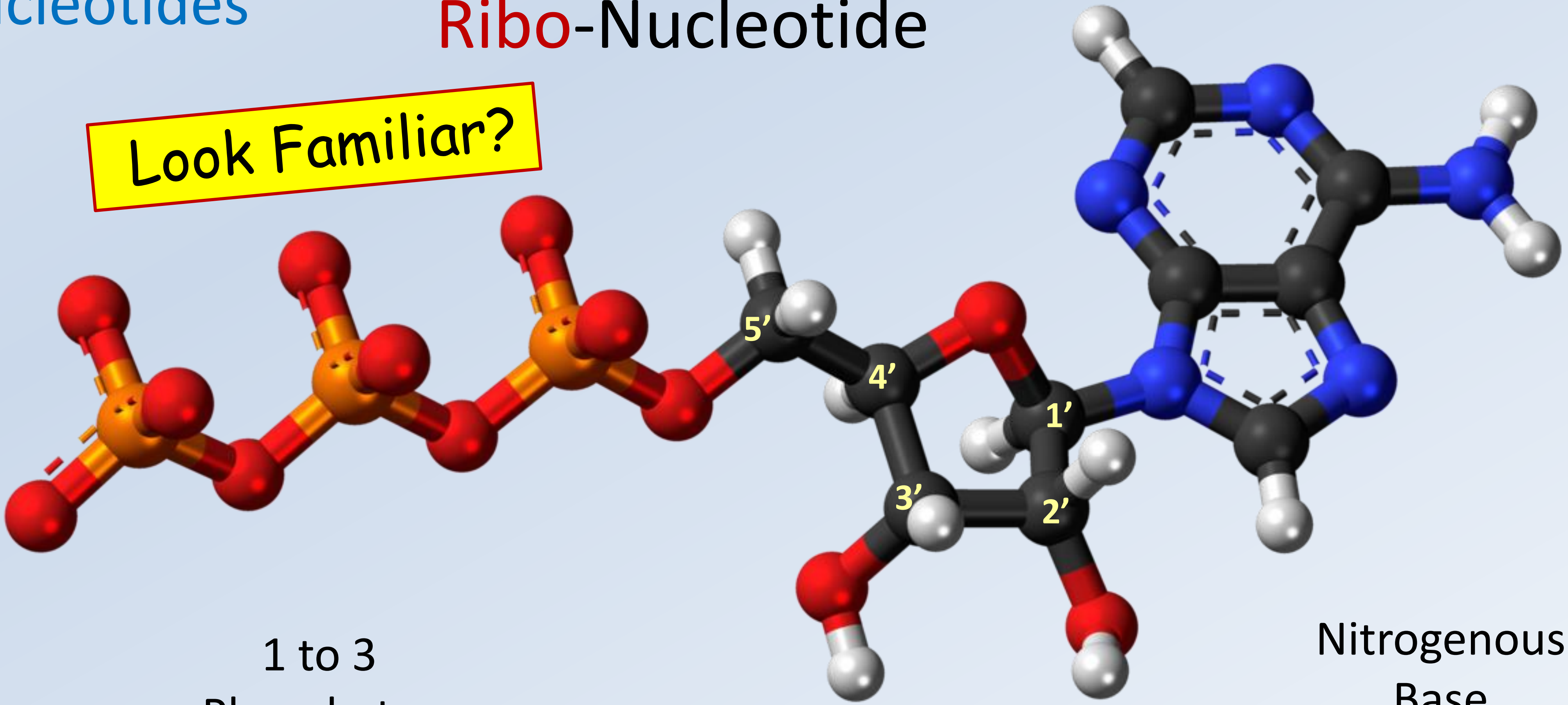
Ribose Core

Nitrogenous
Base
Adenine

Nucleotides

Ribo-Nucleotide

Look Familiar?



1 to 3 Phosphates PO_4

Ribose Core

Nitrogenous Base

Adenine

RNA Component

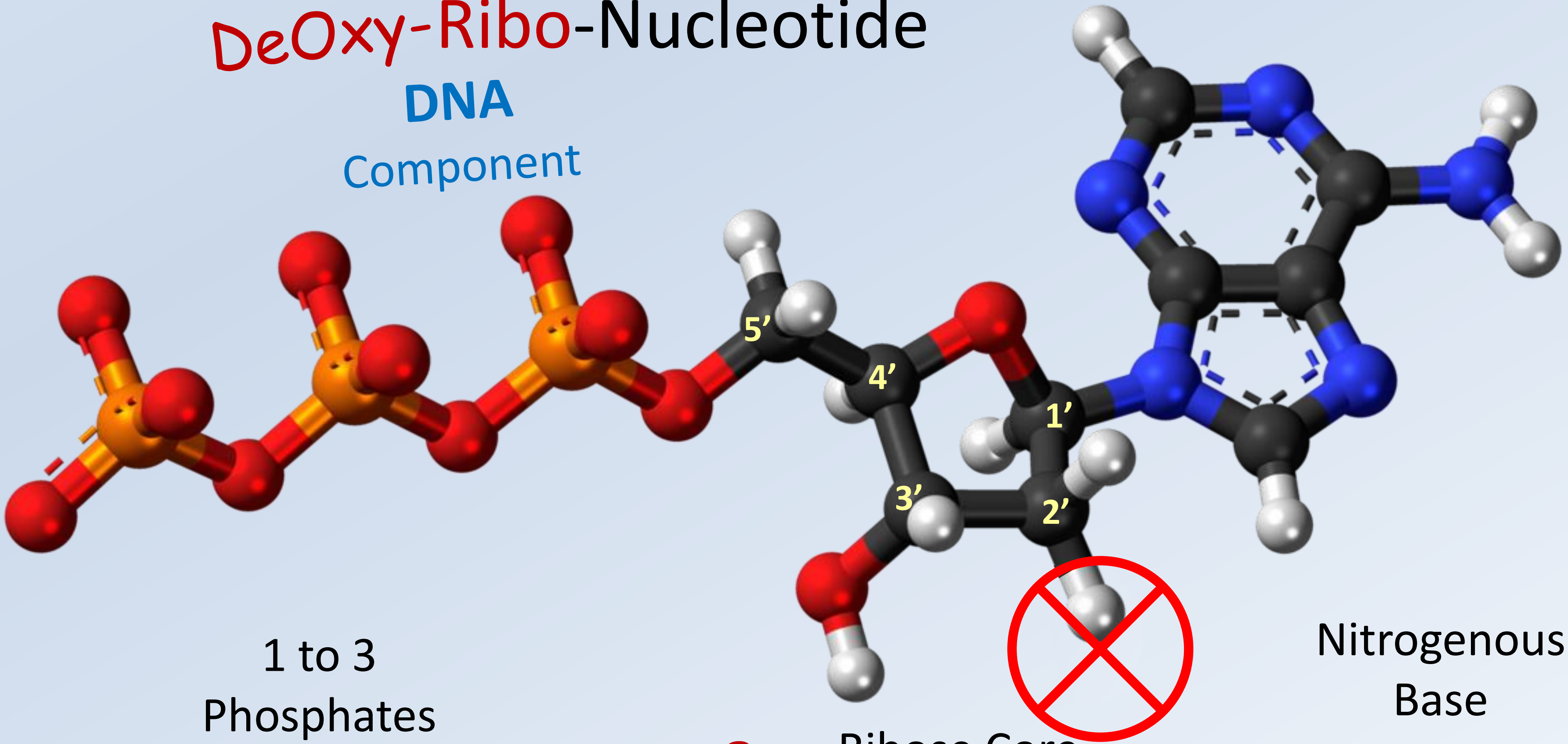
“Adenosine Tri-Phosphate” **ATP**

Nucleotides

DeOxy-Ribo-Nucleotide

DNA

Component



1 to 3
Phosphates
PO_4

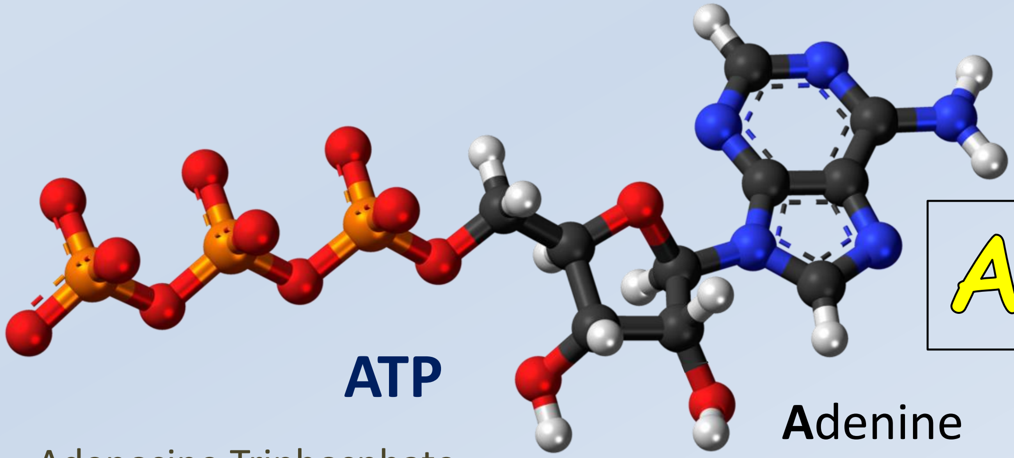
DeOxy-Ribose Core

Nitrogenous
Base

Adenine

dATP

~~4~~ 5 Nucleotides

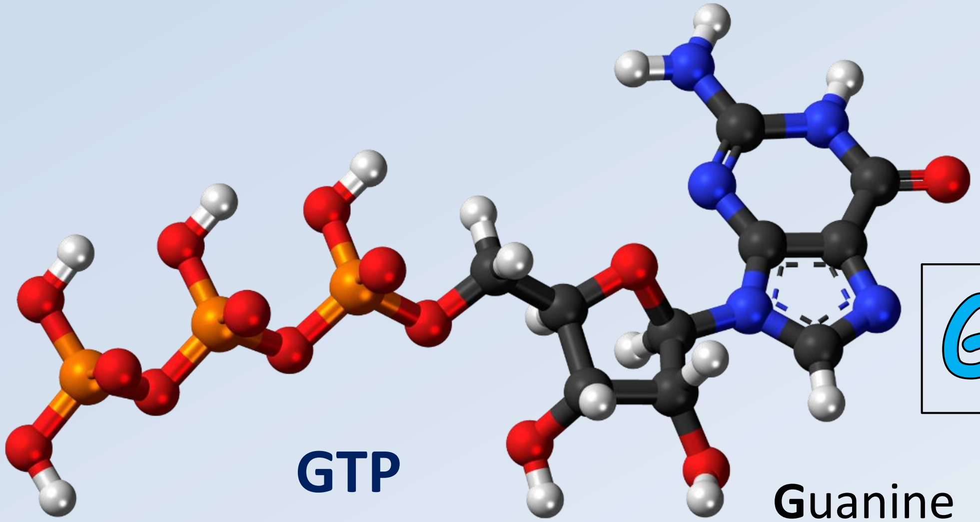


ATP

Adenosine Triphosphate

Adenine

A

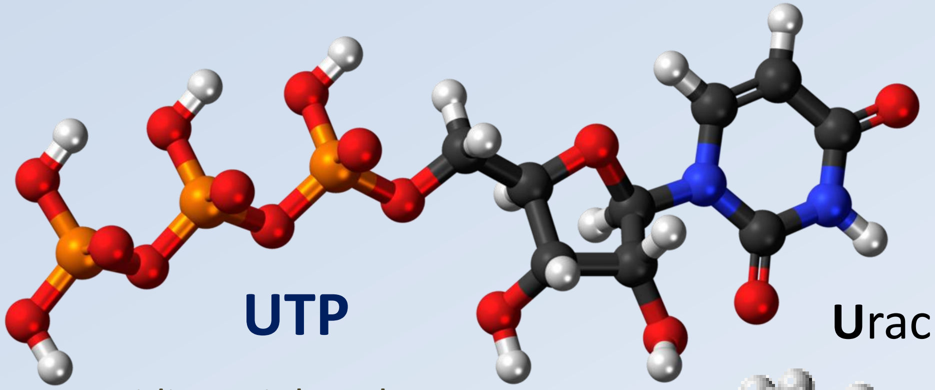


GTP

Guanosine Triphosphate

Guanine

G



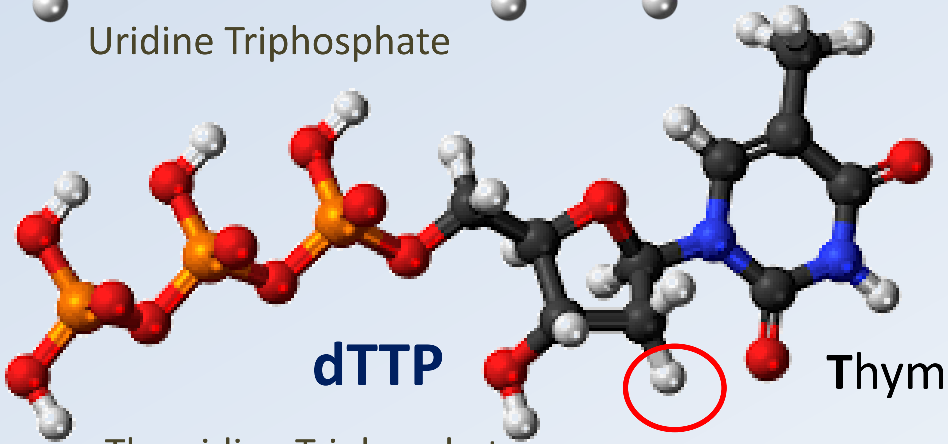
UTP

Uridine Triphosphate

Uracil

U

(RNA only)



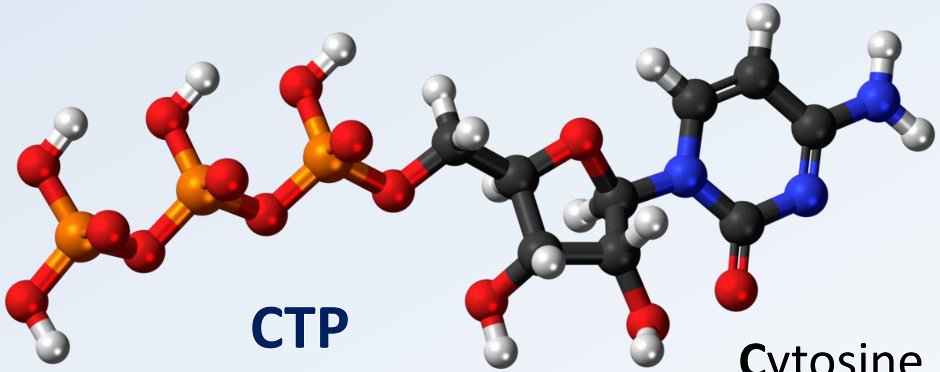
dTTP

Thymidine Triphosphate

Thymine

T

(DNA only)



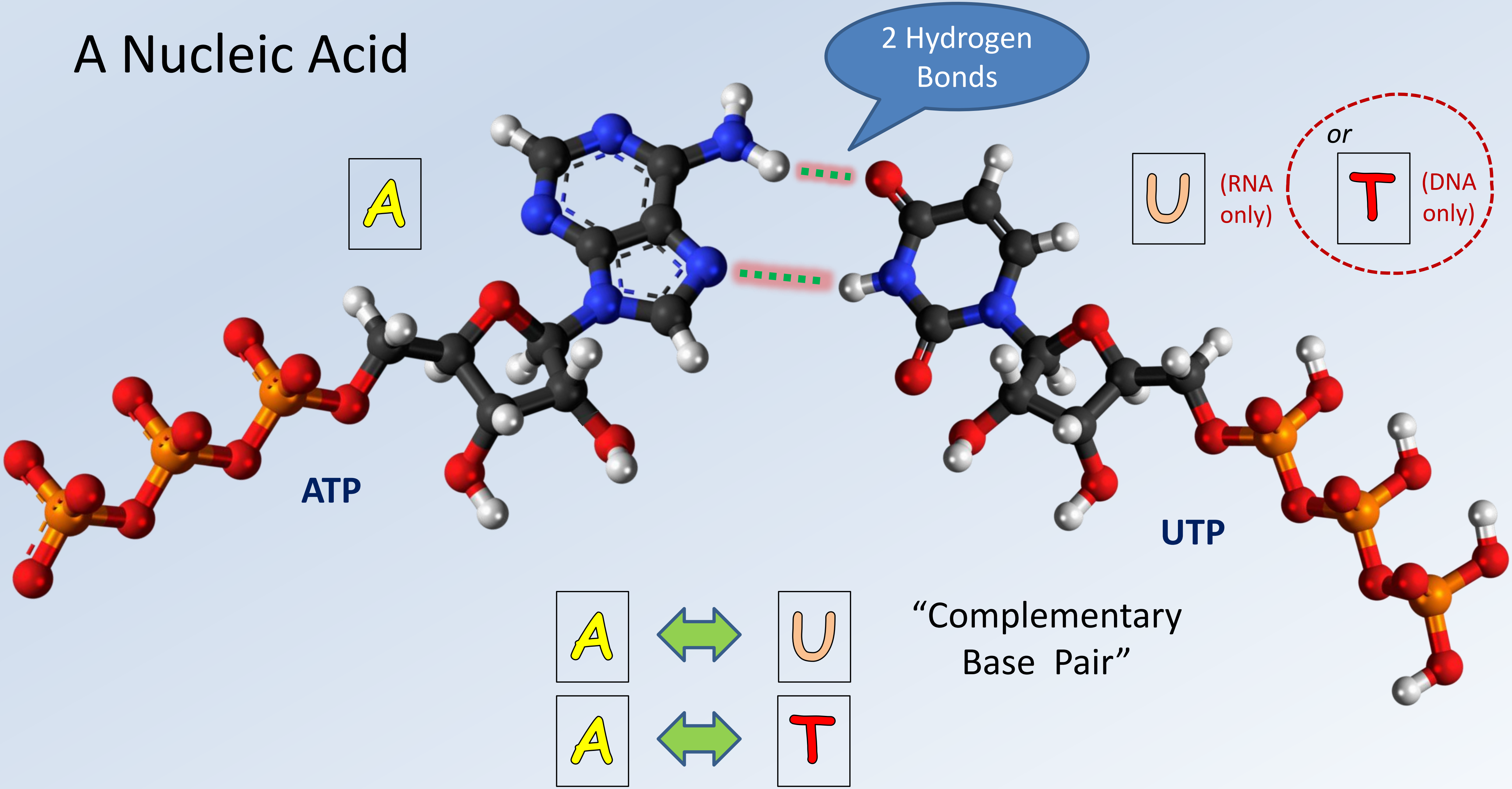
CTP

Cytidine Triphosphate

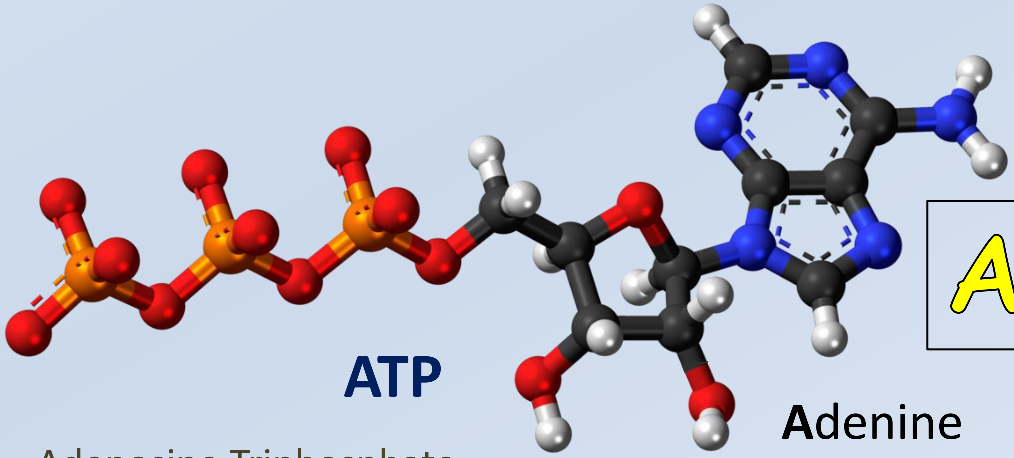
Cytosine

C

A Nucleic Acid



~~4~~ 5 Nucleotides

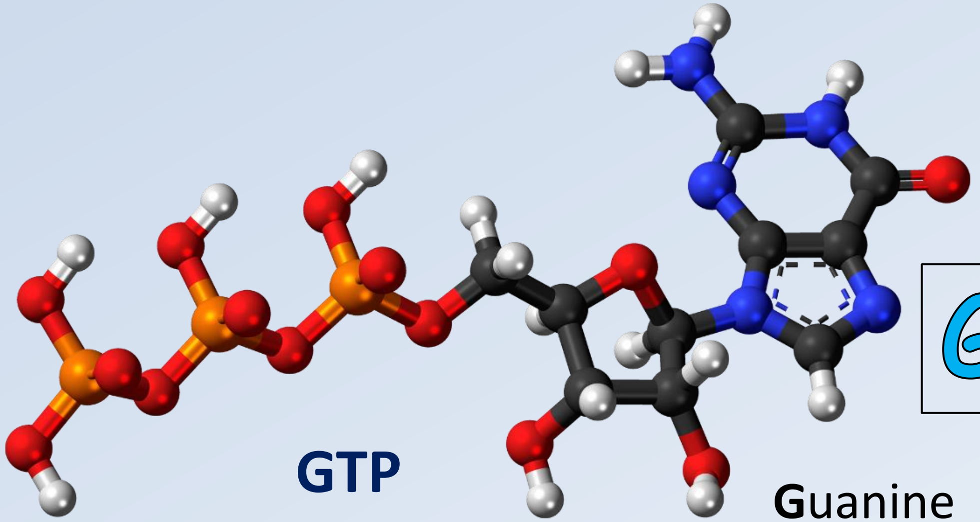


ATP

Adenosine Triphosphate

Adenine

A

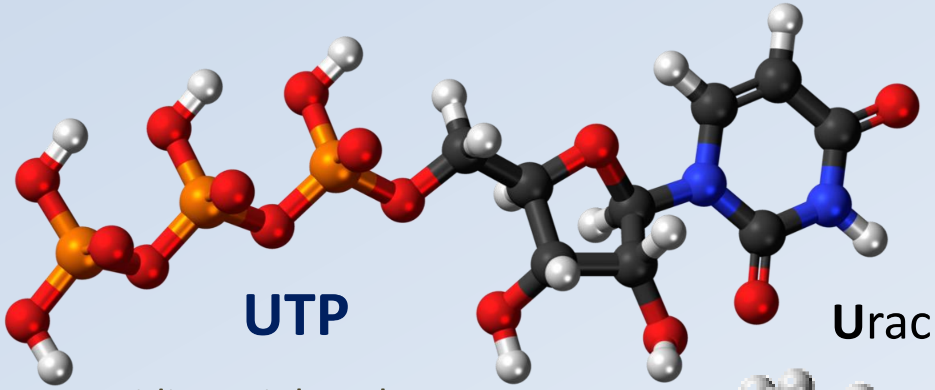


GTP

Guanosine Triphosphate

Guanine

G



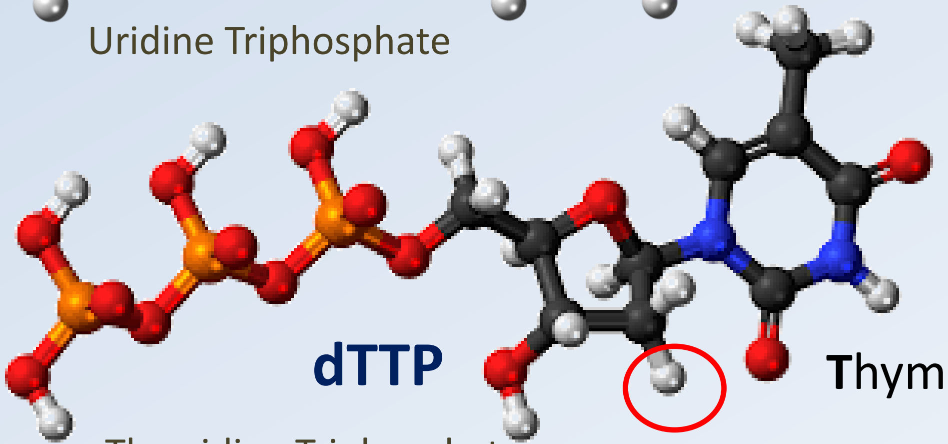
UTP

Uridine Triphosphate

Uracil

U

(RNA only)



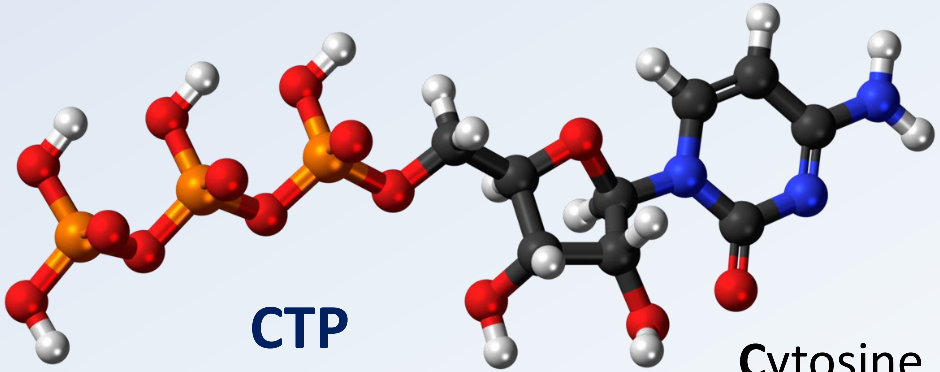
dTTP

Thymidine Triphosphate

Thymine

T

(DNA only)



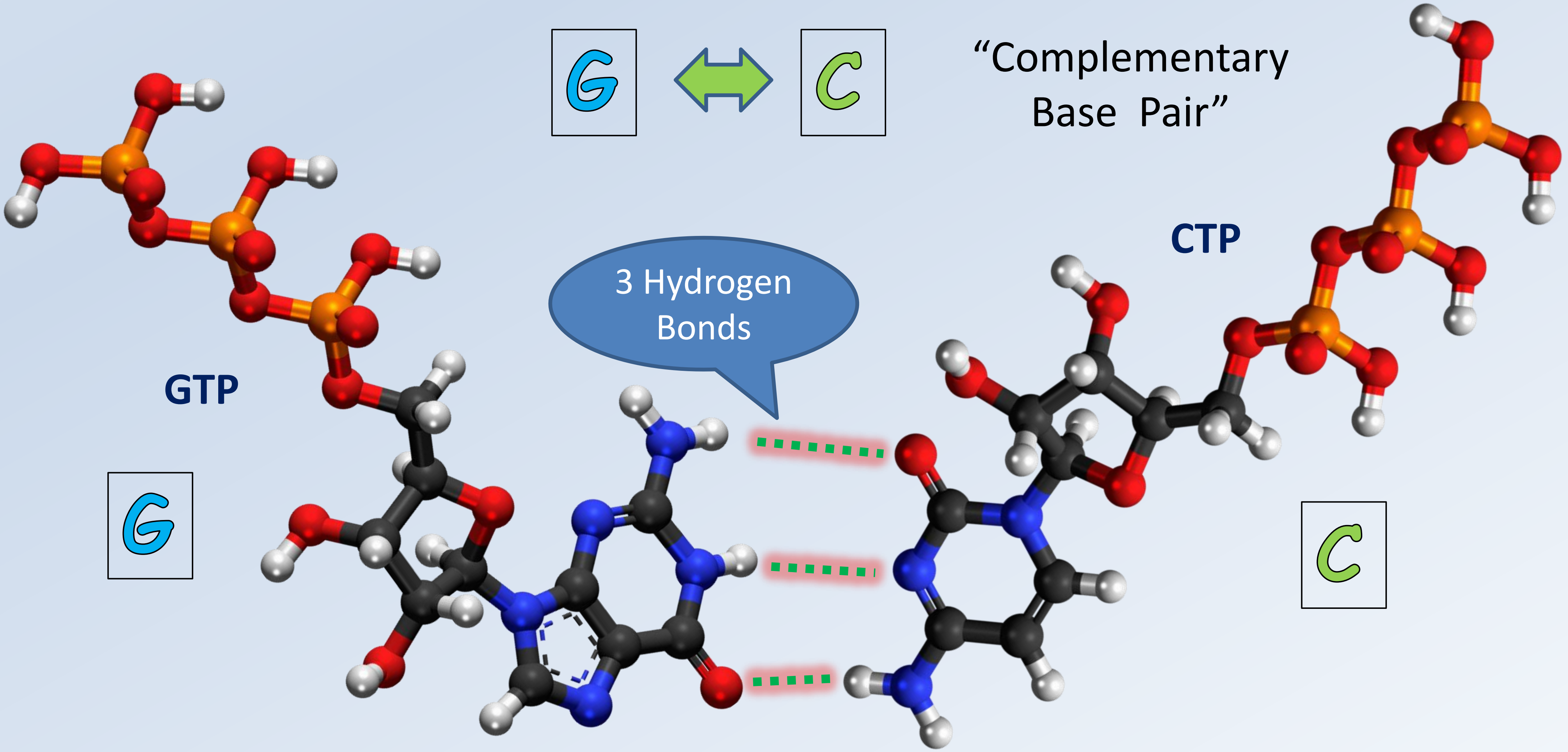
CTP

Cytidine Triphosphate

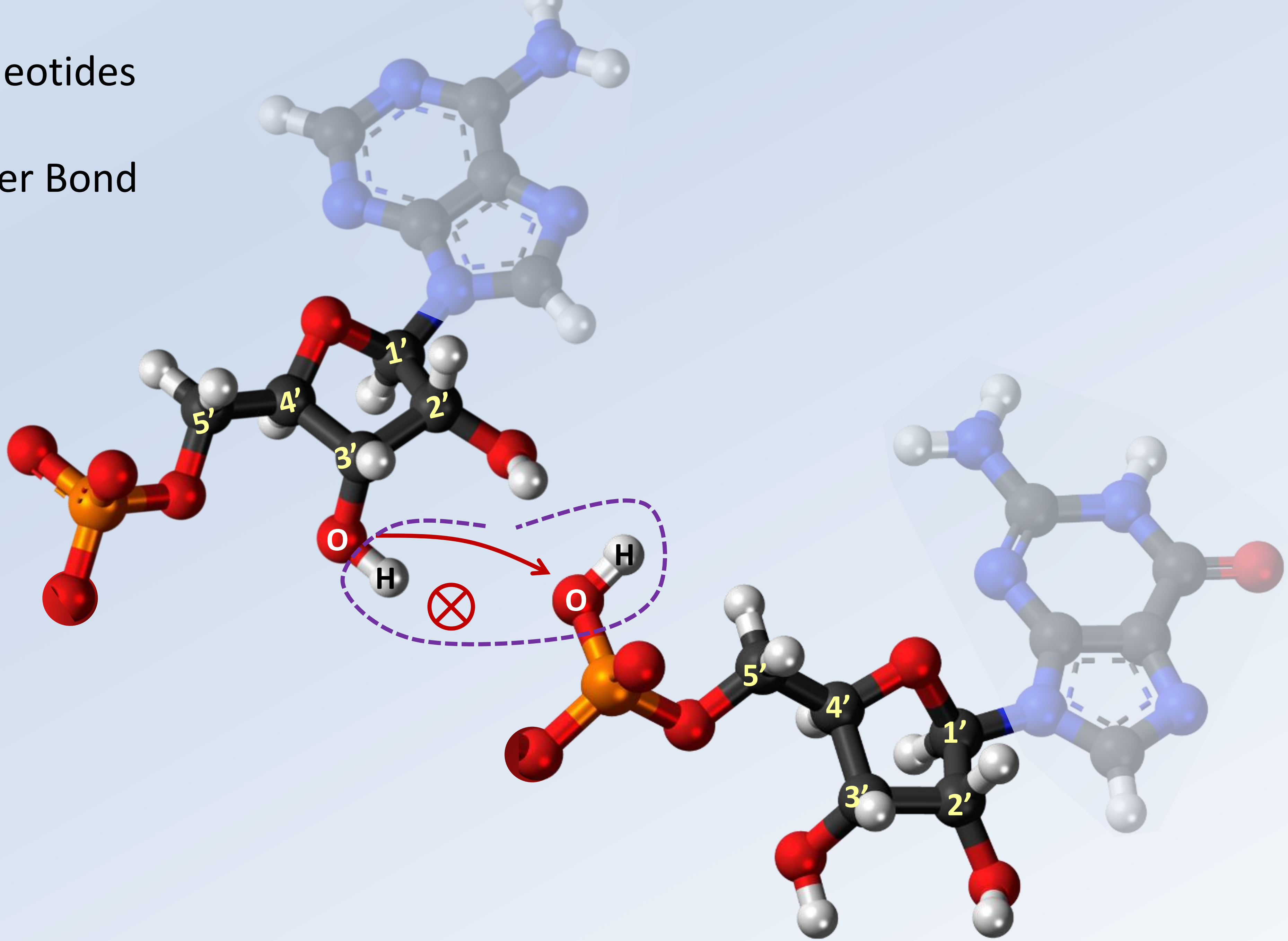
Cytosine

C

Another Nucleic Acid



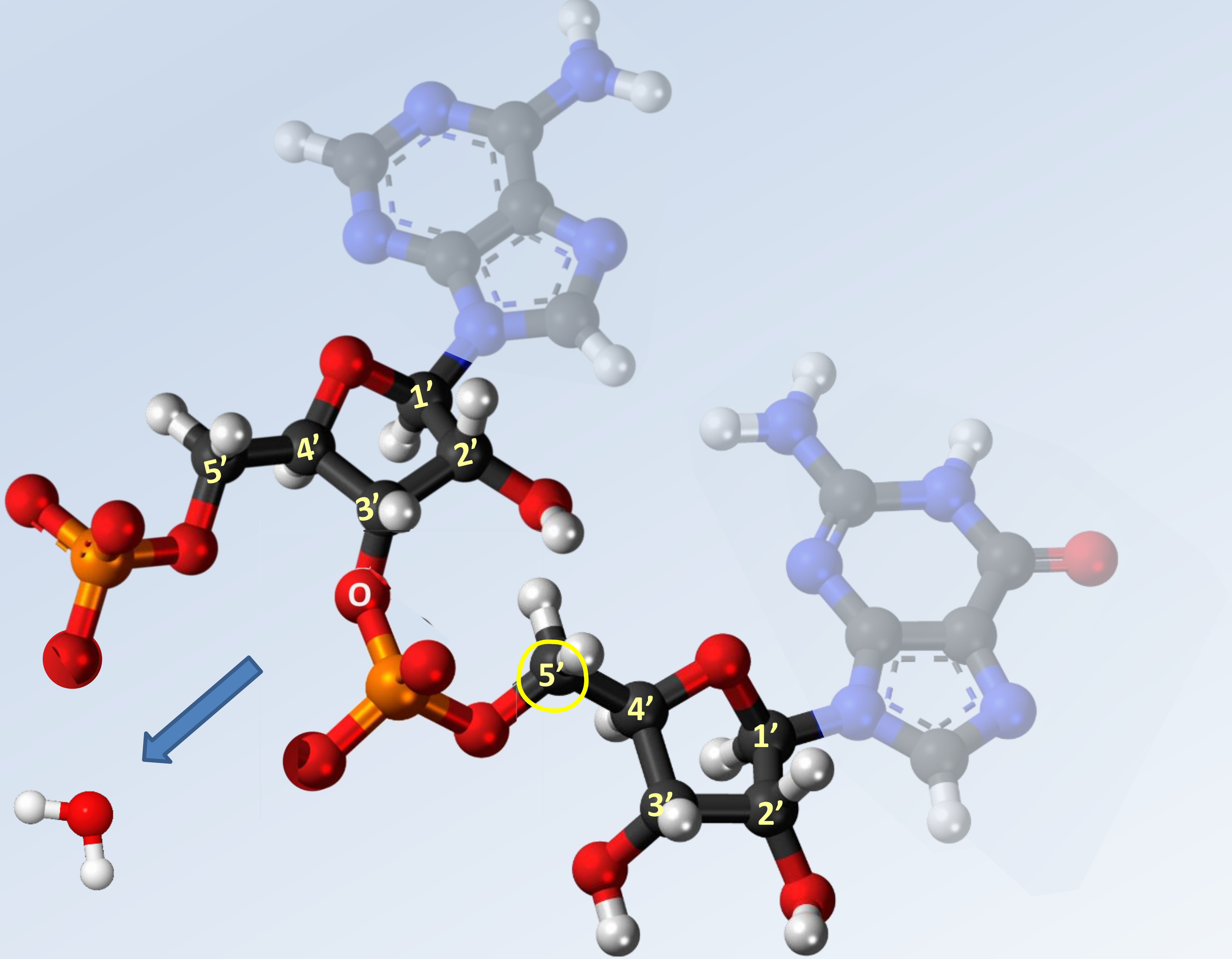
Joining 2 Nucleotides with a Phosphodiester Bond



Joining 2 Nucleotides with a Phosphodiester Bond

Polymerization

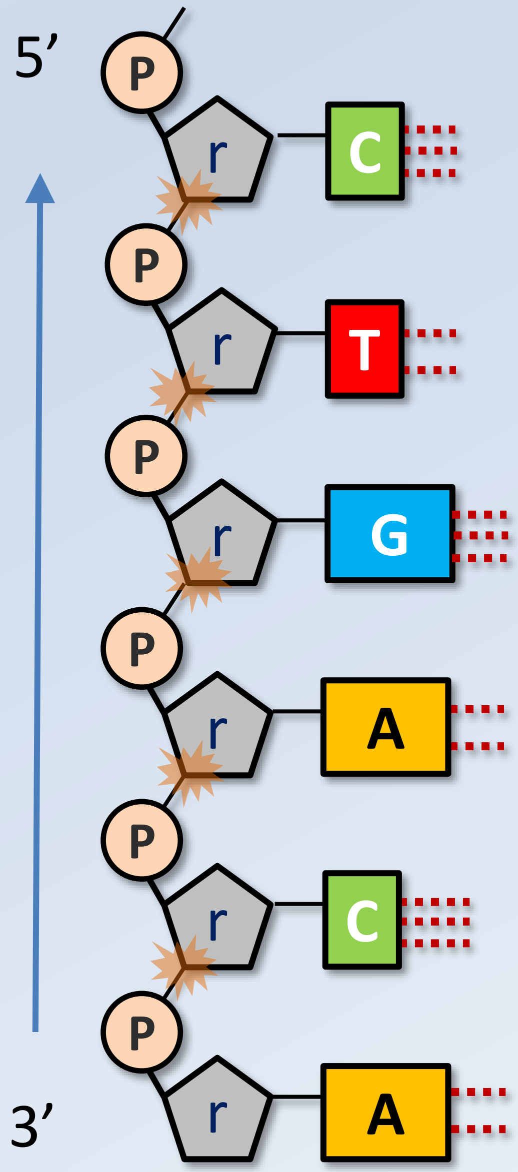
Note:
3’ end of the first nucleotide attaches to **5’** end of the other



Dehydration Reaction

Nucleotide Polymerization

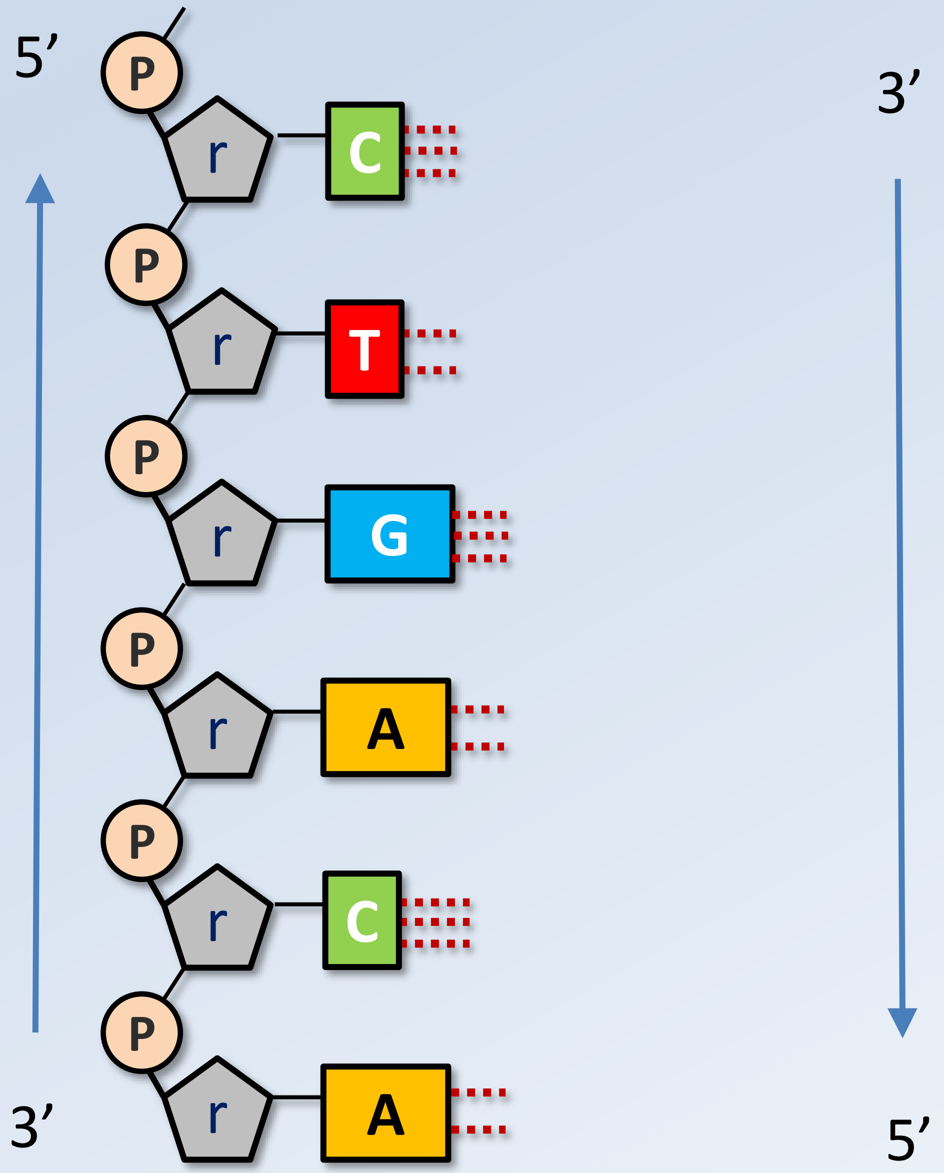
Single Strand
of RNA or DNA



Phosphodiester
Bonds

Nucleotide Polymerization

Single Strand of RNA or DNA



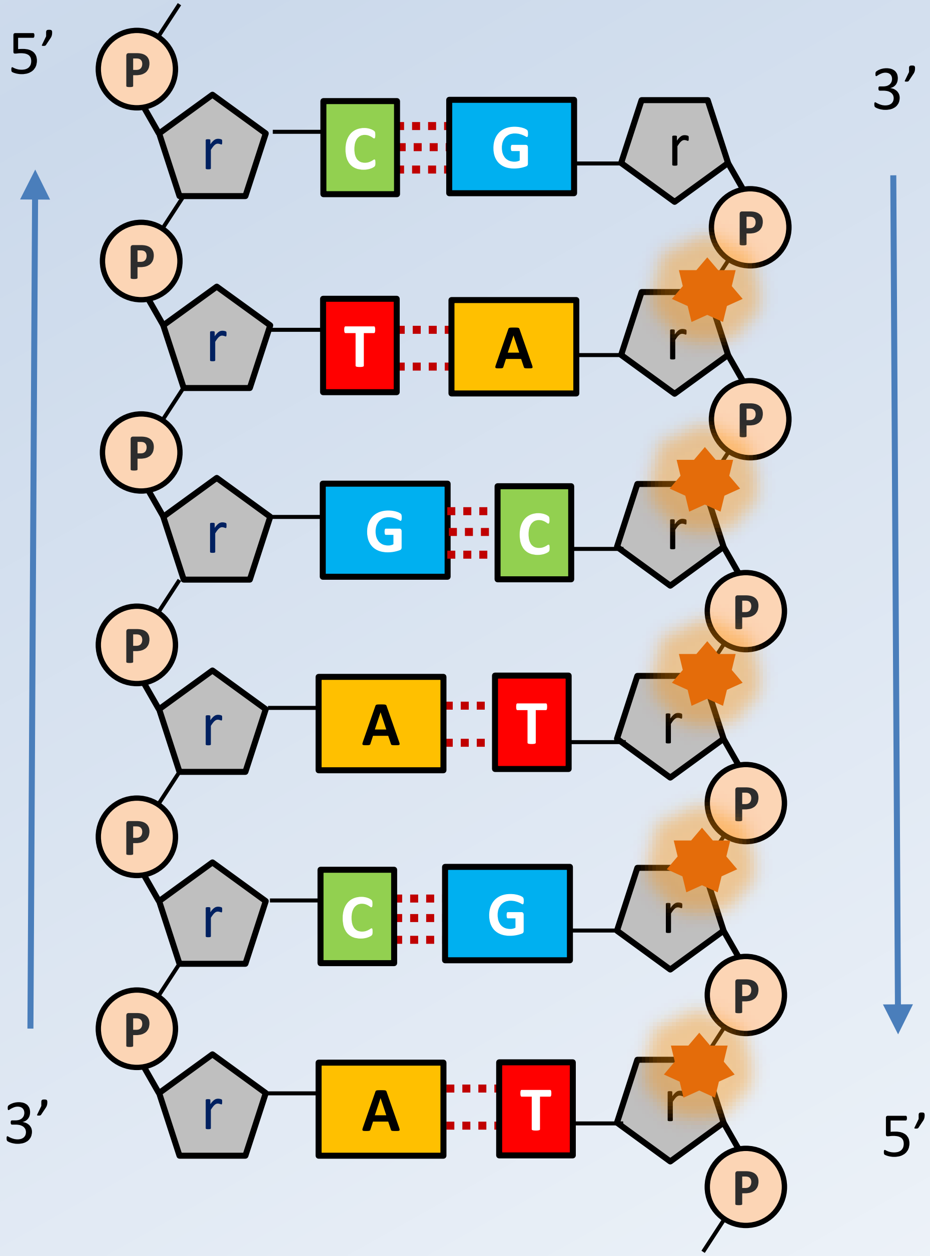
An Exact Complementary Strand will spontaneously find its position and stick!

We now have Double Stranded DNA or RNA

Nucleotide Polymerization

Existing Strand of RNA or DNA

Now add Complementary Bases one by one

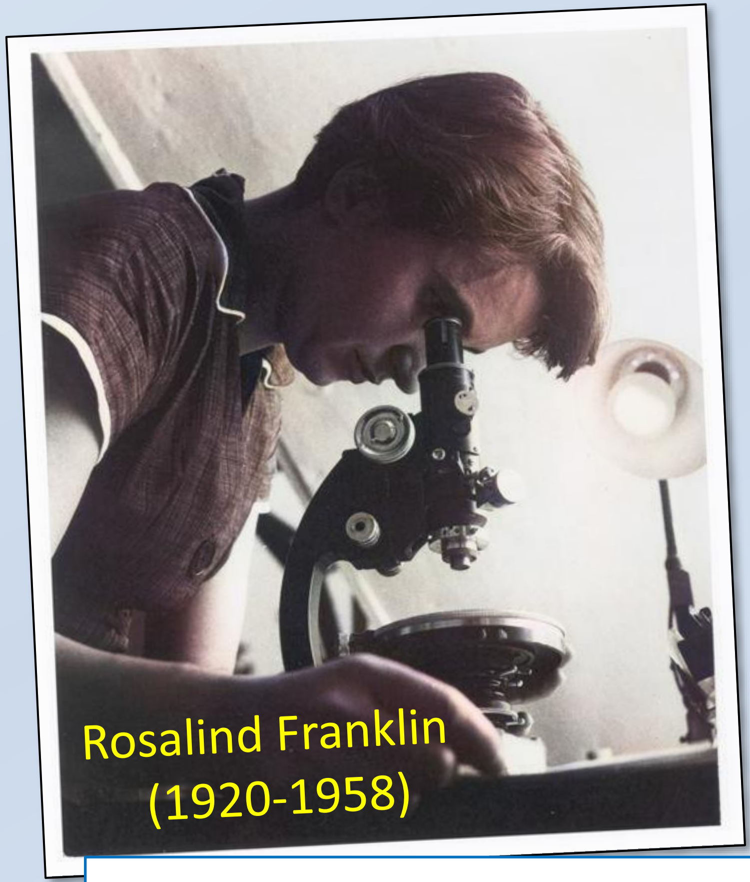


Again, Double Stranded DNA or RNA

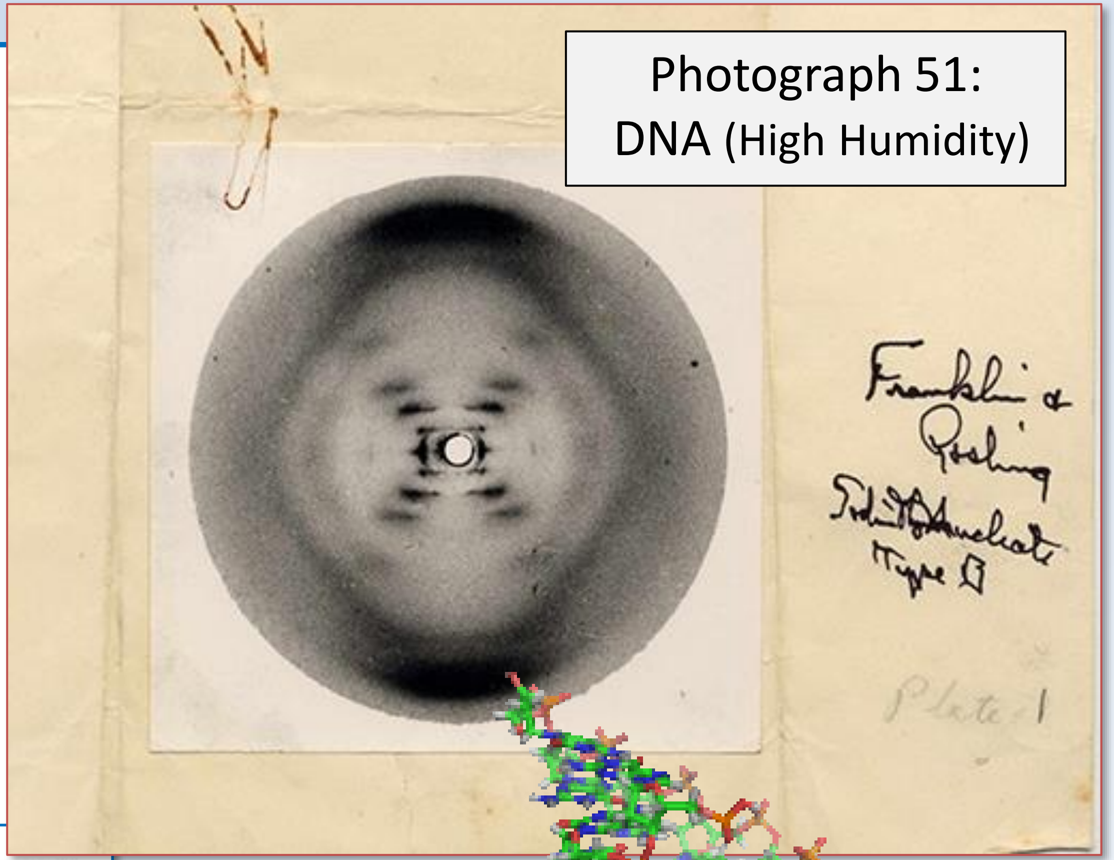
Anti-Parallel

Key to the Discovery of the Structure of DNA

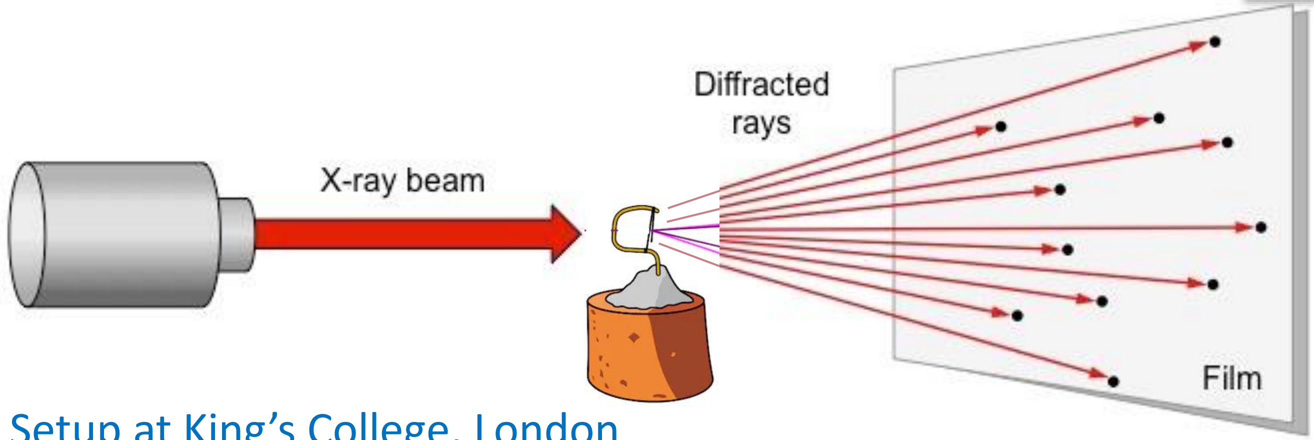
May 1952



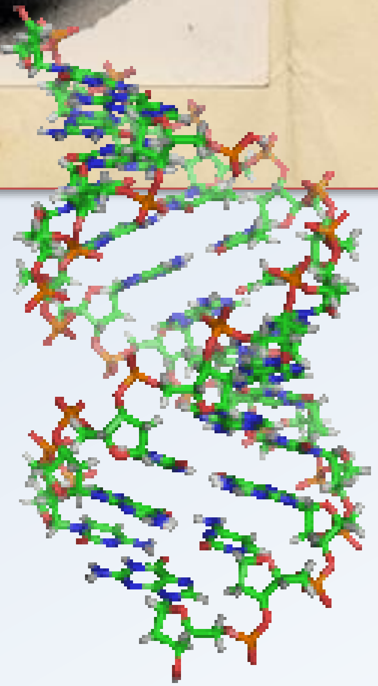
Rosalind Franklin
(1920-1958)



Photograph 51:
DNA (High Humidity)

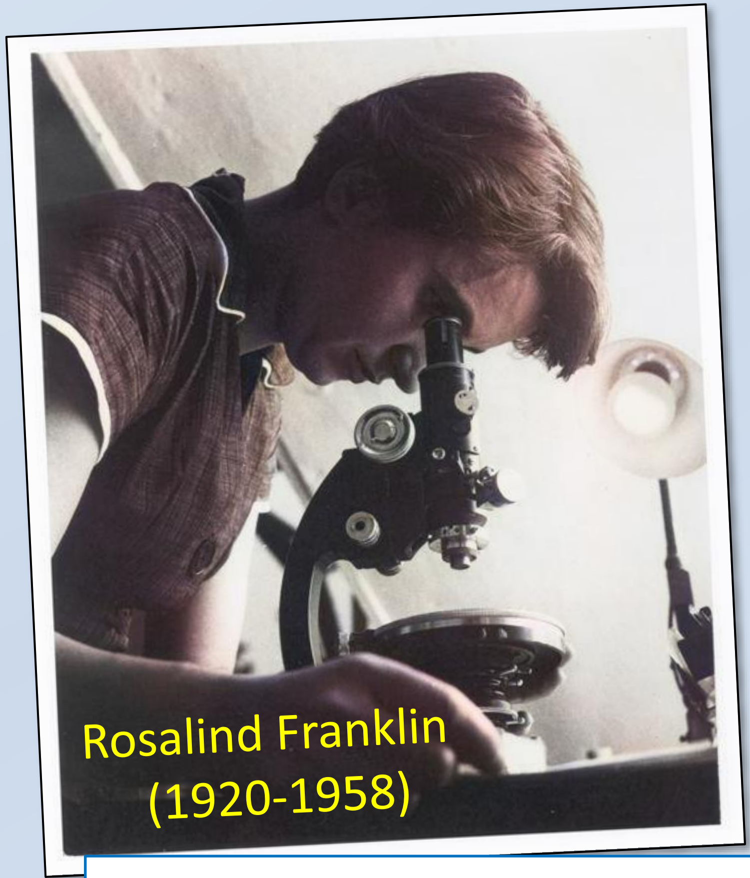


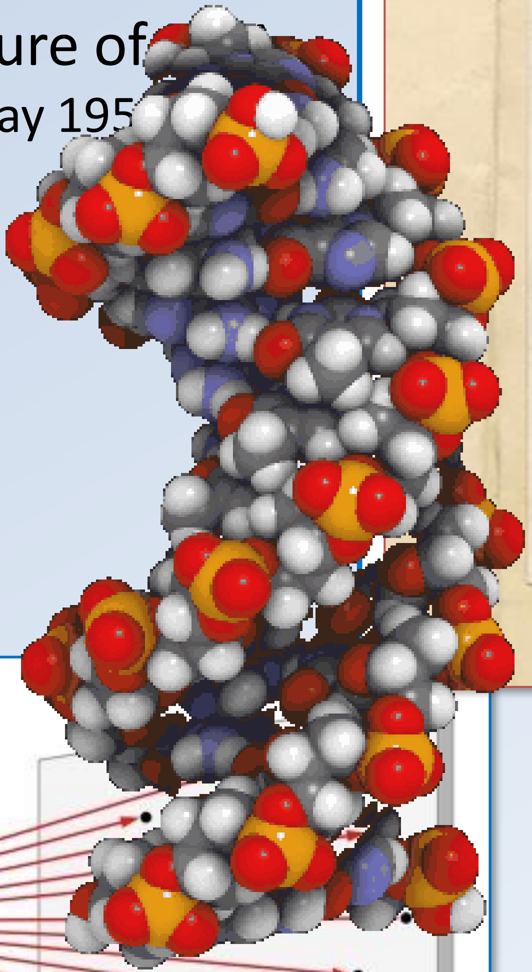
Setup at King's College, London

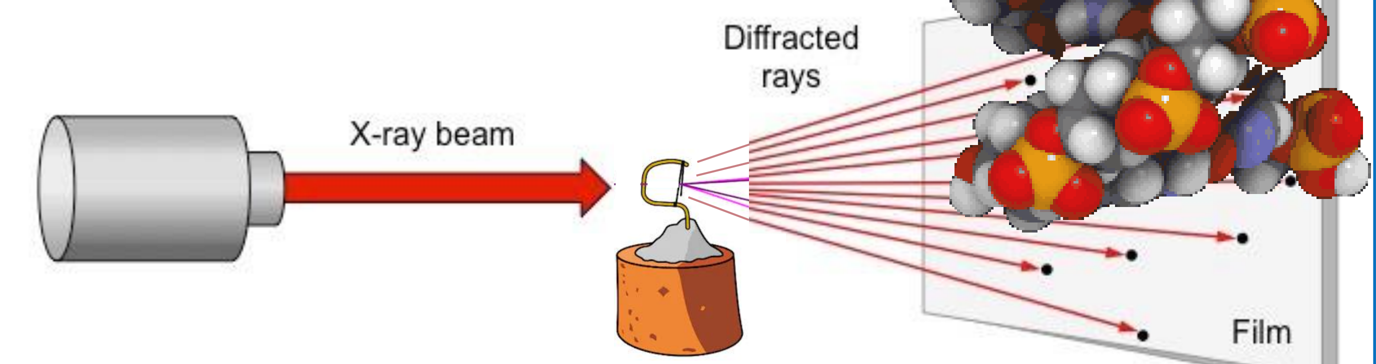


Key to the Discovery of the Structure of

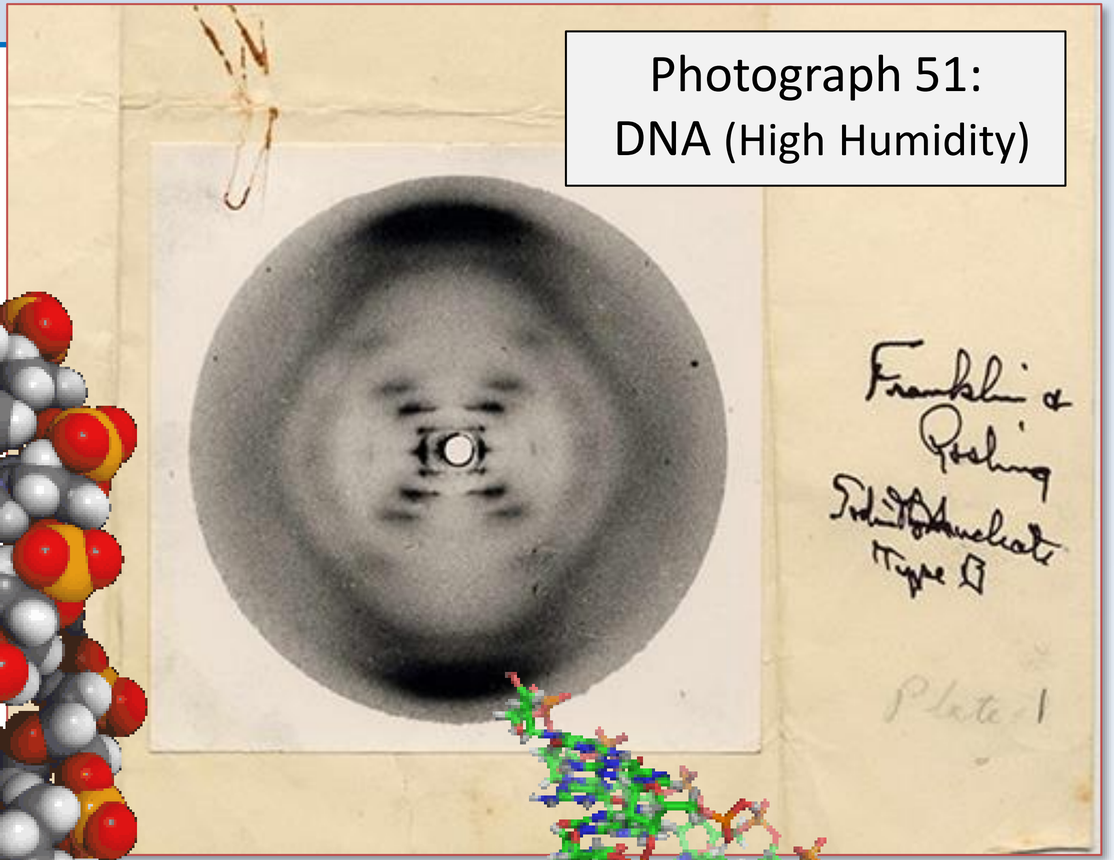
May 195



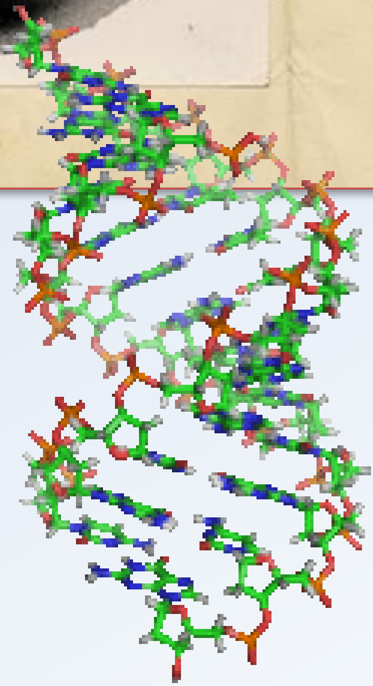




Setup at King's College, London

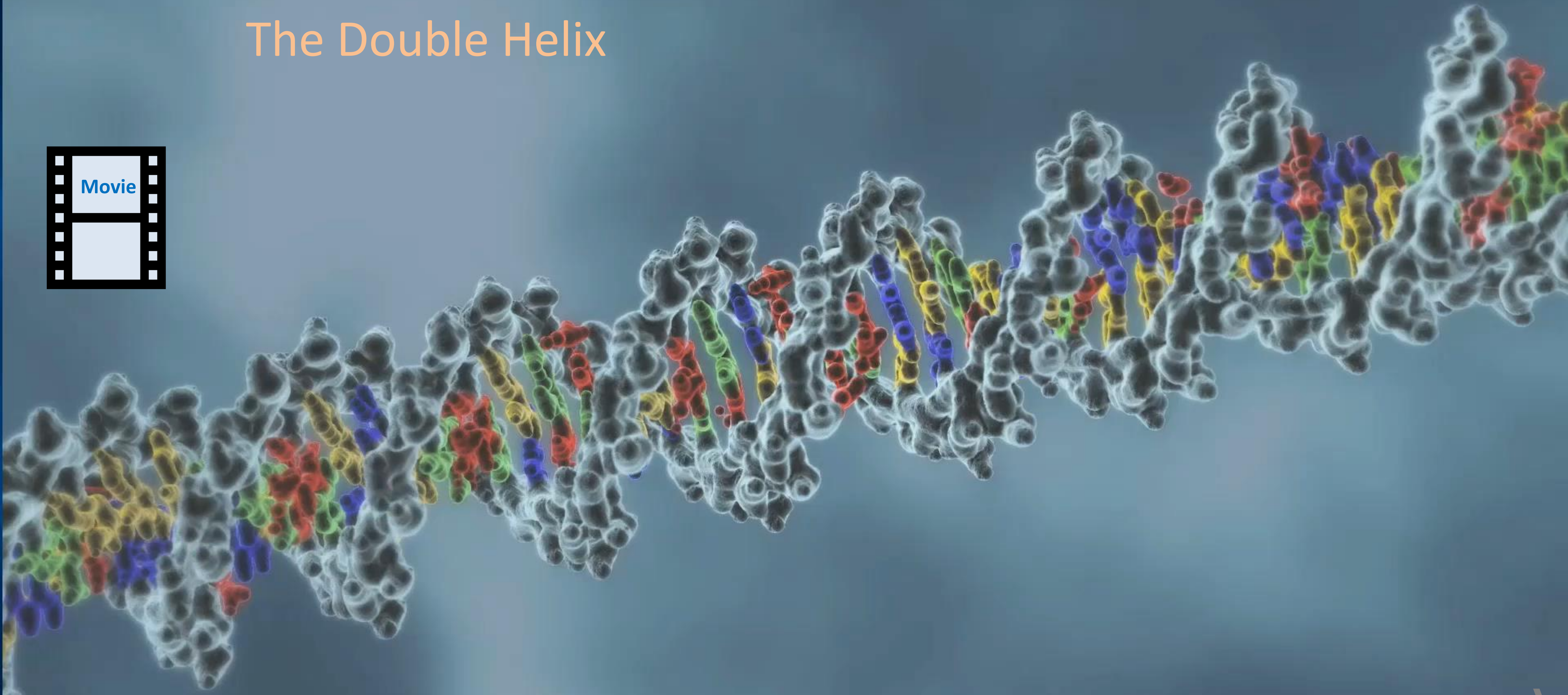


Photograph 51:
DNA (High Humidity)

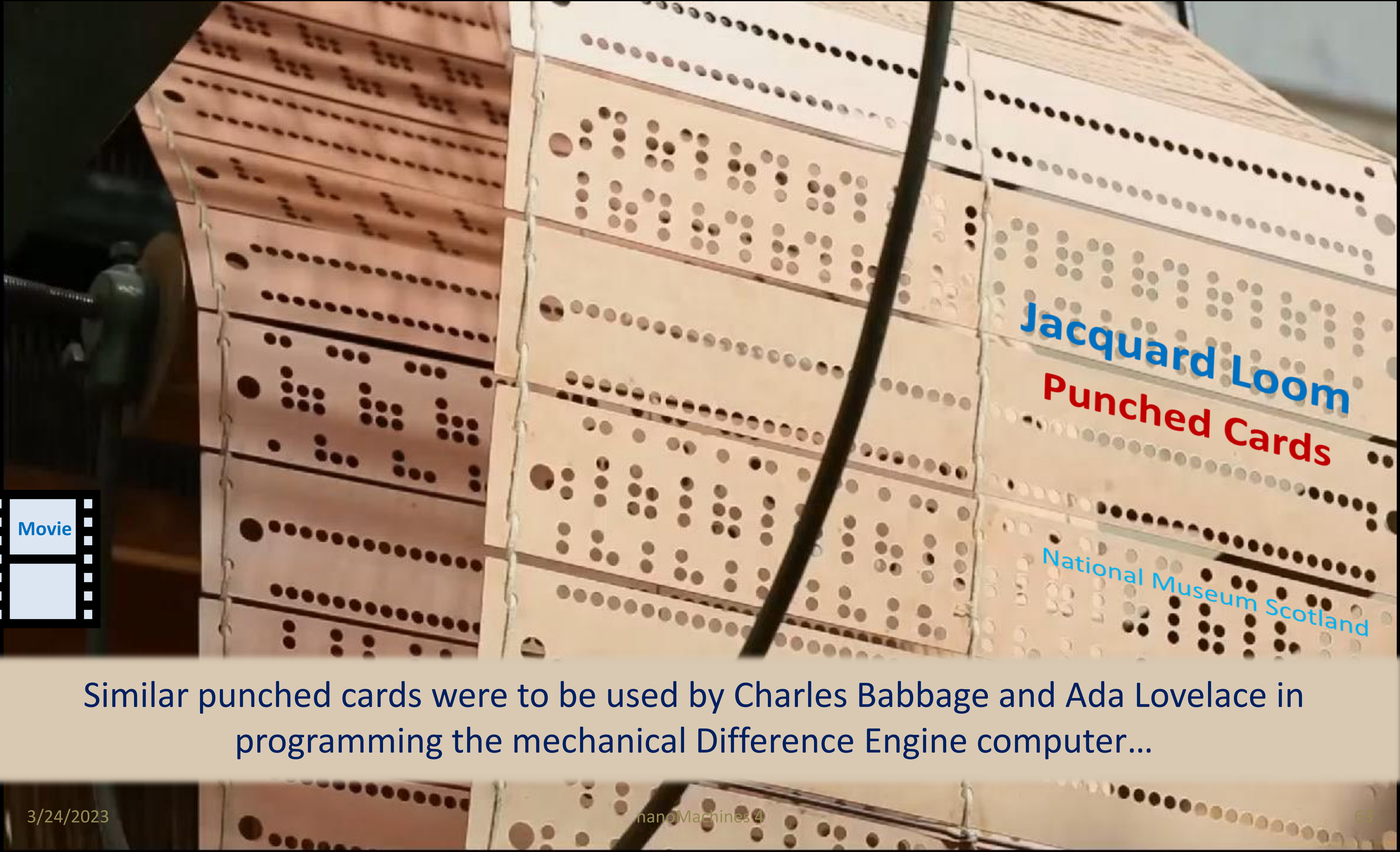


The Double Helix

Movie



Discovery Science (2022)

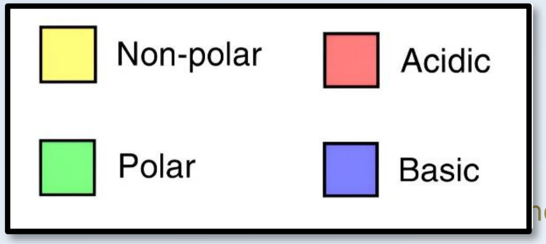


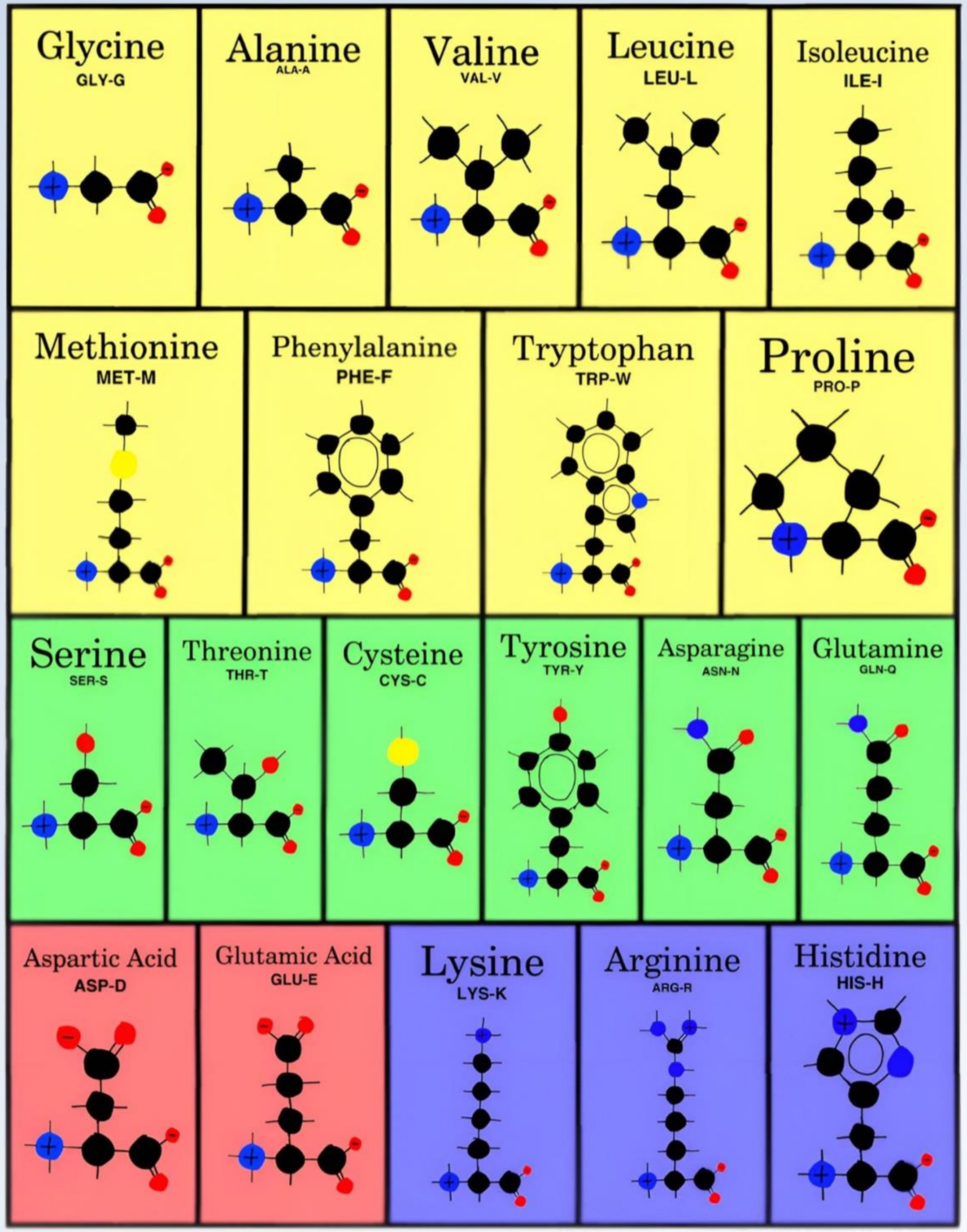
Movie

Similar punched cards were to be used by Charles Babbage and Ada Lovelace in programming the mechanical Difference Engine computer…

The 20 Major α-Amino Acids

G	Glycine	Gly	**P**	Proline	Pro
A	Alanine	Ala	**V**	Valine	Val
L	Leucine	Leu	**I**	Isoleucine	Ile
M	Methionine	Met	**C**	Cysteine	Cys
F	Phenylalanine	Phe	**Y**	Tyrosine	Tyr
W	Tryptophan	Trp	**H**	Histidine	His
K	Lysine	Lys	**R**	Arginine	Arg
Q	Glutamine	Gln	**N**	Asparagine	Asn
E	Glutamic Acid	Glu	**D**	Aspartic Acid	Asp
S	Serine	Ser	**T**	Threonine	Thr





Wikimedia Commons: Spaokobb

The "Universal" Amino Acid Codon Code

Some Amino Acids coded in 2 to 6 different ways

1st base	2nd base: U = Uracil		C = Cytosine		A = Adenine		G = Guanine		3rd base
U	UUU	(Phe/F) Phenylalanine	UCU	(Ser/S) Serine	UAU	(Tyr/Y) Tyrosine	UGU	(Cys/C) Cysteine	U
	UUC		UCC		UAC		UGC		C
	UUA	(Leu/L) Leucine	UCA		UAA	Stop	UGA	Stop[1]	A
	UUG		UCG		UAG		UGG	(Trp/W) Tryptophan	G
C	CUU		CCU	(Pro/P) Proline	CAU	(His/H) Histidine	CGU	(Arg/R) Arginine	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	(Gln/Q) Glutamine	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	(Ile/I) Isoleucine	ACU	(Thr/T) Threonine	AAU	(Asn/N) Asparagine	AGU	(Ser/S) Serine	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	(Lys/K) Lysine	AGA	(Arg/R) Arginine	A
	AUG	(Met/M) Methionine	ACG		AAG		AGG		G
G	GUU	(Val/V) Valine	GCU	(Ala/A) Alanine	GAU	(Asp/D) Aspartic acid	GGU	(Gly/G) Glycine	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	(Glu/E) Glutamic acid	GGA		A
	GUG		GCG		GAG		GGG		G

Start Code

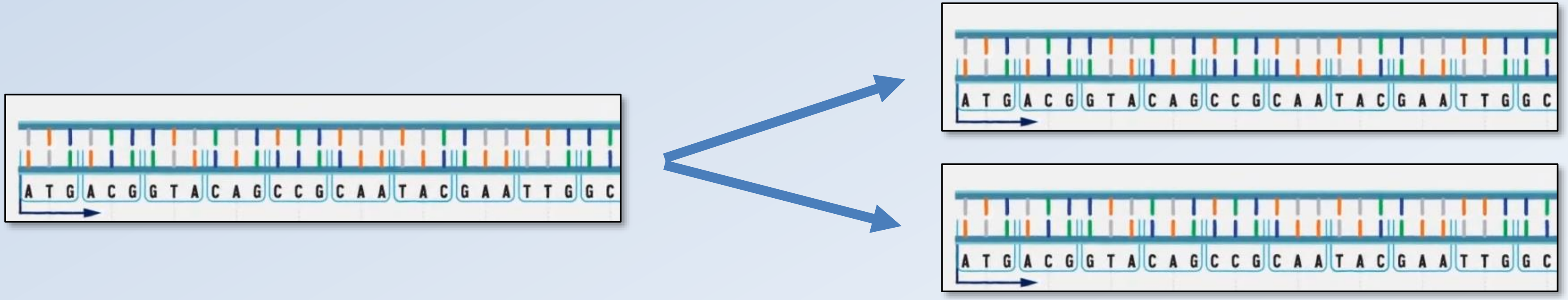
6

Amino-acid properties	Nonpolar	Polar	Basic	Acidic

Wikipedia

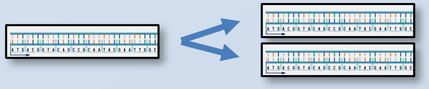
Questions?

① DNA Replication



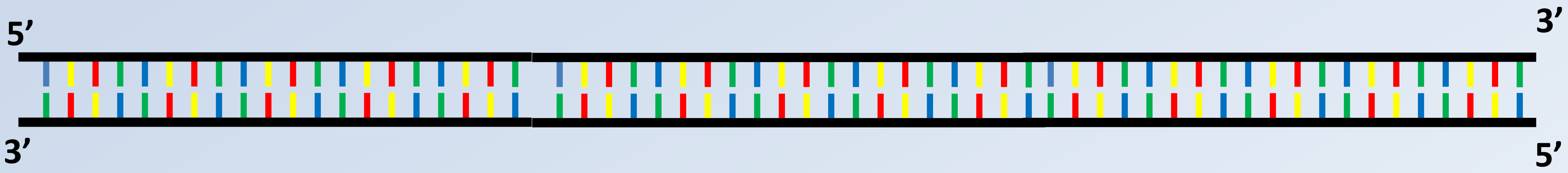
2
Identical
Copies

① DNA Replication



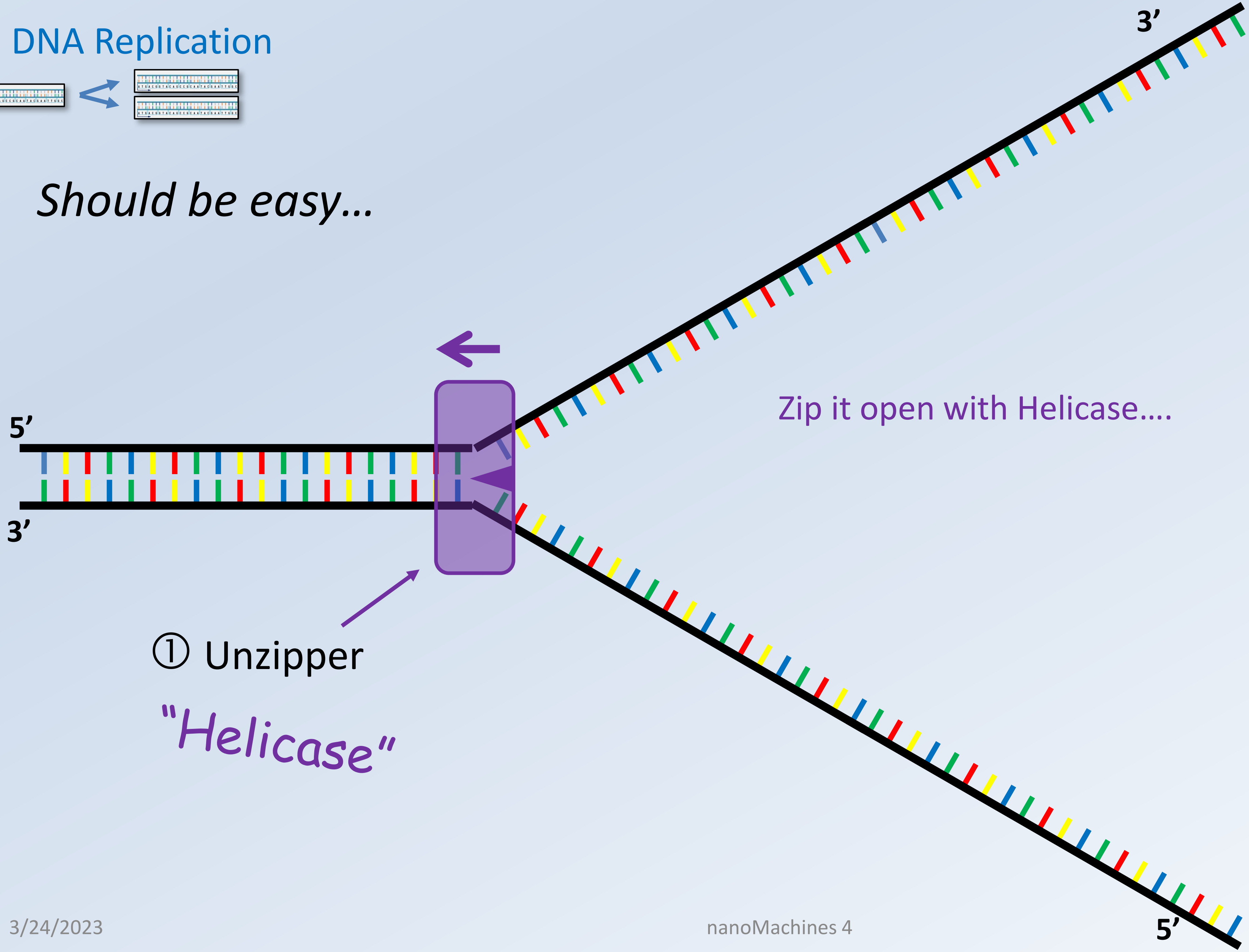
Should be easy…

Start with double stranded DNA ….



① DNA Replication

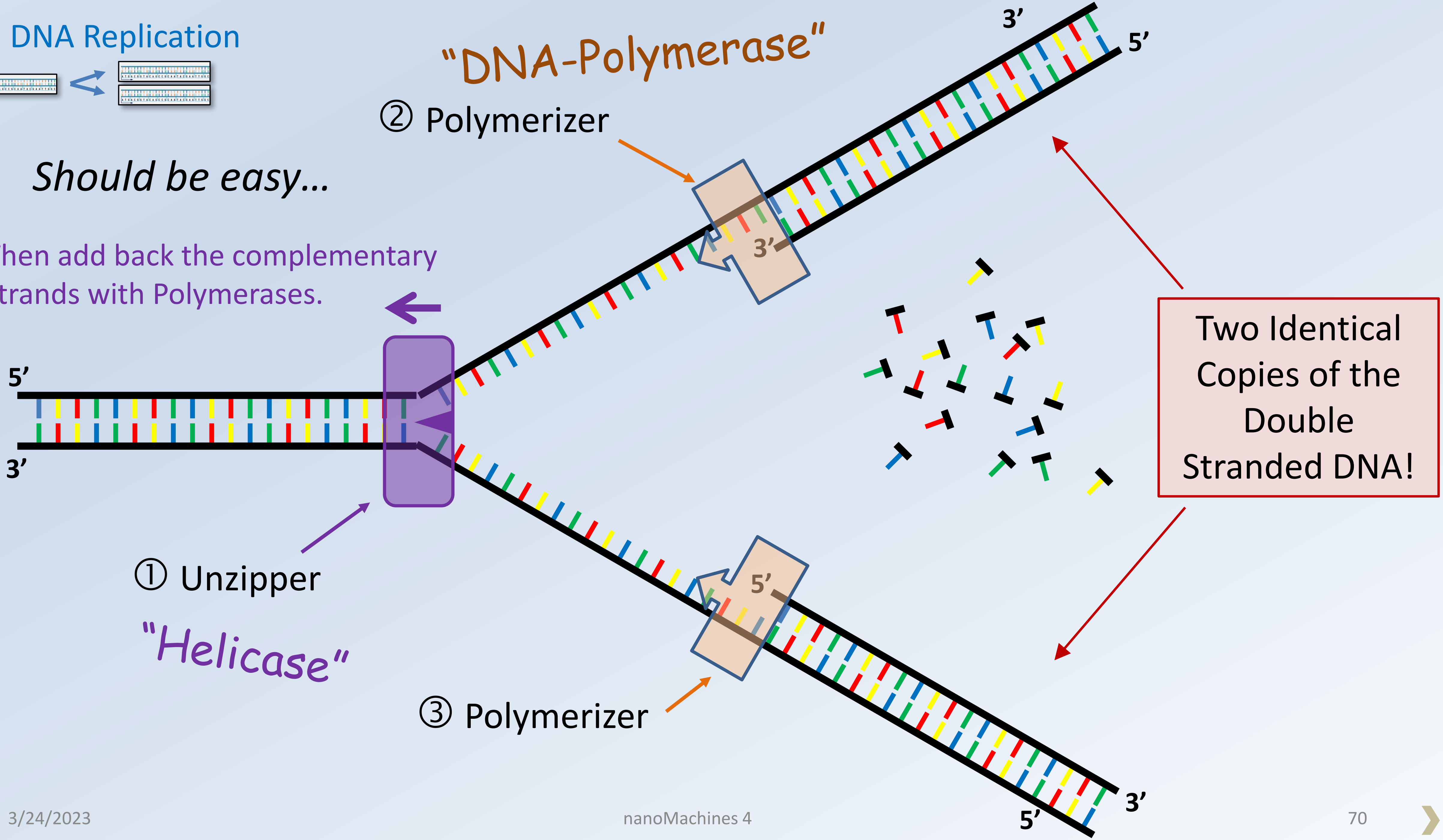
Should be easy…



① DNA Replication

Should be easy…

Then add back the complementary strands with Polymerases.

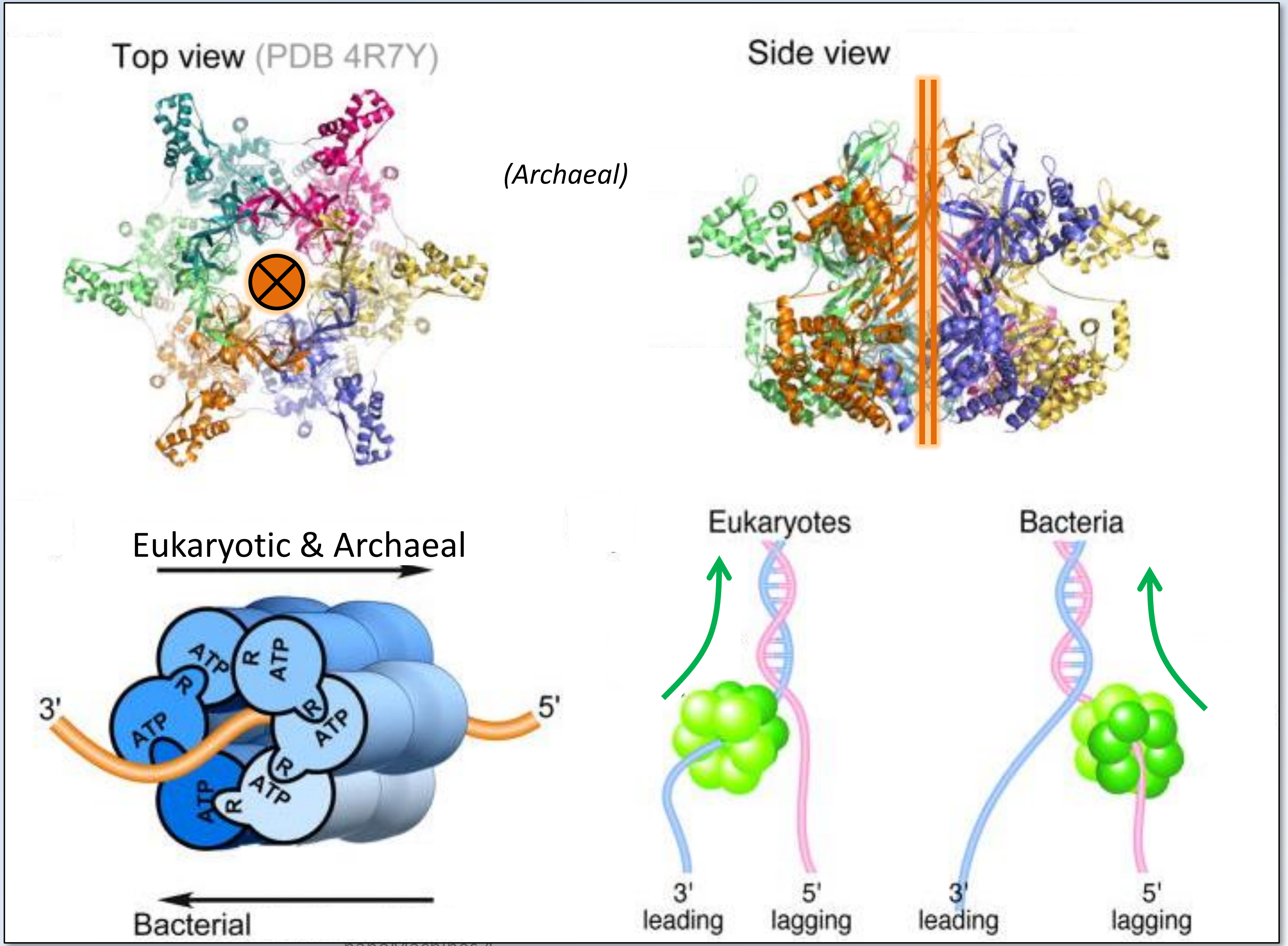


Unzipper

Helicases are completely different in Bacteria *vs.* Eukaryotes

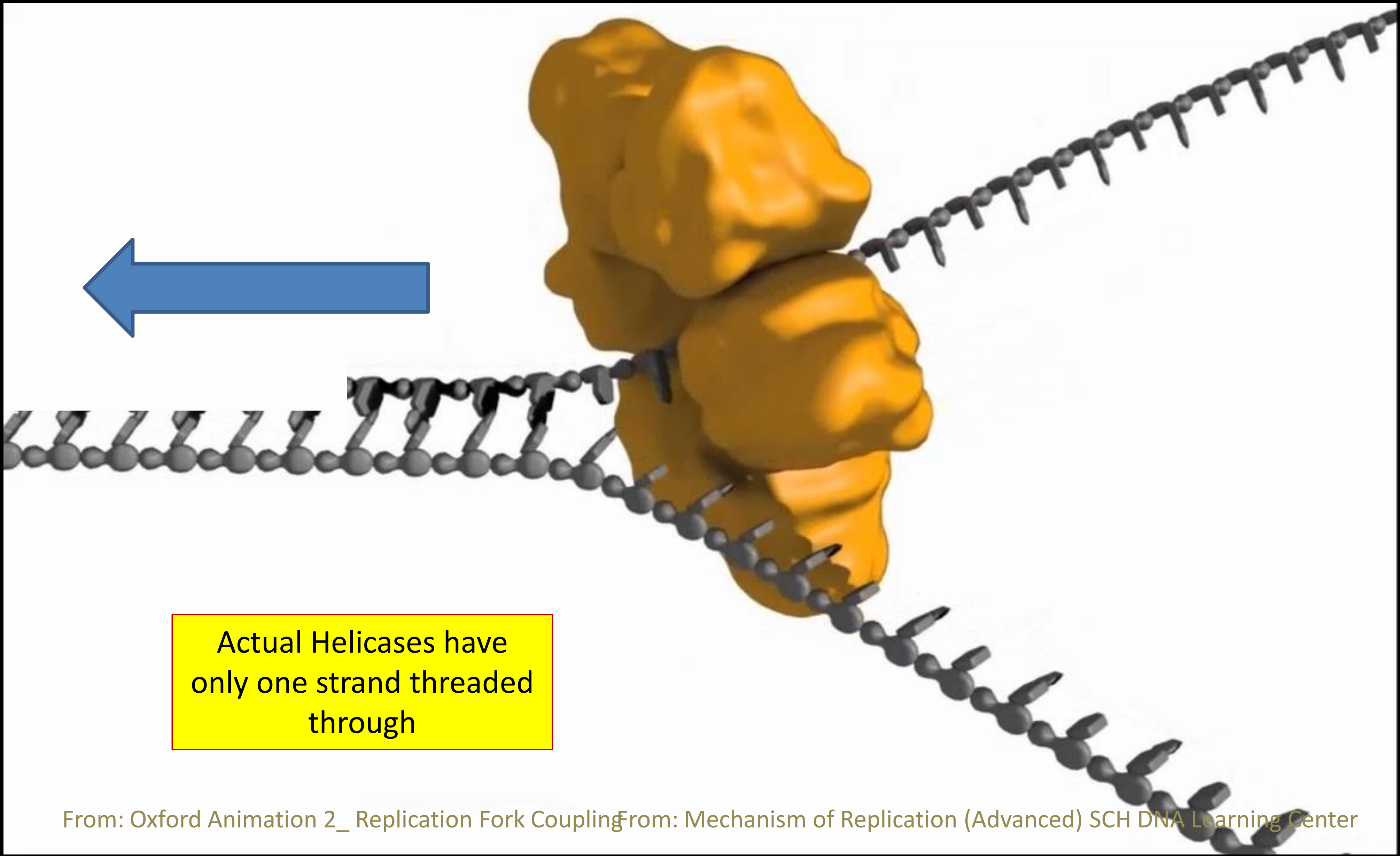
(and Archaea)

- Hexamers
- Burn ATP
- Surround only *one* DNA strand



O'Donnell & Li *(Nature Struc Mol Biol Feb 2018)*

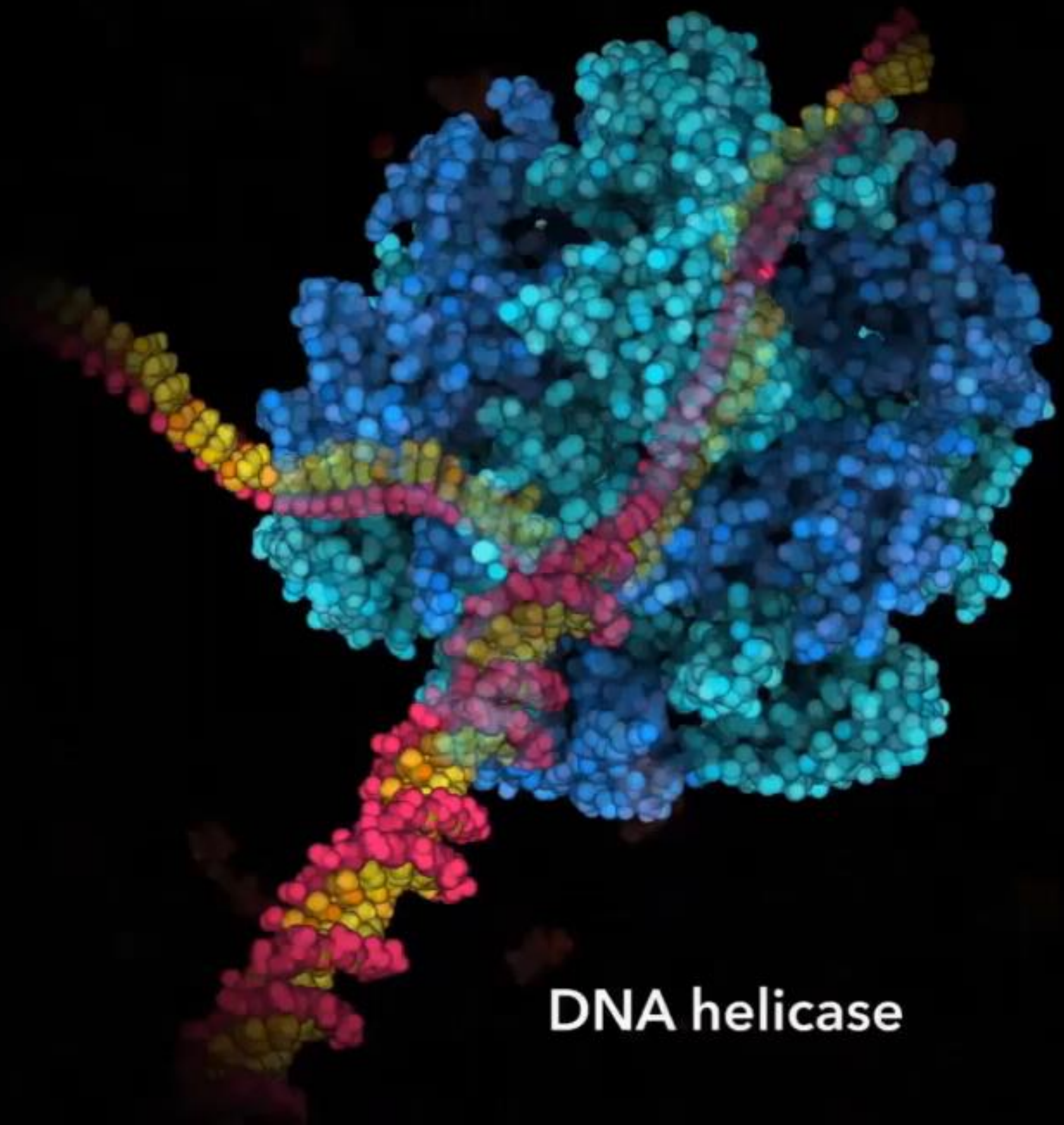
Helicase Animations



Movie

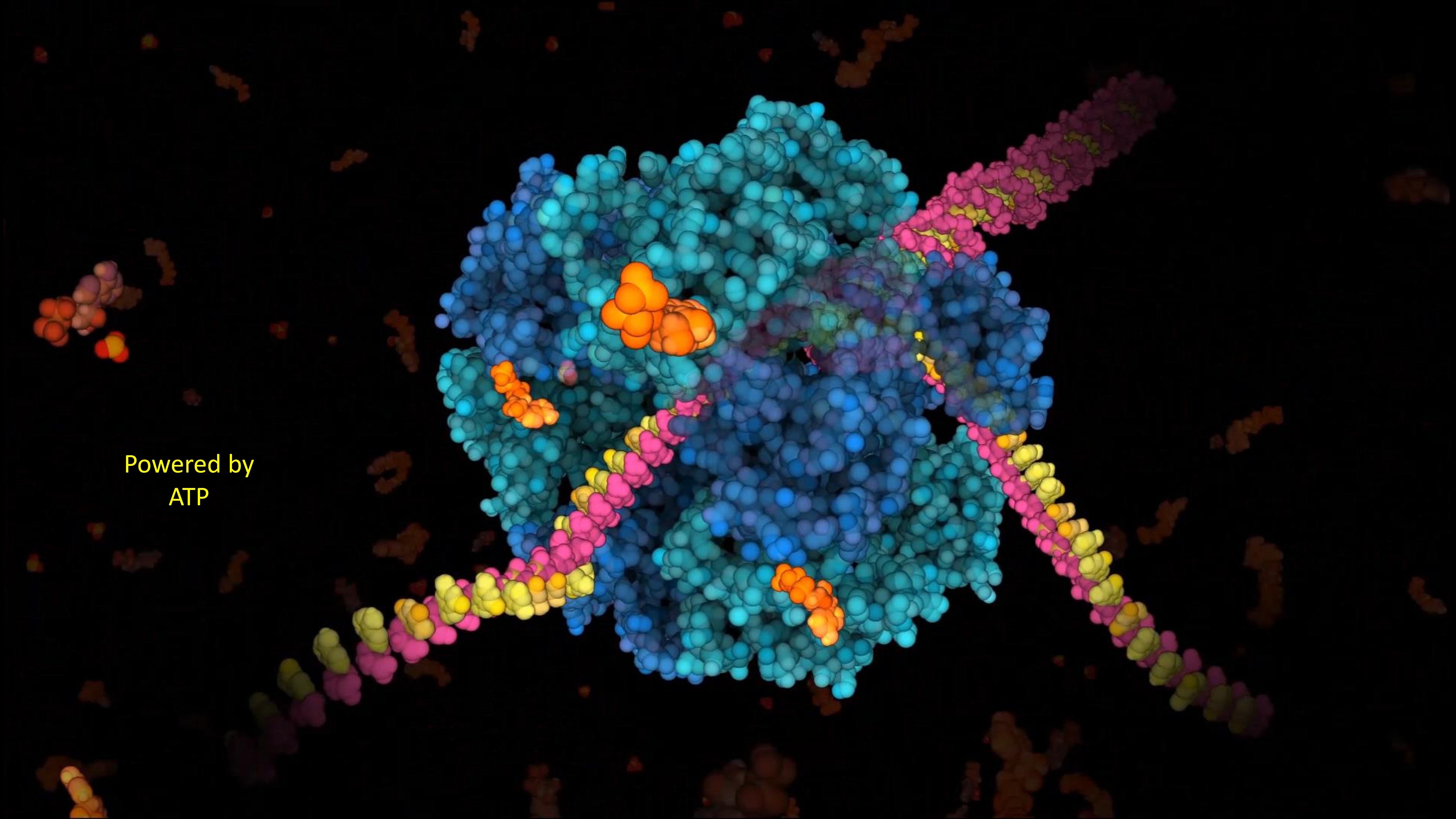
From: Oxford Animation 2_ Replication Fork CouplingFrom: Mechanism of Replication (Advanced) SCH DNA Learning Center

Helicase Animations



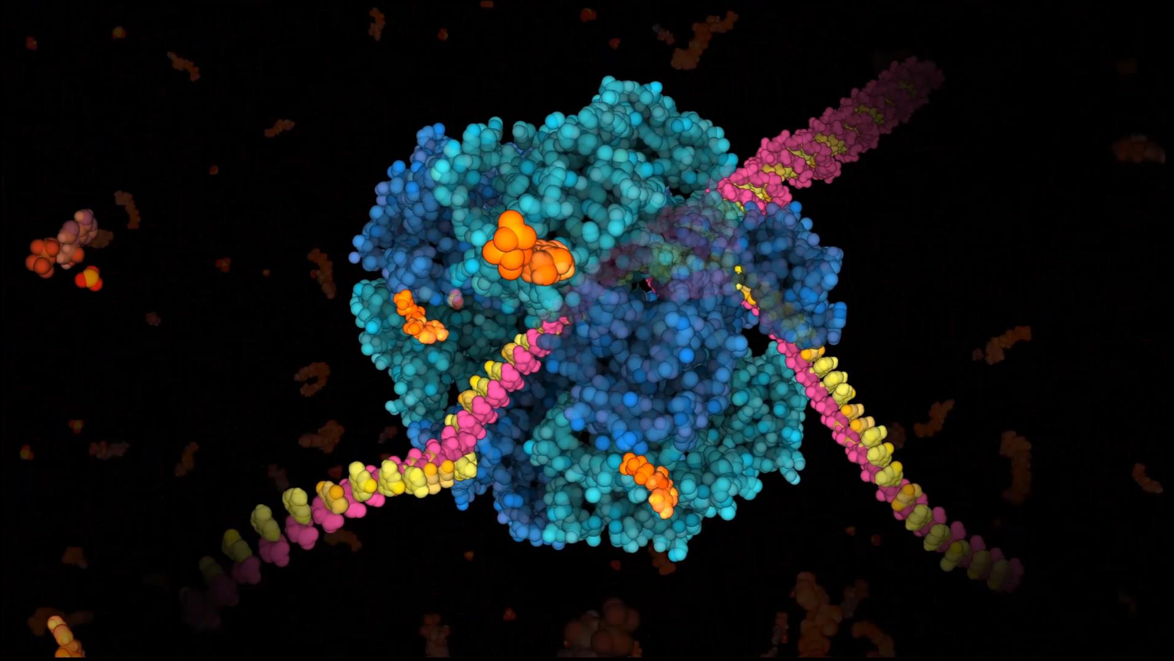
Movie

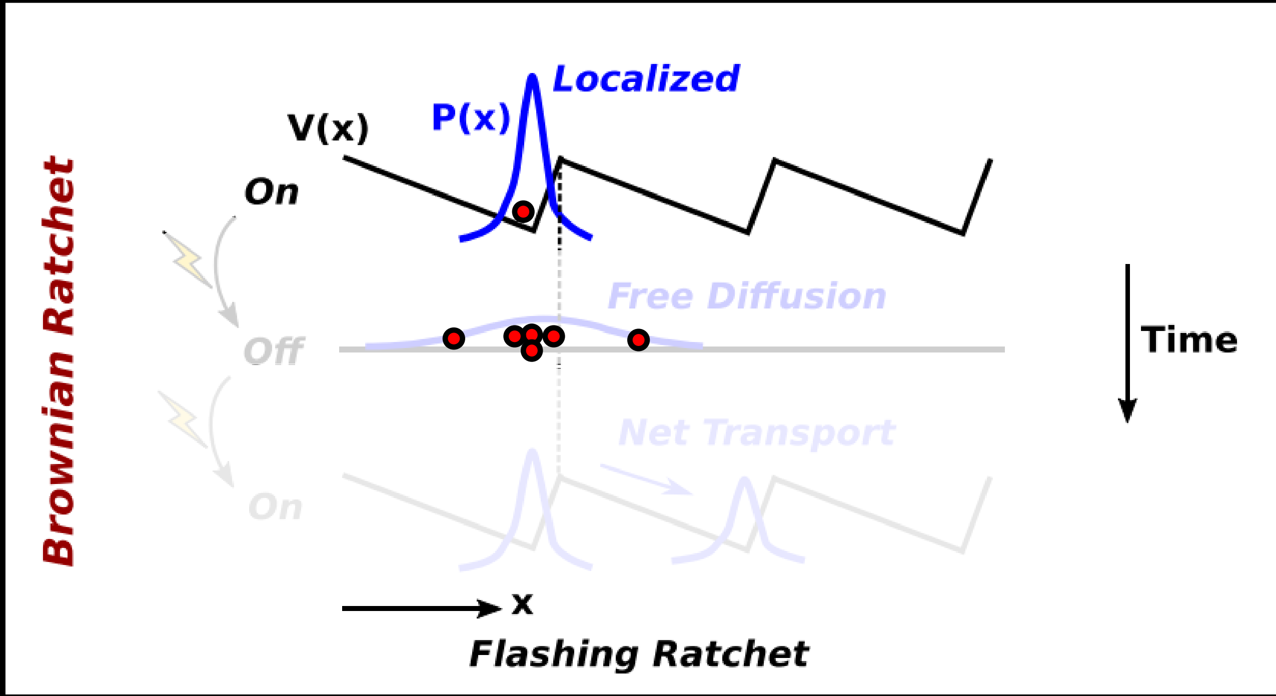
ATP & Helicase (Drew Berry WEHI-tv)


Powered by
ATP

Helicase steps are very small – 0.34 nm per base.

Recent work suggests that Eukaryotic Helicases are likely close to being….

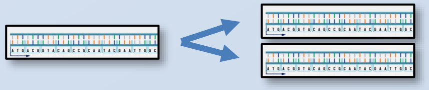




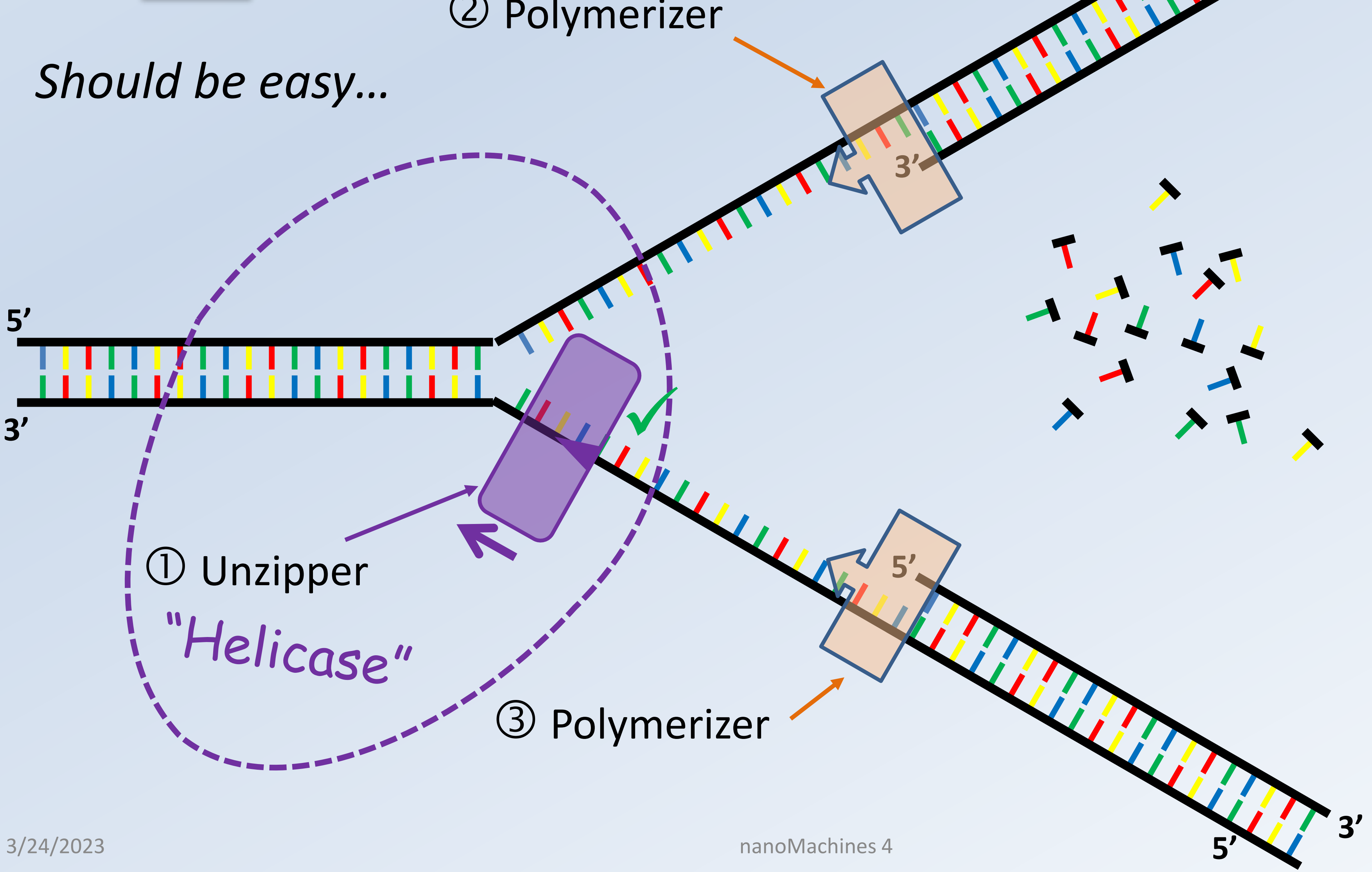
"Brownian Ratchets"

Sometimes observed to take a step backwards

① DNA Replication

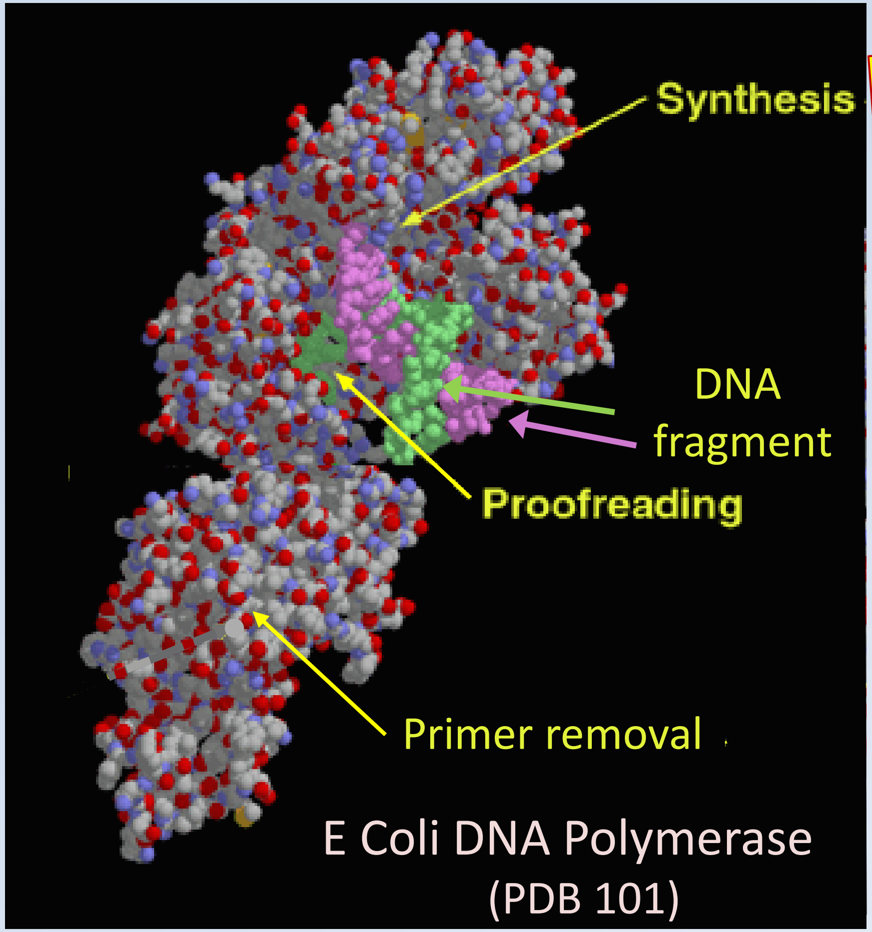


Should be easy…

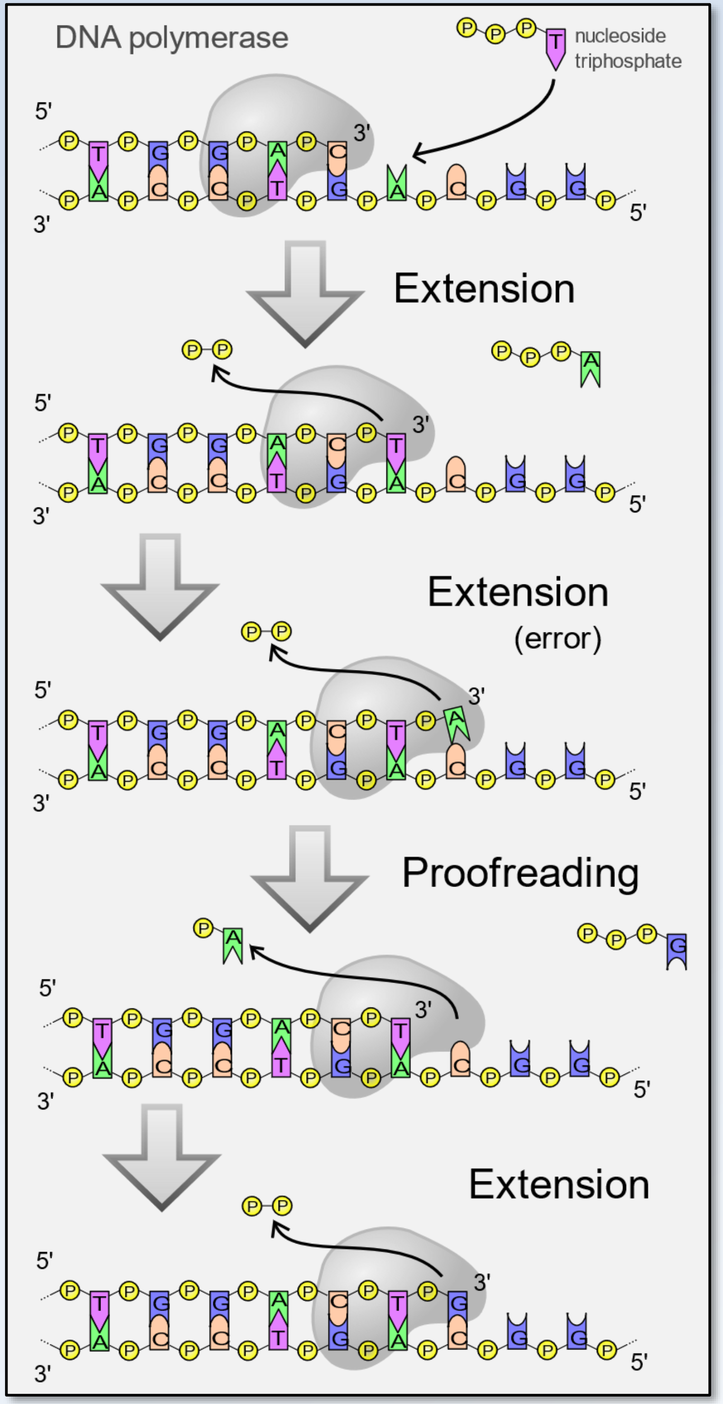


① DNA Replication

DNA-Polymerase

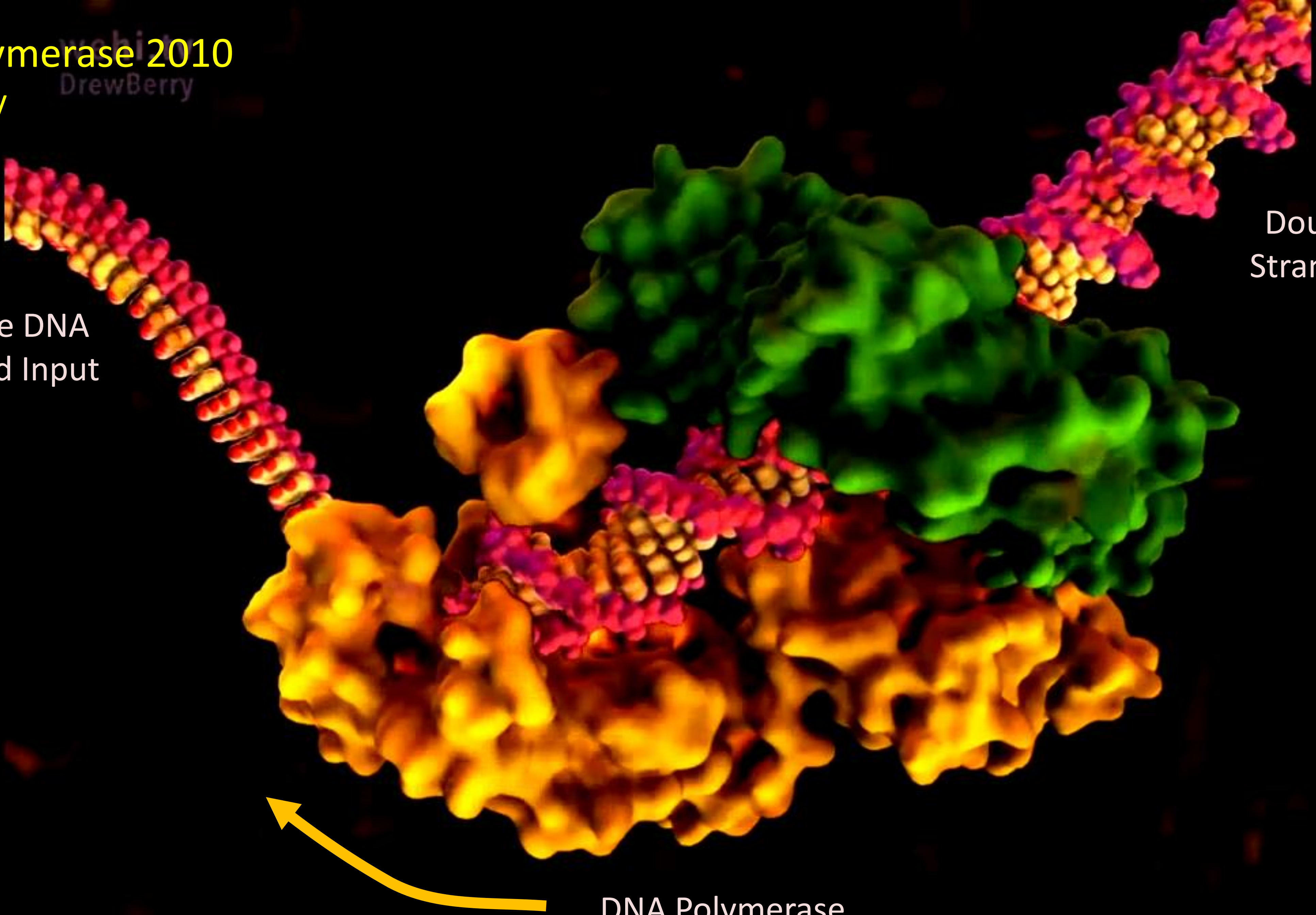


- Active sites are conserved across all life forms
- With **Proofreading**, Error Rates as low as 1 per Billion bases



DNA Polymerase 2010

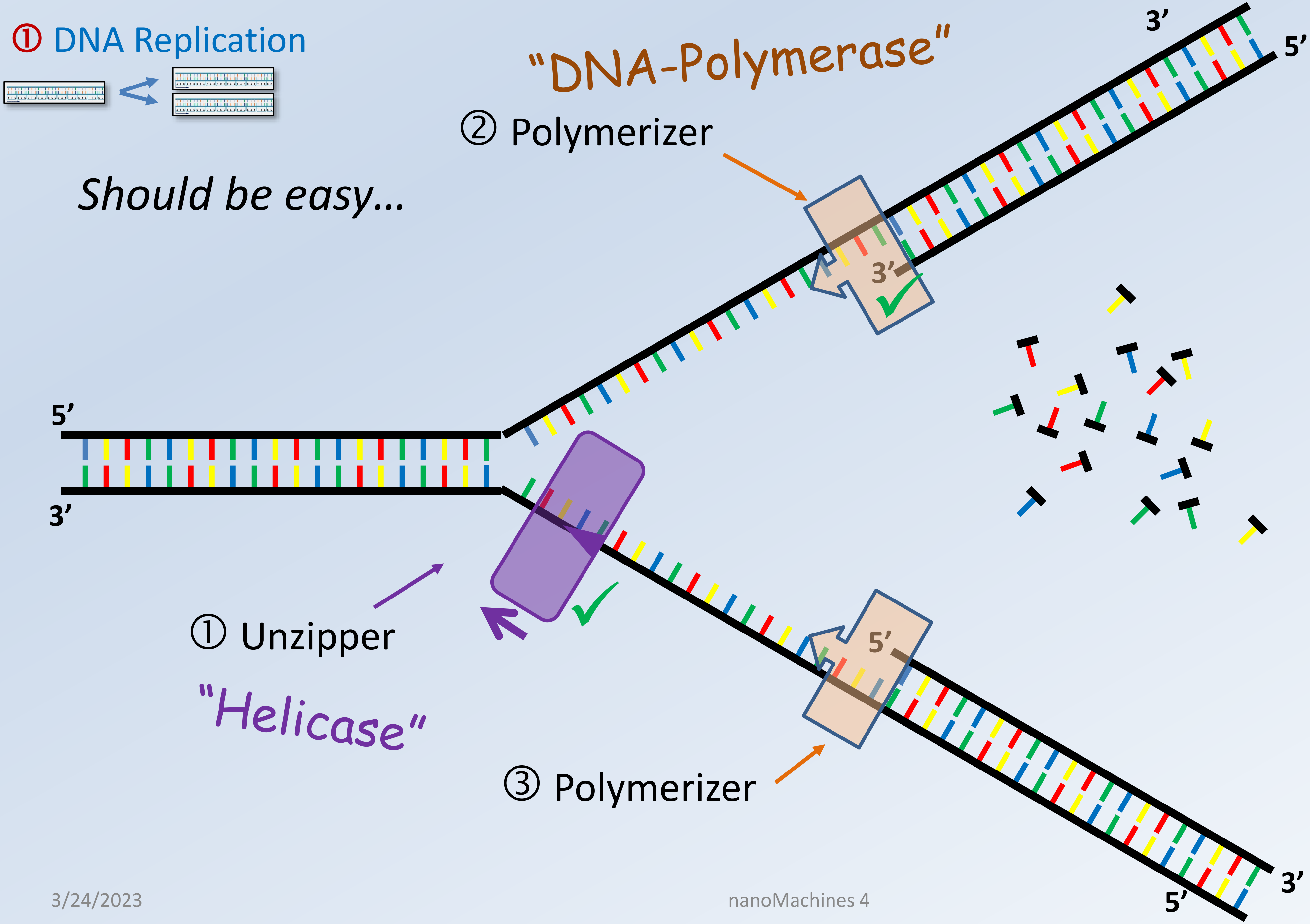
Drew Berry



Movie

① DNA Replication

Should be easy…



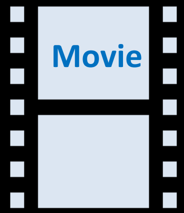
Supercoiling is a result of Helicase unwinding of DNA

Movie

Untangler of Knots

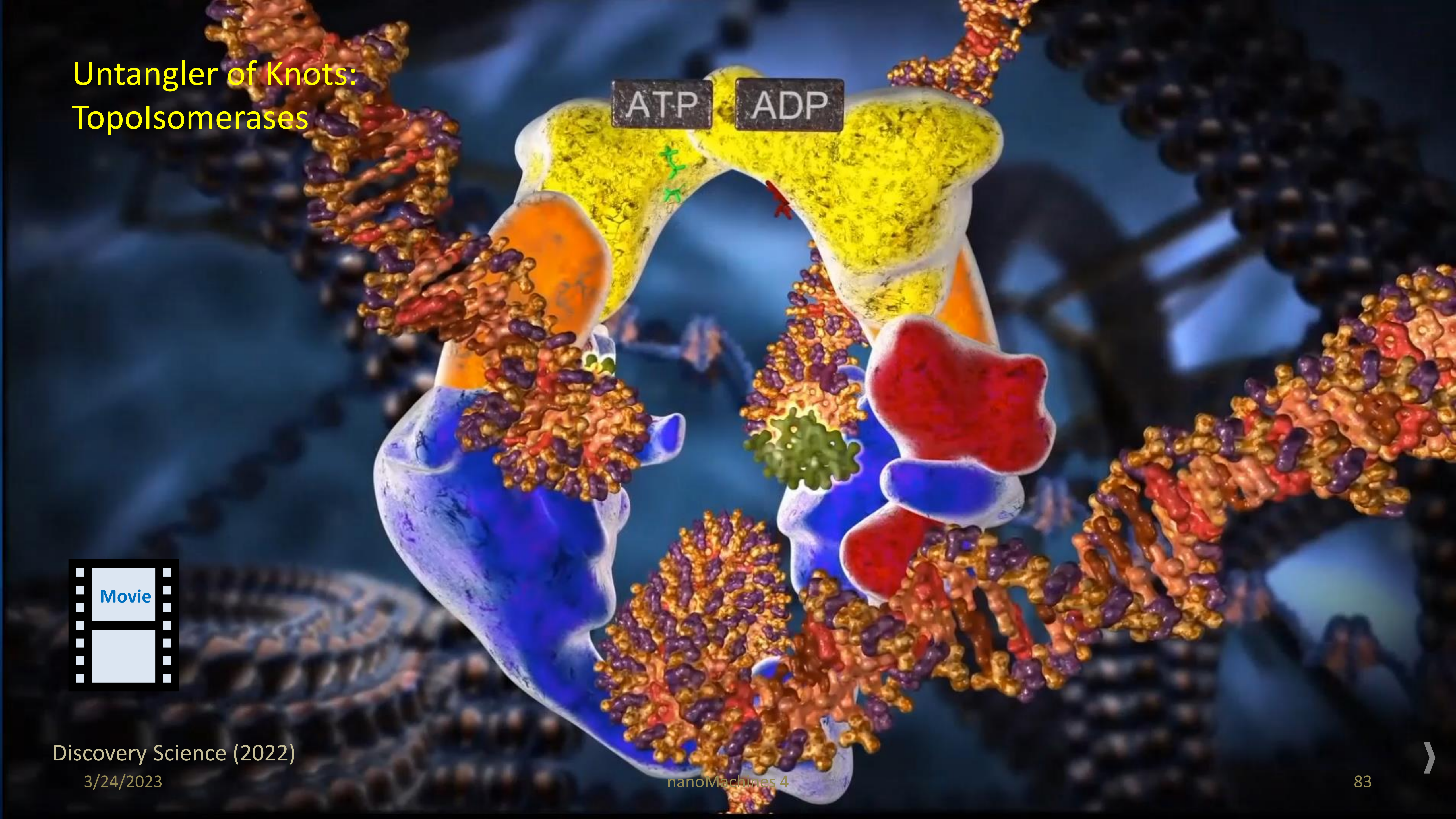
Discovery Science (2022)

Untangler of Knots: TopoIsomerases



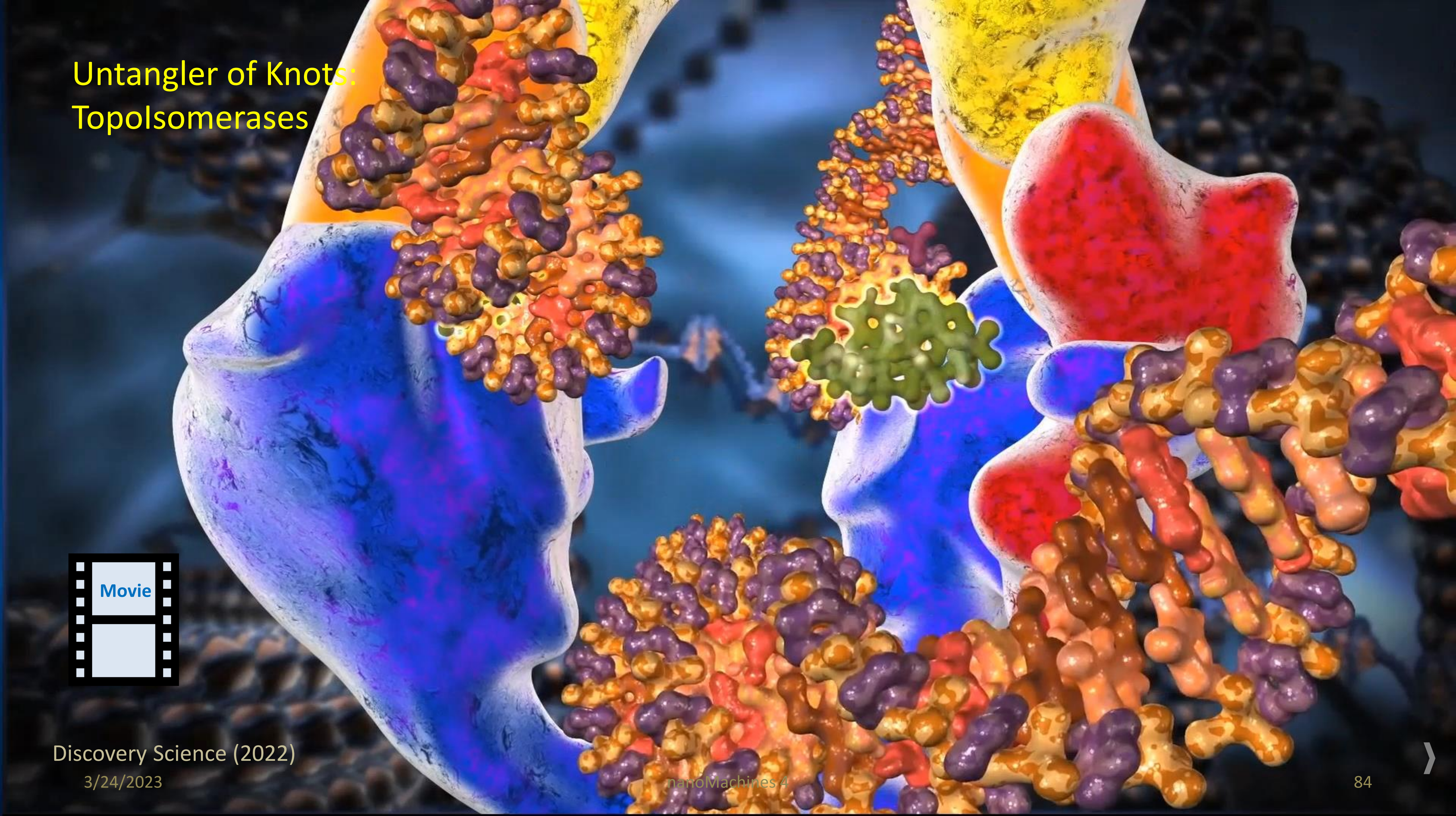
Discovery Science (2022)

Untangler of Knots:
TopoIsomerases



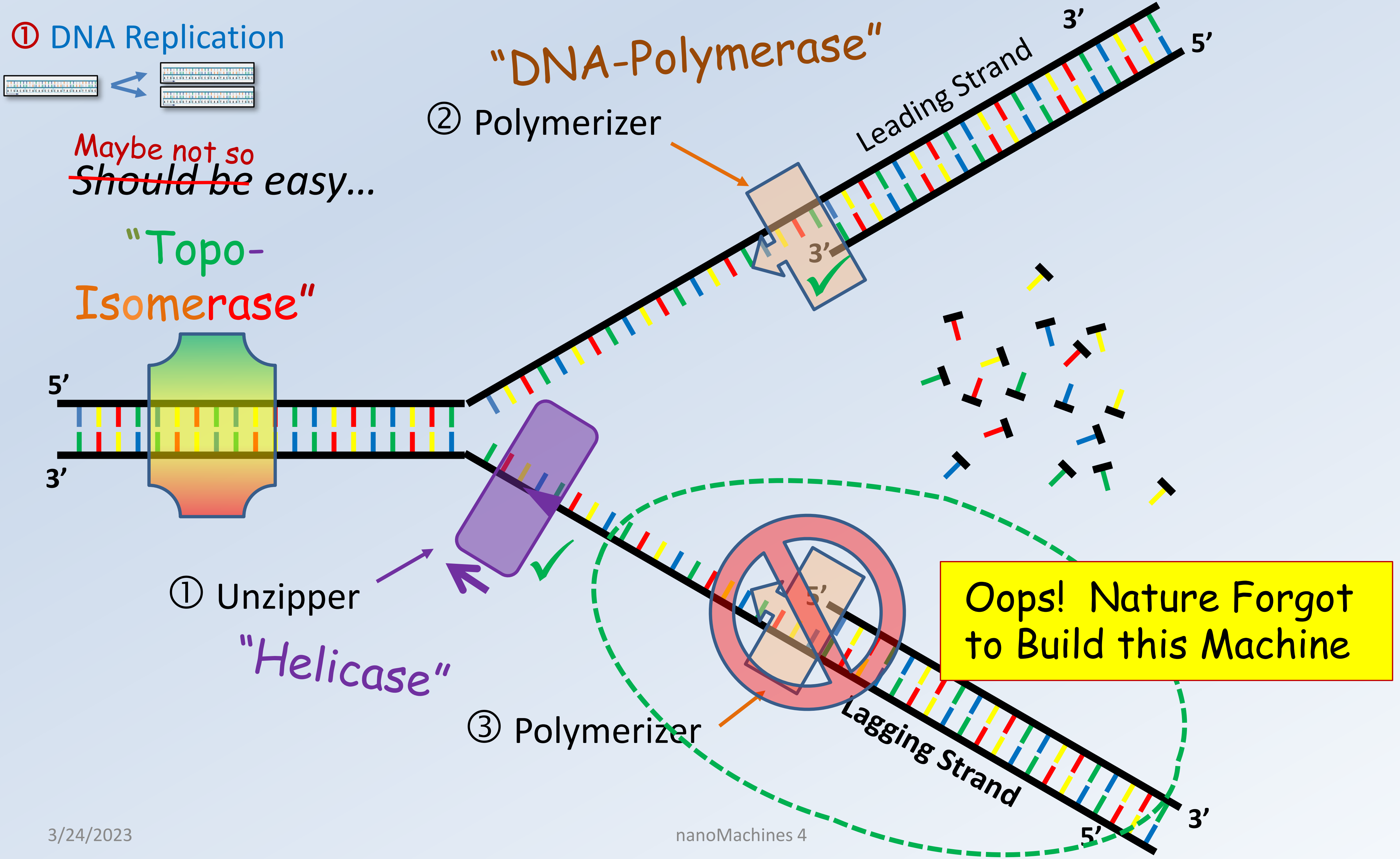
Discovery Science (2022)

Untangler of Knots: TopoIsomerases

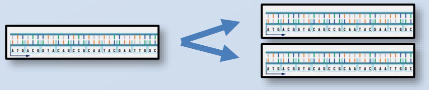


Movie

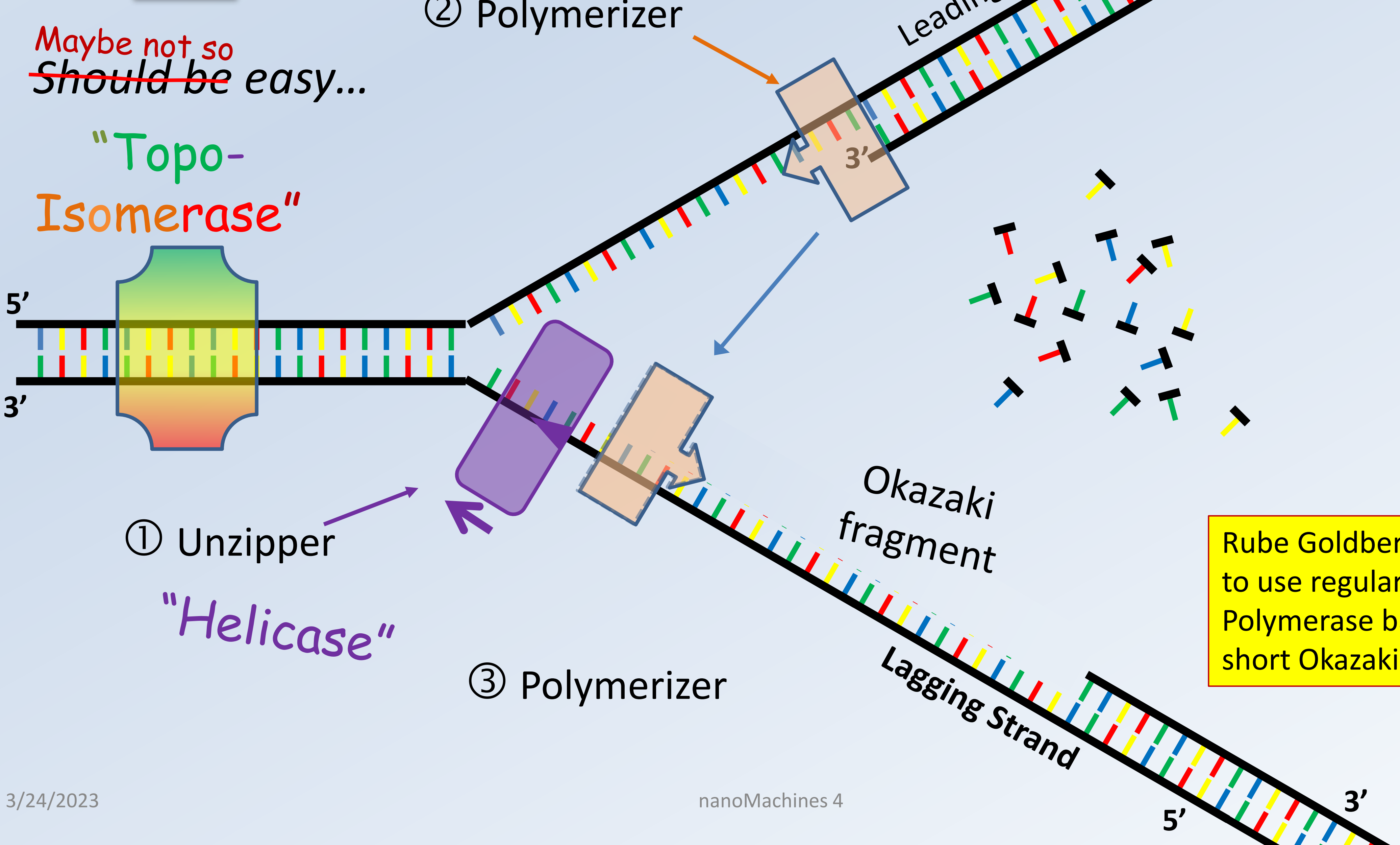
Discovery Science (2022)


① DNA Replication
Maybe not so
Should be easy…
"Topo-
Isomerase"
"DNA-Polymerase"
② Polymerizer
Leading Strand
3'
5'
3'
5'
3'
① Unzipper
"Helicase"
③ Polymerizer
5'
Lagging Strand
Oops! Nature Forgot
to Build this Machine
3'
5'

① DNA Replication



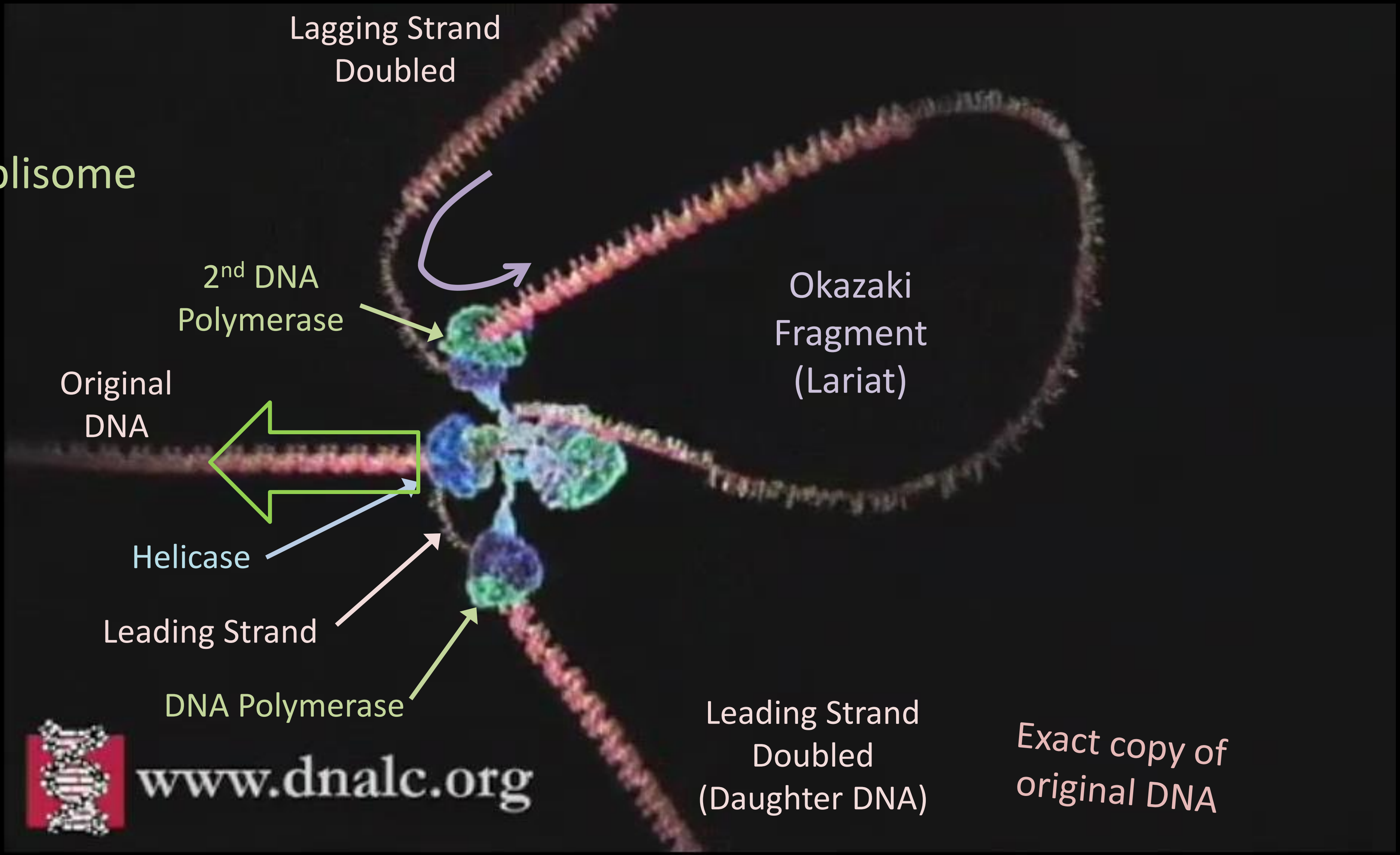
Maybe not so
~~Should be~~ *easy…*



Rube Goldberg solution is to use regular 3'-5' DNA Polymerase backwards in short Okazaki fragments.

Mechanism of Replication

(DNA Learning Center)

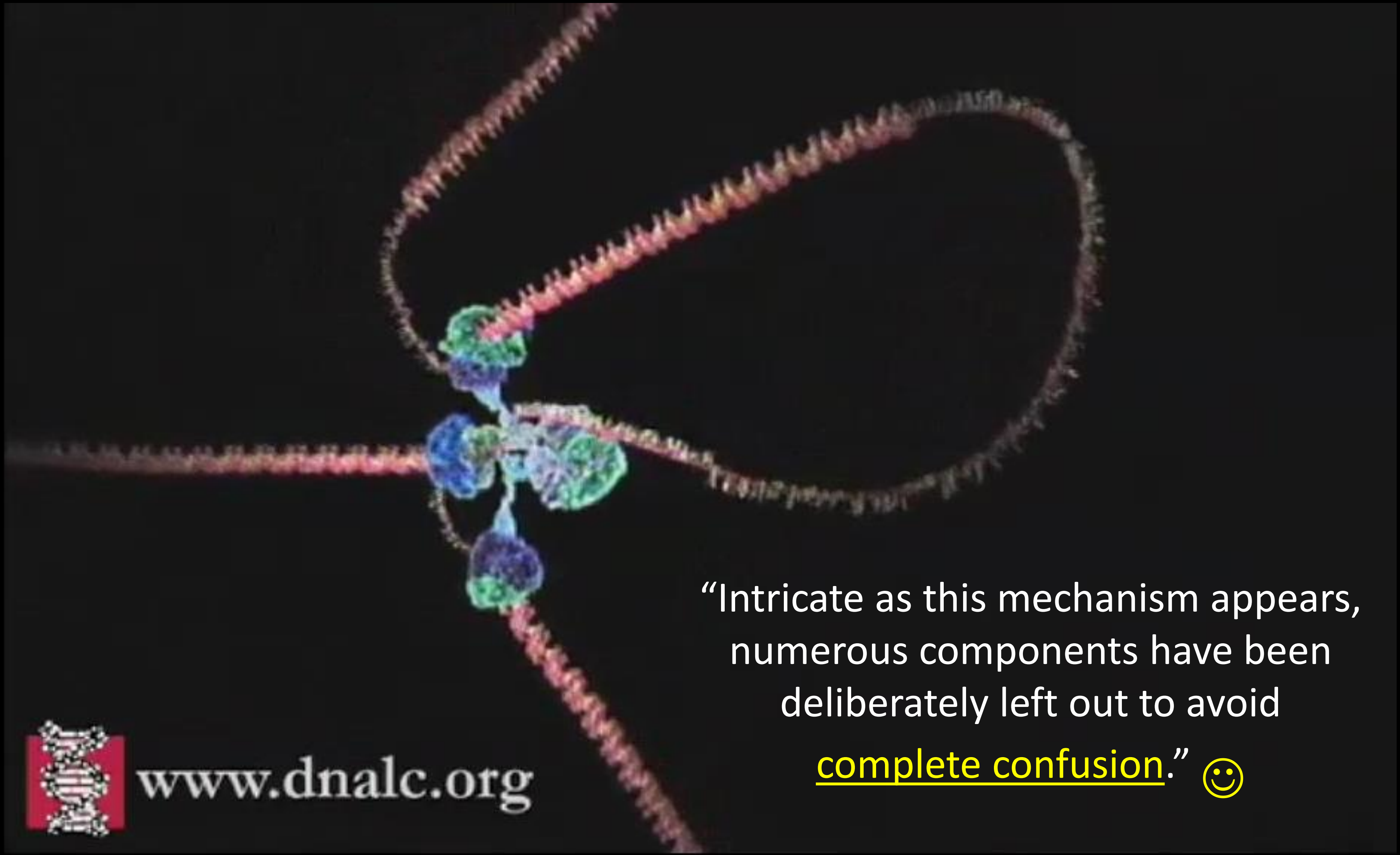


Movie

Mechanism of Replication

(DNA Learning Center)

Replisome

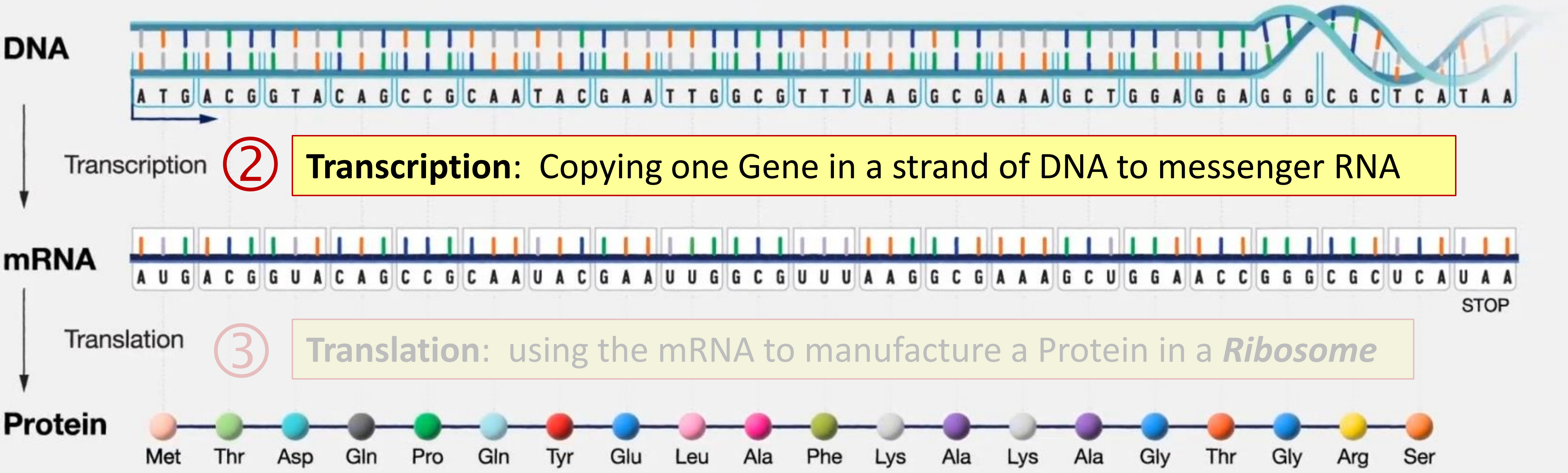


"Intricate as this mechanism appears, numerous components have been deliberately left out to avoid complete confusion." ☺

Questions?

Central Dogma

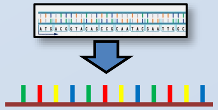
① **Replication**: Duplicating DNA when cells divide.



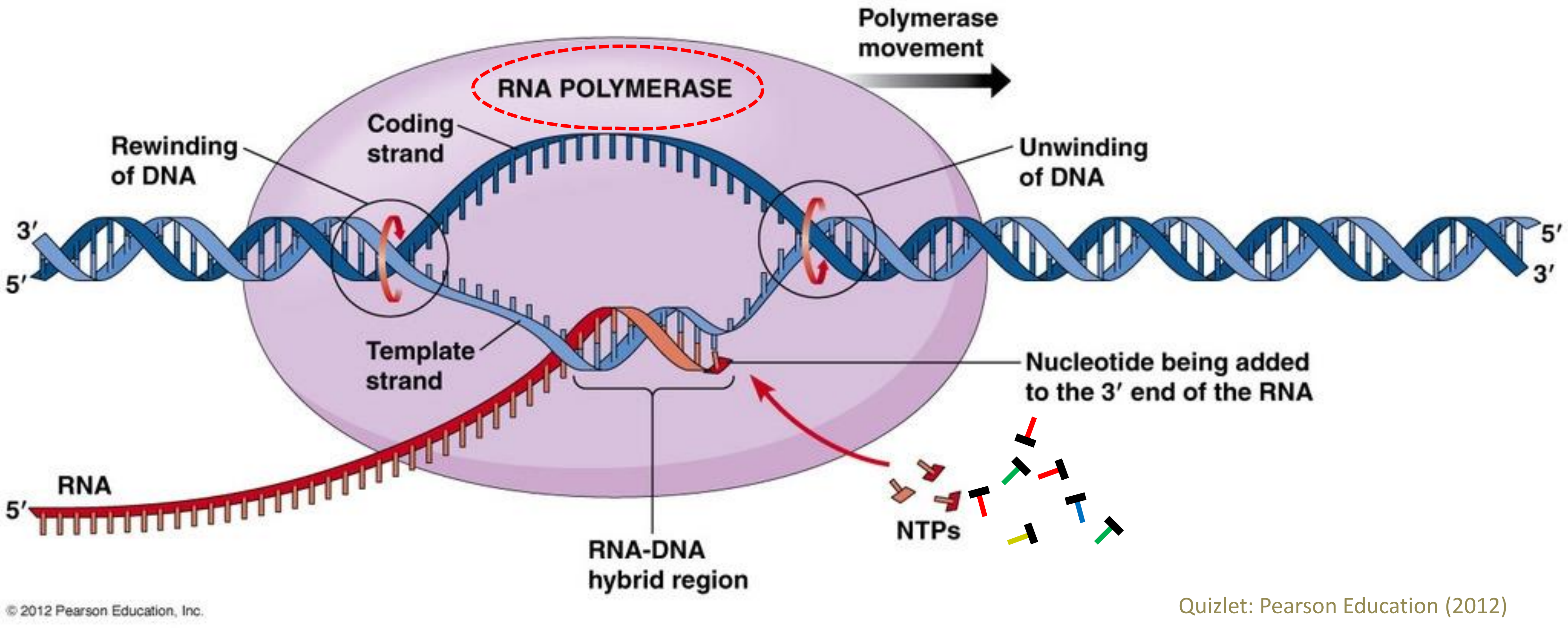
② **Transcription**: Copying one Gene in a strand of DNA to messenger RNA

③ **Translation**: using the mRNA to manufacture a Protein in a ***Ribosome***

National Human Genome Research Institute (2022)

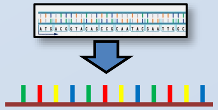


Building Messenger RNA (mRNA)

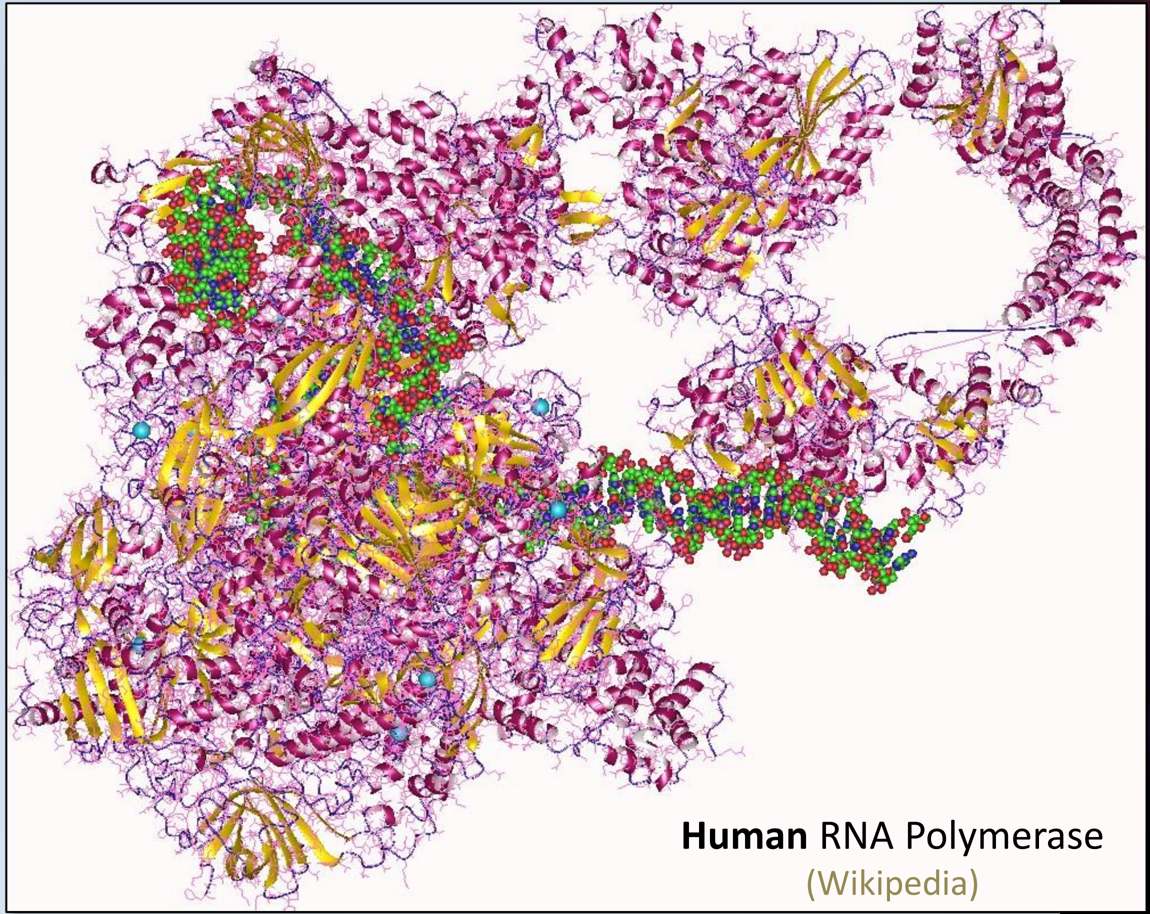


Quizlet: Pearson Education (2012)

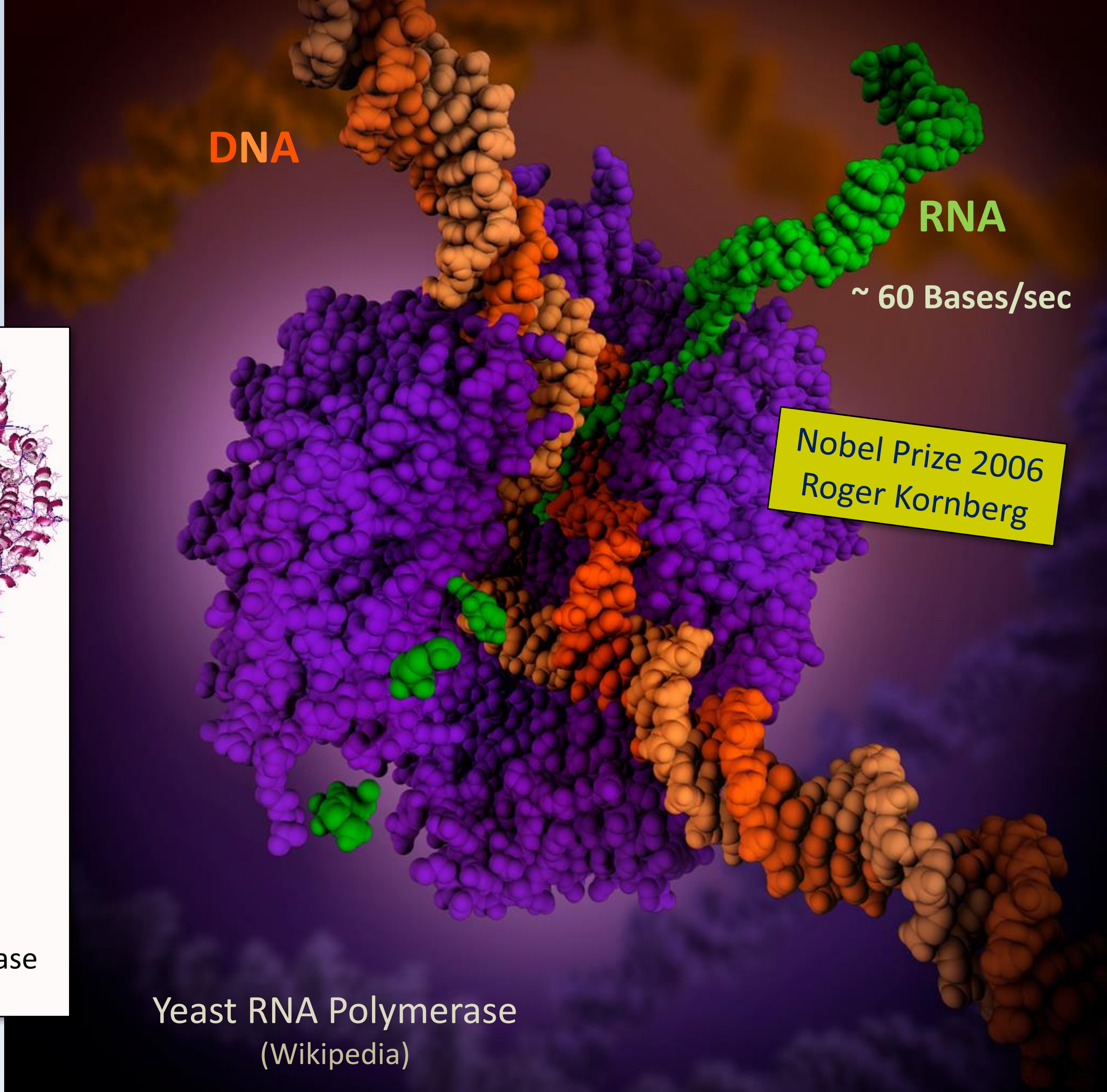
② RNA Transcription



RNA Polymerase



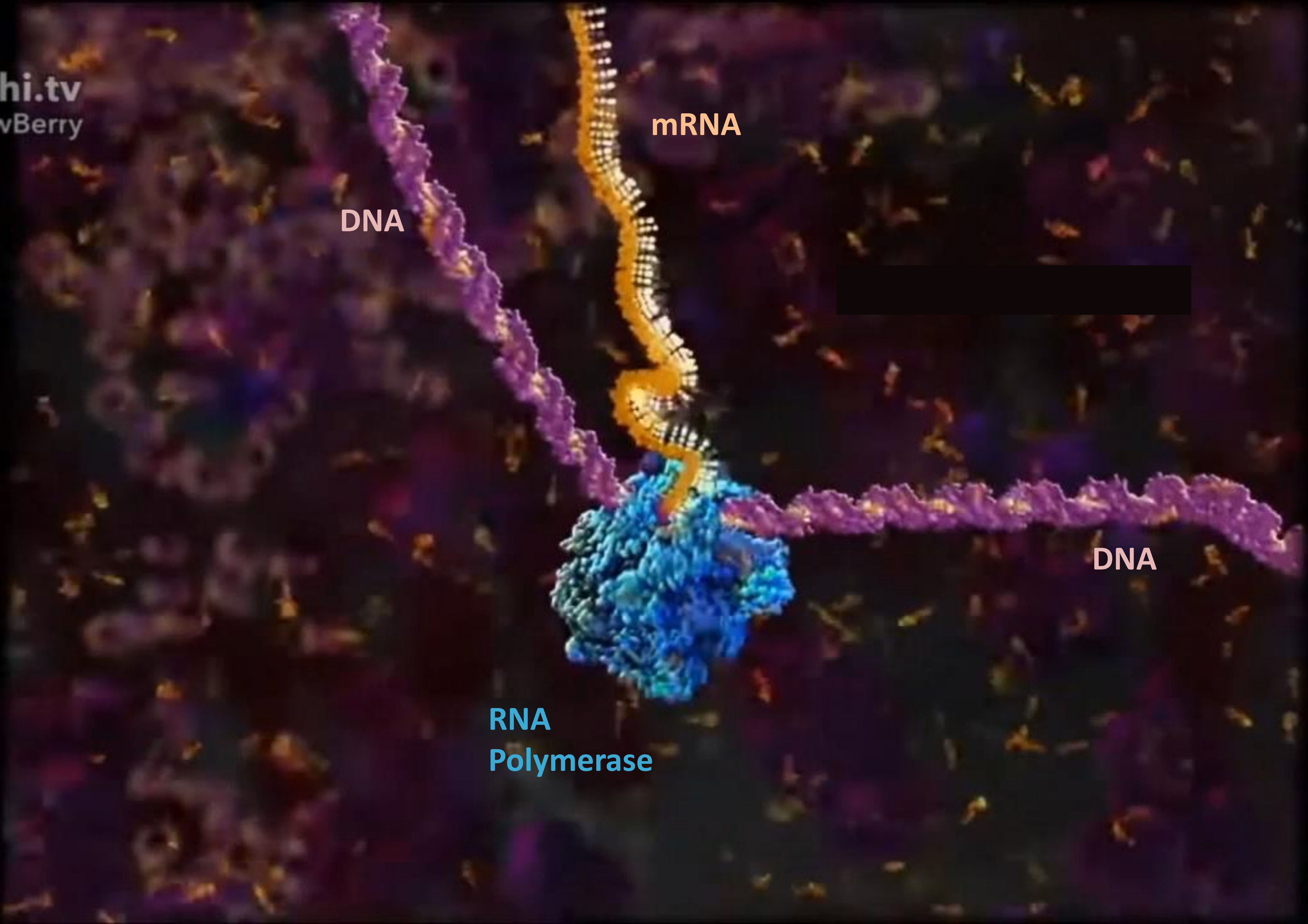
Human RNA Polymerase
(Wikipedia)



Yeast RNA Polymerase
(Wikipedia)

Gene Transcription

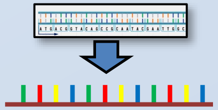
RNA Polymerase



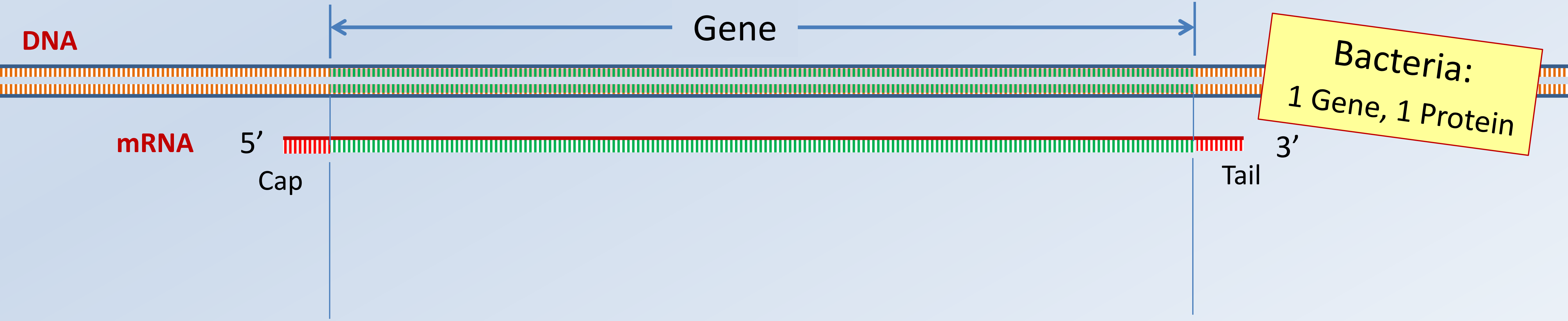
from:
DNA Animation
(2002-2014)
Drew Berry

Movie

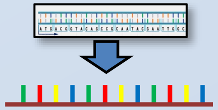
② RNA Transcription



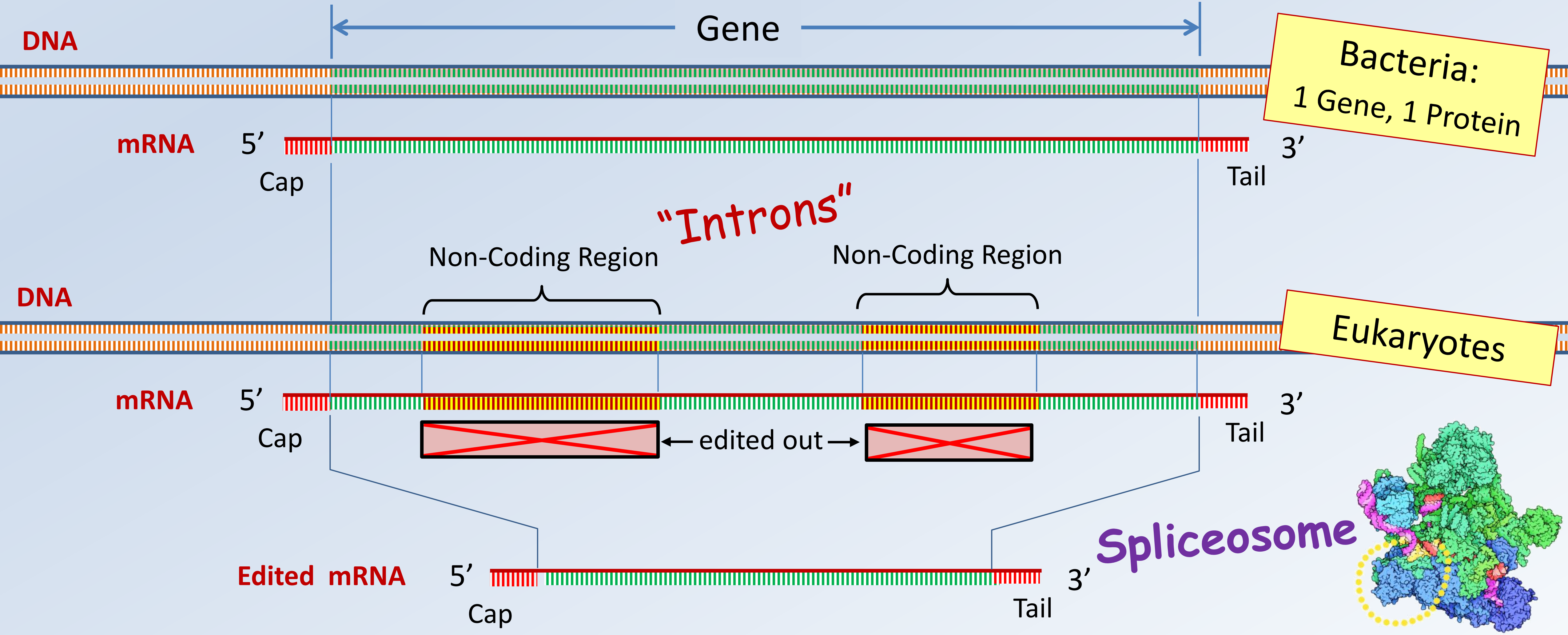
Introns



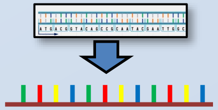
② RNA Transcription



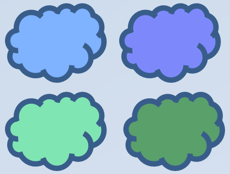
Introns



② RNA Transcription

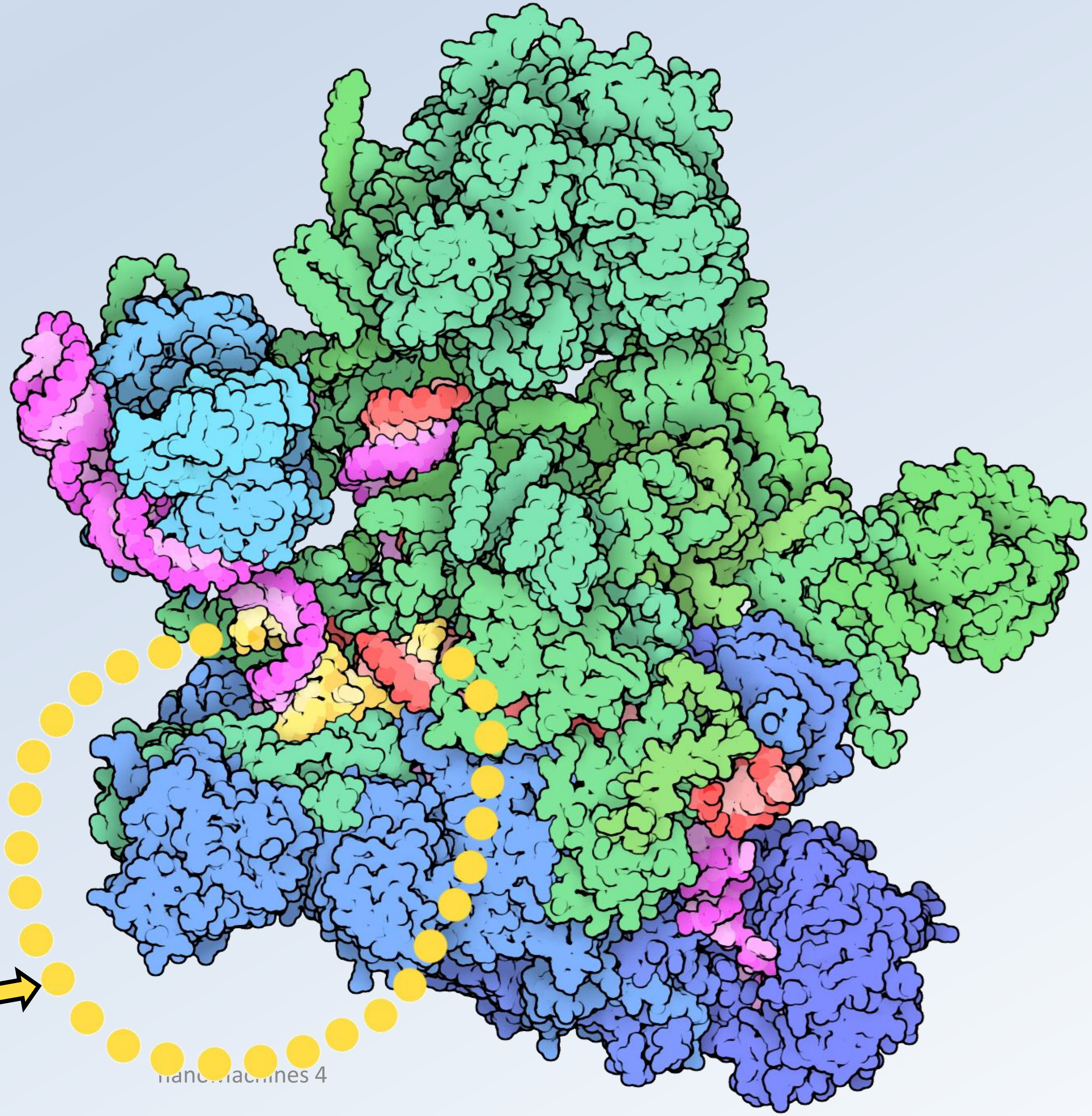


Yeast Spliceosome
(at a particular stage)

Protein subunits 

RNA structural subunits 

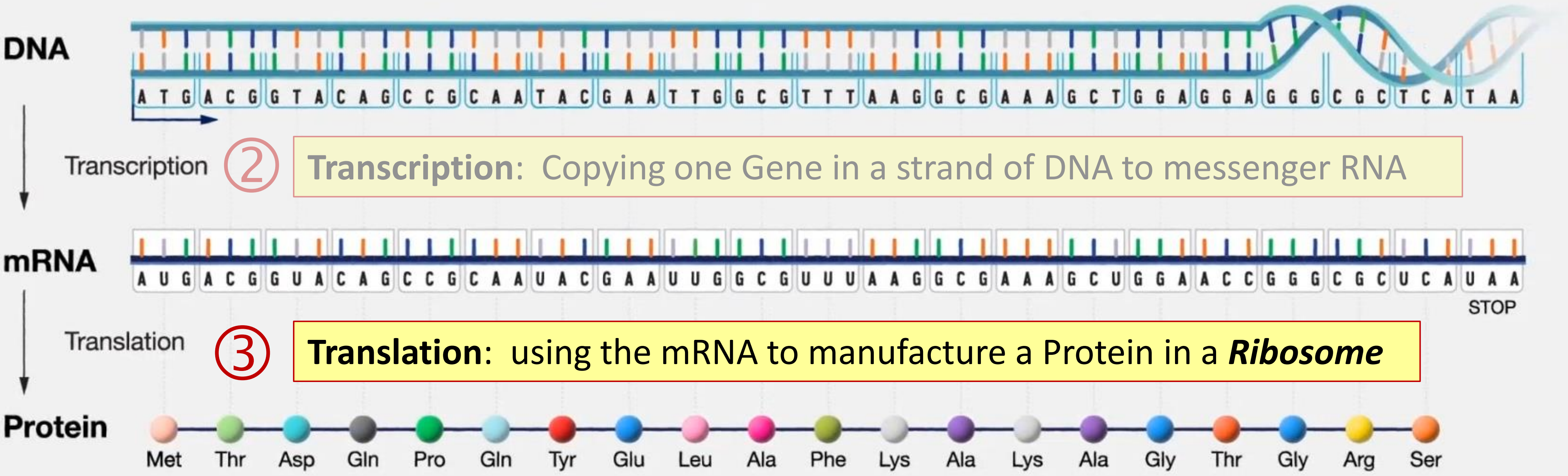
Intron to be excised 



mRNA Intron “Lariat”

Central Dogma

① **Replication**: Duplicating DNA when cells divide.



② **Transcription**: Copying one Gene in a strand of DNA to messenger RNA

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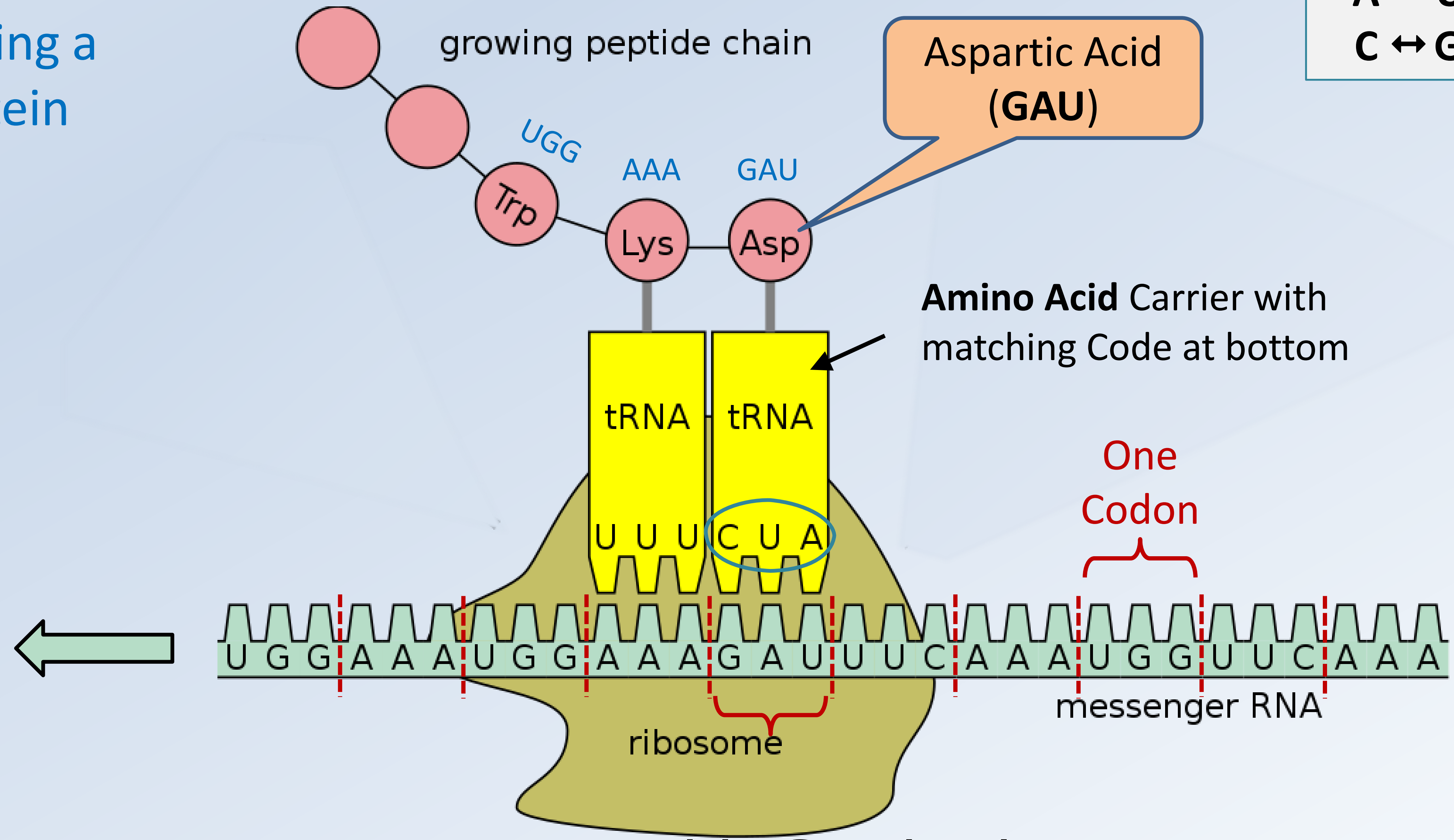
National Human Genome Research Institute (2022)

Ribosome: Building a Protein

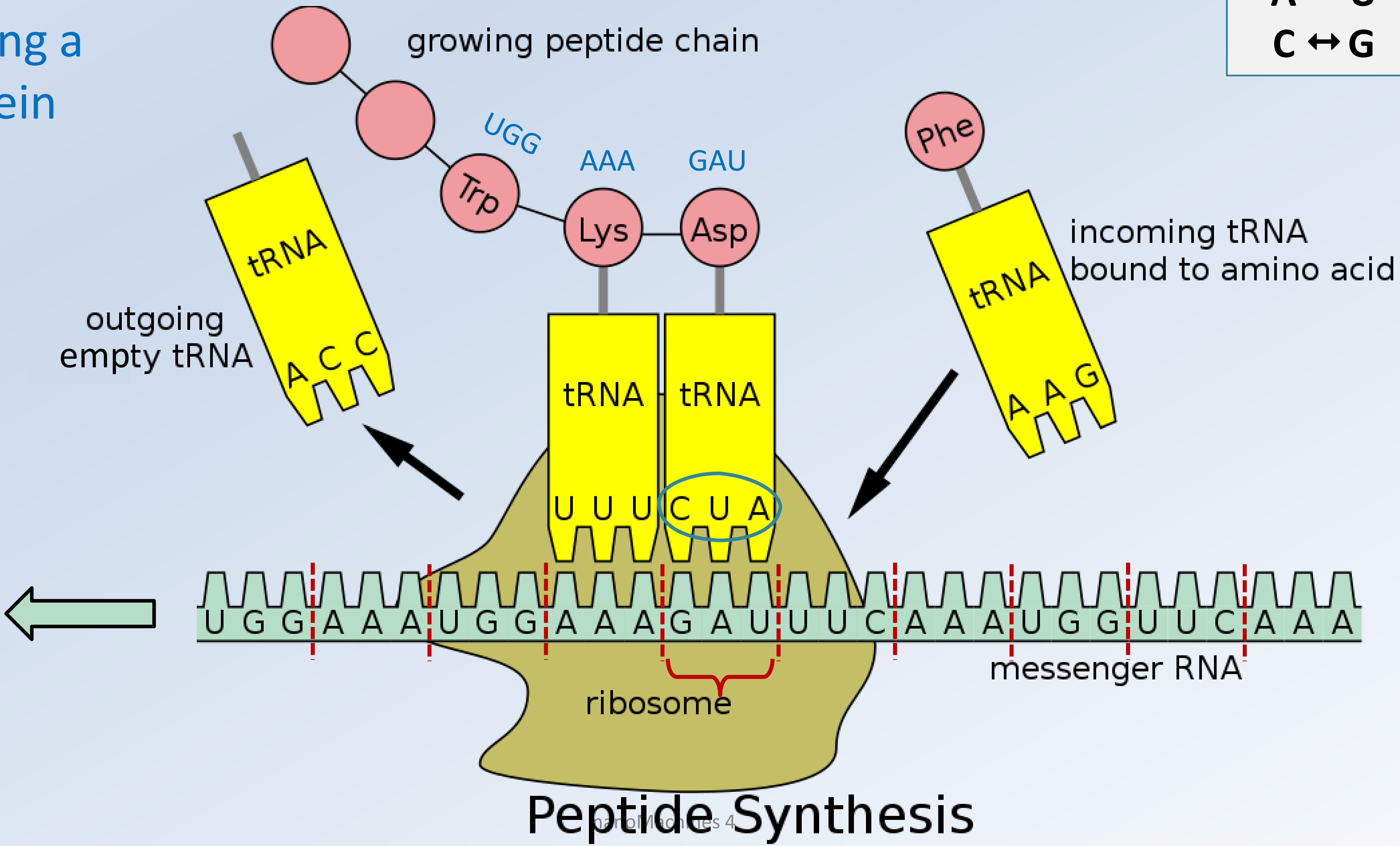
A ↔ U
C ↔ G



Peptide Synthesis

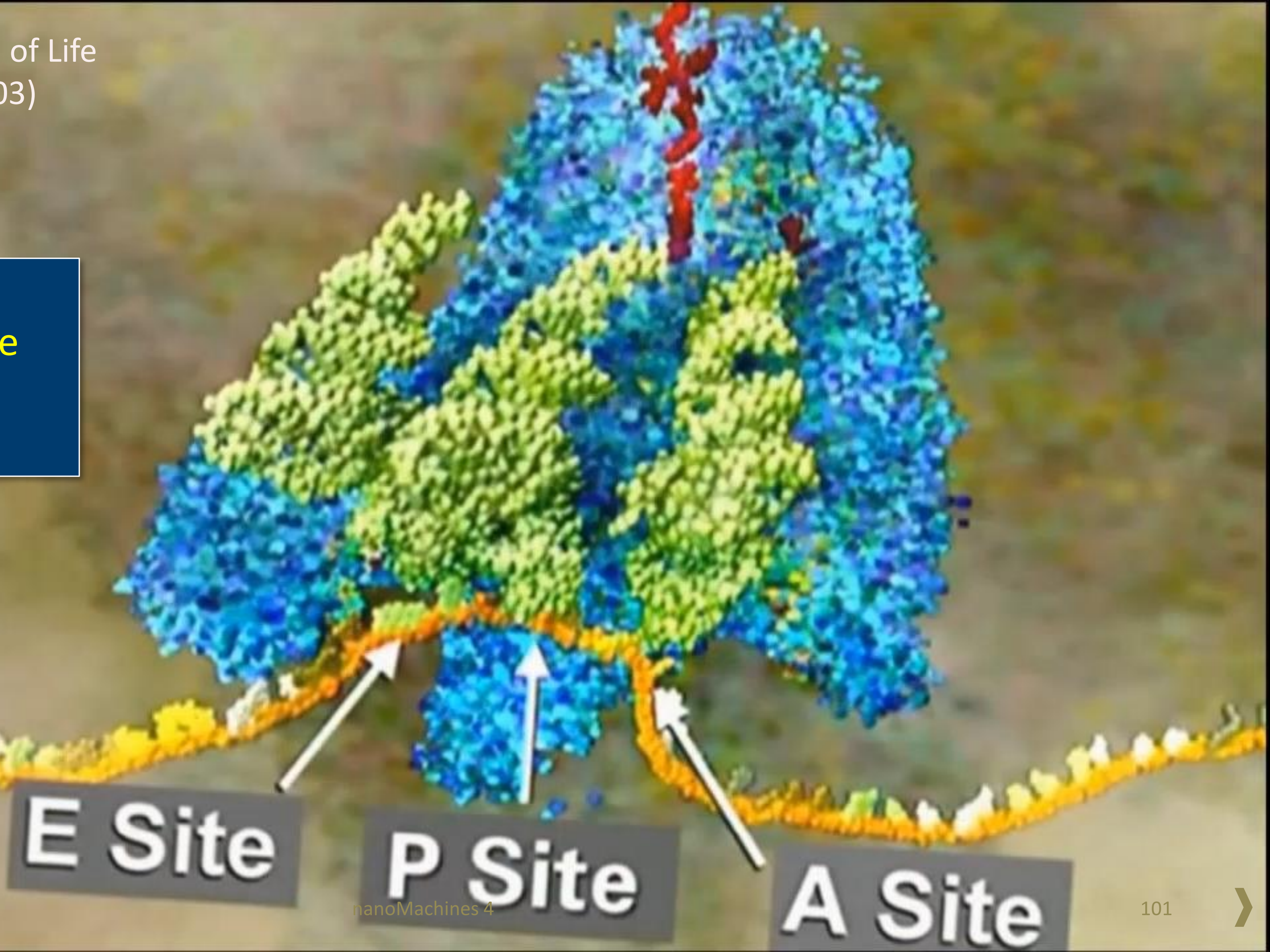
Ribosome: Building a Protein

A ↔ U
C ↔ G



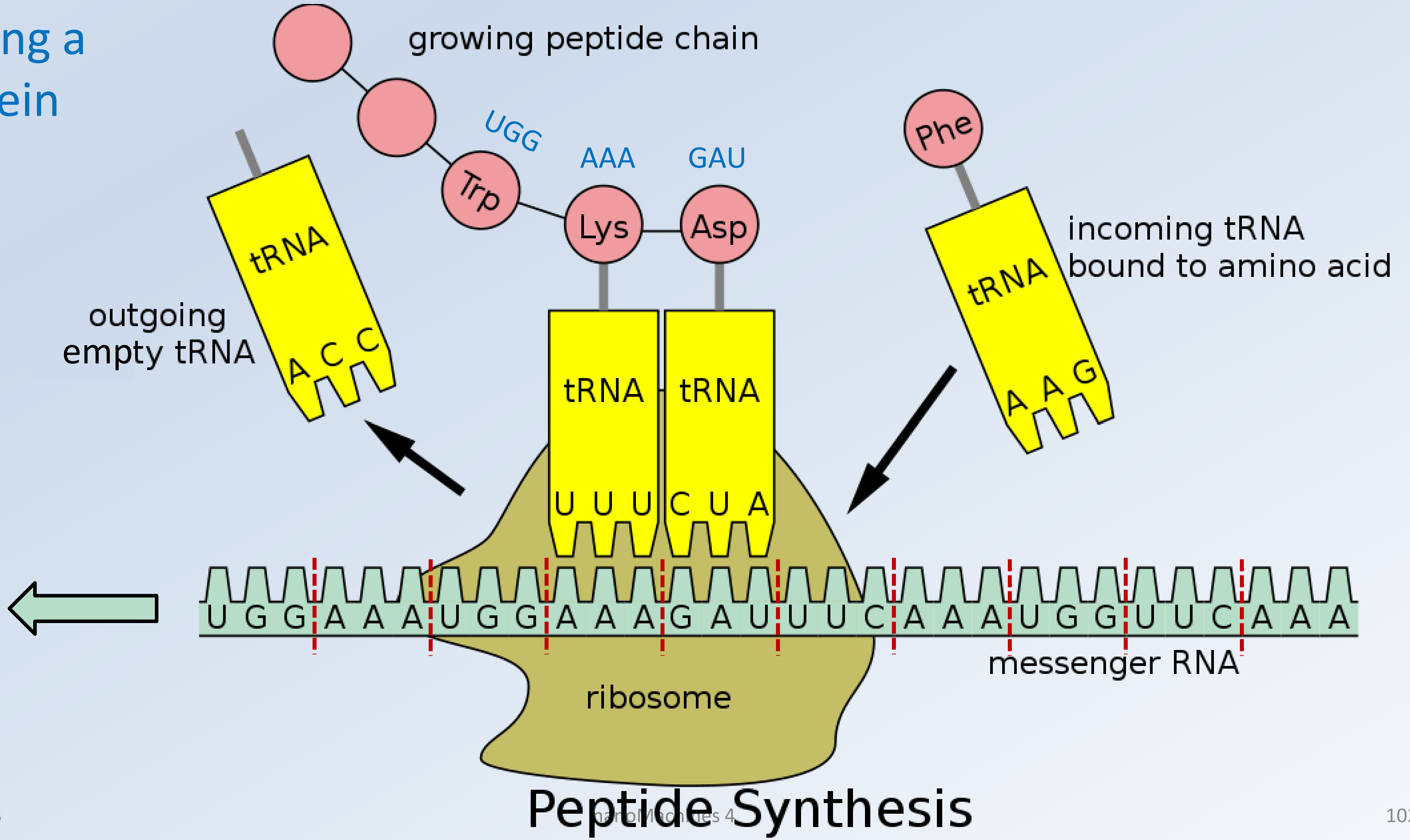
From: The Molecular Basis of Life
(Drake 2016/Berry 2003)

A Simplified
Overview of the
Ribosome
ca 2003

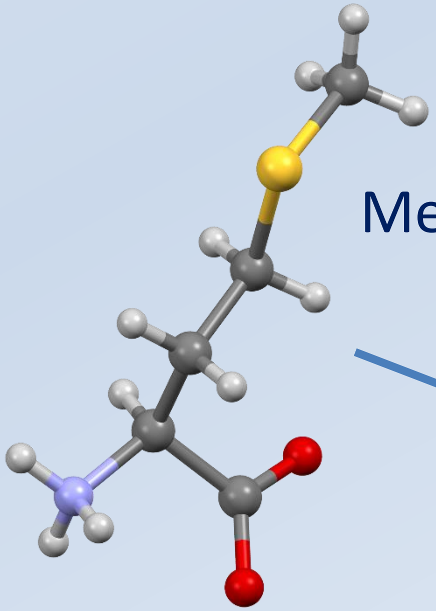


Movie

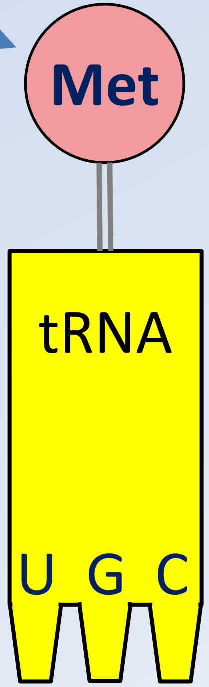
Ribosome: Building a Protein

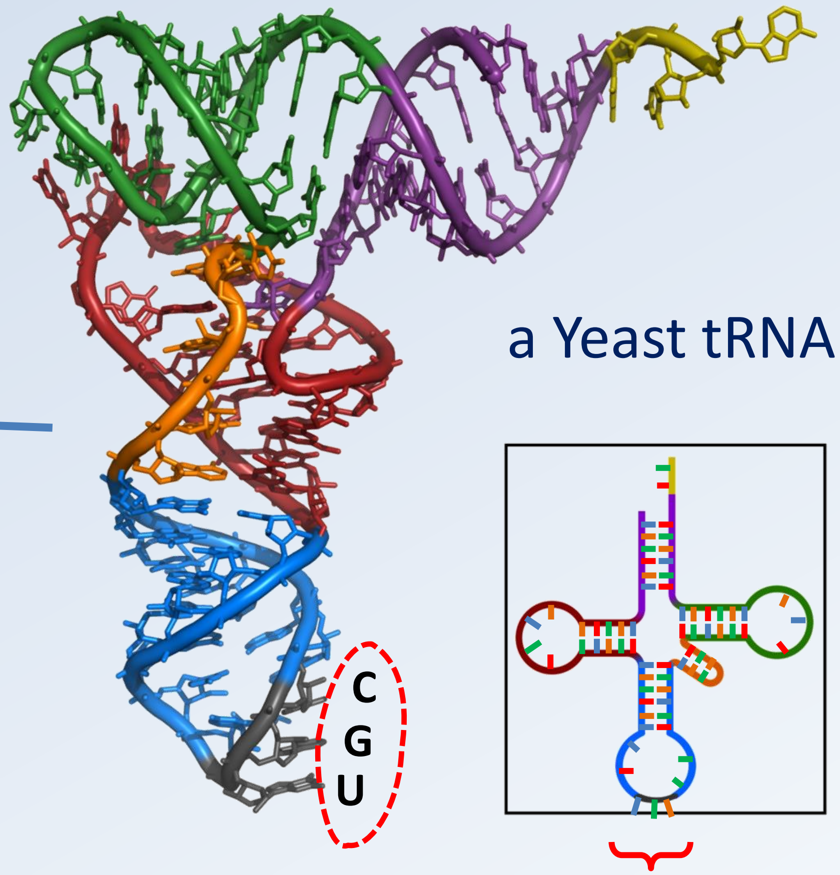


Ribosome: Building a Protein



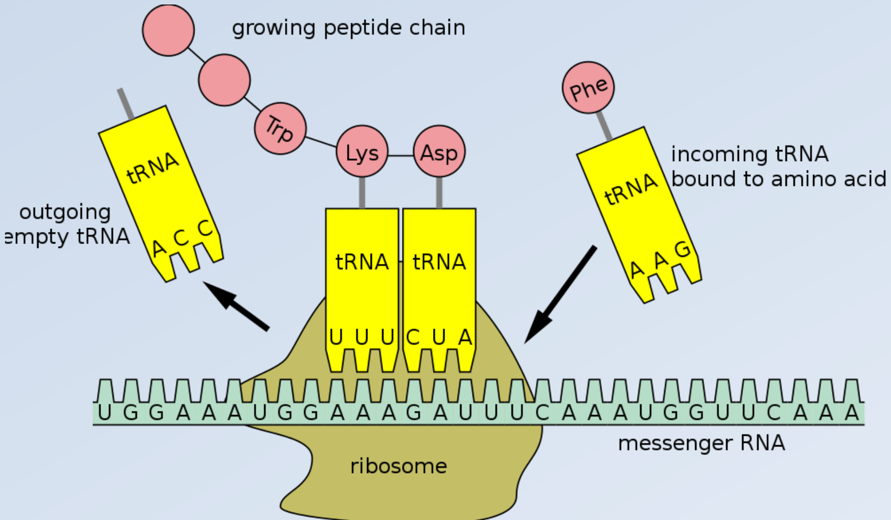
Methionine





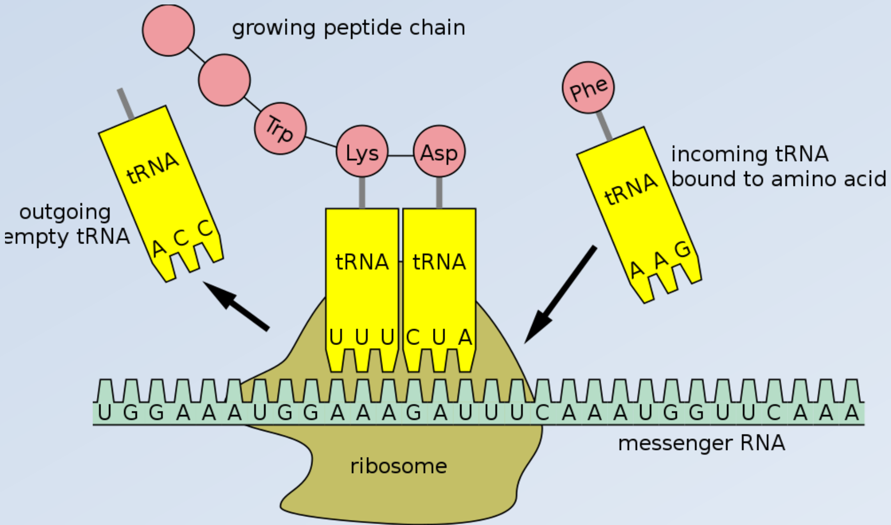
a Yeast tRNA

Wikipedia

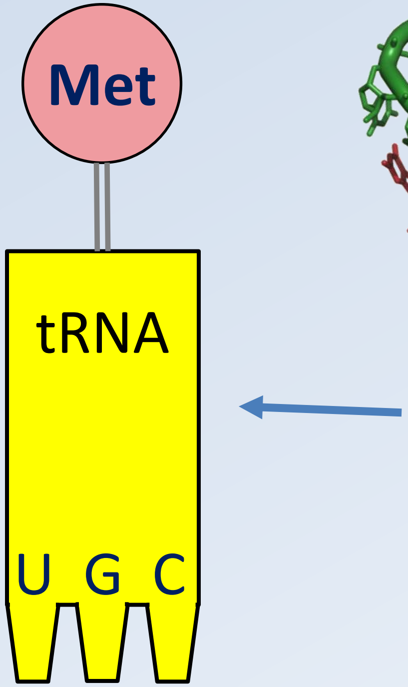


Peptide Synthesis

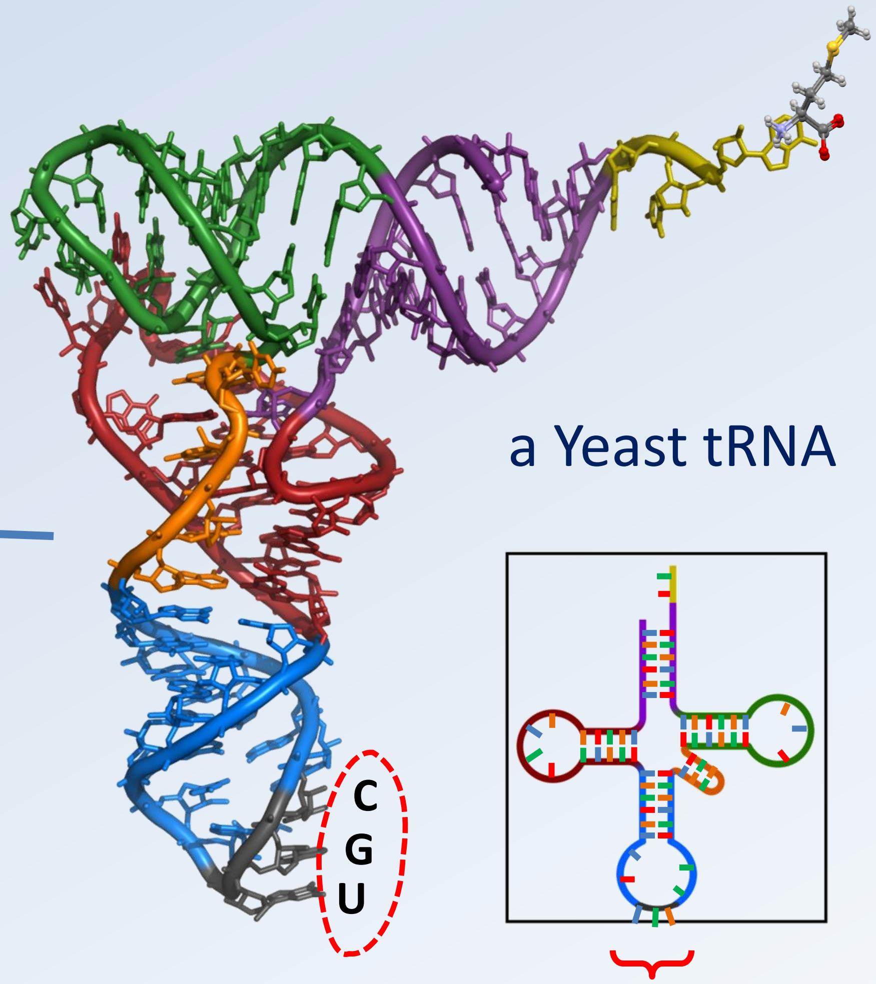
Ribosome: Building a Protein



Peptide Synthesis



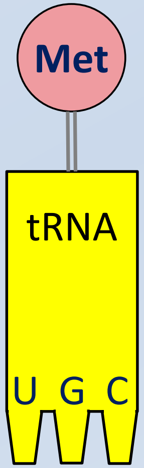
Methionine



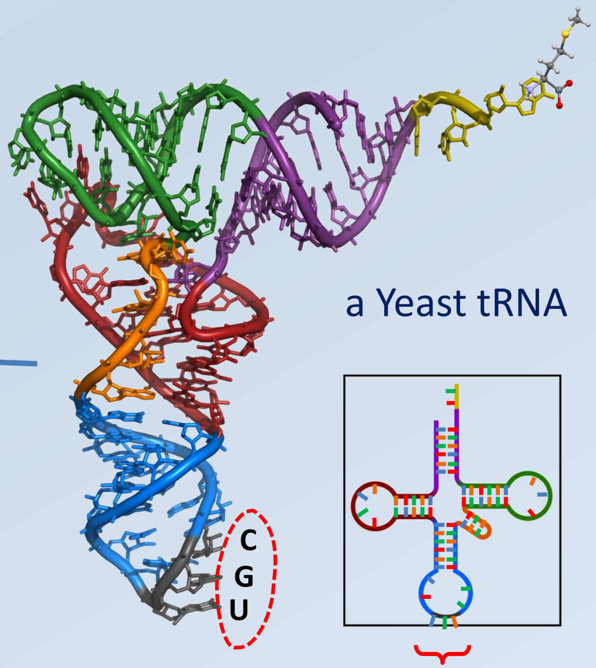
a Yeast tRNA

Wikipedia

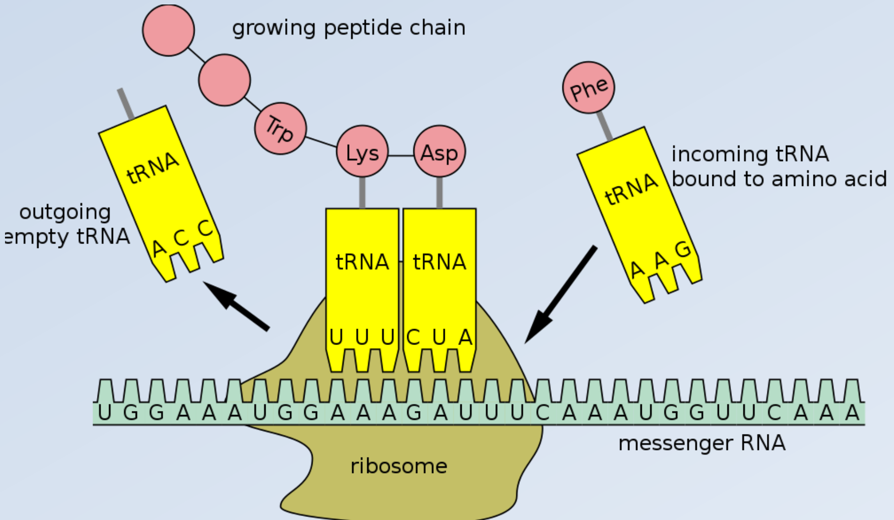
Ribosome: Building a Protein



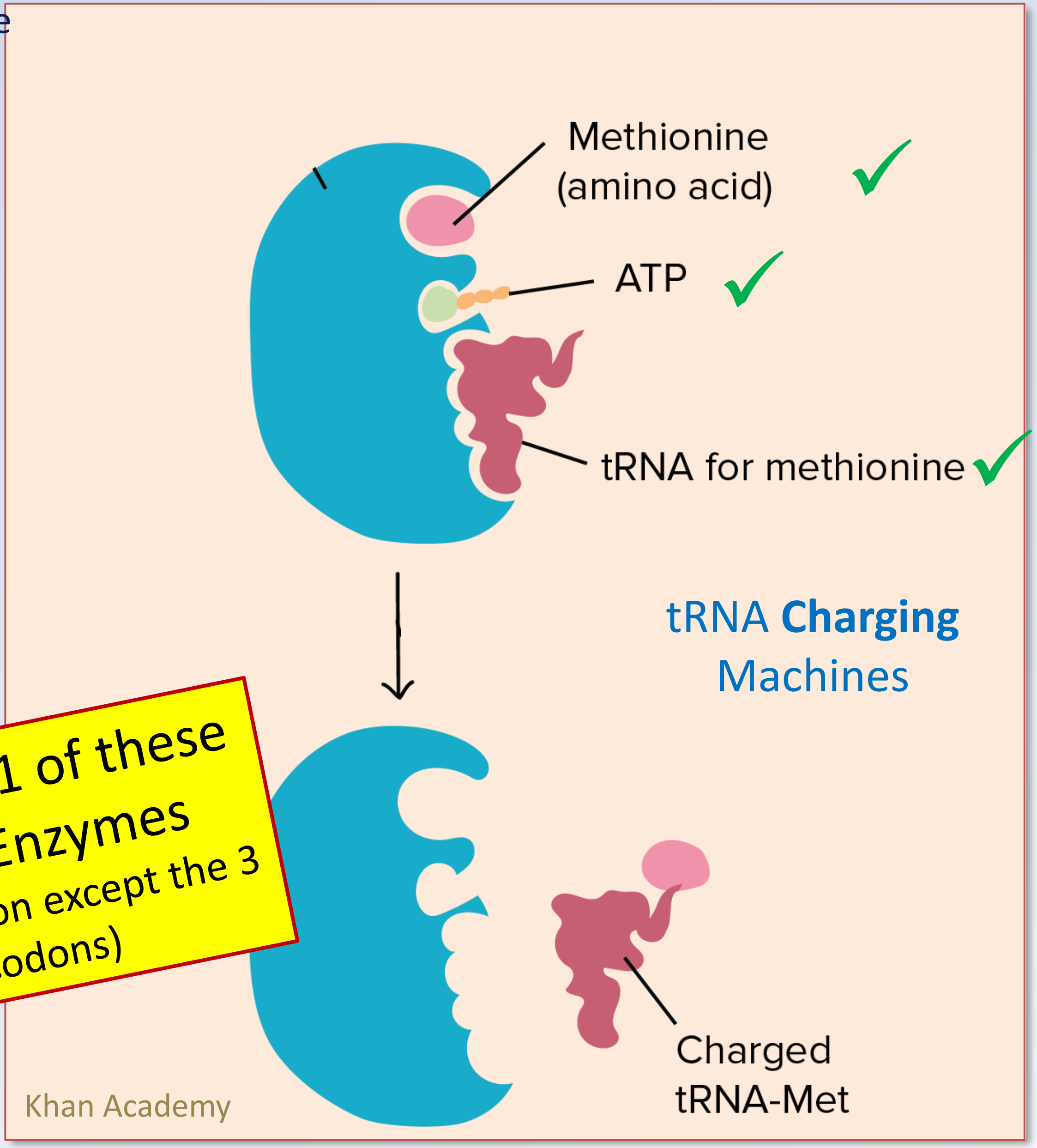
Methionine



a Yeast tRNA



Peptide Synthesis

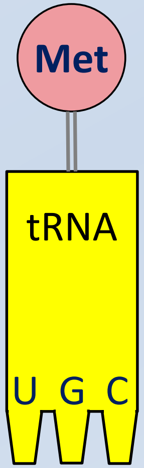


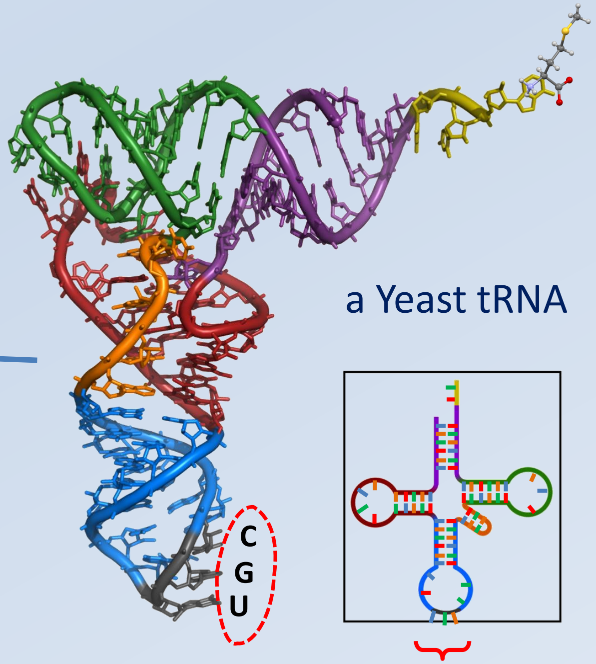
tRNA **Charging** Machines

We'll need 61 of these Charging Enzymes
(1 for each Codon except the 3 STOP Codons)

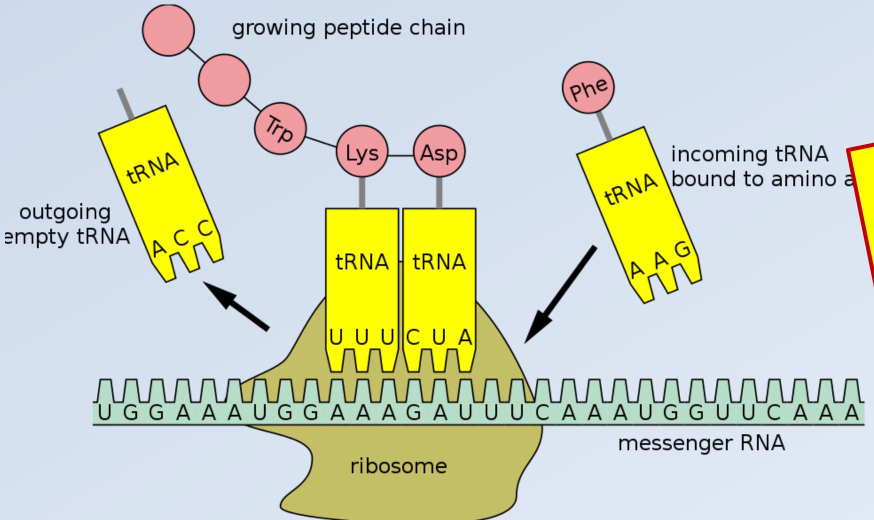
Ribosome: Building a Protein

Methionine

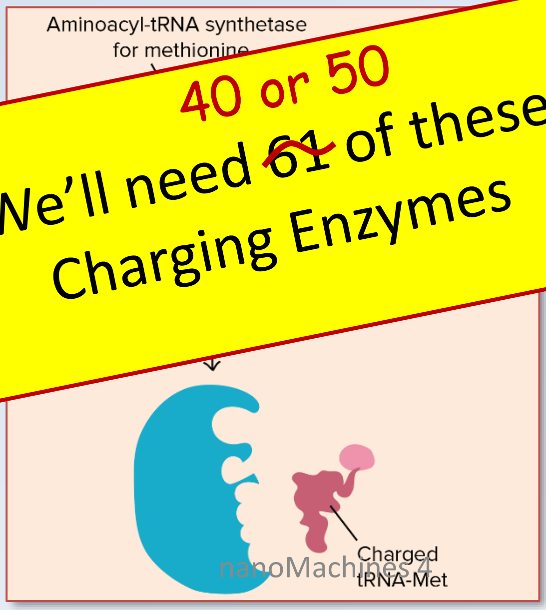




a Yeast tRNA



Peptide Synthesis



40 or 50

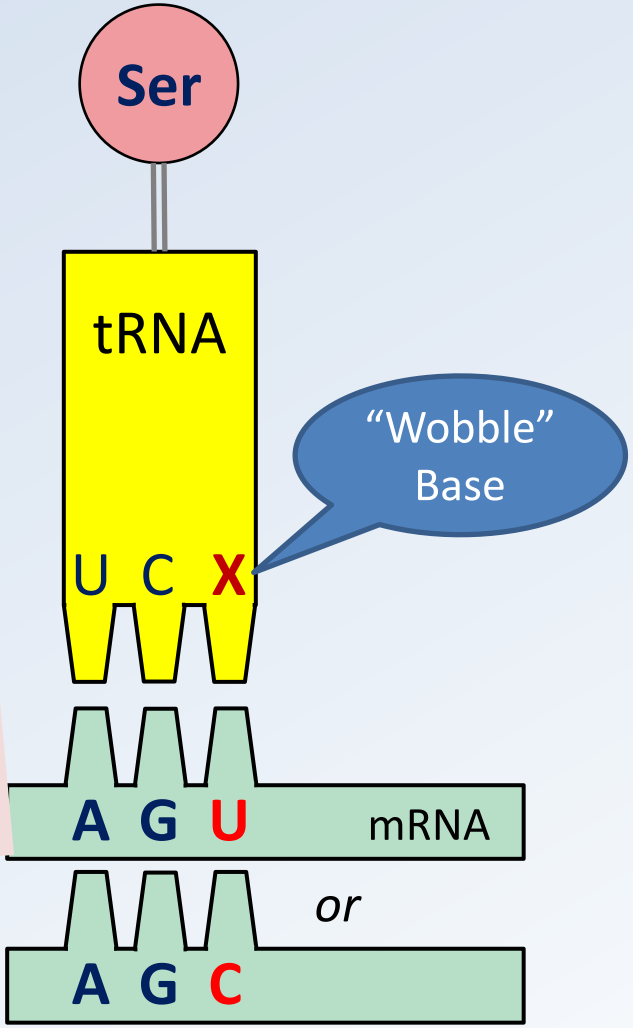
We'll need ~~61~~ of these Charging Enzymes

Example: **Serine** **AGU** **AGC** } 2 codes

A ↔ U
C ↔ G

Nature's Hack

Using special "Wobble" bases, some tRNA's can cover several Codons for same Amino Acid

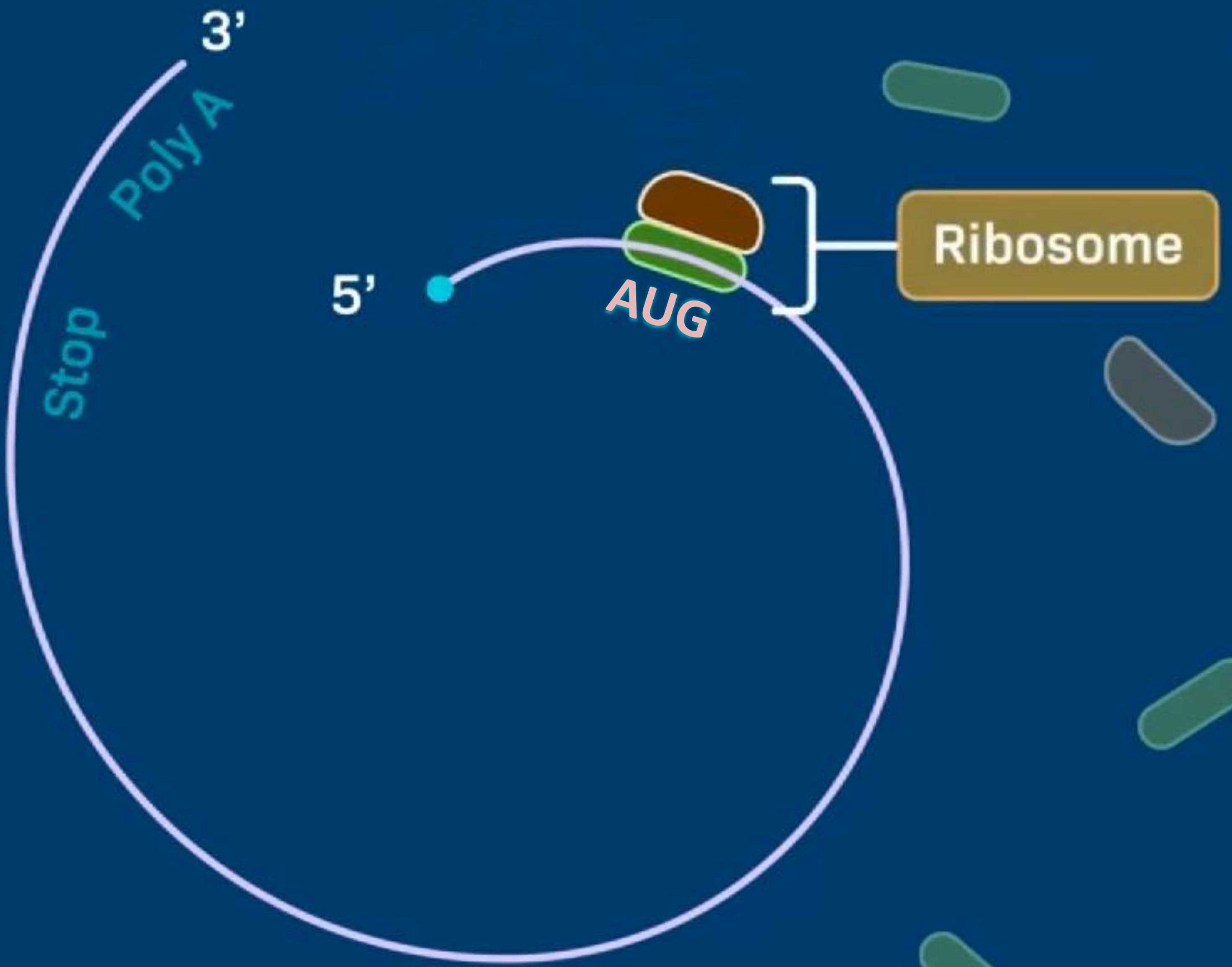


Polyribosome
(LabExchange 2022)

UAA, UAG or UGA

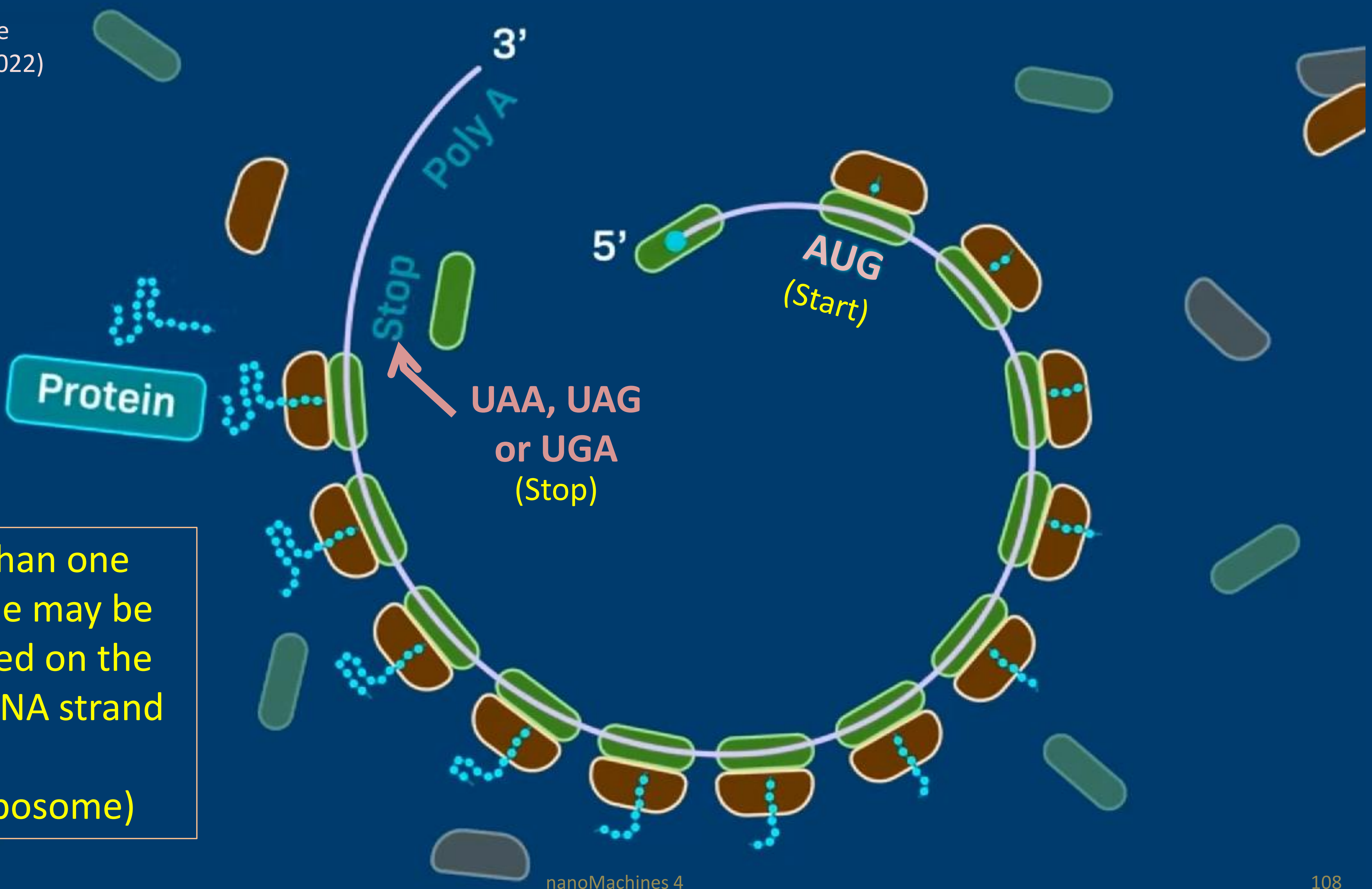
More than one Ribosome may be assembled on the same mRNA strand

(PolyRibosome)



Movie

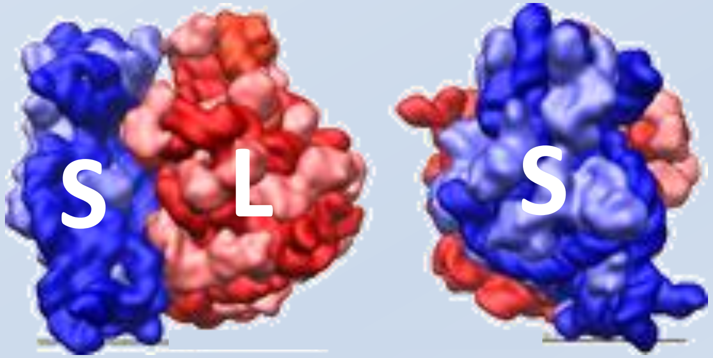
Polyribosome
(LabExchange 2022)



More than one Ribosome may be assembled on the same mRNA strand

(PolyRibosome)

Ribosome constructed of 2 Subunits: Large and Small



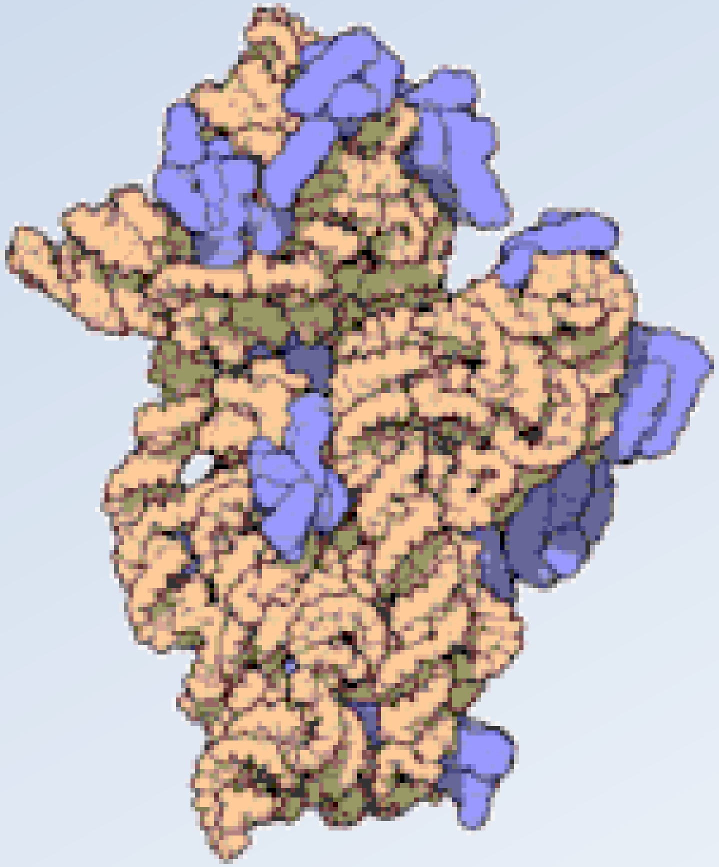
- Proteins ☐ 53 *79*
- rRNA's ☐☐☐ 5 6
  - Nucleotides 4343 *5107*

Eukaryotes

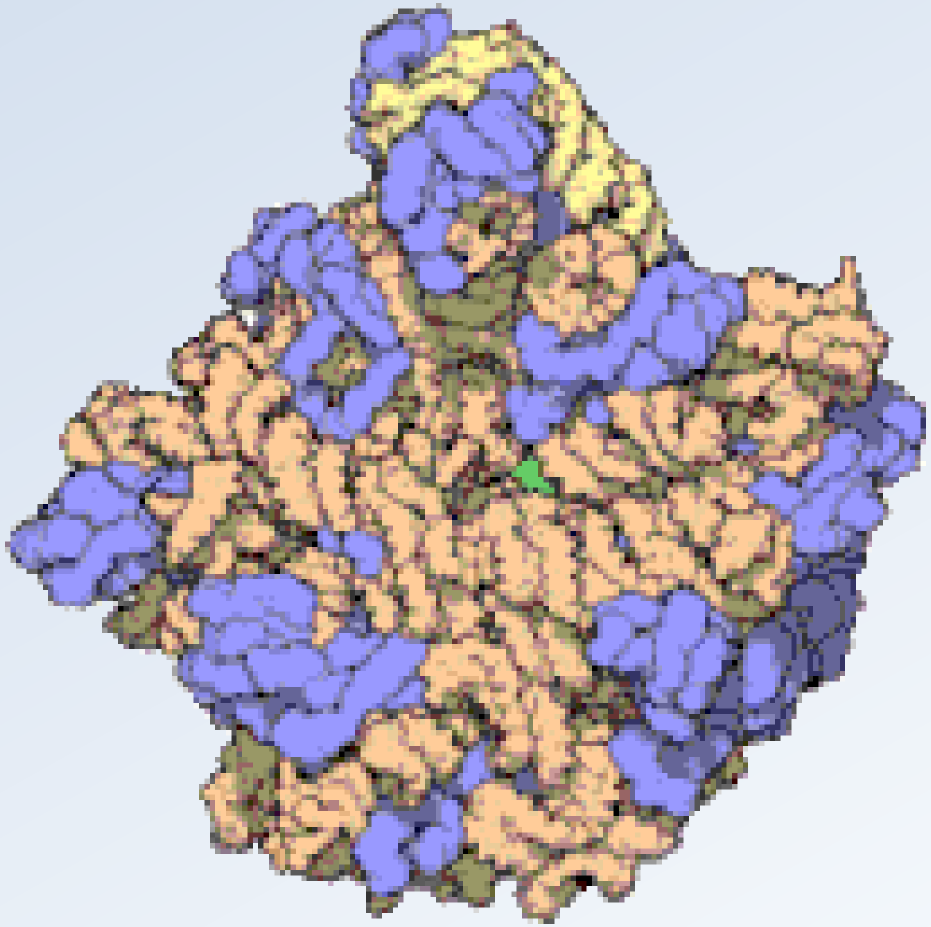
~65% RNA

100% of key components are RNA's!

Small subunit

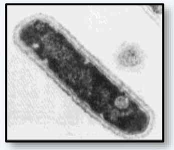


Large subunit



Thermus thermophilus

Wikipedia

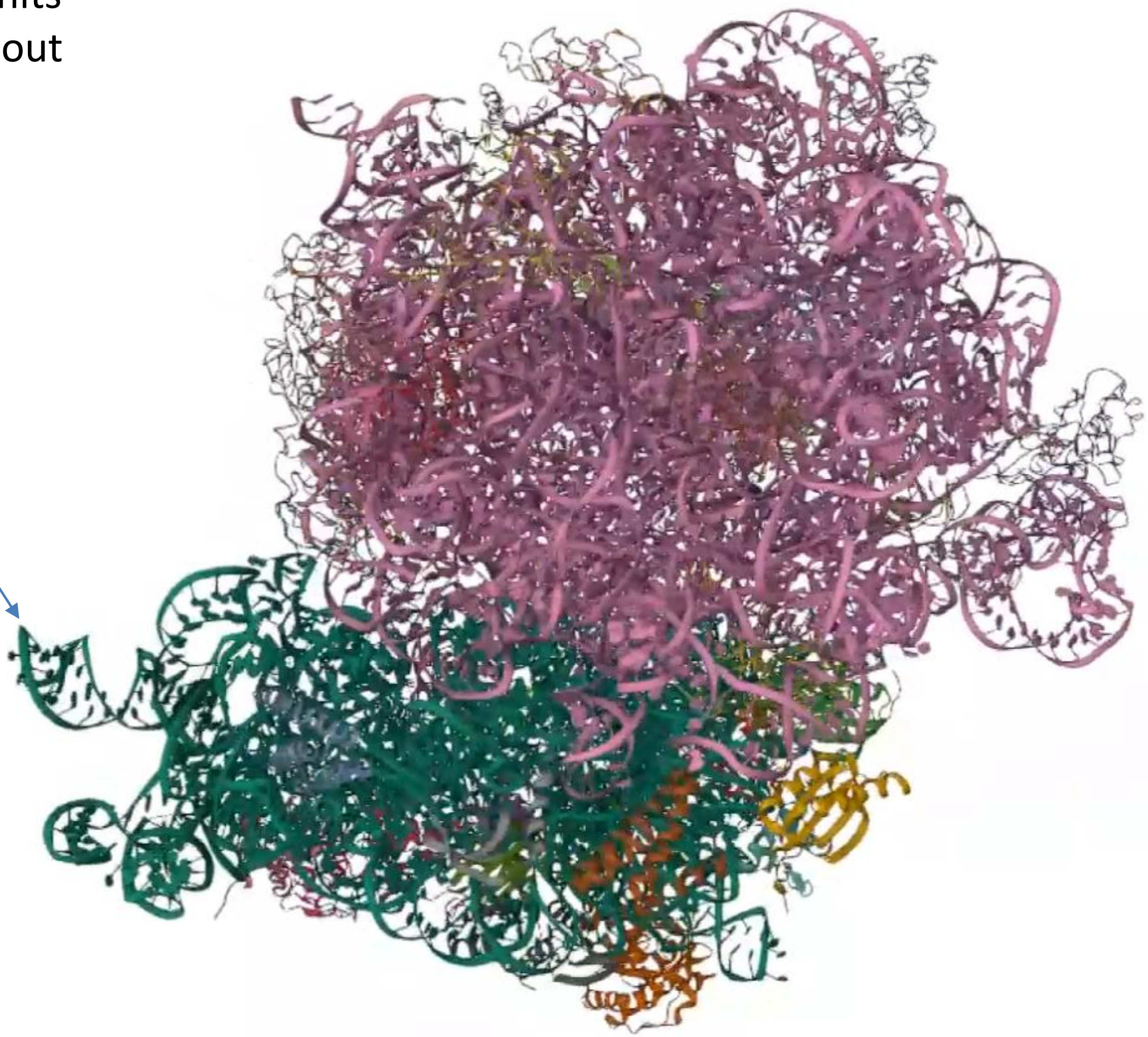


Interface residues buried fraction [biojava-structure-6.0.5] | value: 0.33 | Position: 1489 [auth: 1496]

E Coli Ribosome

(Large & Small Subunits assembled, but without tRNA’s or mRNA)

Note RNA elements



Settings / Controls Info

Click to close.

Animate	Spin
Camera	Perspective
Background	
Occlusion	✓ On
Shadow	✕ Off
Outline	✕ Off
Fog	✓ On
Clipping	74
Layout	1 of 1

Moving in 3D

Rotate
Drag using *left mouse button*

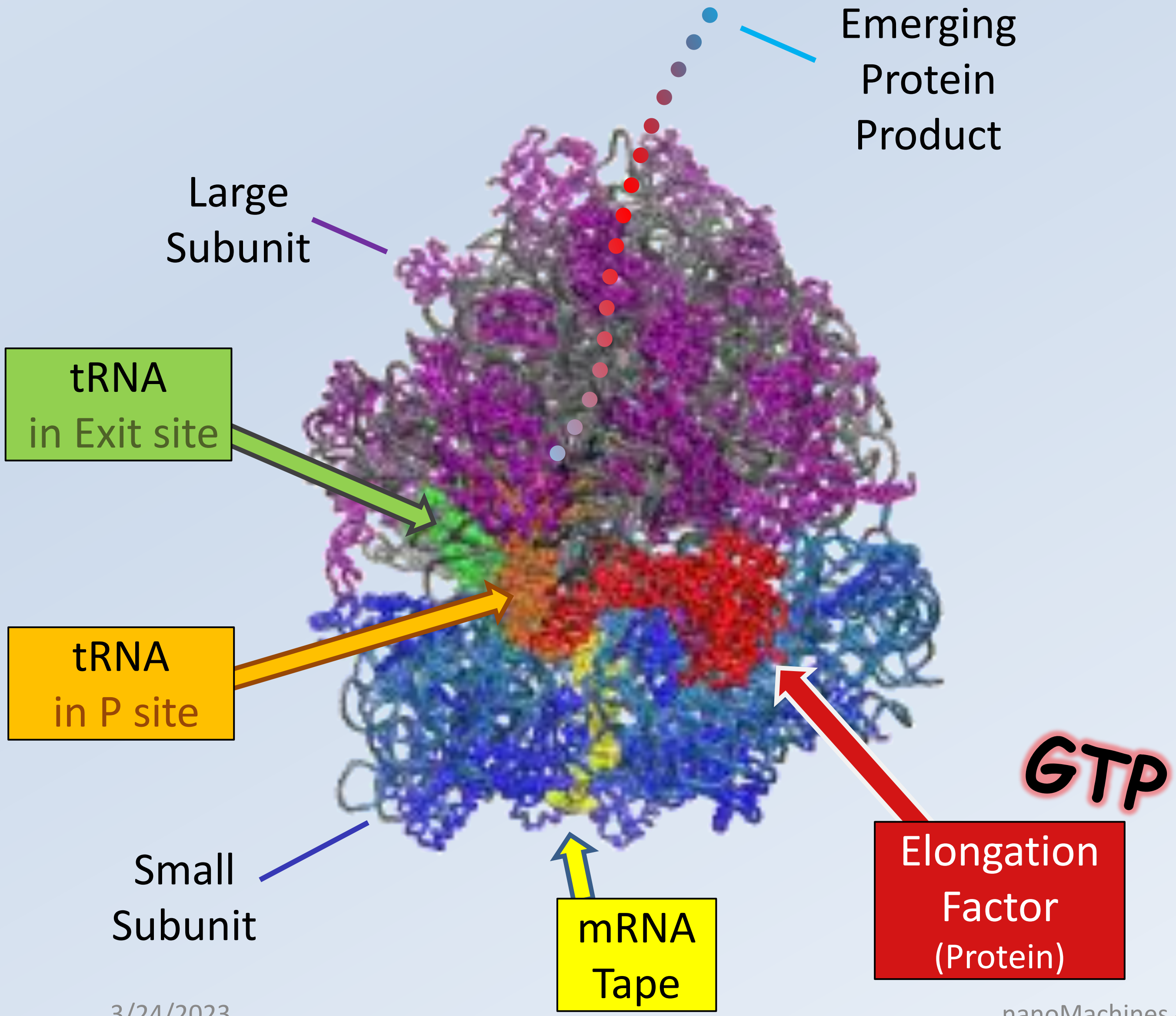
Rotate around z-axis

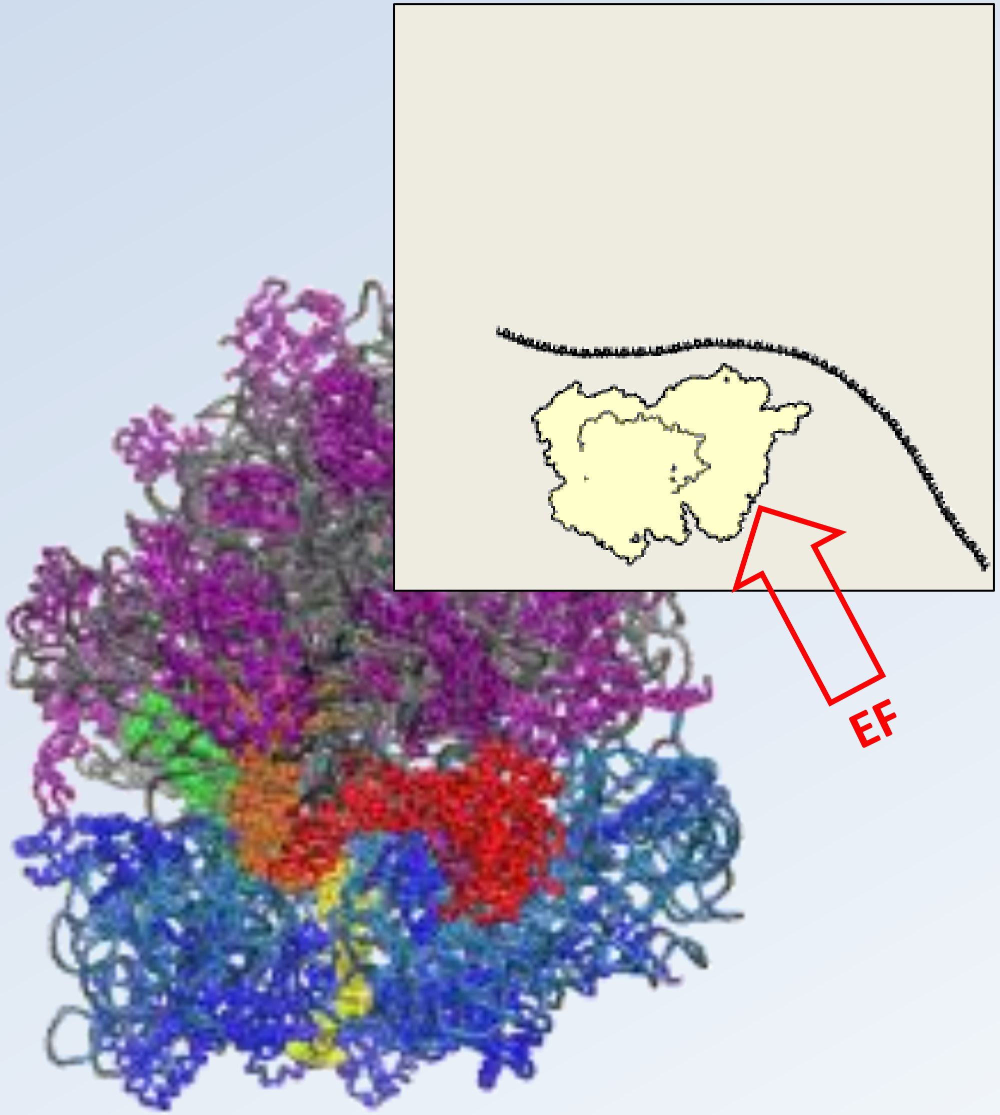
PDB: code 4v4a

Credit: RCSB.org

Ribosome "Innards" in Action

Thermus thermophilus Bacterium



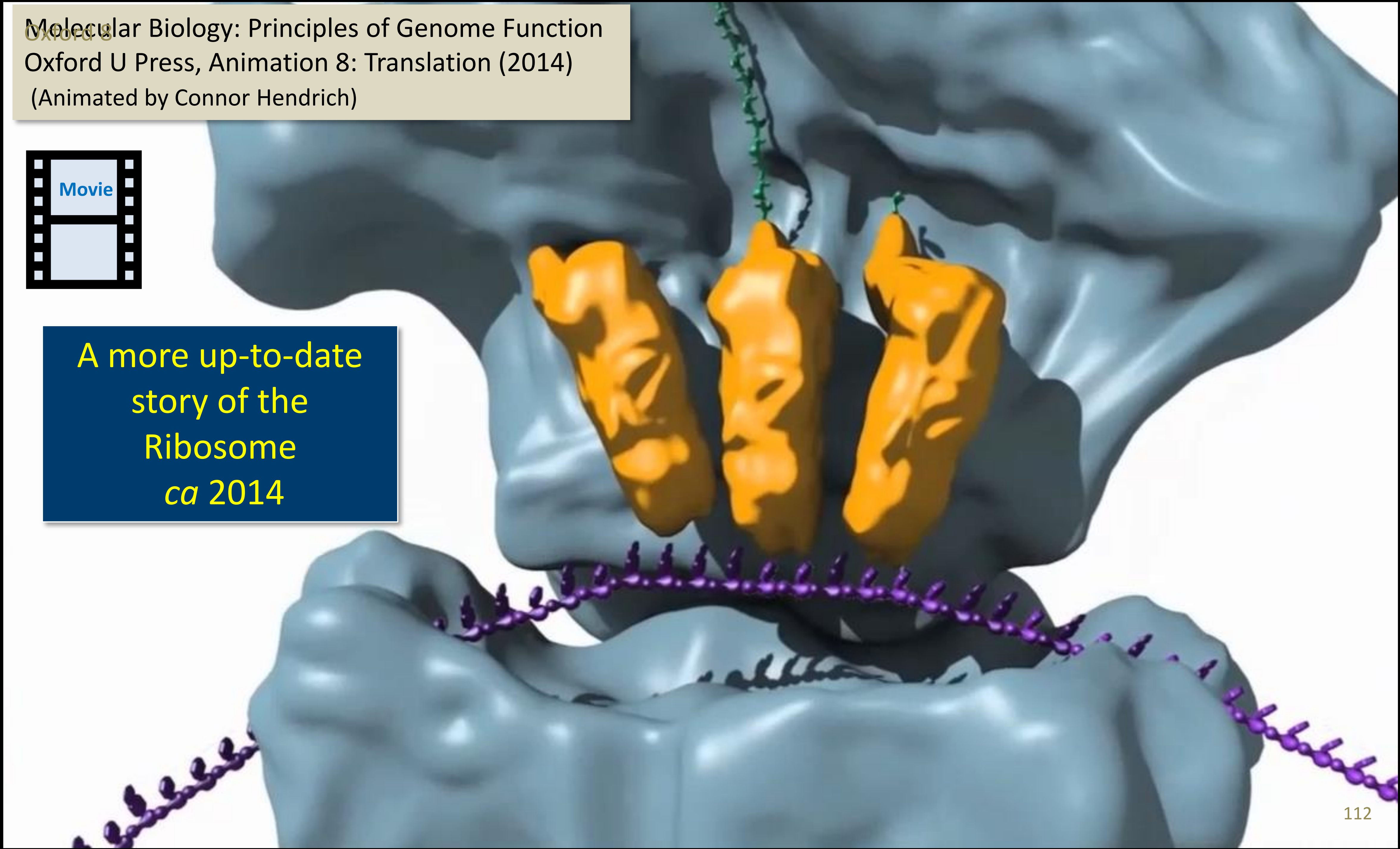


Wikipedia

Oxford 8

Molecular Biology: Principles of Genome Function
Oxford U Press, Animation 8: Translation (2014)
(Animated by Connor Hendrich)

Movie



A more up-to-date story of the Ribosome *ca* 2014

Questions?