# Advanced Cell Biology. Lecture 15

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# Outline

How proteins are controlled

How proteins are studied

DNA replication

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DNA replication

# Previous final question: the answer

Which way of sedimentation employs differences between molecular sizes?

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Which way of sedimentation employs differences between molecular sizes?

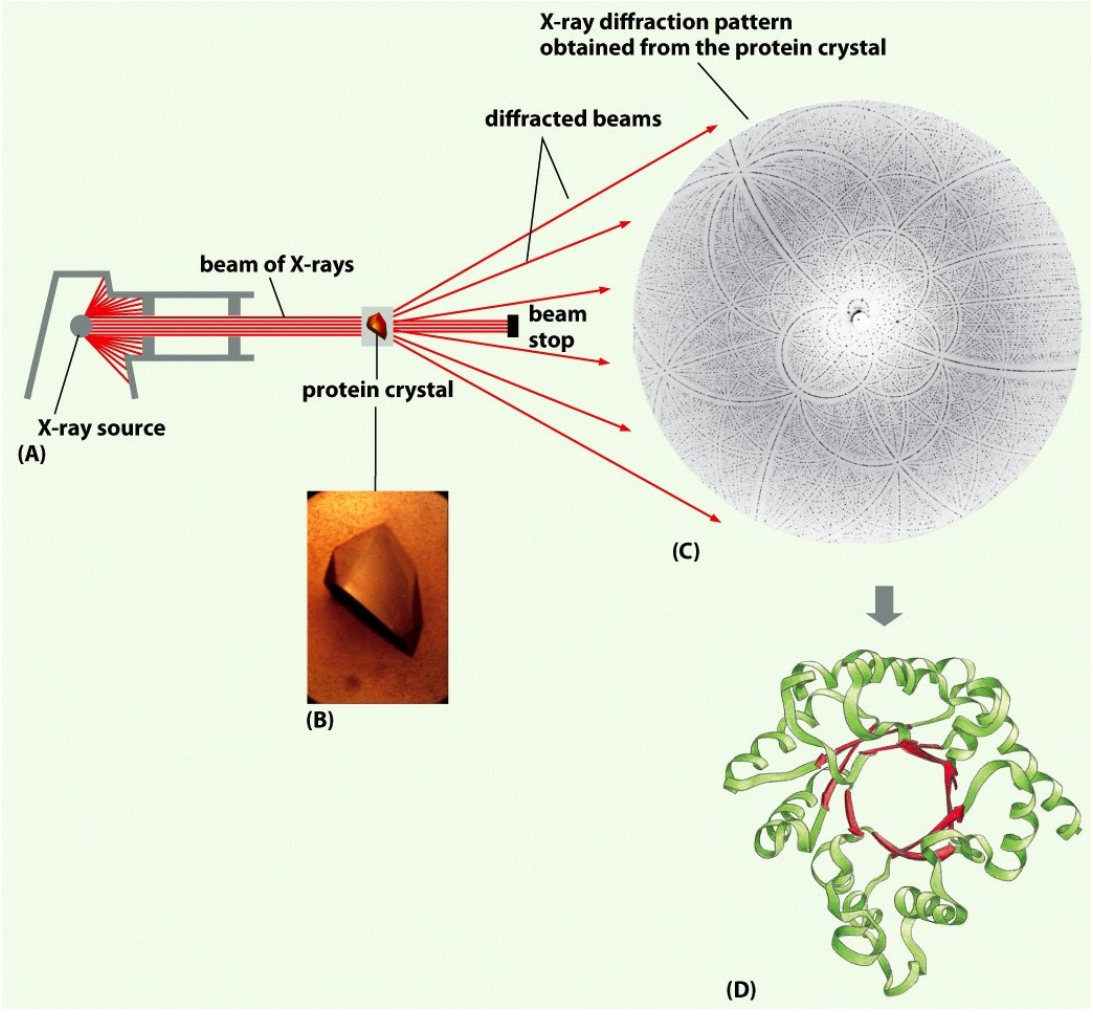
- Velocity sedimentation: larger proteins sediment faster

Safe crackers animation

# X-rays crystallography

- Based on the ability of proteins to form crystals
- These crystals will scatter X-rays and we will see difraction pattern, different for different proteins
- Useful for prediction of protein 3D structure

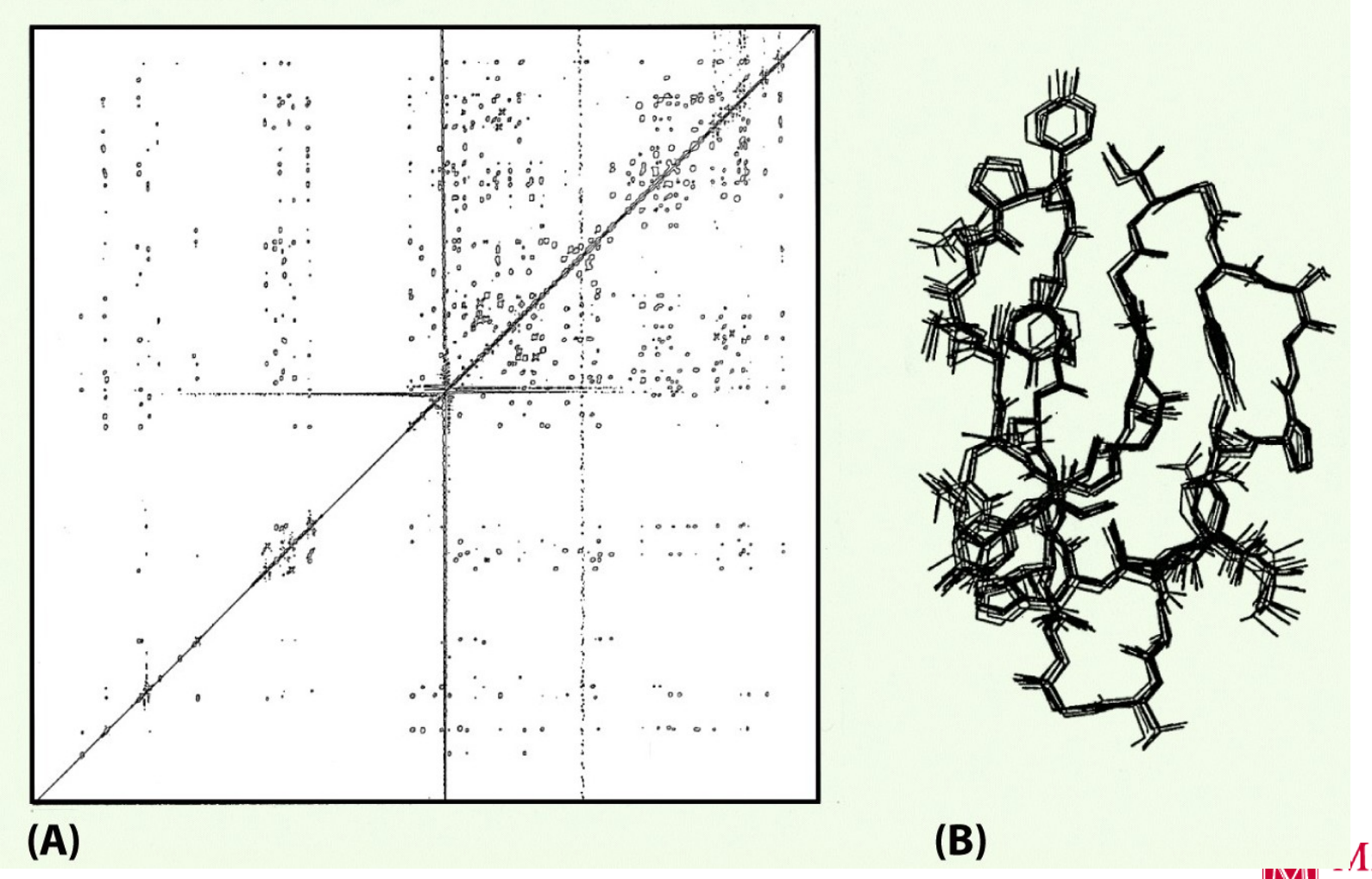
# X-rays crystallography



# Nuclear magnetic resonance (NMR)

- Unfortunately, not all proteins form crystals and it is not always possible to obtain enough protein for crystallization
- Method is based on the response of nuclei to radio waves
- Again, it is used mostly for understanding conformation of protein

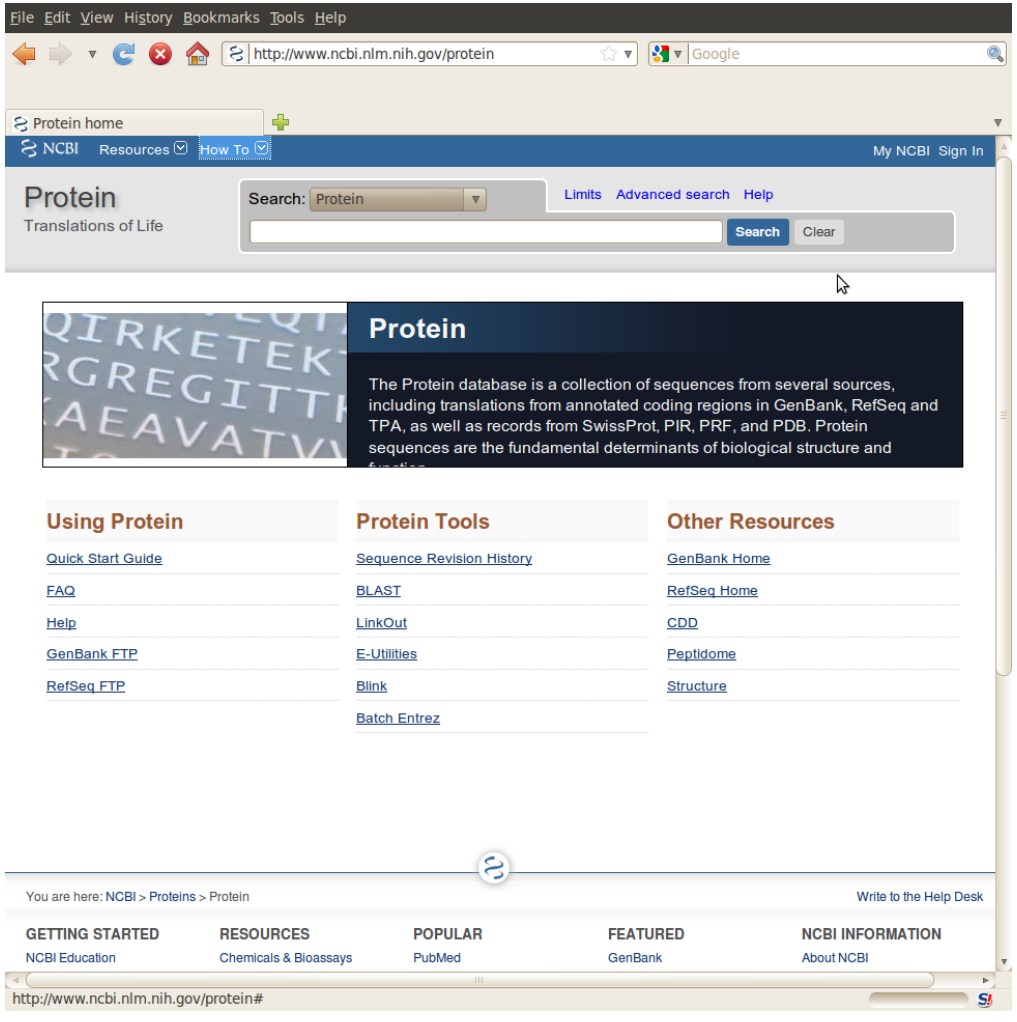
# Nuclear magnetic resonance



# Protein databases

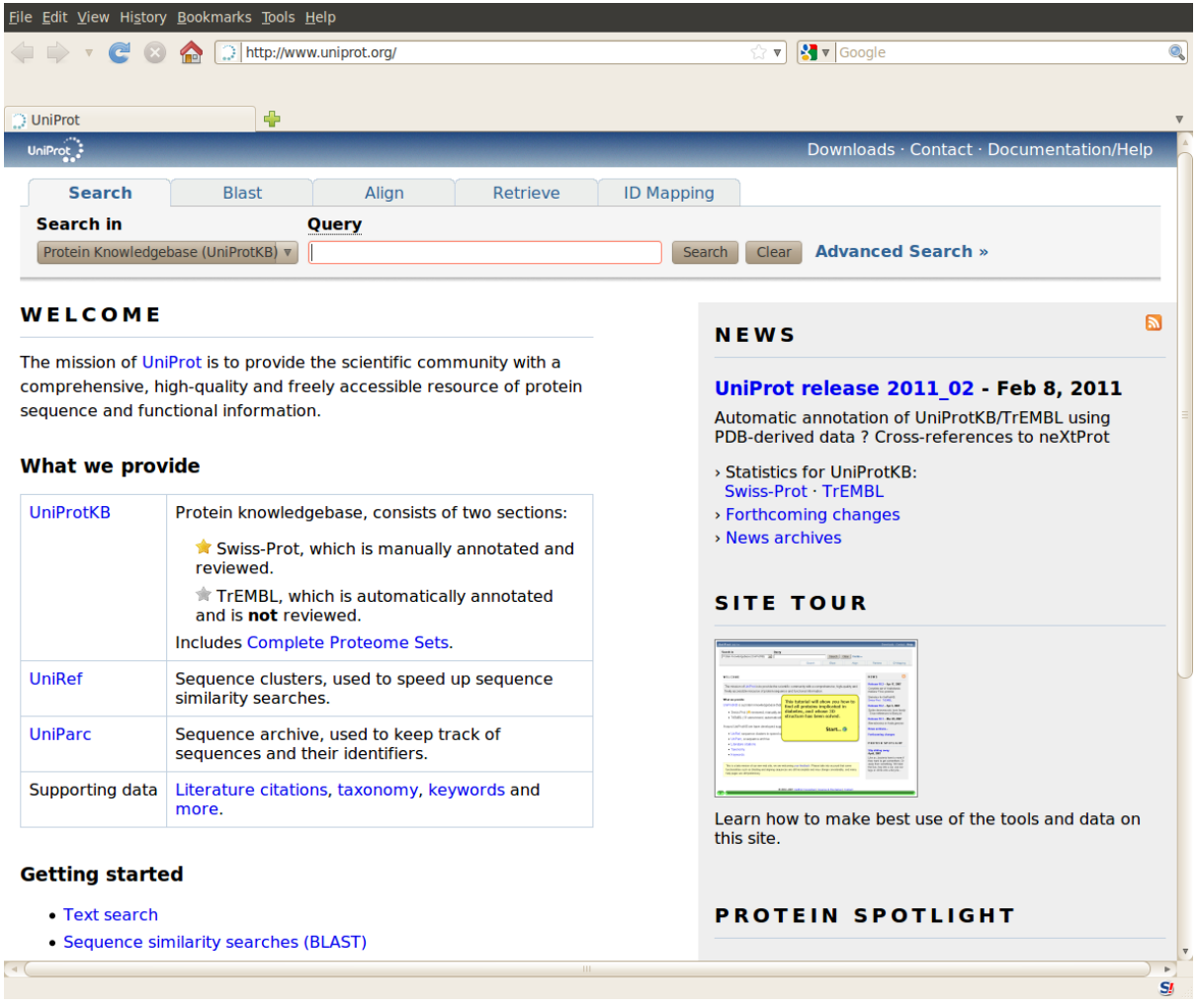
- UniProt
- NCBI protein
- RCSB

# NCBI Protein database



# RCSB database

# UniProt database



# Template

- Both DNA strands may act as a template for the synthesis of other strand
- Template hypothesis was first expressed by Nikolaj Koltsov in 1927
- Replication is semiconservative

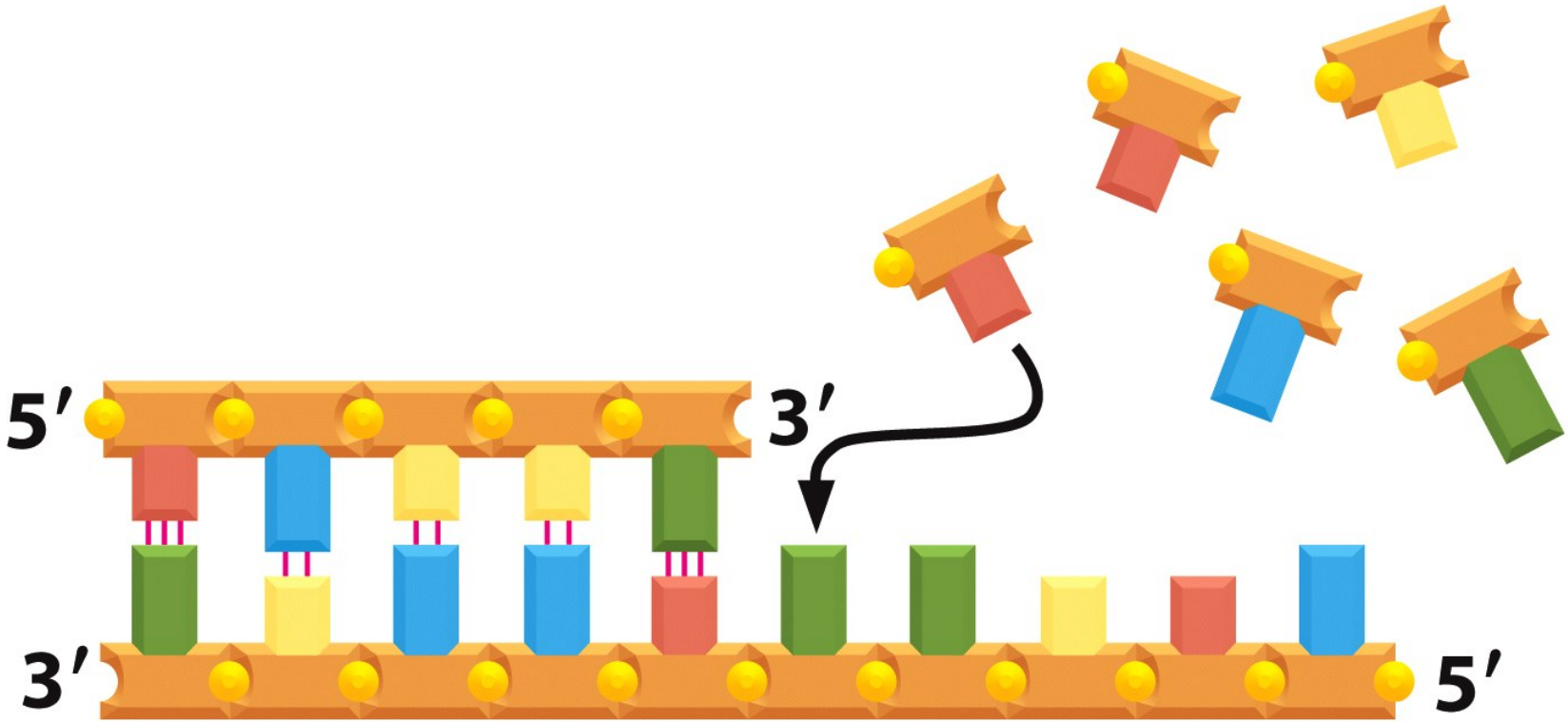



Figure 6-2 Essential Cell Biology 3/e (© Garland Science 2010)

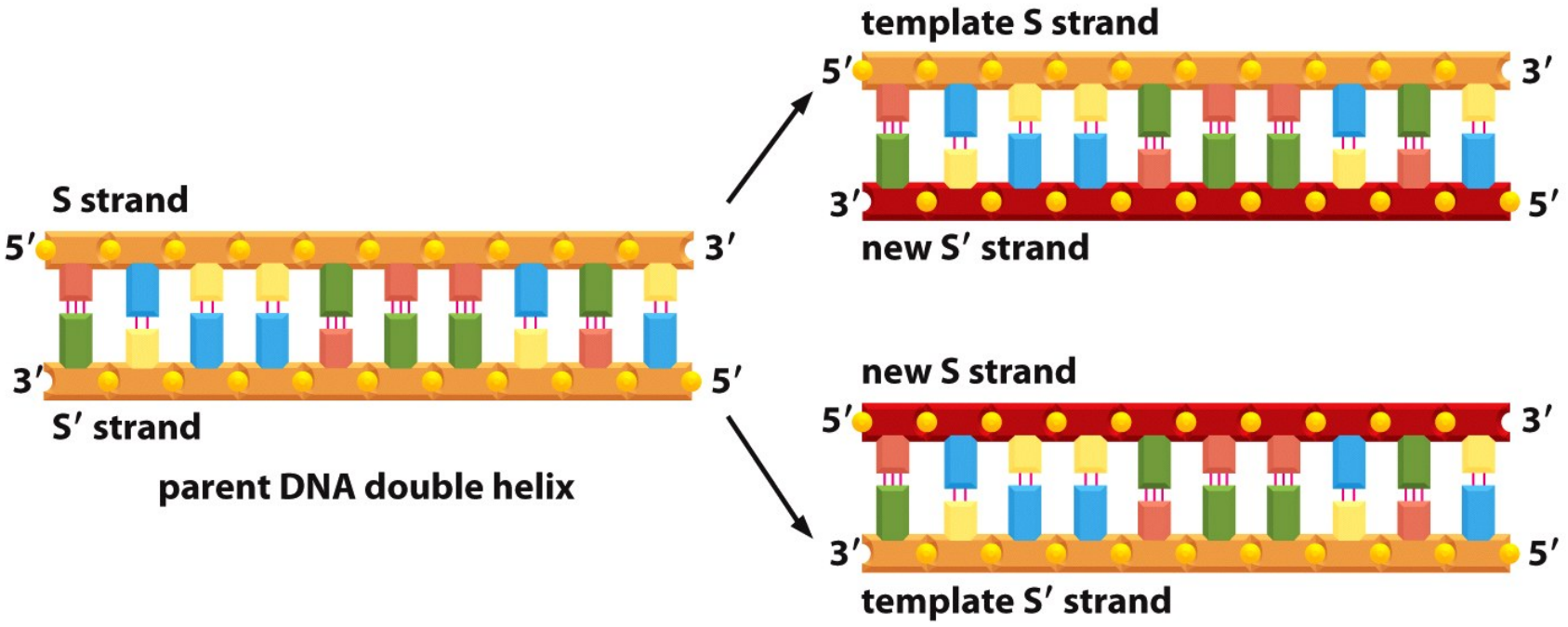



Figure 6-3 Essential Cell Biology 3/e (© Garland Science 2010)

# Meselson-Stahl experiment (1958)

- Theoretically, three variants of replications were possible: semiconservative, dispersive and conservative
- Experiment was based on two bacterial cultures which grew on different media: with normal nitrogen $^{14}N$, and with heavy nitrogen, $^{15}N$
- After growing for 20' on heavy medium, bacteria produce DNA molecules with intermediate weight *only*
- That ruled out conservative hypothesis.

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- After growing for 20' on heavy medium, bacteria produce DNA molecules with intermediate weight *only*
- That ruled out conservative hypothesis. How to rule out dispersive hypothesis?—Explanation has been given on lecture.

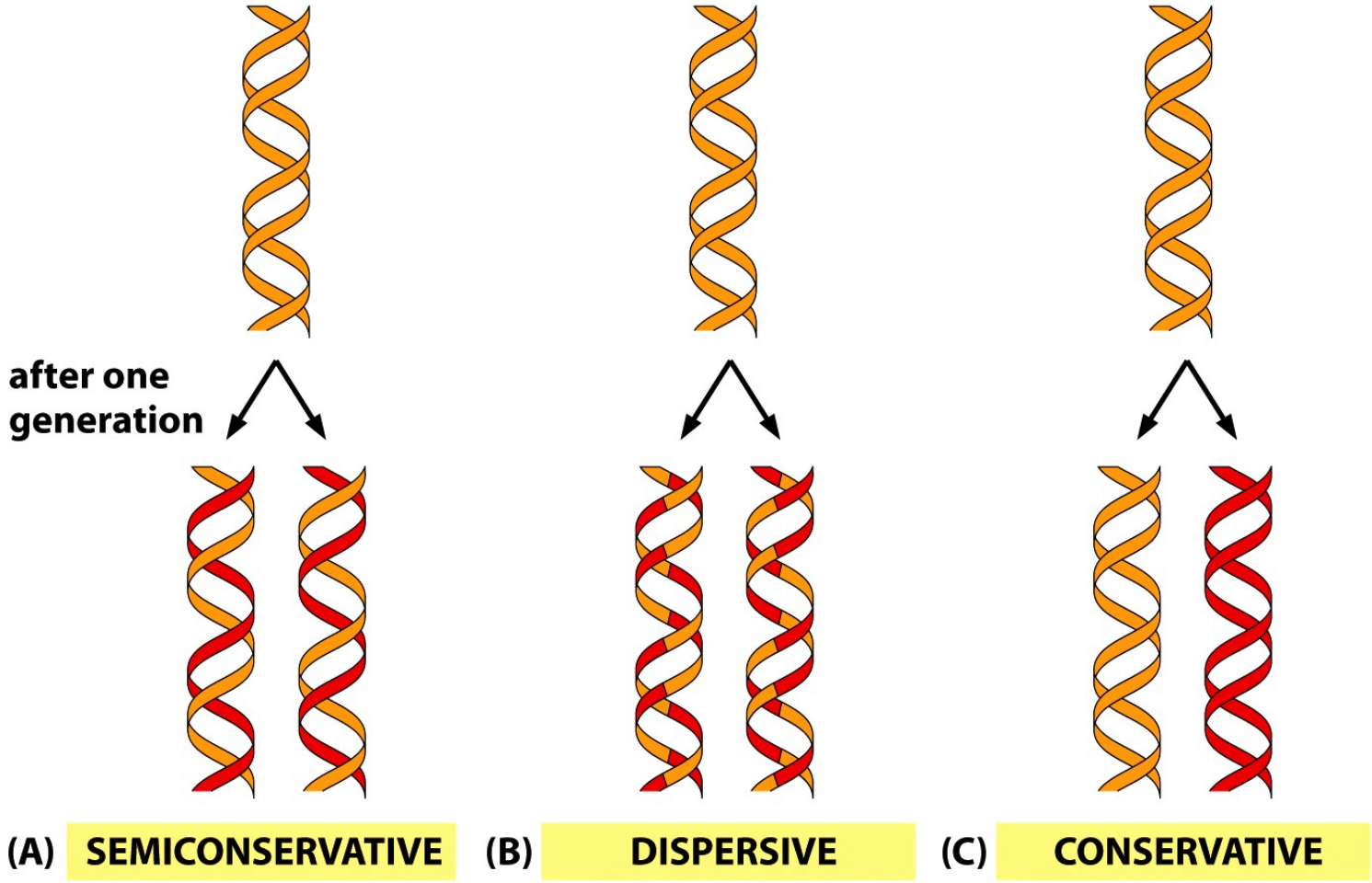


Figure 6-6 Essential Cell Biology 3/e (© Garland Science 2010)

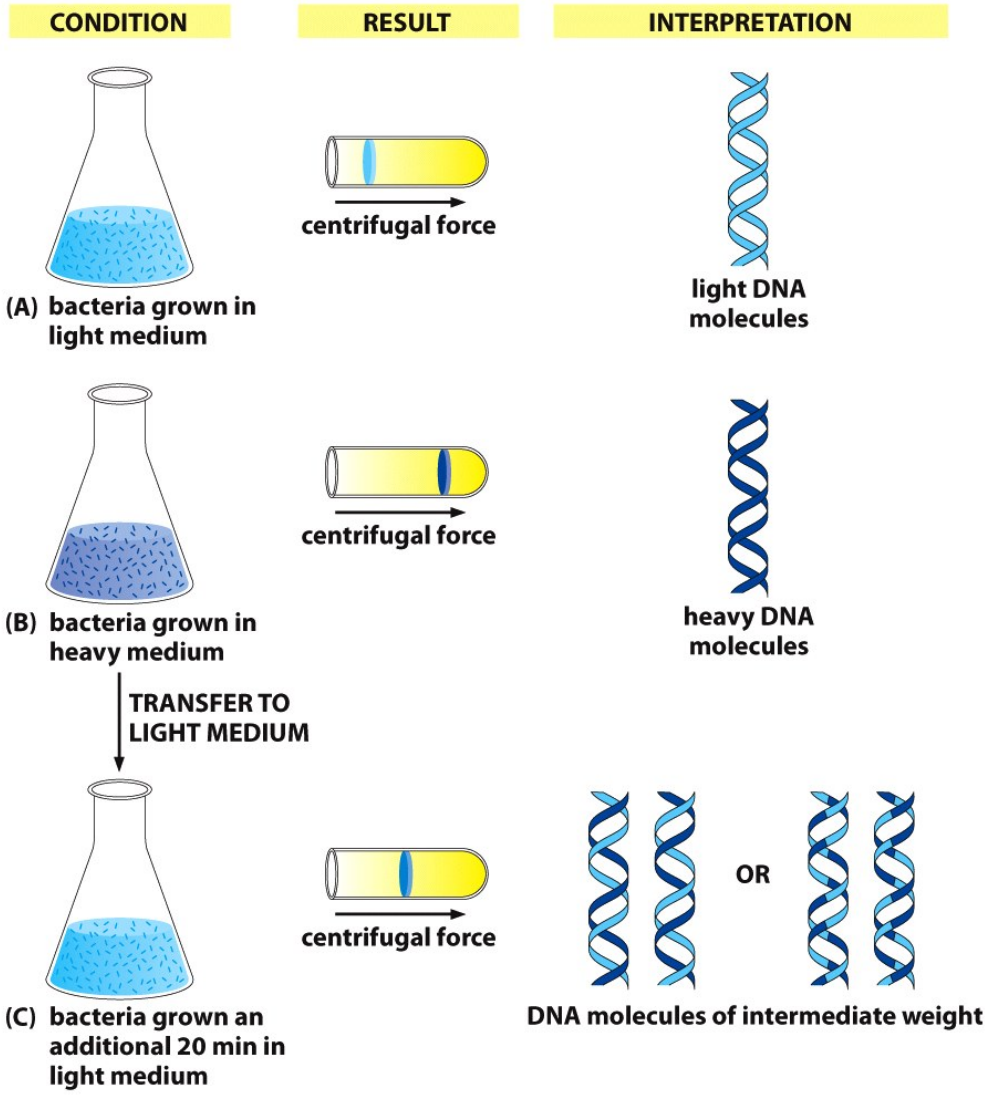


Figure 6-8 Essential Cell Biology 3/e (© Garland Science 2010)

# Replication origin and forks

- DNA double helix should open for replication: this is a replication origin place
- When replication starts, these openings will grow and form replication forks which are visible under microscope

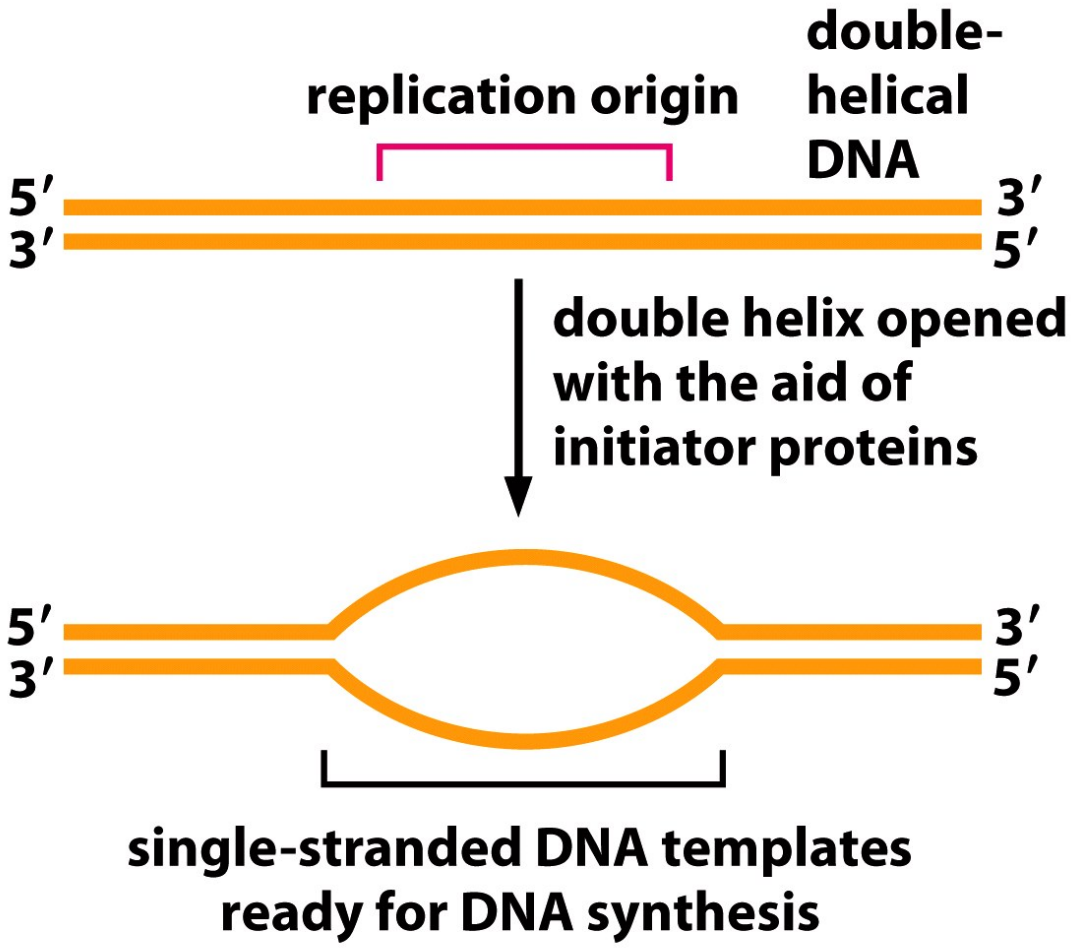


Figure 6-5 Essential Cell Biology 3/e (© Garland Science 2010)

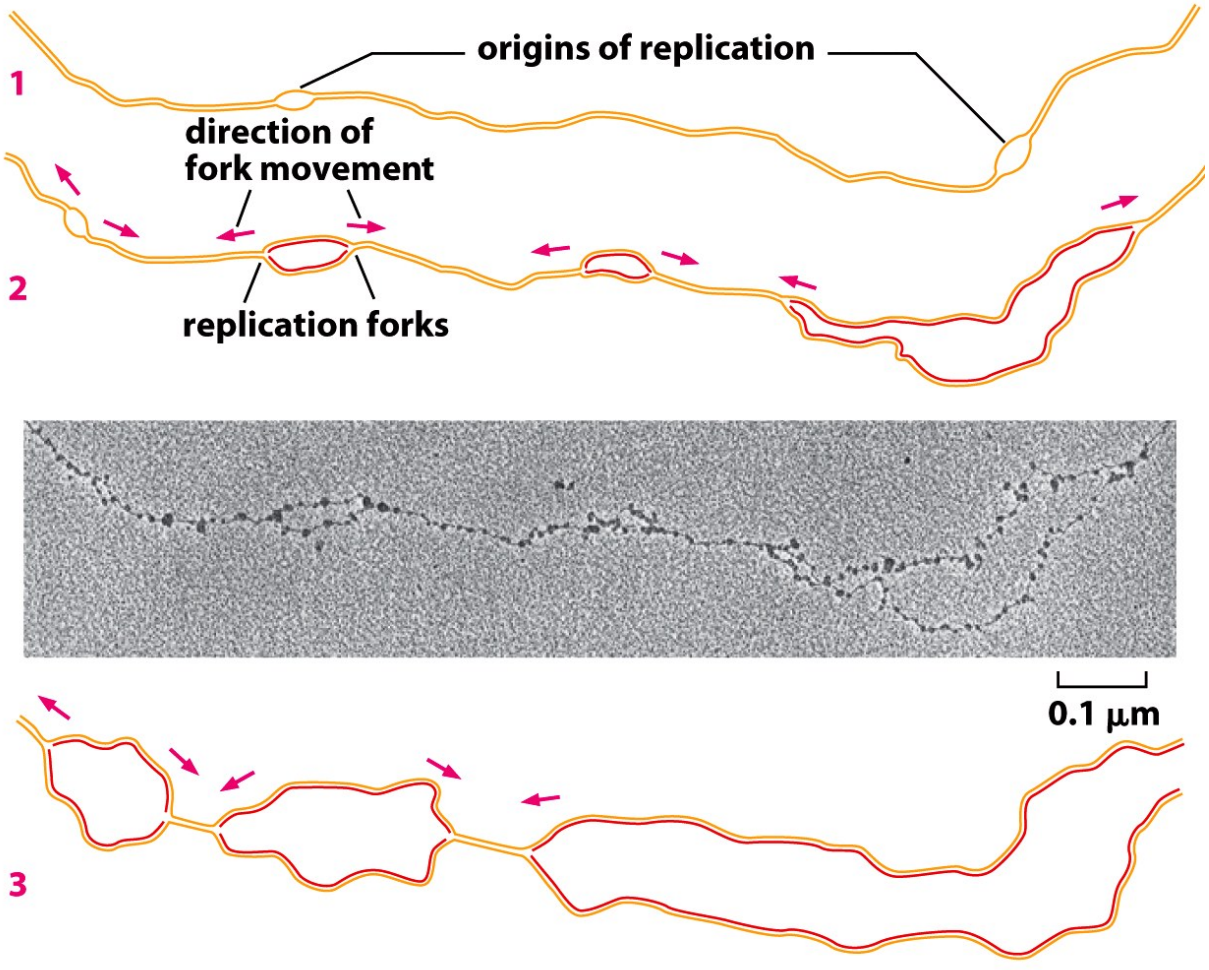


Figure 6-9 Essential Cell Biology 3/e (© Garland Science 2010)

# Lagging and leading strands

- **DNA is synthesized only in 5'-to-3' direction**. Structure of DNA and complexity of replication do not allow the other direction.
- Therefore, fork is asymmetrical: one strand is replicating smoothly whereas other strand (lagging) replicated by "leaps", still 5'-to-3'
- Every "leap" produce one Okazaki fragment which are later joined

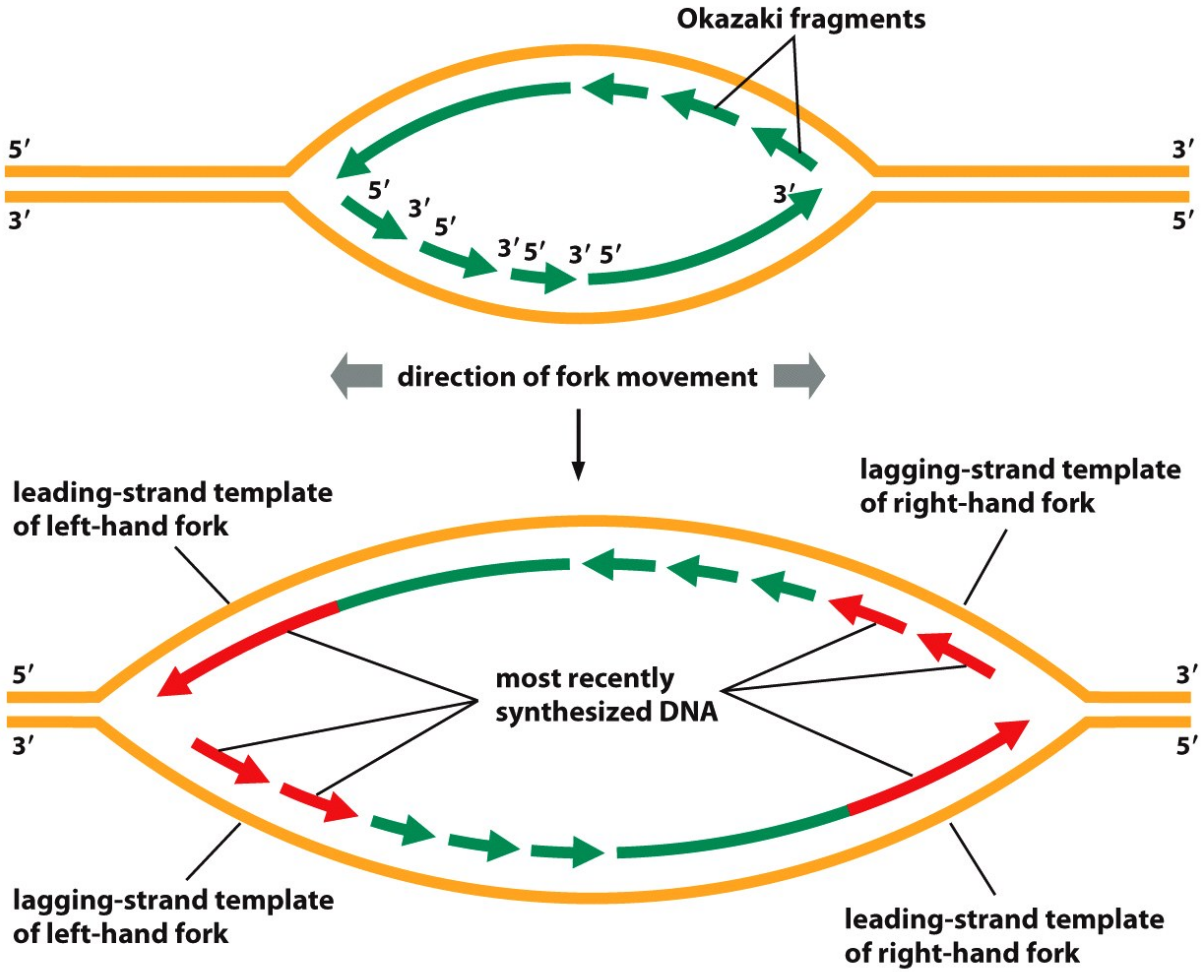


Figure 6-12 Essential Cell Biology 3/e (© Garland Science 2010)

# DNA proofreading

- DNA polymerase in the main replication enzyme with error rate of $\approx$0.00000001
- Every time DNA polymerase adds new nucleotide, it checks if previous was correctly placed (if not, it removes the wrong nucleotide)
- Two different binding sites in DNA polymerase work for synthesis and proofreading
- Proofreading goes in opposite, 3'-to-5' direction

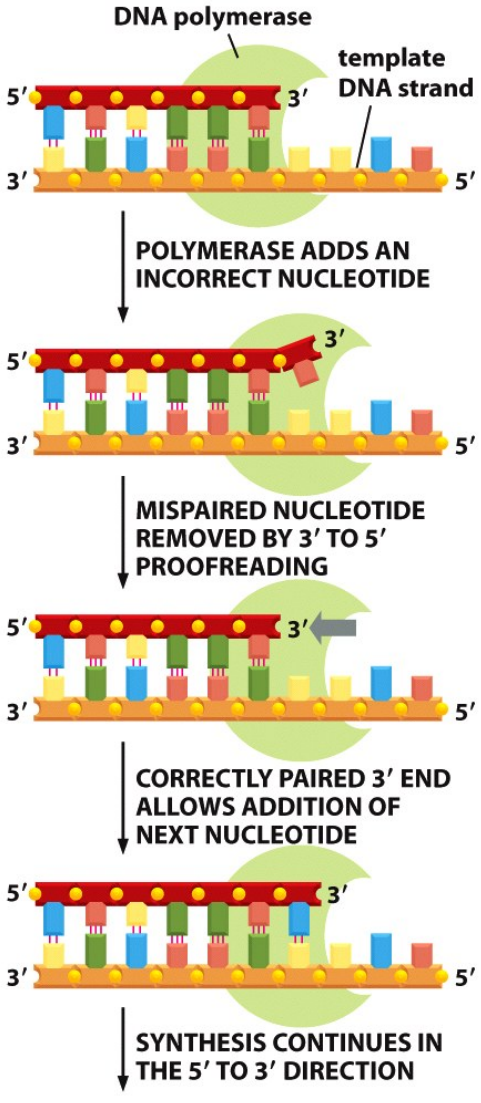


Figure 6-13 Essential Cell Biology 3/e (© Garland Science 2010)

DNA polymerase movie

# RNA primers

- DNA polymerase cannot start nucleotide chain itself
- Instead, primase enzyme synthesize small RNA primer ($\approx$10 nucleotides) which used as a starting point
- Primase error rate is $\approx$0.0001 (!)
- In lagging strand, RNA sites are interleaving with DNA fragments
- Then RNA sites are erasing, and DNA ligase joins fragments together

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- Then RNA sites are erasing, and DNA ligase joins fragments together
- Why primer is RNA?

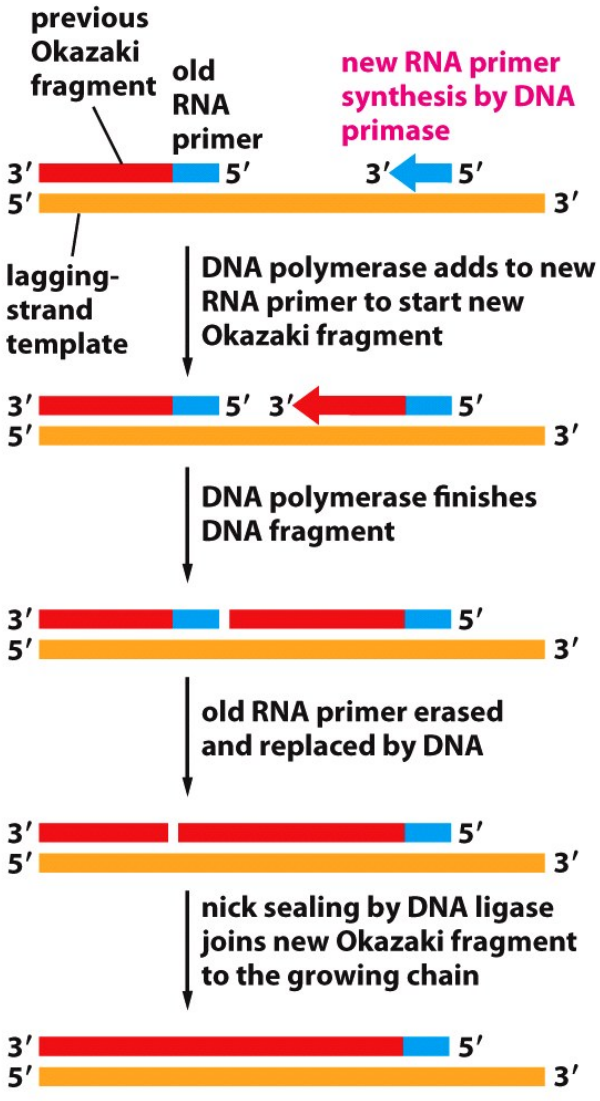


Figure 6-16 Essential Cell Biology 3/e (© Garland Science 2010)

# Final question (2 points)

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Why cells use RNA as DNA replication primers?

# Summary

- DNA replication is a semiconservative process
- DNA replication could go only in one direction
- Proofreading and RNA priming are helping in replication

# For Further Reading

A. Shipunov.
*Advanced Cell Biology* [Electronic resource].
2011—onwards.
Mode of access: `http://ashipunov.info/shipunov/school/biol_250`

B. Alberts et al.
*Essential Cell Biology*. 3rd edition.
Garland Science, 2009.
*Chapter 6*, pages 198–208.