

Division of Labor at the Eukaryotic Replication Fork

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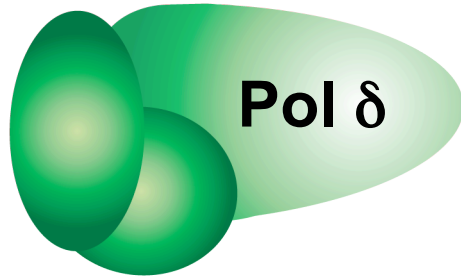
Laboratory of Molecular Genetics

Laboratory of Structural Biology

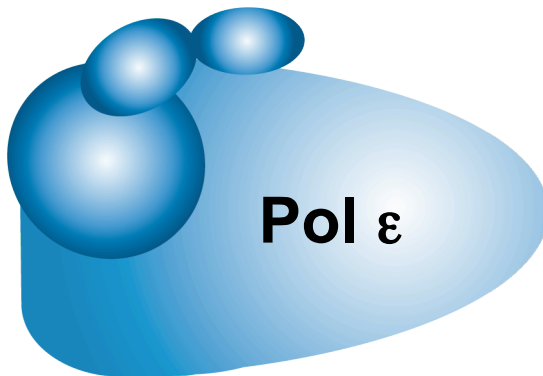
National Institute of Environmental Health Sciences

Division of Labor at the Replication Fork

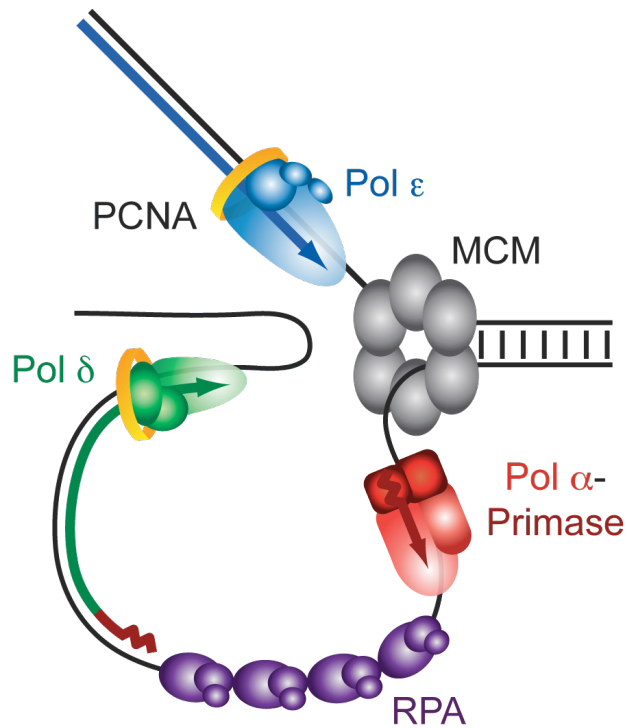
Pol δ and **Pol ϵ** are both required for replication



What is the division of labor between **Pol δ** and **Pol ϵ** ?

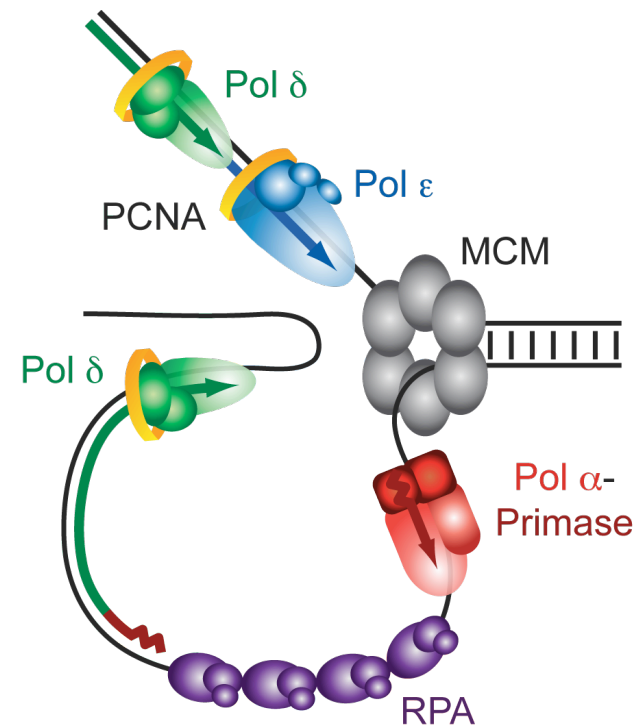


Division of Labor at the Replication Fork



Leading = Pol ϵ

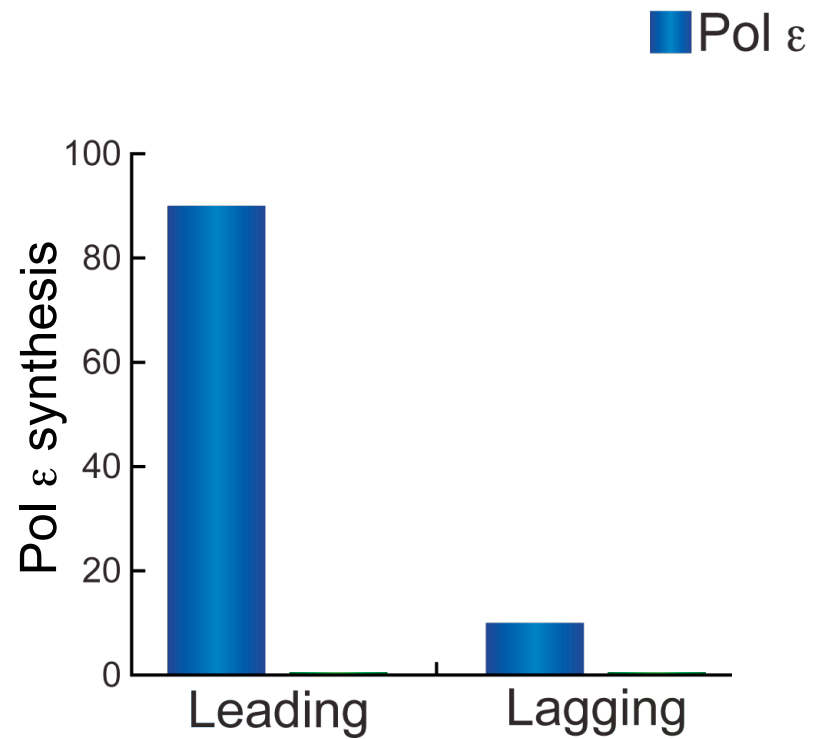
Lagging = Pol δ



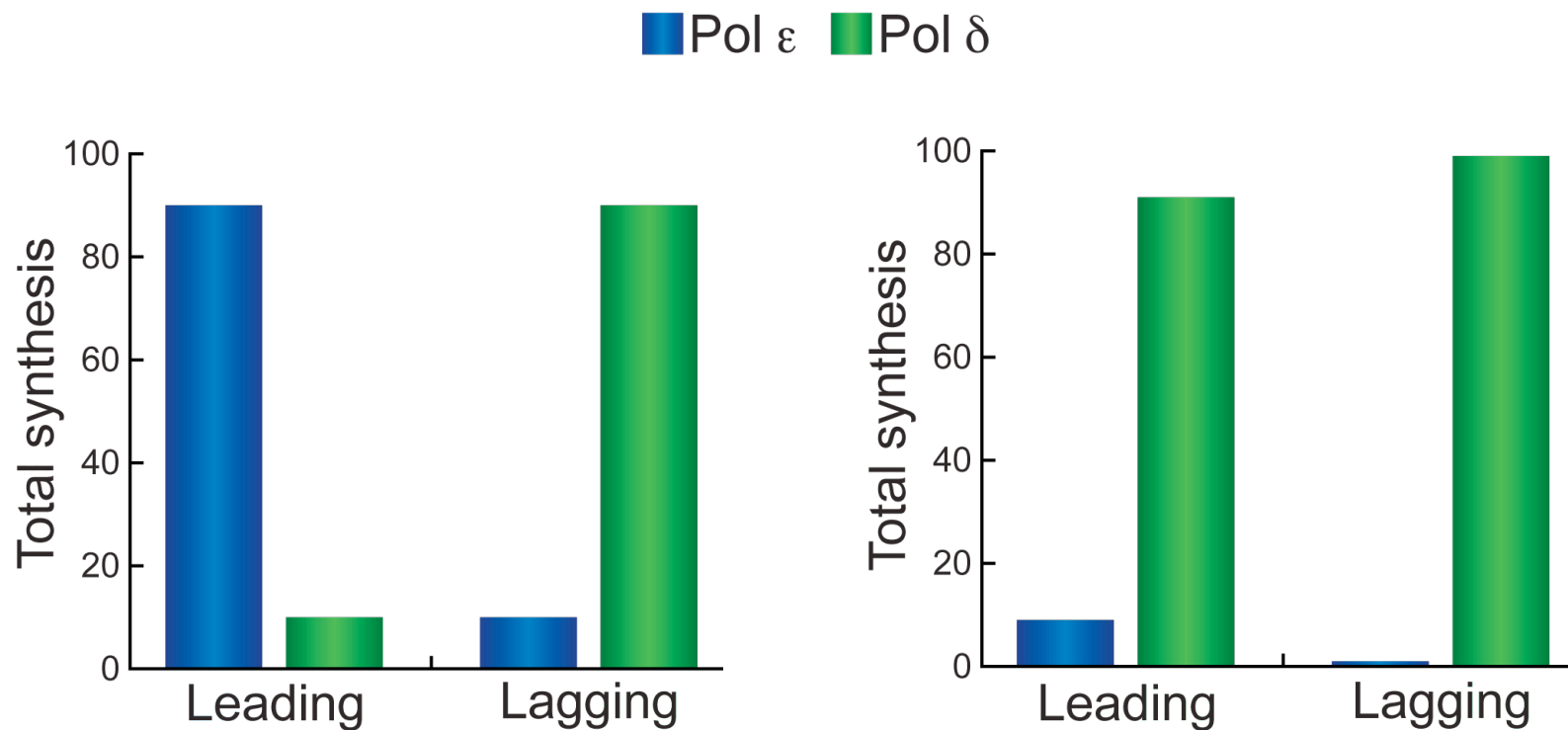
Leading = Pol ϵ + δ

Lagging = Pol δ

What is the Division of Labor at the Fork?



What is the Division of Labor at the Fork?



How is Pol δ synthesis distributed between the leading and lagging strand during replication?

Strand Assignment of Pol δ During Replication

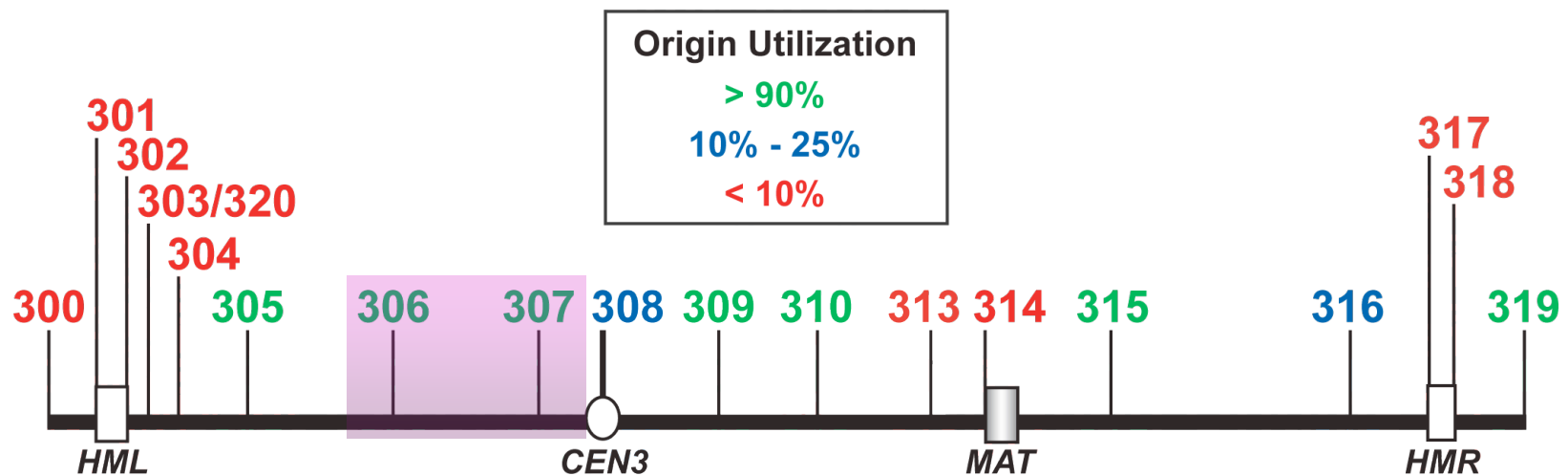
Approach:

Track the activity of Pol δ during replication *in vivo*

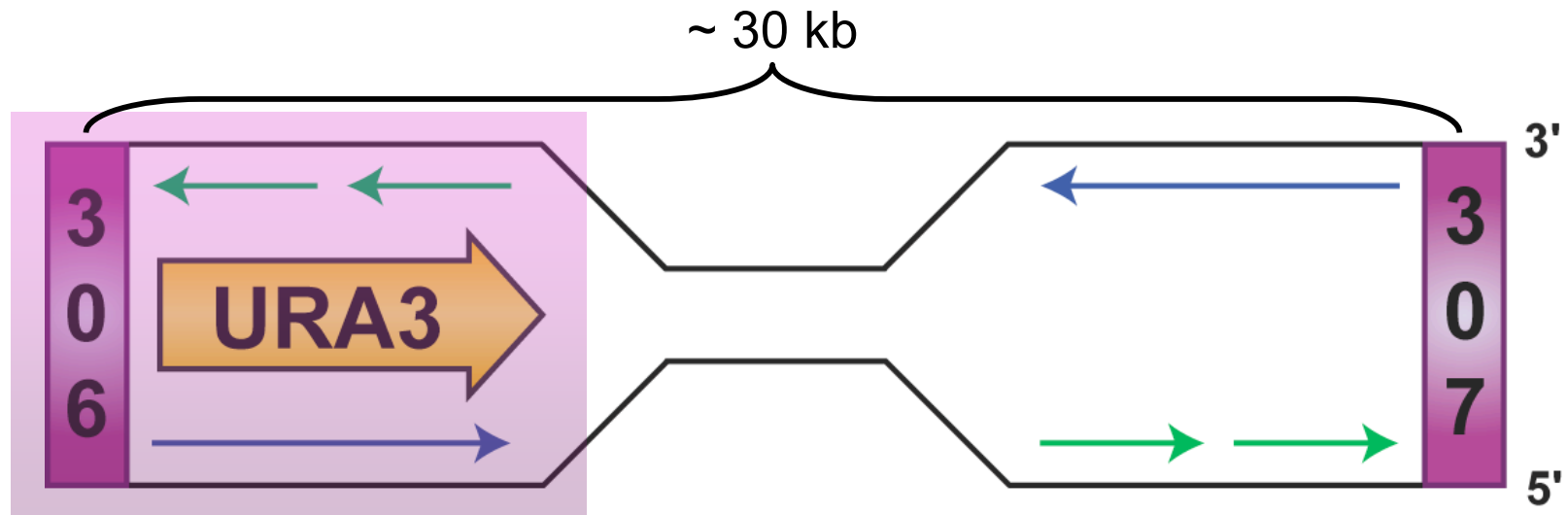
1. Identify leading and lagging strands during *in vivo* replication
2. Mark sites of Pol δ synthesis using a mutator allele
3. Assign mutator Pol δ to leading and/or lagging strand according to mutational bias

Identify Leading and Lagging Strands

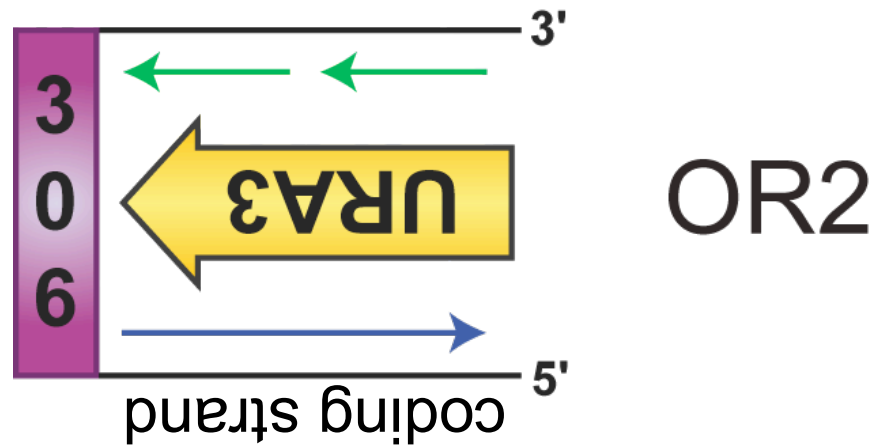
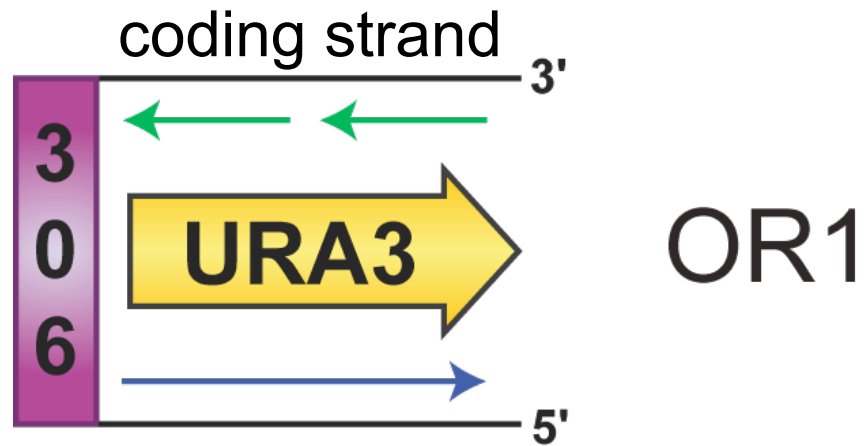
Yeast Chromosome III Replication Origins



Identify Leading and Lagging Strands



Identify Leading and Lagging Strands



Mutator Pol δ to Mark Sites of Synthesis

L612M Pol δ

Nick McElhinny et al.,
JBC 2007

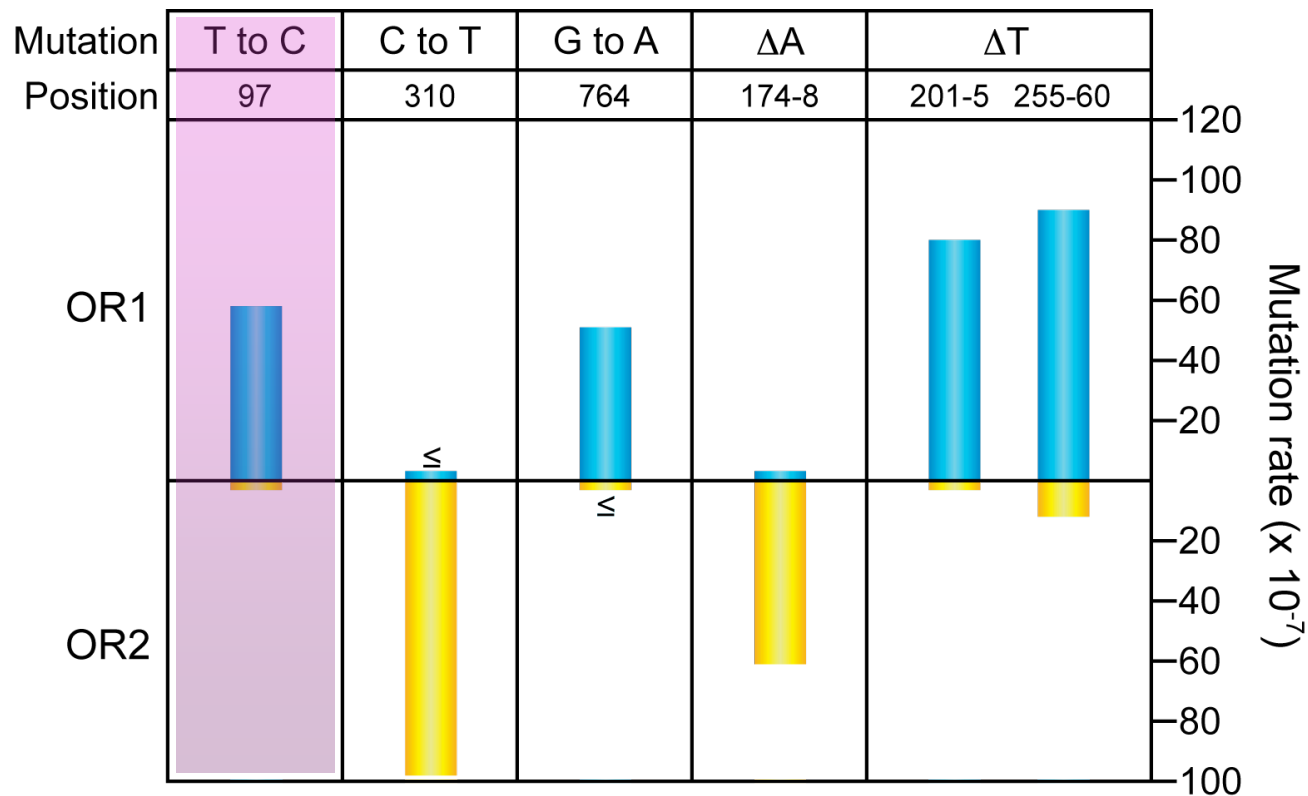
1. Wild-type catalytic activity
2. Intact exonuclease activity
3. Normal cell growth
4. Reduced fidelity *in vitro*
5. Mutator phenotype *in vivo*
6. Mutational bias

Pol active site Motif A

RB69	410	F D L T S	L	Y P S I I R Q V N	424
S.cer α	863	M D F N S	L	Y P S I I Q E F N	877
S.cer δ	607	L D F N S	L	Y P S I M M A H N	621
S.cer ϵ	639	V D V A S	M	Y P N I M T T N R	653
S.cer ζ	974	L D F Q S	L	Y P S I M I G Y N	988

L612M Pol δ Exhibits an Orientation Bias in Mutagenesis

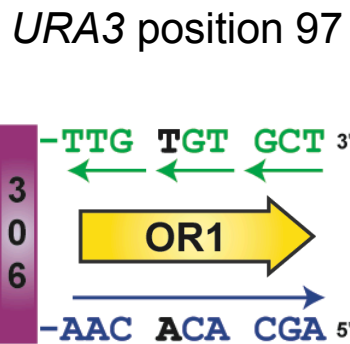
Orientation Bias implies Strand Bias



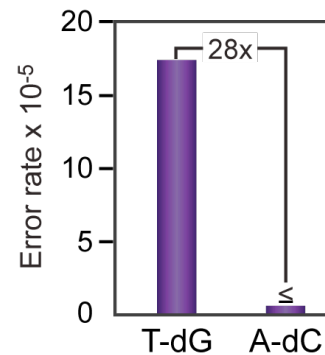
Strand Assignment of Pol δ via Mutational Bias

Mutations
observed:

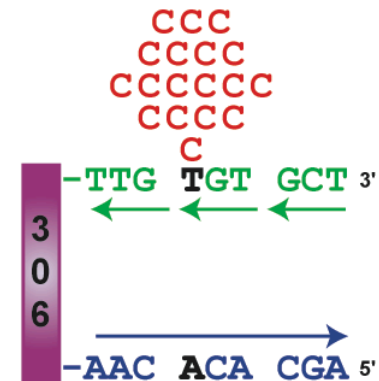
18 T-A to C-G



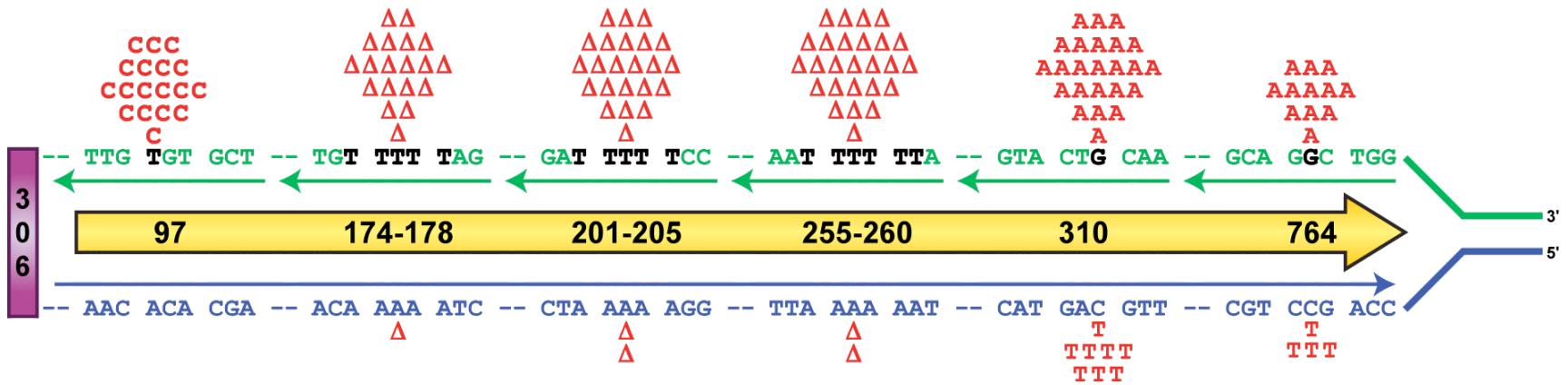
Mutational Bias of
L612M Pol δ



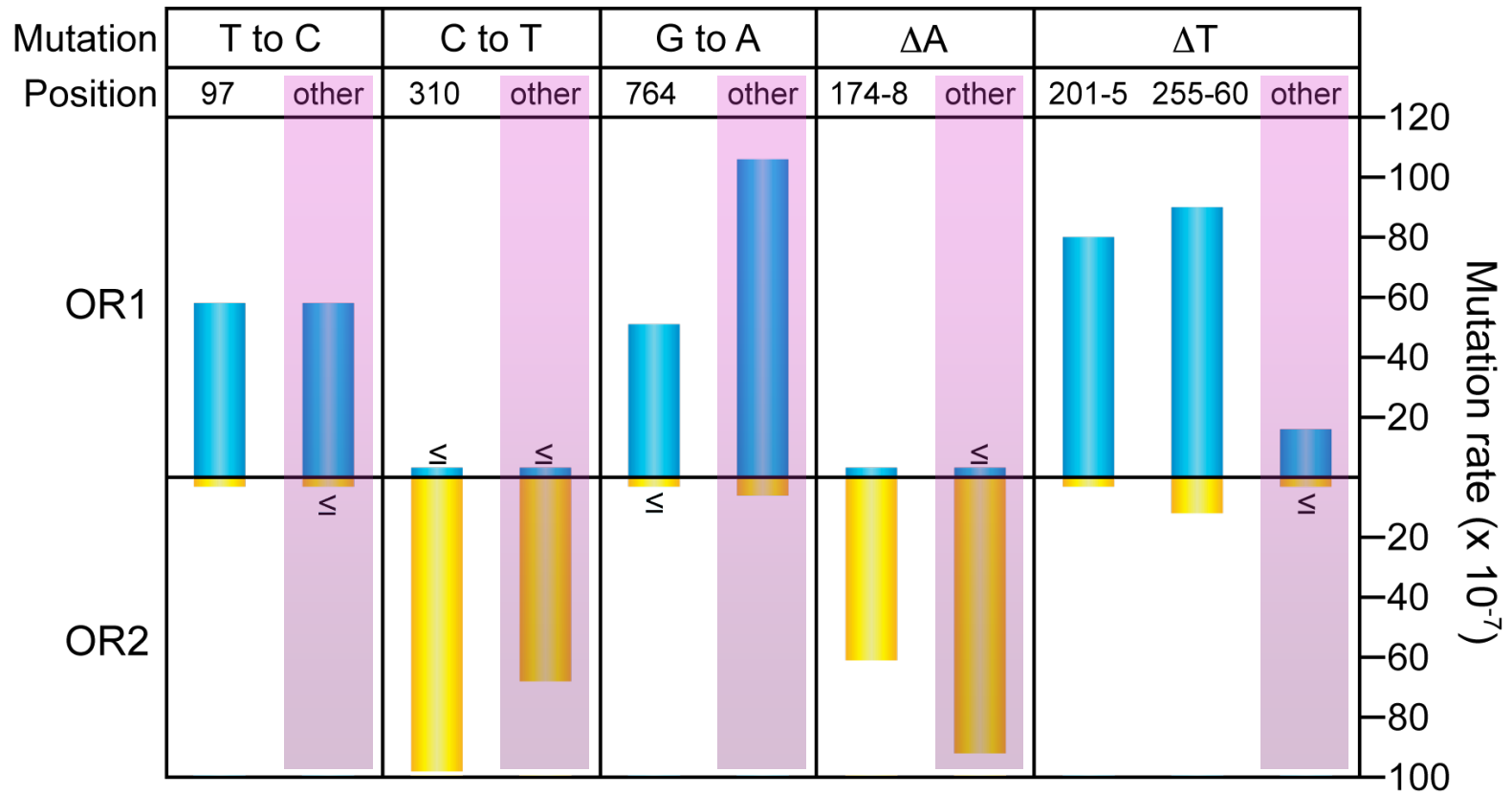
28 T-dG : 1 A-dC



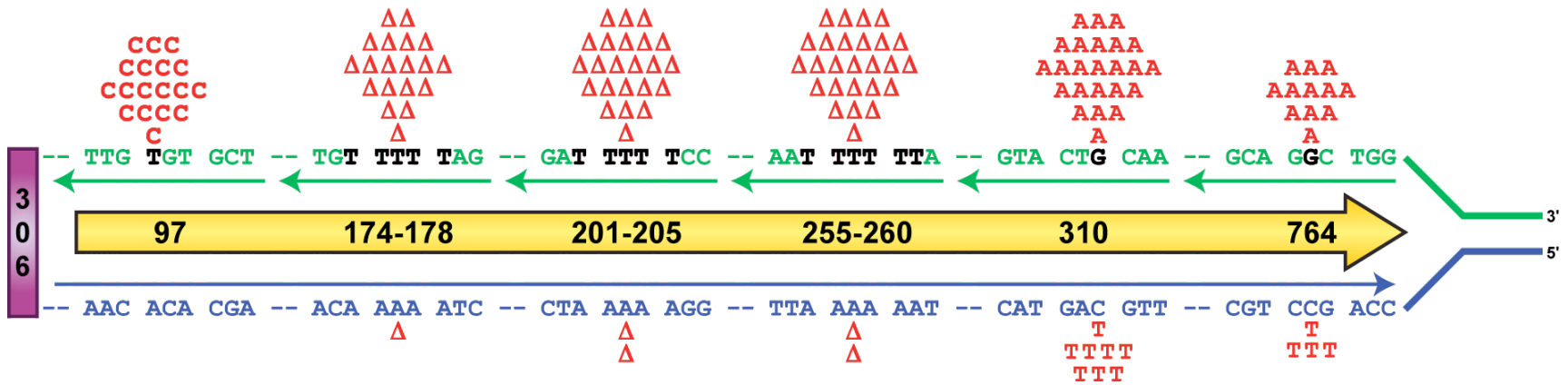
Pol δ Primarily Replicates the Lagging Strand



Bias of Pol δ for Lagging Strand is not Limited to Hotspots



Pol δ Primarily Replicates the Lagging Strand



Conclusions

Nearly equal division of labor at the eukaryotic replication fork

Pol δ primarily replicates the **lagging strand** template

Pol ϵ primarily replicates the **leading strand** template

