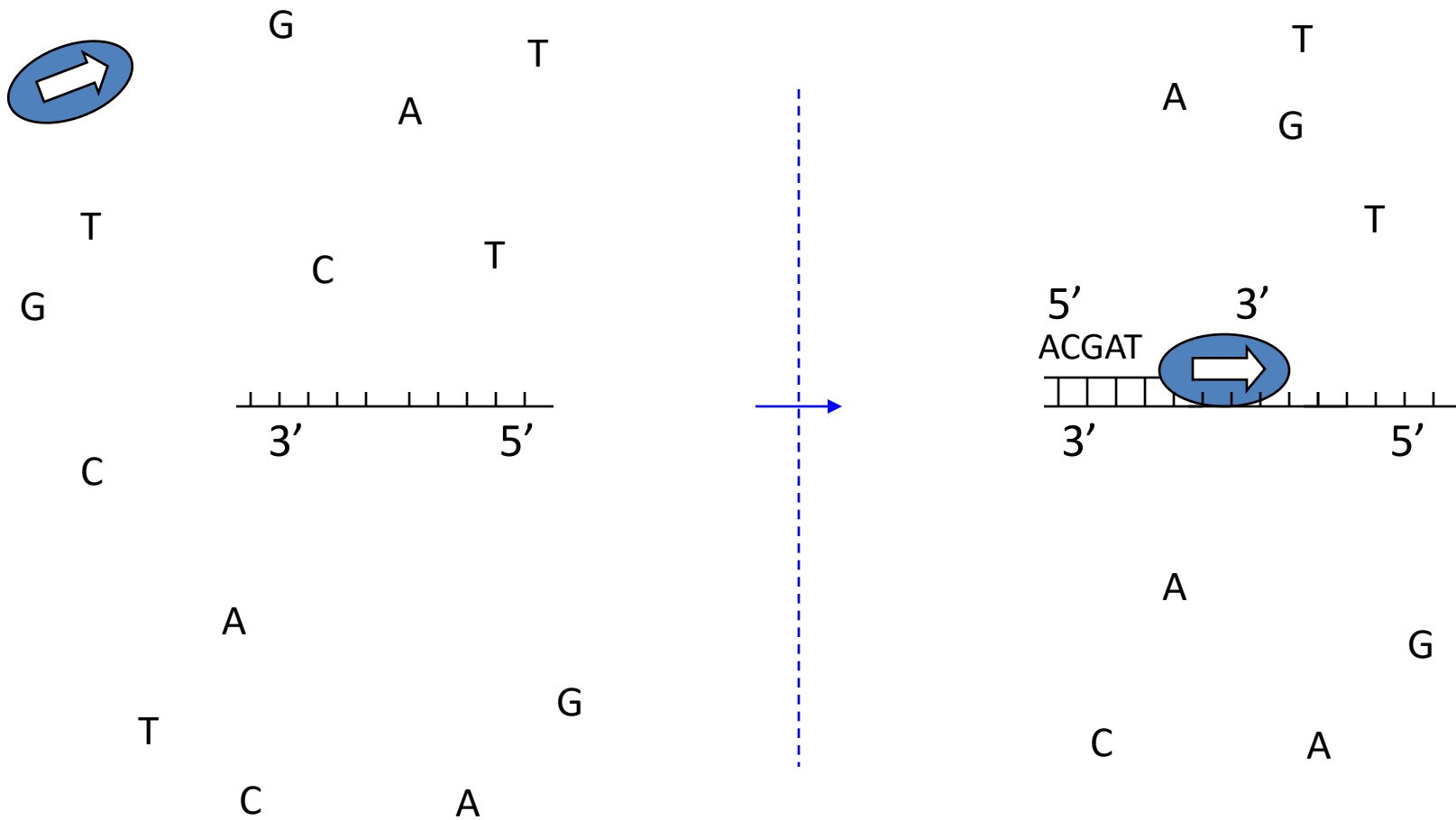


# Bio Interlude

DNA Replication

# DNA Replication: Basics



# Issues & Complications, I

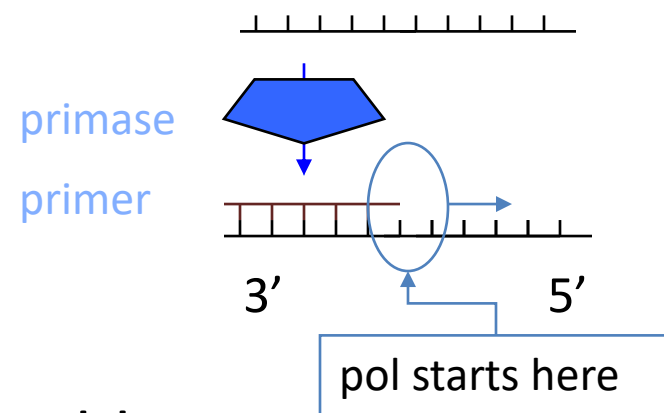
1st ~10 nt's added are called the *primer*

In simple model, DNA pol has 2 jobs: prime & extend

Priming is error-prone

So, specialized *primase* does the priming; pol specialized for fast, accurate extension

Still doesn't solve the accuracy problem (hint: primase makes an *RNA* primer)



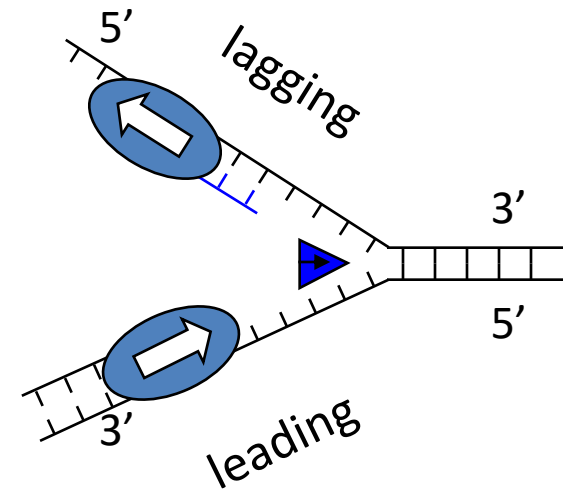
# Issue 2: Rep Forks & Helices

“Replication Fork”: DNA double helix is progressively unwound by a DNA **helicase**, and both resulting single strands are duplicated

DNA **polymerase** synthesizes new strand 5' -> 3' (reading its template strand 3' -> 5')

That means on one (the “leading”) strand, DNA pol is chasing/pushing the replication fork

But on the other “lagging” strand, DNA pol is running away from it.



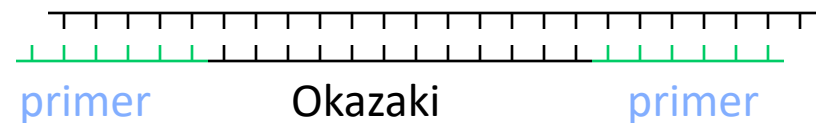
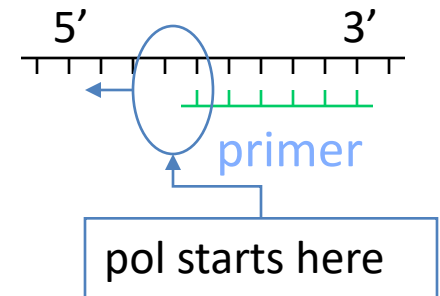
# Issue 3: Fragments

Lagging strand gets a series of “Okazaki fragments” of DNA (~200nt in eukaryotes) following each primer

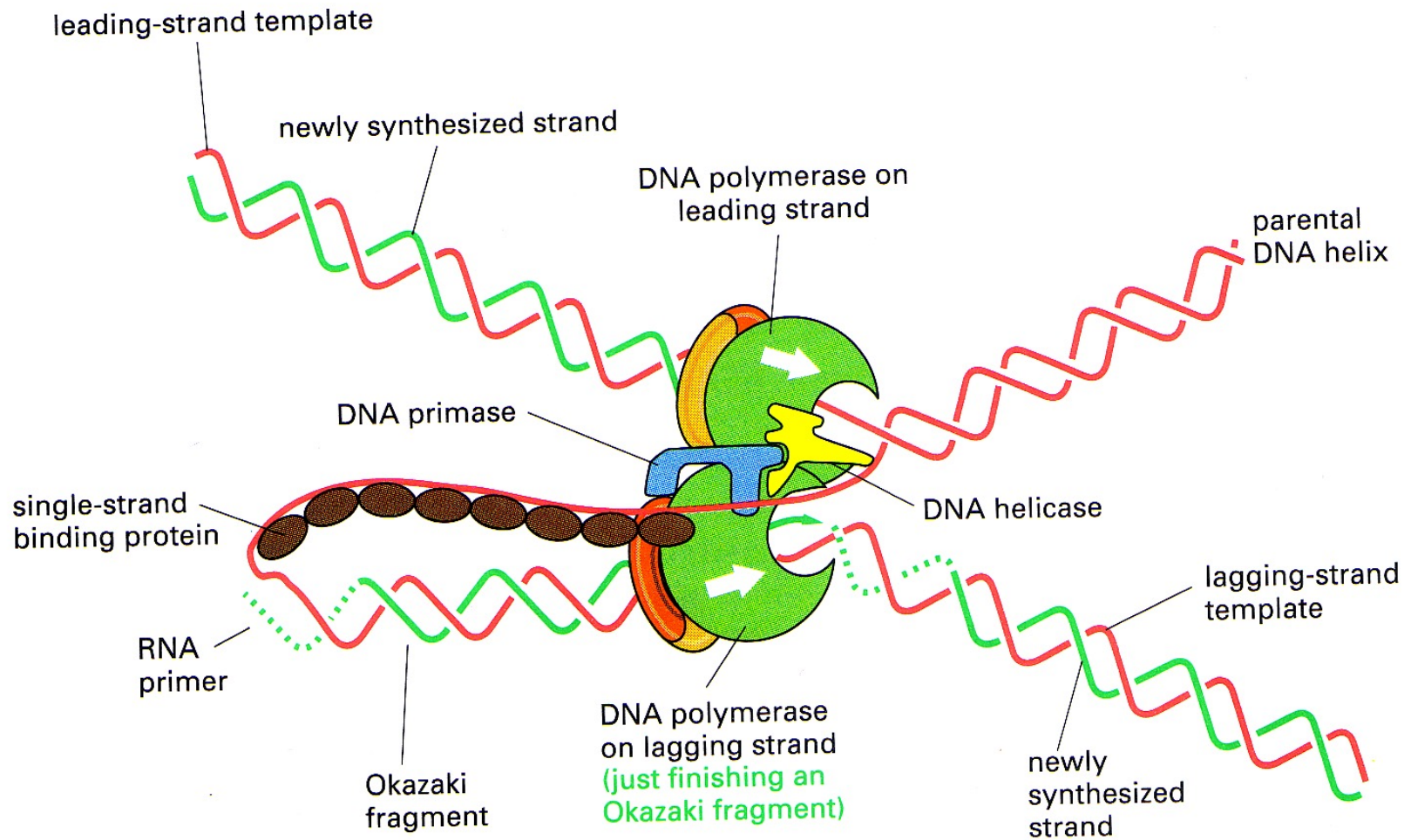
The RNA primers are later removed by a *nuclease* and *DNA pol*

fills gaps (more accurate than primase; primed by *DNA* from adjacent Okazaki frag

Fragments joined by *ligase*



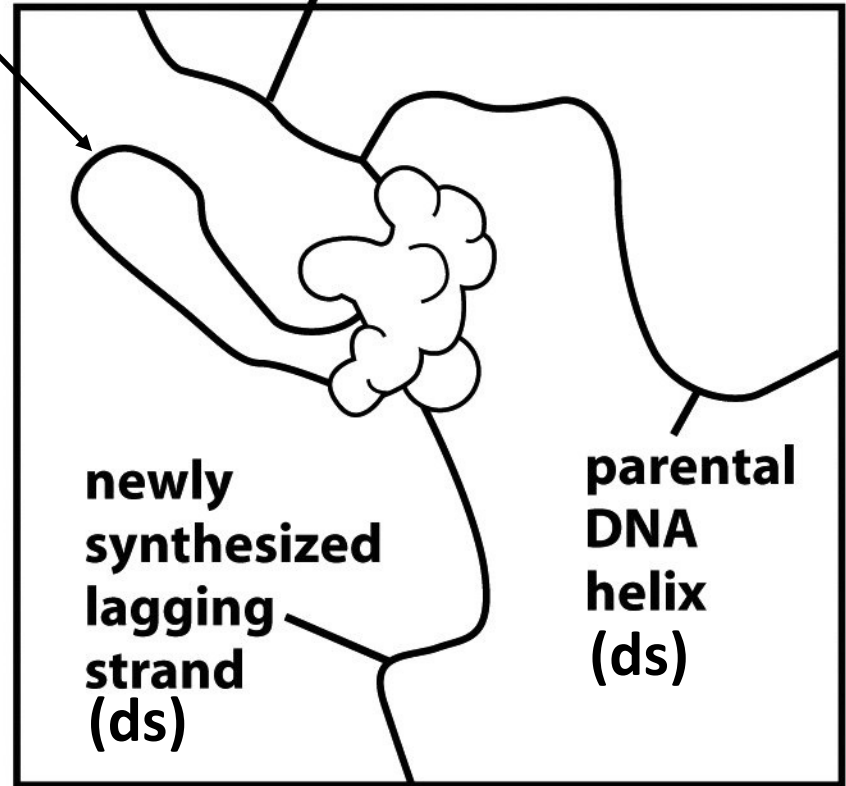
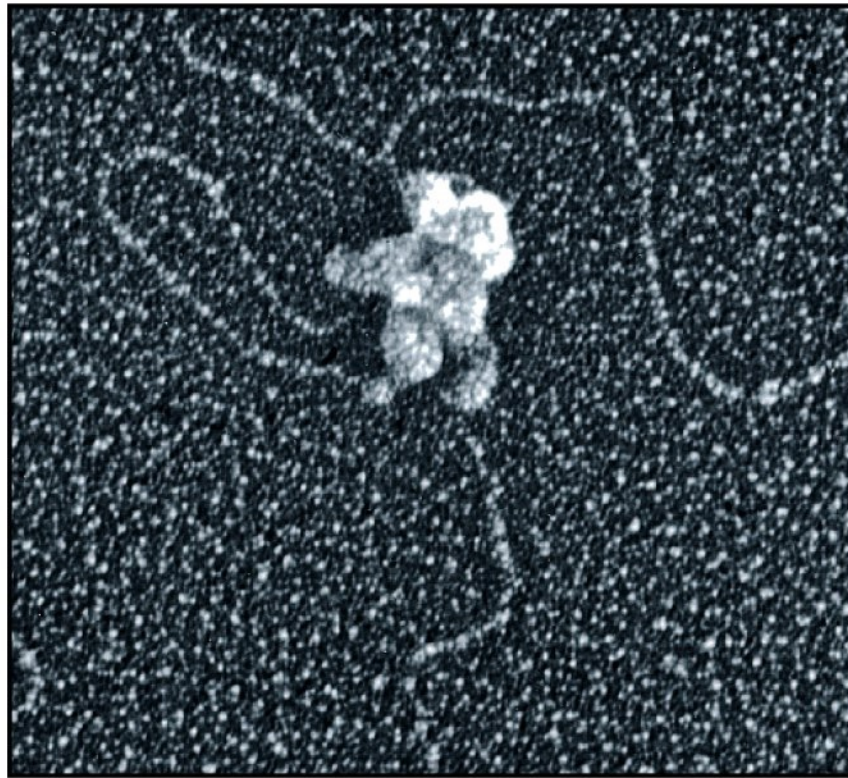
# Issue 4: Coord of Leading/Lagging



Alberts et al., Mol. Biol. of the Cell, 3rd ed, p258

Uncopied lagging  
stranded loop (ss)

newly synthesized  
leading strand (ds)



**(B)**

**(C)**

Figure 5-19bc Molecular Biology of the Cell 5/e (© Garland Science 2008)

# Very Nice DNA Repl. Animation

[https://www.youtube.com/watch?v=yqESR7E4b\\_8](https://www.youtube.com/watch?v=yqESR7E4b_8)

(Replication at about 1:41 – 2:50)





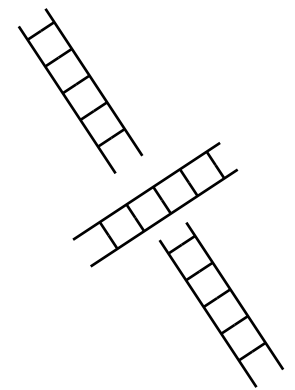
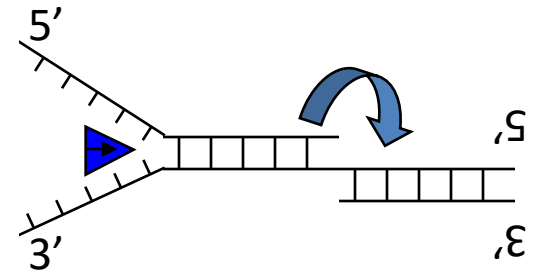
[www.wehi.edu.au](http://www.wehi.edu.au)

# Issue 5: Twirls & Tangles

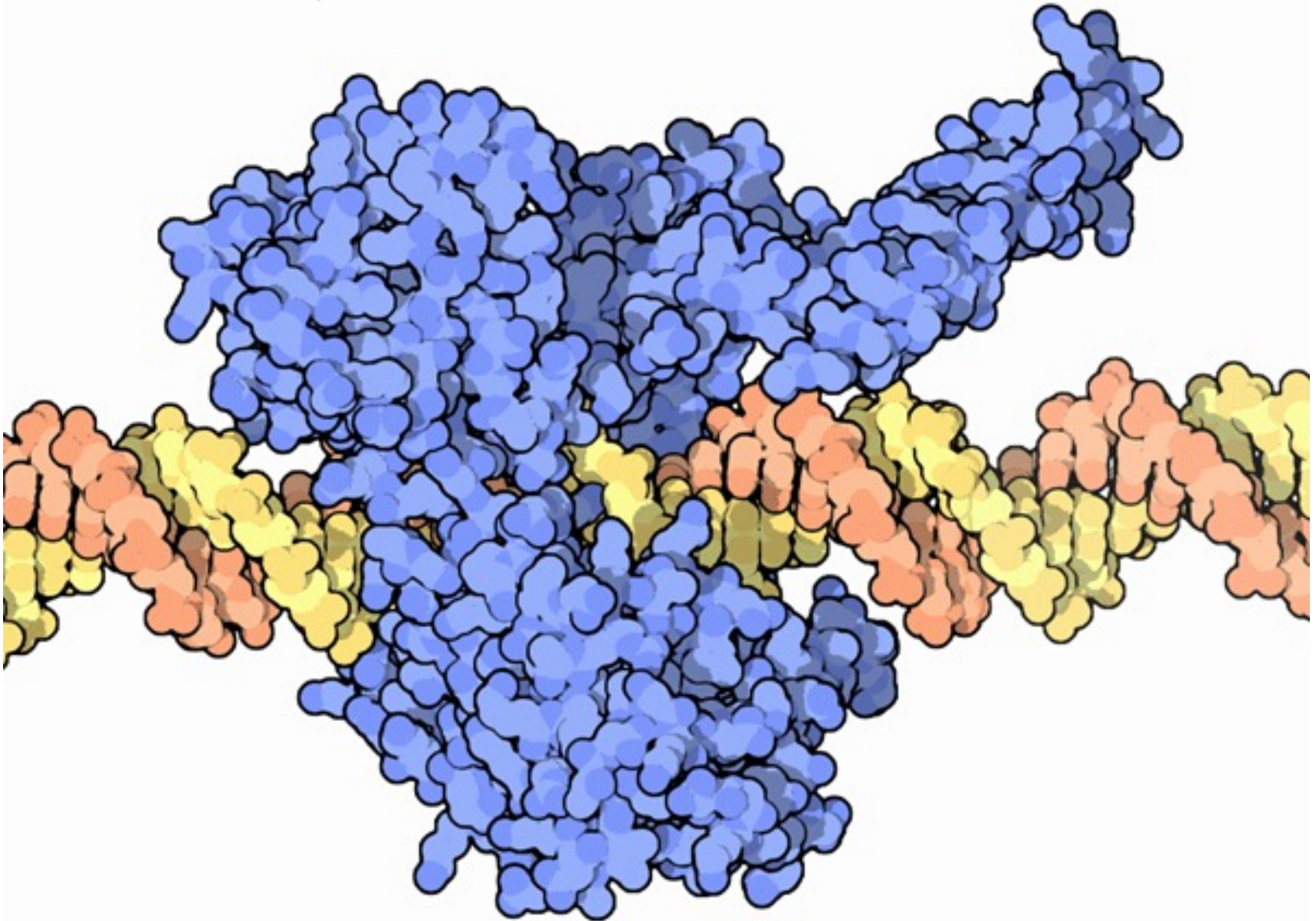
Unwinding helix (~10 nucleotides per turn) would cause stress.

*Topoisomerase I* cuts DNA backbone on *one* strand, allowing it to spin about the remaining bond, relieving stress

*Topoisomerase II* can cut & rejoin *both* strands, after allowing another double strand to pass through the gap, de-tangling it.



# Topoisomerase I + DNA



# Issue 6: Proofreading

Error rate of pol itself is  $\sim 10^{-4}$ , but overall rate is  $\approx 10^{-8} - 10^{-9}$ , due to proofreading & repair, e.g.

- pol itself can back up & cut off a mismatched base if one happens to be inserted

- priming the new strand is hard to do accurately, hence RNA primers, later removed & replaced

- other enzymes scan helix for “bulges” caused by base mismatch, figure out which strand is original, cut away new (faulty) copy; DNA pol fills gap

- which strand is original? Bacteria: “methylate” some A’s, eventually. Euks: strand nicking

# Replication Summary

Speed: 50 (eukaryotes) to  
500 (prokaryotes) bp/sec

Accuracy: 1 error per  $10^9$  bp

Complex & highly optimized

Highly similar across all living cells

More info:

Alberts et al., *Mol. Biol. of the Cell*