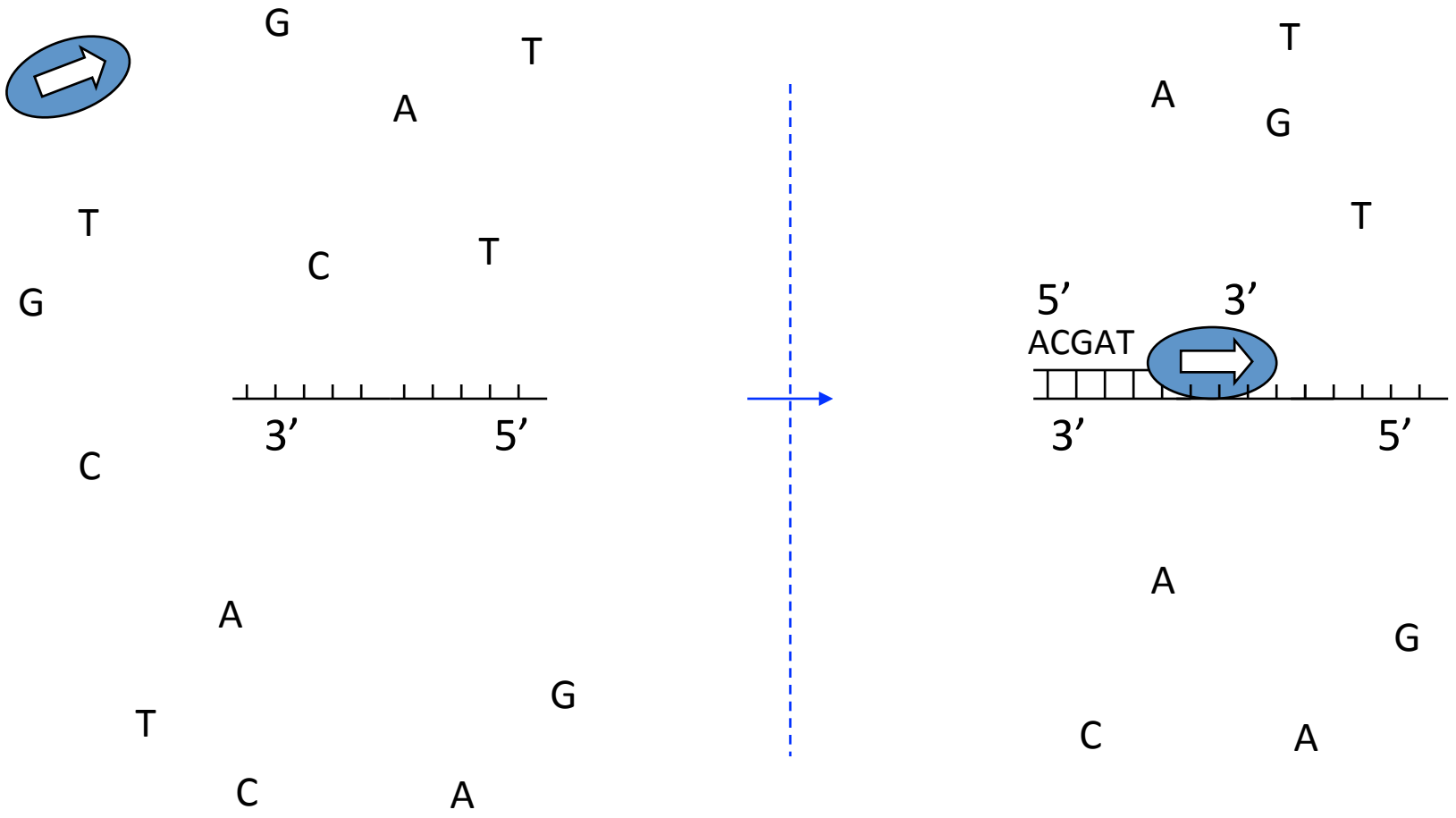
Bio Interlude

DNA Replication

DNA Replication: Basics



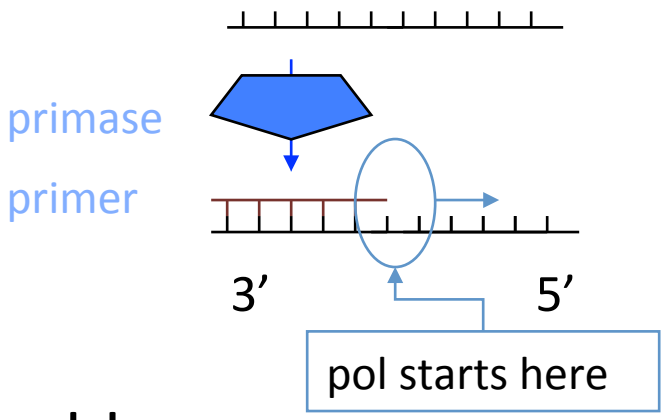
Issues & Complications, I

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)

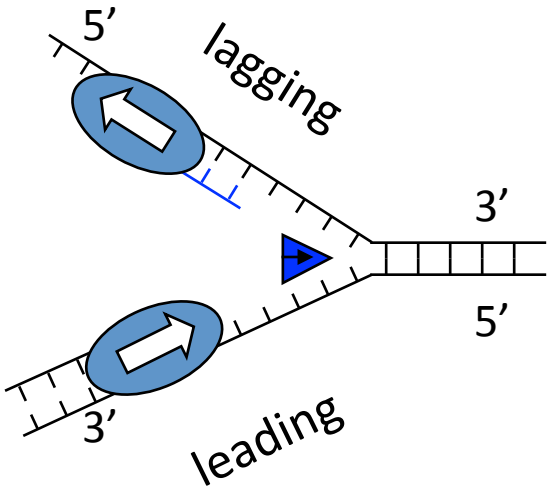
Issue 2: Rep Forks & Helices

"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')

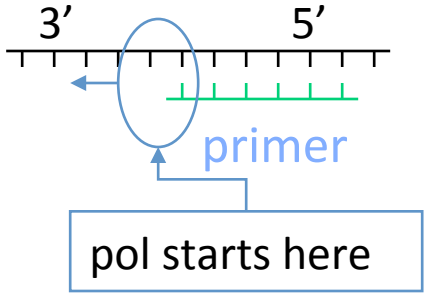
That means on one (the "leading") strand, DNA pol is chasing/pushing the replication fork

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

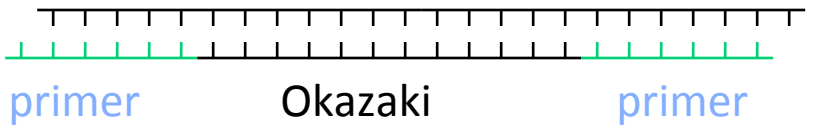


Issue 3: Fragments

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

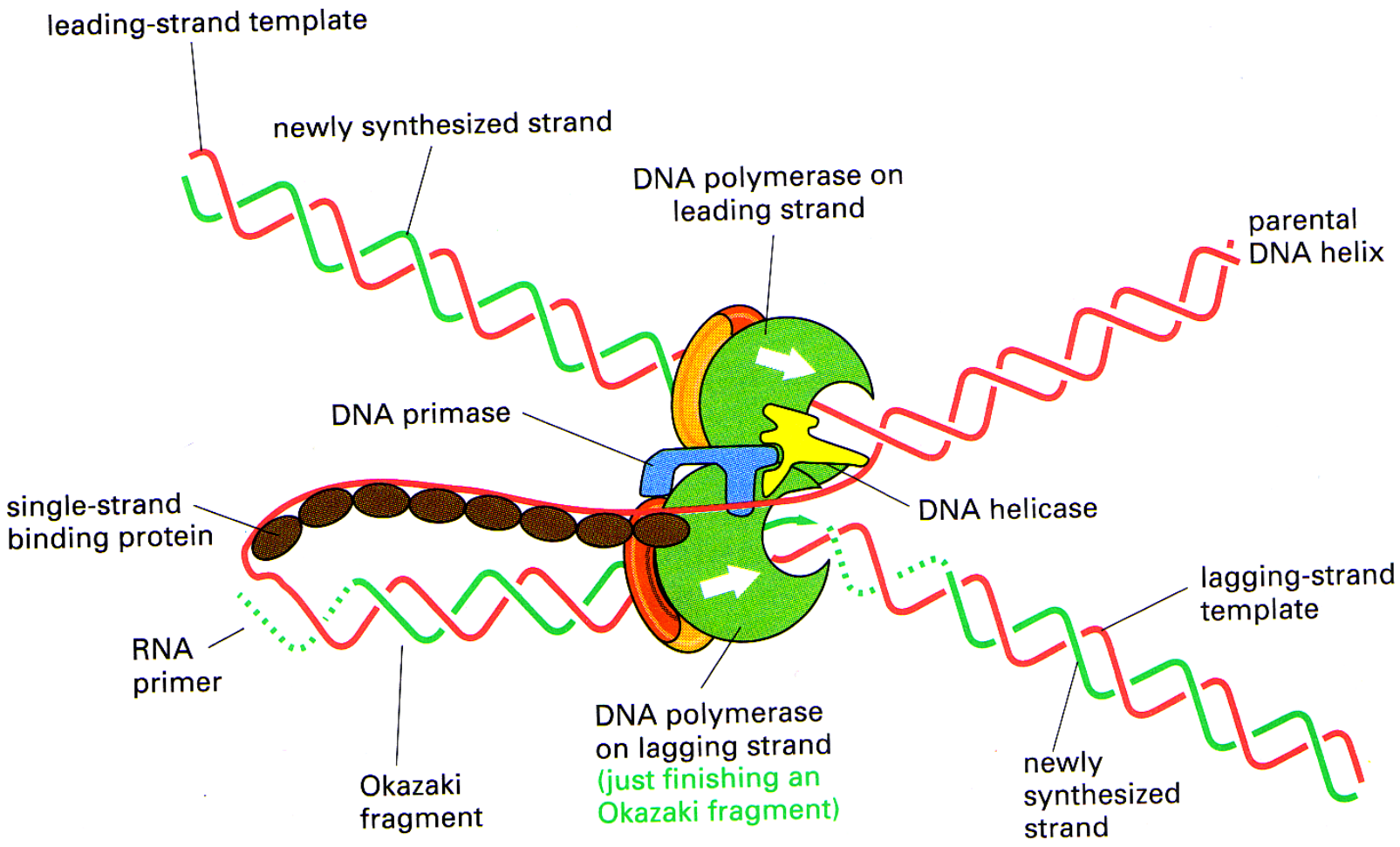


The RNA primers are later removed by a *nuclease* and *DNA* pol fills gaps (more accurate than primase; primed by *DNA* from adjacent Okazaki frag

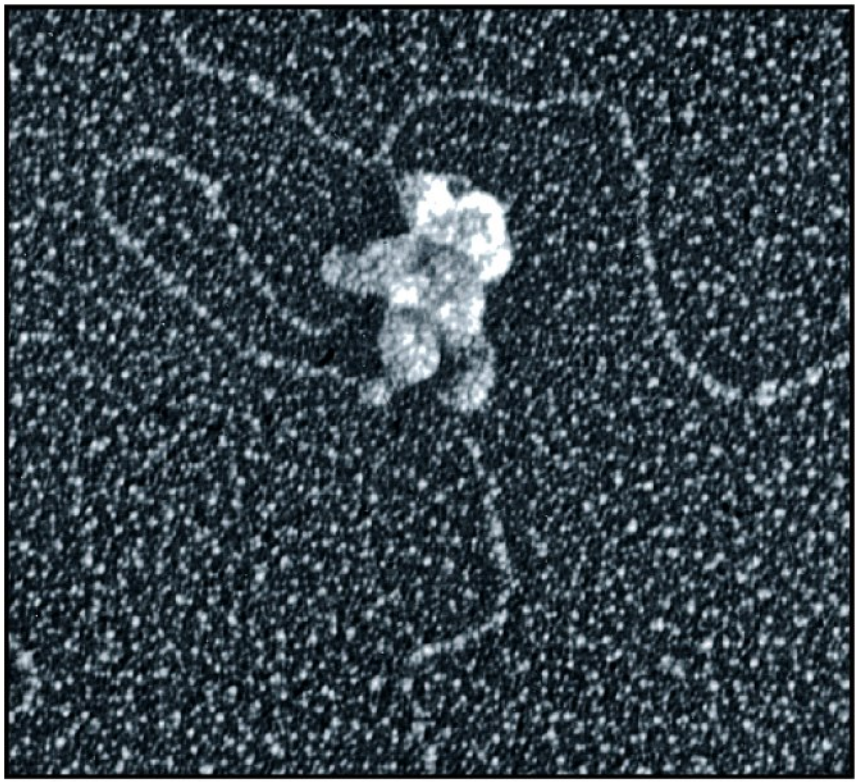


Fragments joined by *ligase*

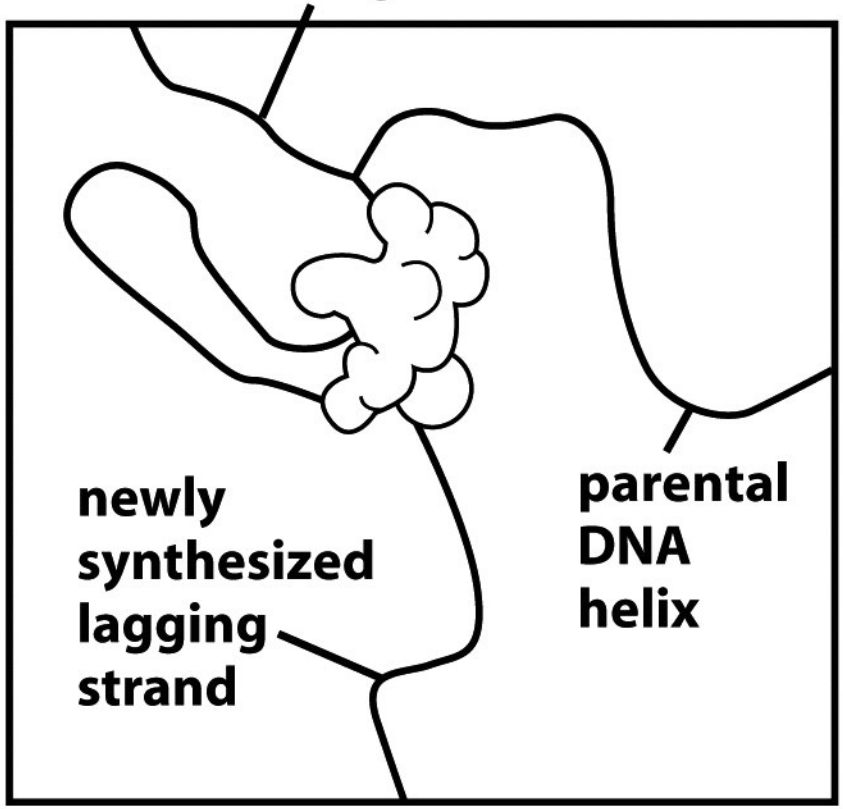
Issue 4: Coord of Leading/Lagging



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



(B)



(C)

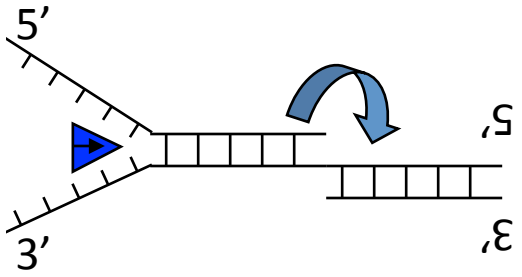
Figure 5-19bc Molecular Biology of the Cell 5/e (© Garland Science 2008)

https://www.youtube.com/watch?v=yqESR7E4b_8

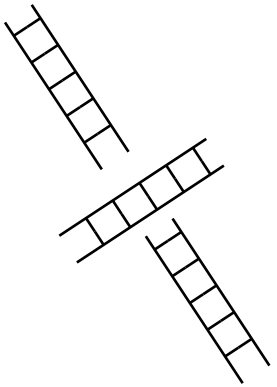
(Replication starts at about 1:40)

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.



Issue 6: Proofreading

Error rate of pol itself is ~10^{-4}, but overall rate is 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

- Nobel prize in chem 2015, for DNA damage repair

- http://www.nytimes.com/2015/10/08/science/tomas-lindahl-paul-modrich-aziz-sancarn-nobel-chemistry.html?_r=0

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*