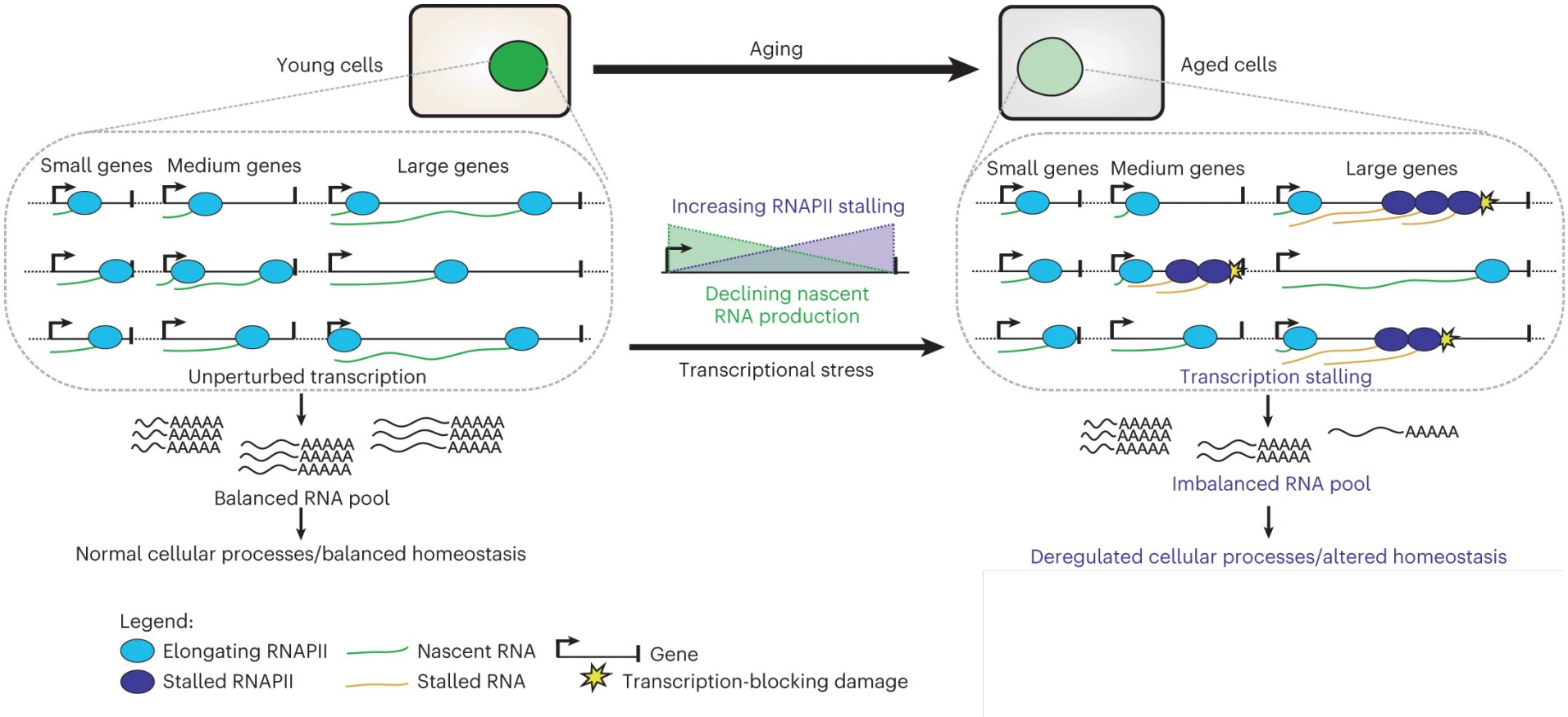
Unit 4: DNA Repair

We all get old...partly because cells can't deal with all the DNA damages that accumulate with age, which block RNA polymerases transcribing (long) genes...



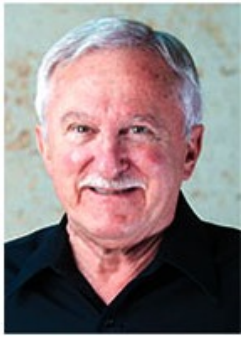
Gyenis et al. Nature Genetics 2023

DNA Damage Tolerance and Repair

1: Dealing with problems that occur during DNA replication

- Ribonucleotides incorporated during DNA replication
- Mutations resulting from errors made during DNA replication
  - Mismatch Repair Pathway

(P.Modrich/Nobel Prize Chemistry 2015)



2: Dealing with problems caused by DNA damage unrelated to DNA replication

- Non-exhaustive list of damages
- Strategies and mechanisms of DNA damage tolerance and repair:
  - Bypass/Translesion DNA polymerases
  - Direct Reversal

- Base Excision Repair (T.Lindahl/Nobel Prize Chemistry 2015)

- Nucleotide Excision Repair

(A.Sancar/Nobel Prize Chemistry 2015)

We will not cover:
Double-stranded break repair

Learning outcomes:
What you need to know/understand after this unit

Understand the errors that can occur during replication and the mechanisms involved in fixing these errors

Know the major type of damages studied in lecture and their consequences on DNA polymerases (block/mutations)

Understand how translesion DNA polymerases ensure replication over damaged DNA

Understand and know the 3 type of mechanisms that correct DNA damages and what type of damages they typically correct

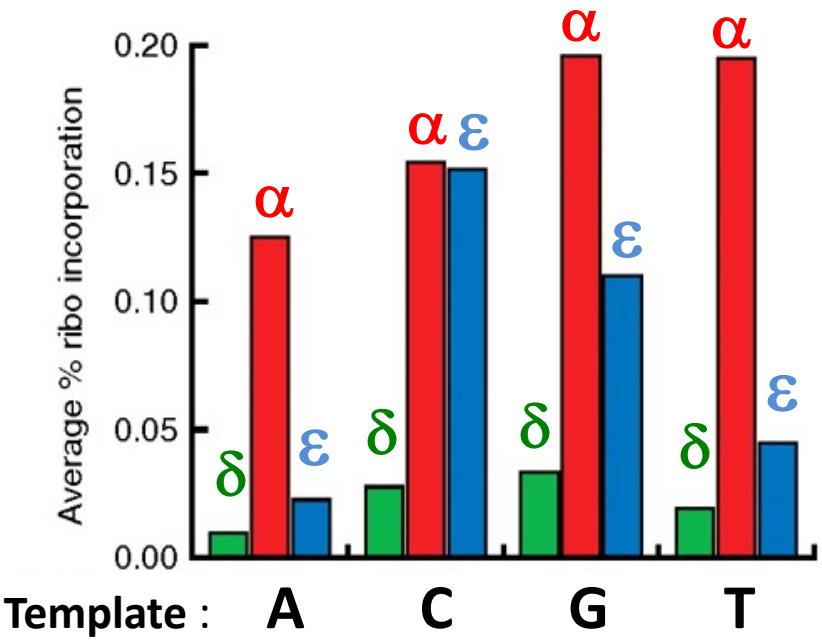
Understand how DNA repair enzymes recognize damaged DNA: General principles and examples studied

The problem of ribonucleotide incorporation by DNA polymerases

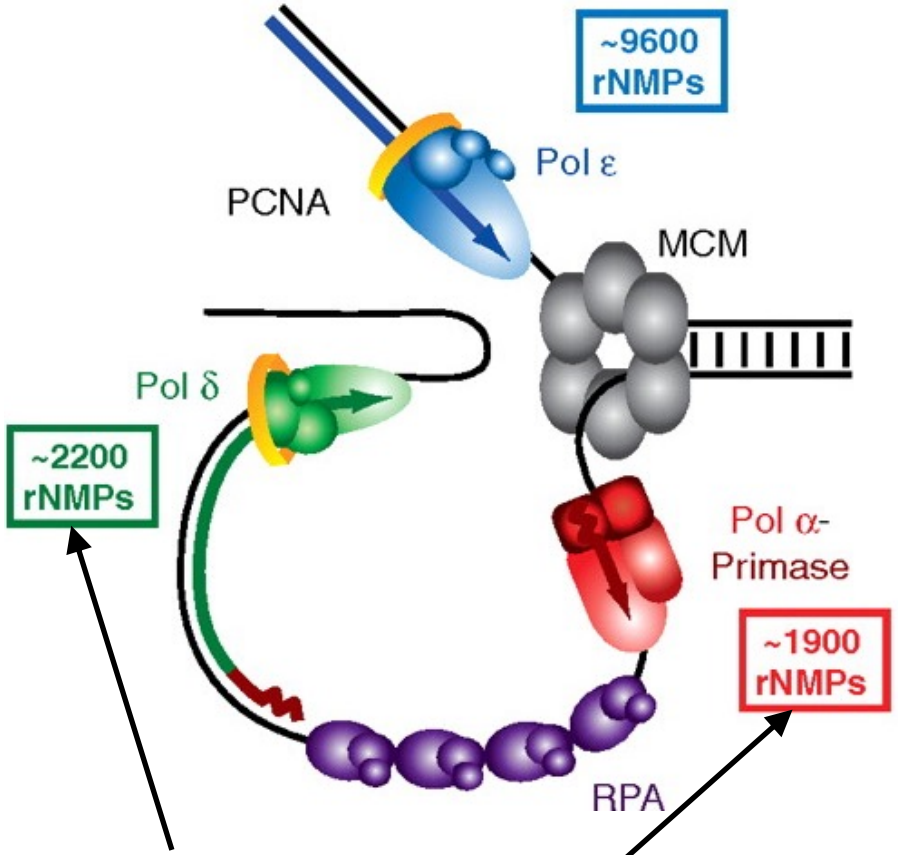
Why wouldn’t we want rNTPs incorporated during DNA replication?

The problem of ribonucleotide incorporation by DNA polymerases

- **DNA polymerases discriminate deoxy vs riboNTP, but not at 100%**
- **[rNTPs] >> [dNTPs] in vivo → this leads to some riboNTP incorporation in newly synthesized DNA (sugar discrimination by DNA polymerases is not 100%)**

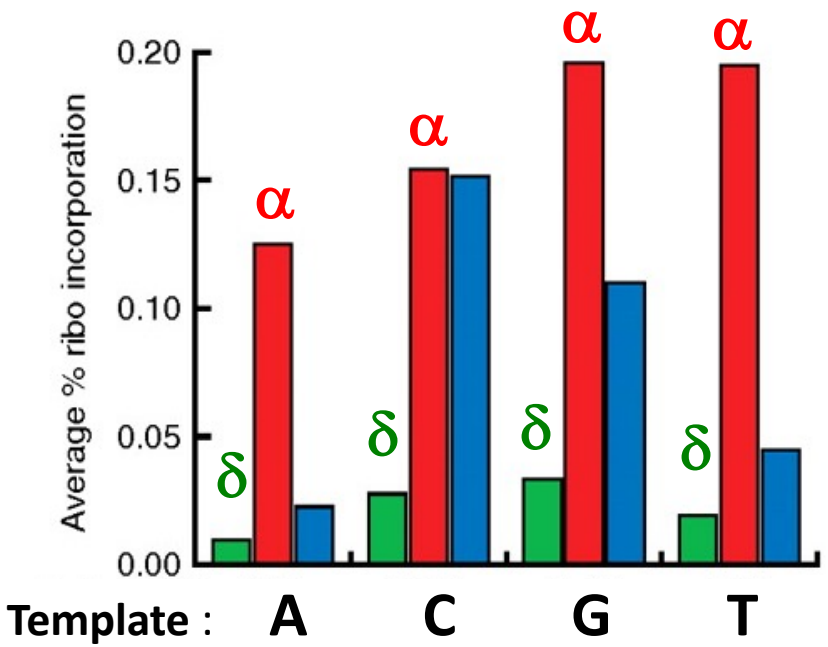


(PNAS 107, p4950, 2010)



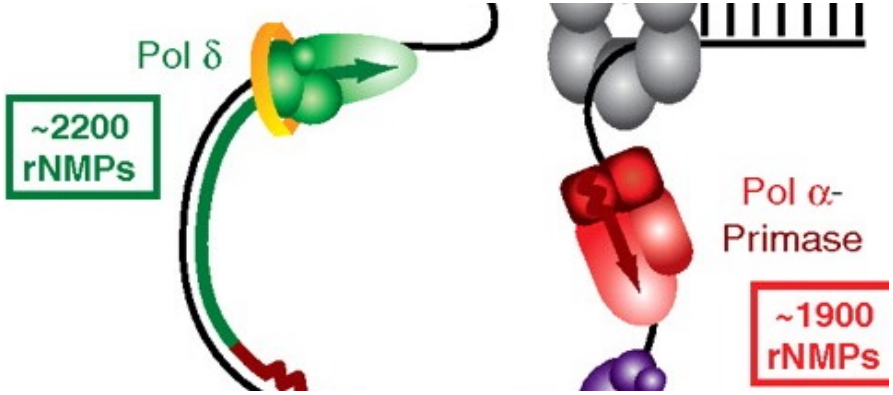
Total number of rNMPs incorporated when replicating the whole yeast genome

Why is Pol δ incorporating more rNMPs than α while its avg % rNTP incorporation is lower?



A: α synthesizes primers which are then removed by processing of Okazaki fragments so less rNMPs overall

B: Rates of misincorporation measured in vitro don´t reflect polymerases activities in vivo

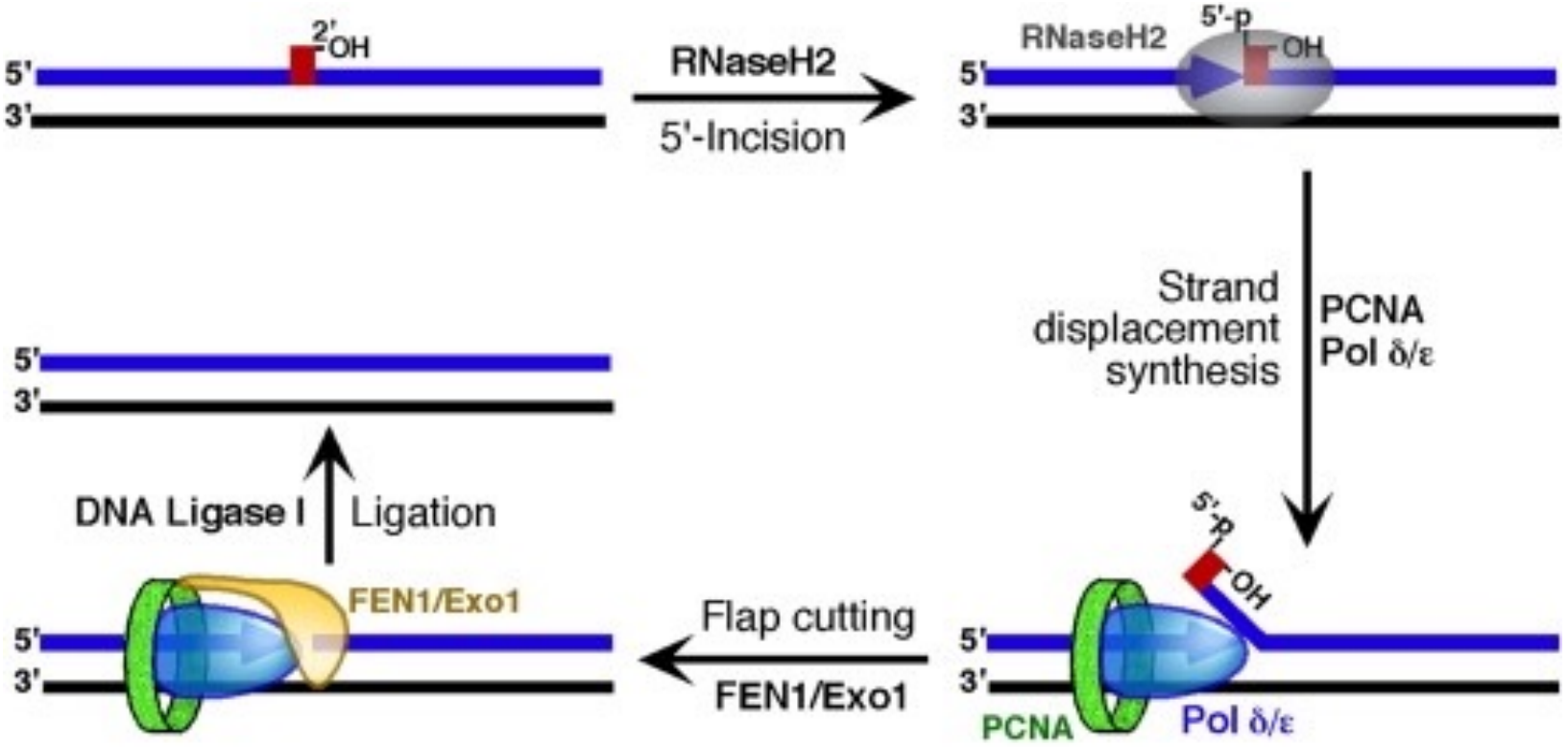


C: α has a riboNMP proofreading activity which delta does not have

D: δ synthesizes more DNA length so this results in more rNMP per round of replication despite lower %

Enzymes that deal with removal of RNA primers in Okazaki fragments also remove riboNTPs *mis*incorporated into DNA:

Mainly RNase H + Enzymes that process Okazaki fragments

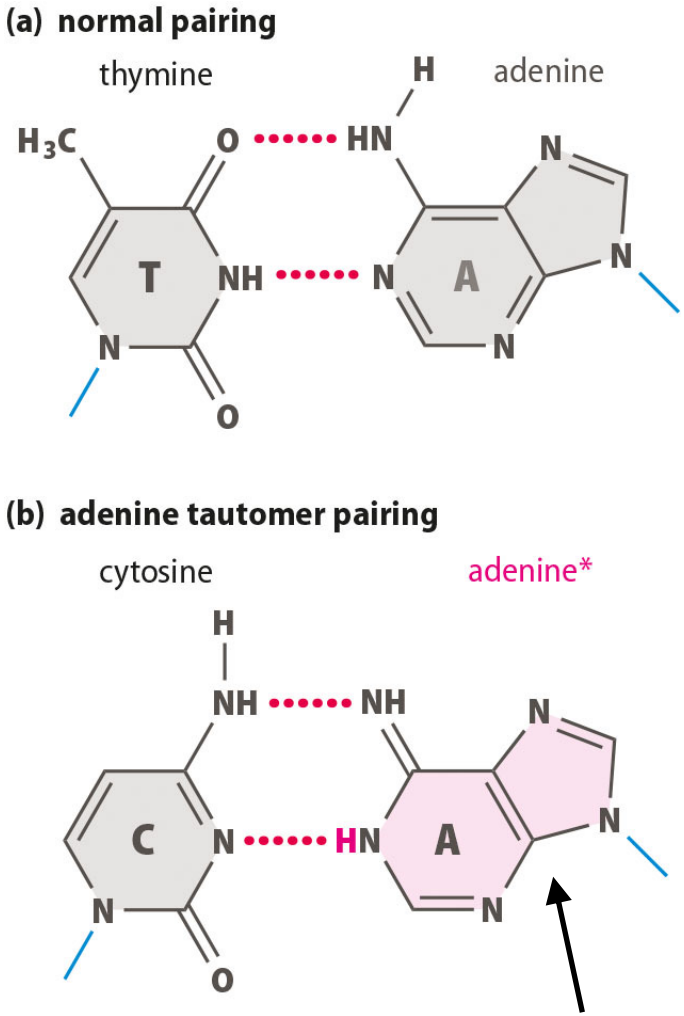


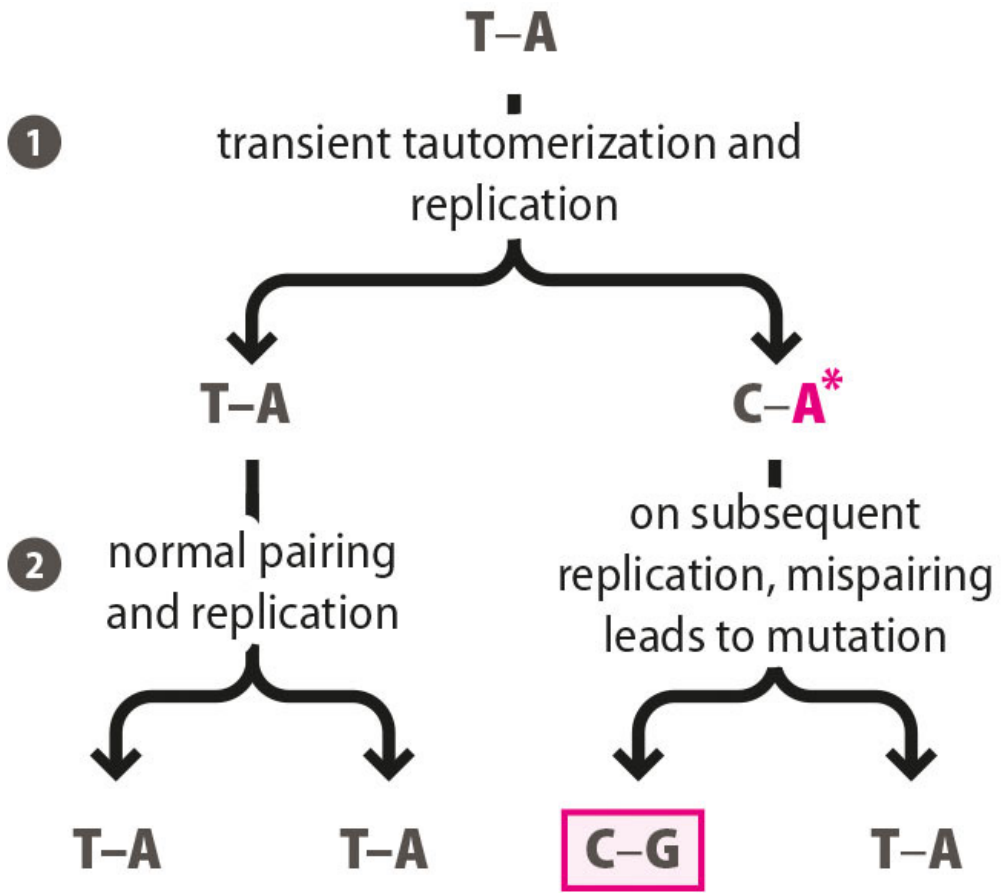
Sparks et al. Mol. Cell 2012

Why might a DNA polymerase make a mistake?

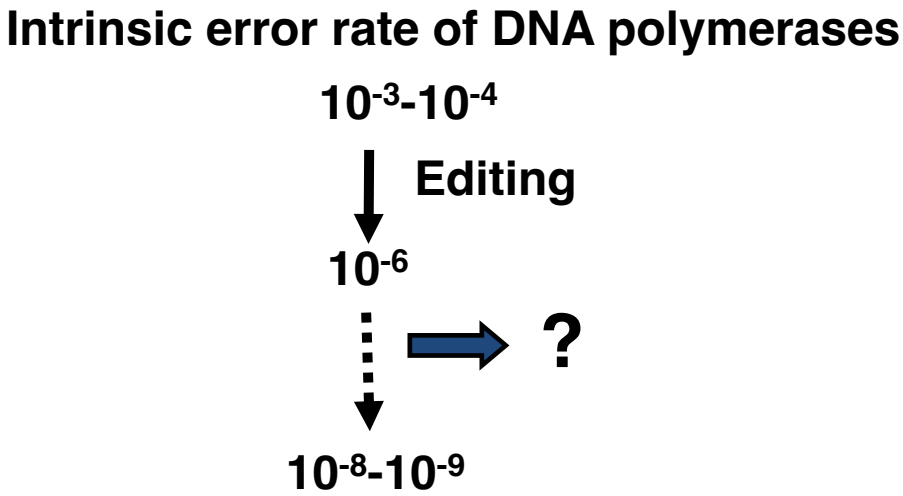
Mistakes can occur due to tautomerization

Transient tautomerization happens with probability of ~10^{-3}-10^{-5}





How do we get to the 10^{-8}-10^{-9} error rates observed for DNA replication?



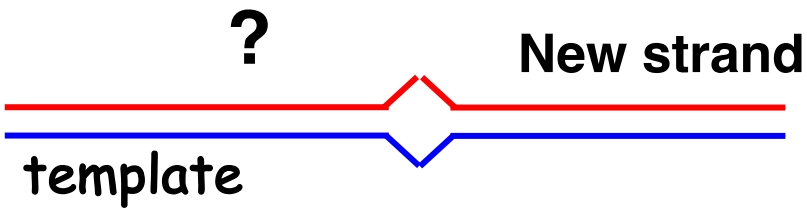
Increasing replication fidelity by mismatch repair
(2015 Nobel Prize: Paul Modrich)

Suppose a mistake is made:

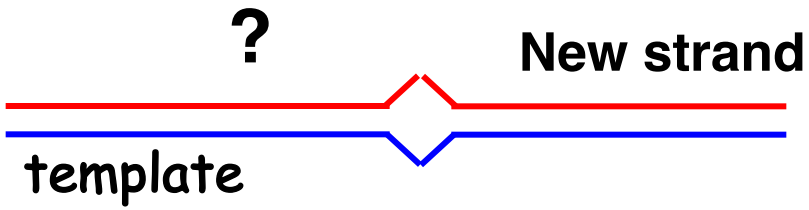
How would a cell know that there is a mistake?

Once we identify that there's a mismatch, how do we know which of the two nucleotides is correct?

How do we differentiate between the template strand and the new strand?

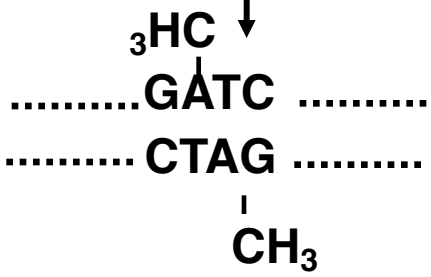


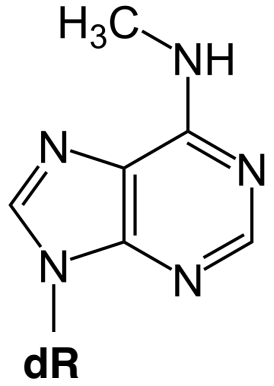
Increasing replication fidelity by mismatch repair in bacteria



1) DNA is methylated at specific palindromic sequences (adenosine N6)

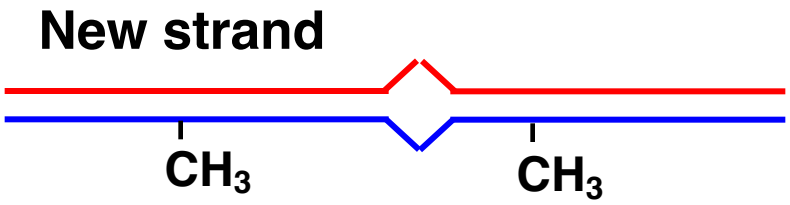
Dam methylase





Dam methylase methylates adenosines using a methyl group from S-adenosyl methionine (SAM)

2) DNA methylation is delayed after replication



How frequently are hemimethylated GATC sites encountered on average in chromosomes (assuming 25% A, T, G, C)?

A: every 256 base pairs (4^4)

B: every 64 base pairs (4x16)

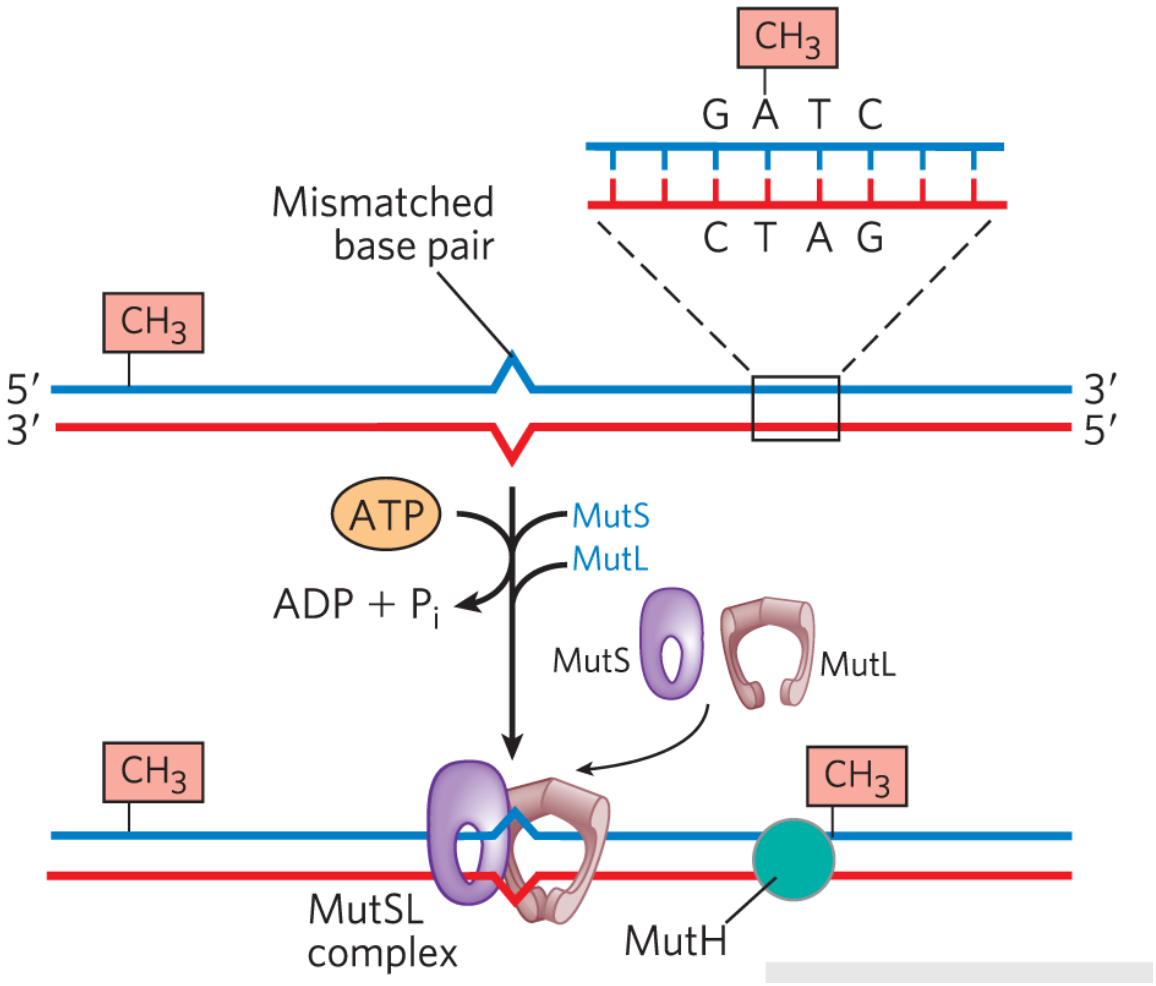
C: every 16 base pairs (2^4)

D: every 65536 base pairs (16^4)

Mismatch repair in *E. coli*

- **The proteins involved in mismatch repair are called Mut proteins, where Mut stands for mutator**
  - **Inactivation of Mut proteins causes a high mutation rate (a "mutator phenotype")**
- **3 key proteins:**
  - **(1) MutS: recognizes and binds mismatched base pairs**
  - **(2) MutL:**
  - **(3) MutH: an endonuclease that nicks the new DNA strand**

Mismatch repair in *E. coli*

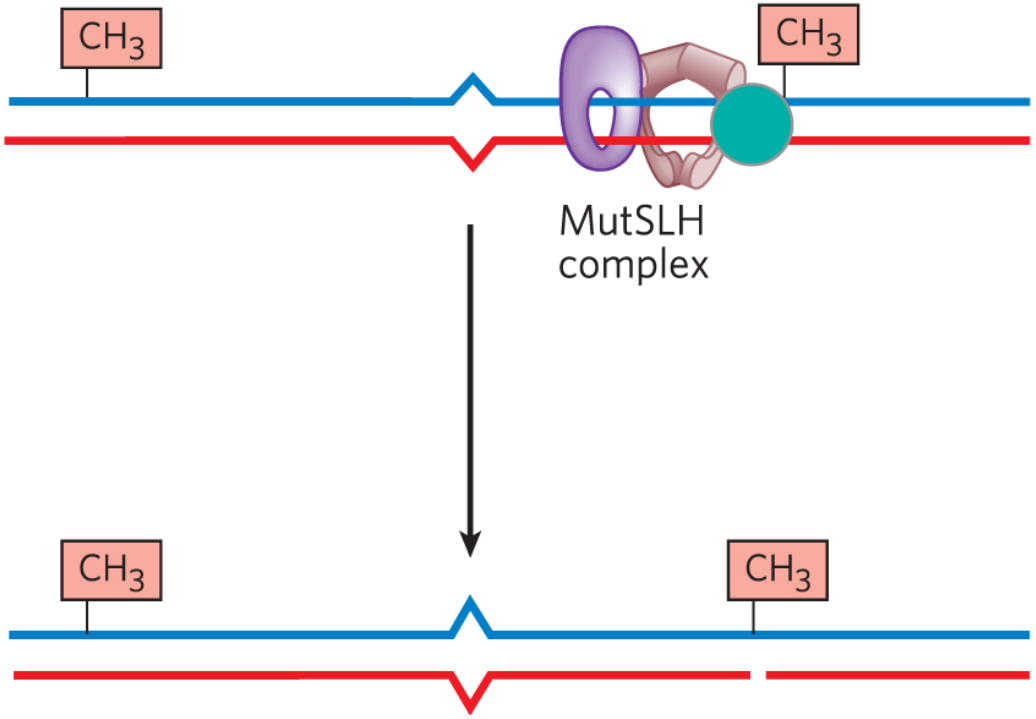


Step 1: MutH binds to hemimethylated GATC

Step 2: MutS scans the DNA and forms a clamplike complex upon encountering a mismatch (detects all but C-C)

Step 3: MutL binds to MutS → MutSL complex

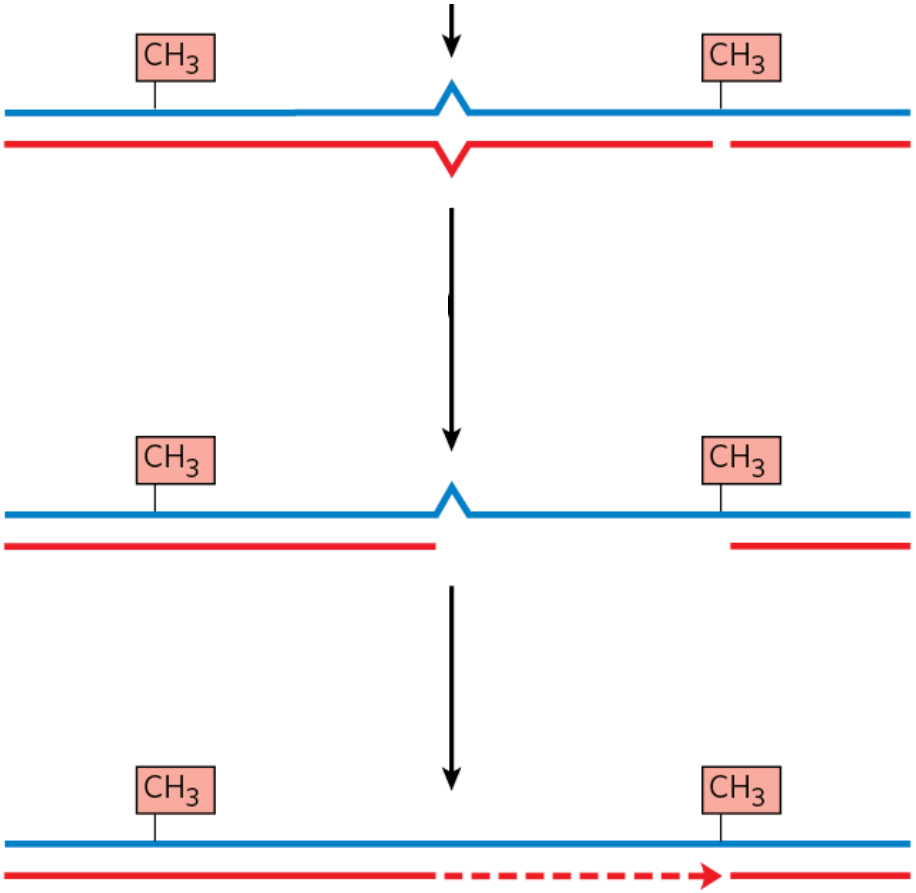
Mismatch repair in *E. coli*



<u>Step 4</u>: MutSL complex slides along DNA to MutH

<u>Step 5</u>: MutH catalyzes cleavage of the unmethylated strand on the side of the G in GATC

Mismatch repair in *E. coli*



Step 6: DNA helicase II (UvrD helicase) unwinds the unmethylated DNA, SSB binds the ssDNA

Step 7: An exonuclease degrades the unwound unmethylated ssDNA

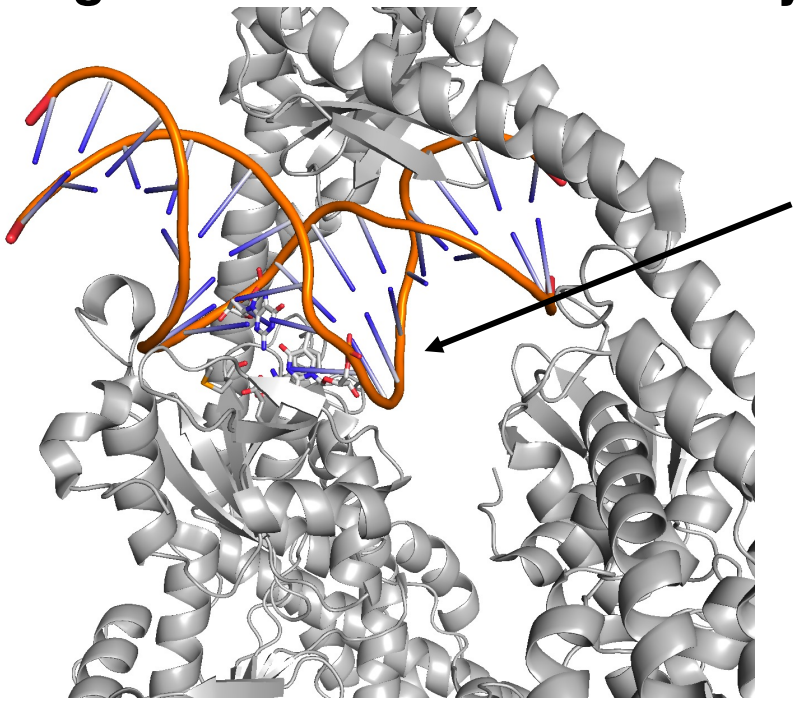
Step 8: DNA pol III fills in the gap with new DNA

Step 9: DNA ligase seals the nick

Mismatch repair in other organisms

- **Key steps in mismatch repair are the same in prokaryotes and eukaryotes**
  - **(1) Recognition of the mismatched base pair**
  - **(2) Removal of the incorrect nucleotide on the newly synthesized strand**
  - **(3) Resynthesis using the parental DNA strand as a template**
- **What differs between organisms:**
  - **(1) Strategies for recognition of the newly synthesized strand**
  - **(2) Cleavage around the incorrect nucleotide**

Recognition of a G-T mismatch by MutS in prokaryotes

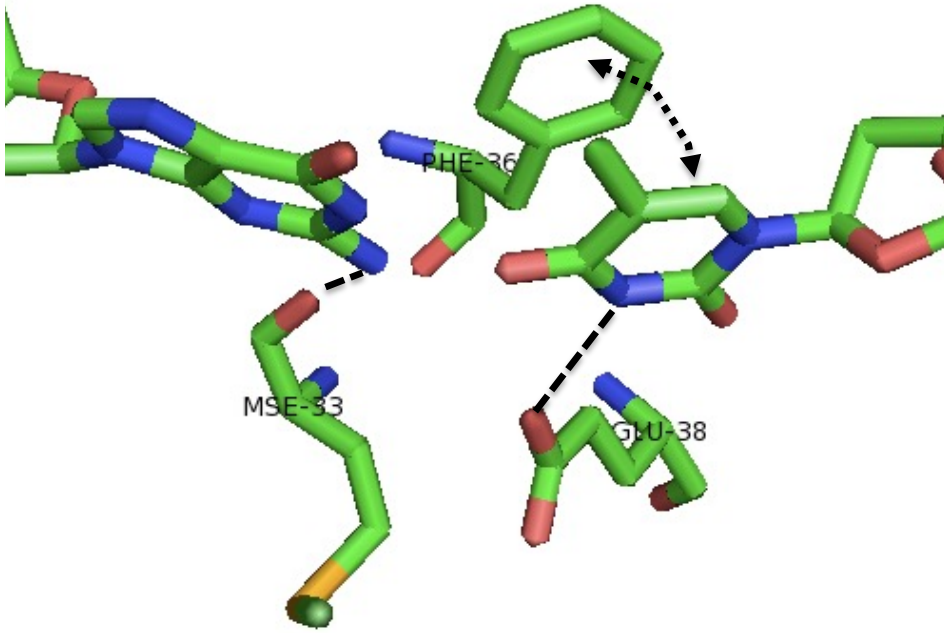


- DNA is kinked at site of mismatch

- Recognition of mismatch by:

- H bonding of MutS residues with mismatched bases (MET33 with G, GLU38 with T)

- stacking on extruded base (PHE36)



PyMol: MismatchRecognitionbyMutS-2.pse

Lamers et al. Nature 2000