ZOO 203 – MOLECULAR BIOLOGY
UNIT 1: DNA REPLICATION
(PART –IV)

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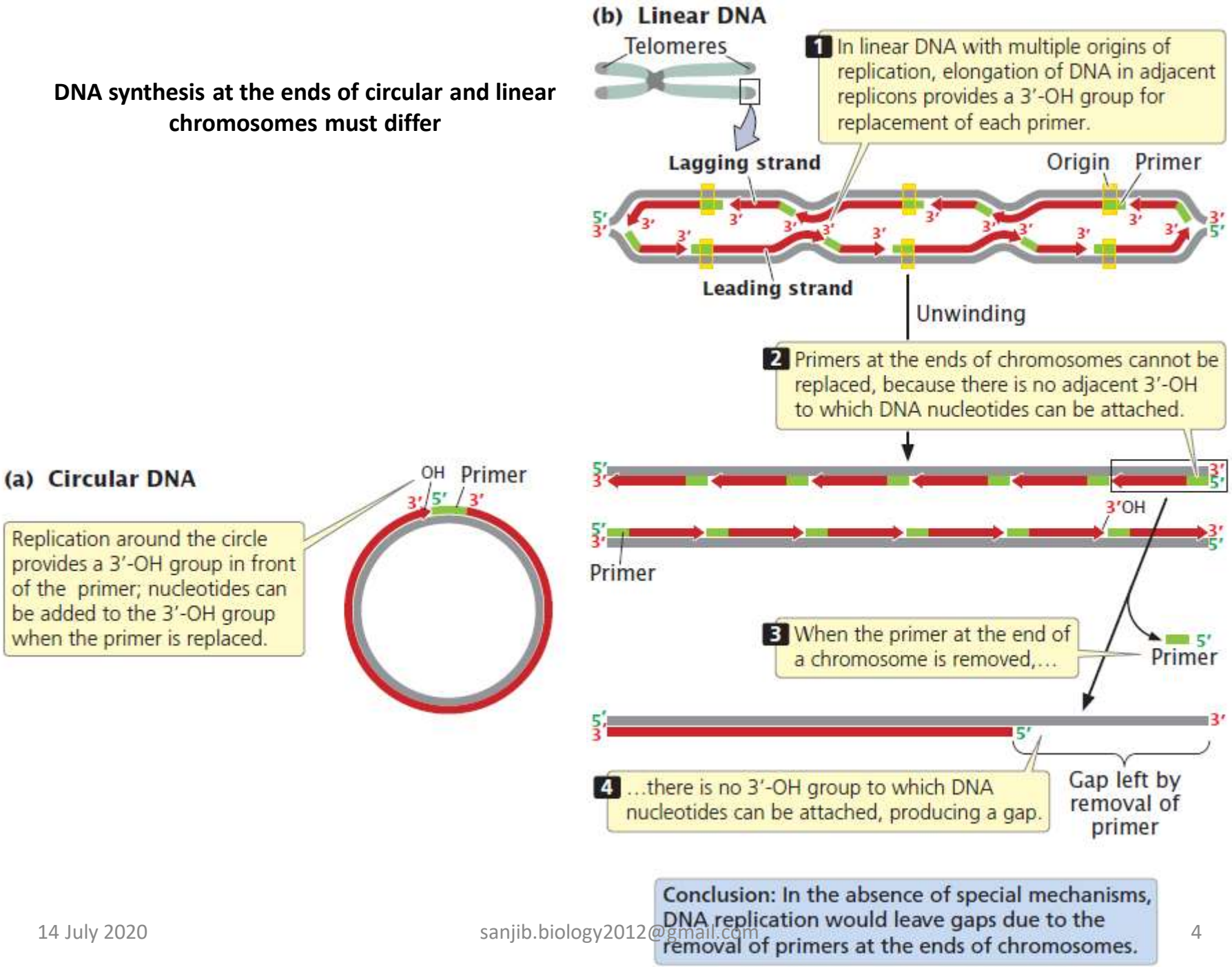
FINISHING REPLICATION

- Completion of DNA replication requires a set of specific events.
- These events are different for **circular** versus **linear** chromosomes.
- For a circular chromosome, the conventional replication fork machinery replicates the entire molecule, but the resulting daughter molecules are topologically linked to each other.
- In contrast, the replication fork machinery cannot complete replication of the very ends of linear chromosomes.
- Therefore, organisms containing linear chromosomes have developed novel strategies to replicate their chromosome ends.

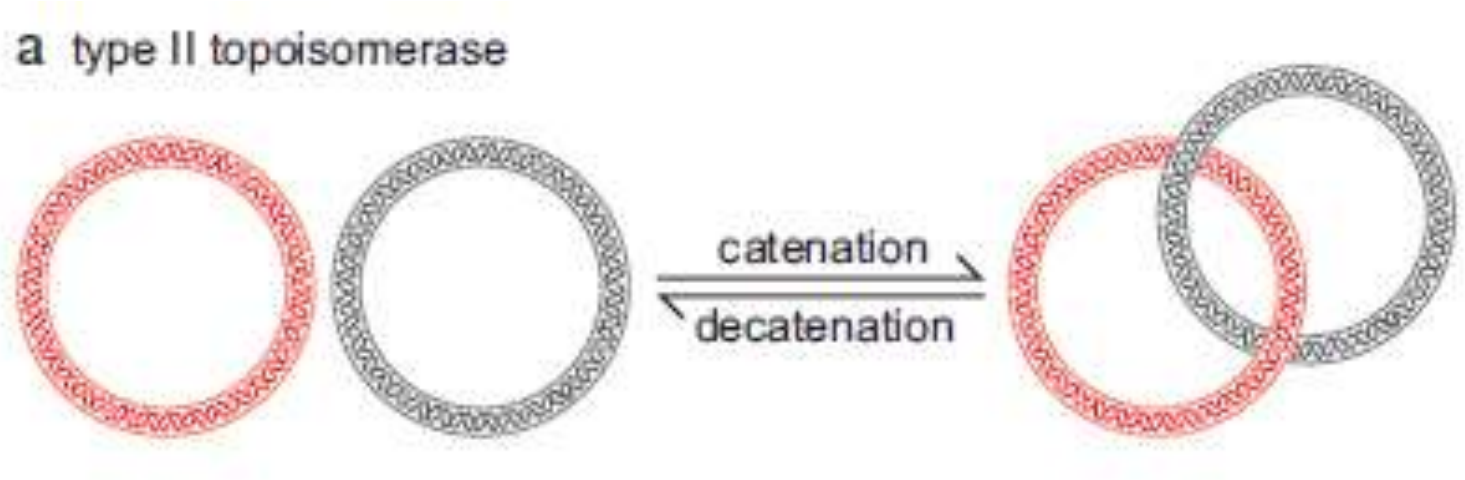
DNA synthesis at the ends of circular and linear chromosomes must differ



Type II Topoisomerases Are Required to Separate Daughter DNA Molecules

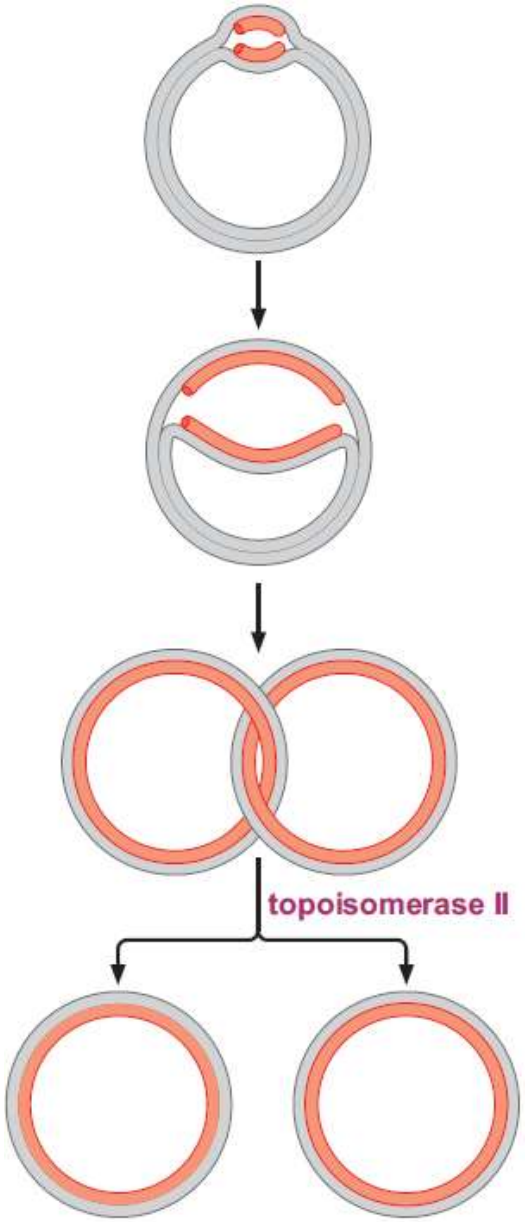
- Type II topoisomerases can catenate and decatenate covalently closed, circular DNA molecules by introducing a double strand break in one DNA and passing the other DNA molecule through the break.
- Catenane is the general term for two circles that are linked (similar to links in a chain).
- After replication of a circular chromosome is complete, the resulting daughter DNA molecules remain linked together as **catenanes.**
- To segregate these chromosomes into separate daughter cells, the two circular DNA molecules must be disengaged from each other or “decatenated.” This separation is accomplished by the action of Type II Topoisomerases .

- Type II Topoisomerases have the ability to break a dsDNA molecule and pass a second dsDNA molecule through this break. This reaction can easily decatenate the two circular daughter chromosomes by breaking one DNA circle and passing the second through the break, allowing their segregation into separate cells.



Topoisomerase II catalyzes the decatenation of replication products.

After a circular DNA molecule is replicated, the resulting complete daughter DNA molecules remain linked to each other. Type II DNA topoisomerases can efficiently separate (or decatenate) these DNA circles



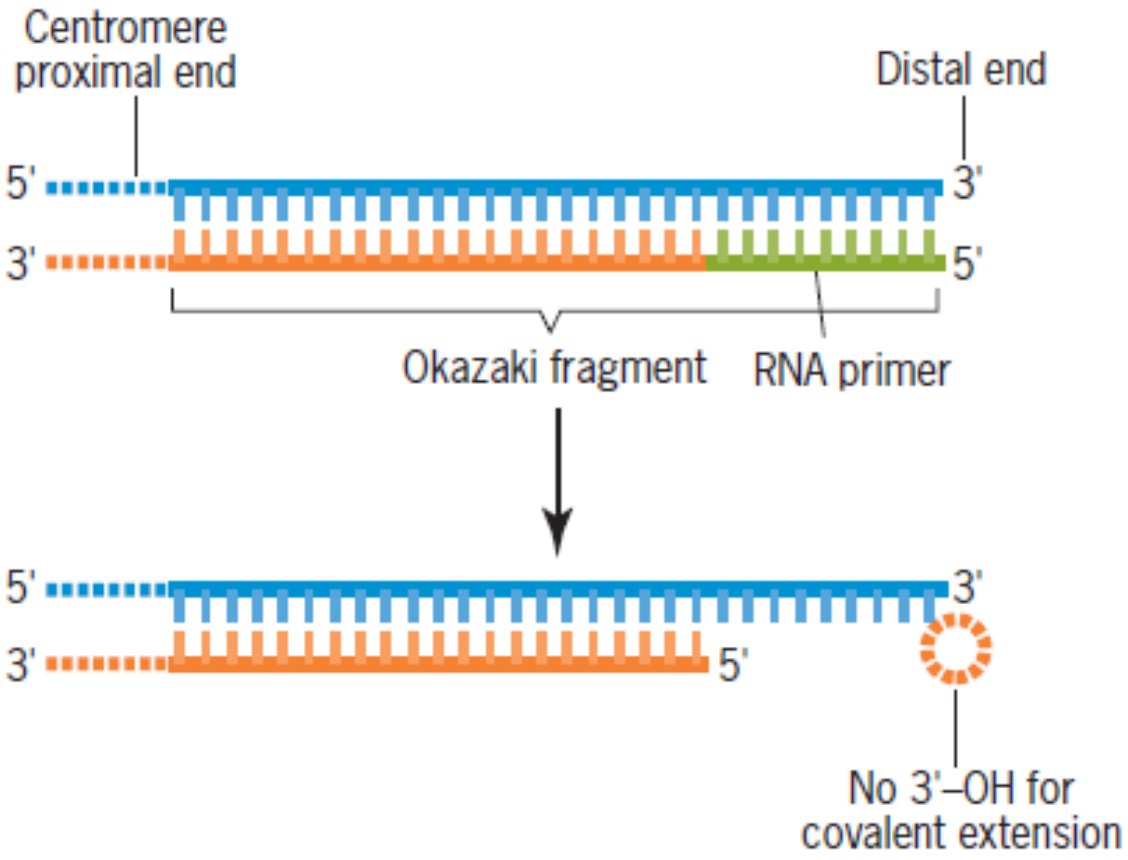
The end-replication problem

- At the very end of a linear chromosome, however, there is no adjacent stretch of replicated DNA to provide this crucial 3′-OH group. When the primer at the end of the chromosome has been removed, it cannot be replaced by DNA nucleotides, which produces a gap at the end of the chromosome, suggesting that the chromosome should become progressively shorter with each round of replication. Chromosome shortening would mean that, when an organism reproduced, it would pass on shorter chromosomes than it had inherited. Chromosomes would become shorter with each new generation and would eventually destabilize. This situation has been termed the **end-replication problem**. Chromosome shortening does in fact take place in many somatic cells but, in single-celled organisms, germ cells, and early embryonic cells, chromosmes do not shorten and self-destruct. So how are the ends of linear chromosomes replicated?

Lagging-Strand Synthesis Is Unable to Copy the Extreme Ends of Linear Chromosomes

- The requirement for an RNA primer to initiate all new DNA synthesis creates a dilemma for the replication of the ends of linear chromosomes, called The **end replication problem.**
- This difficulty is not observed during the duplication of the **leading-strand** template. In that case, a single internal RNA primer can direct the initiation of a DNA strand that can be extended to the extreme 5’ terminus of its template.
- In contrast, the requirement for multiple primers to complete lagging-strand synthesis means that a complete copy of its template cannot be made. Even if the end of the last RNA primer for Okazaki fragment synthesis anneals to the final base of the lagging-strand template,
- once this RNA molecule is removed, there will remain a short region (the size of the RNA primer) of **unreplicated ssDNA** at the end of the chromosome.
- Although this shortening would only occur on oneof the two strands of the daughter molecule, after the next round of replication occurs both strands of the daughter molecule would be shorter.
- This means that each round of DNA replication would result in the shortening of one of the two daughter DNA molecules. Obviously, this scenario would disrupt the complete propagation of the genetic material from generation to generation. Slowly, but surely, genes at the end of the chromosomes would be lost.

The telomere lagging-strand primer problem.



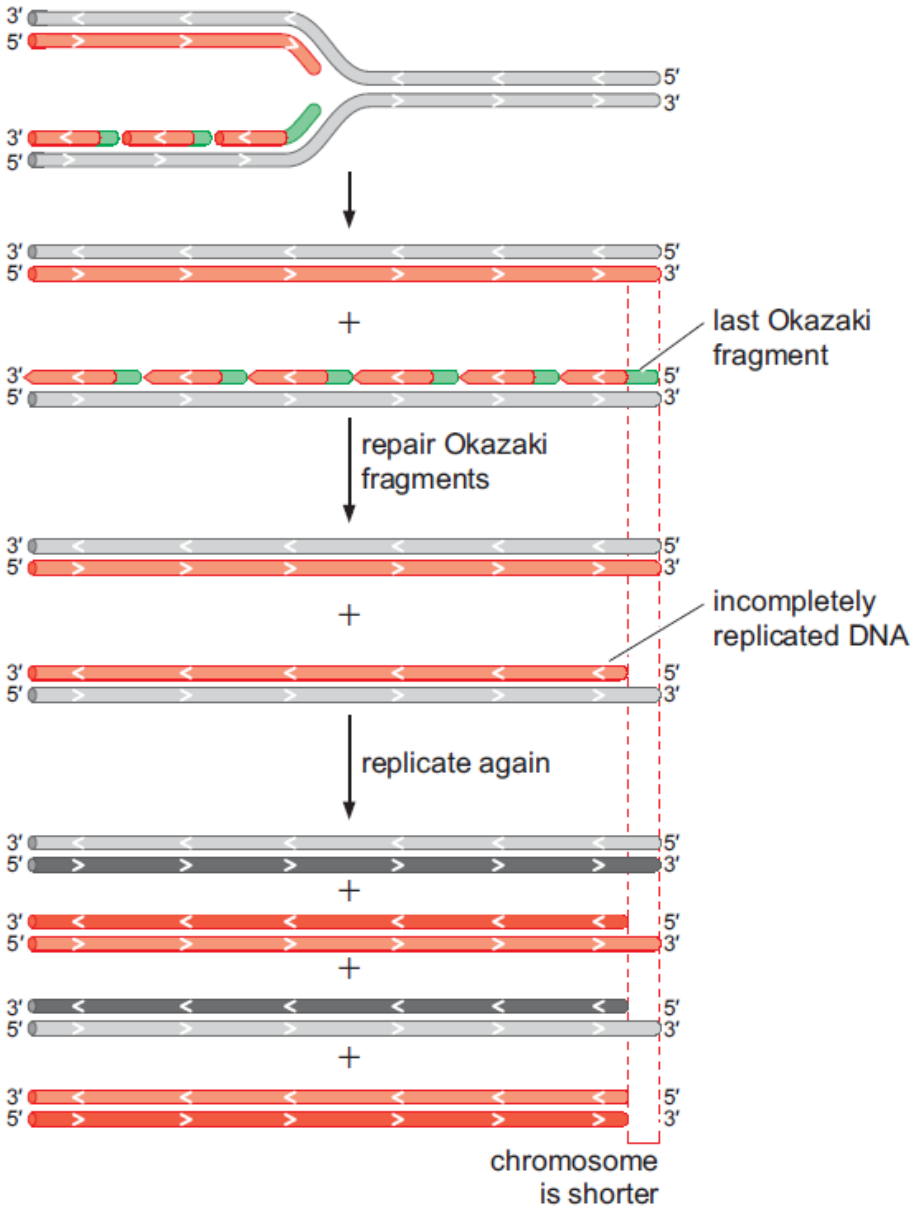
The end replication problem.

As the lagging-strand replication machinery reaches the end of the chromosome, at some point, primase no longer has sufficient space to synthesize a new RNA primer.

This results in incomplete replication

and a short ssDNA region at the 3’ end of the lagging-strand DNA product.

When this DNA product is replicated in the next round, one of the two products will be shortened and will lack the region that was not fully copied in the previous round of replication.

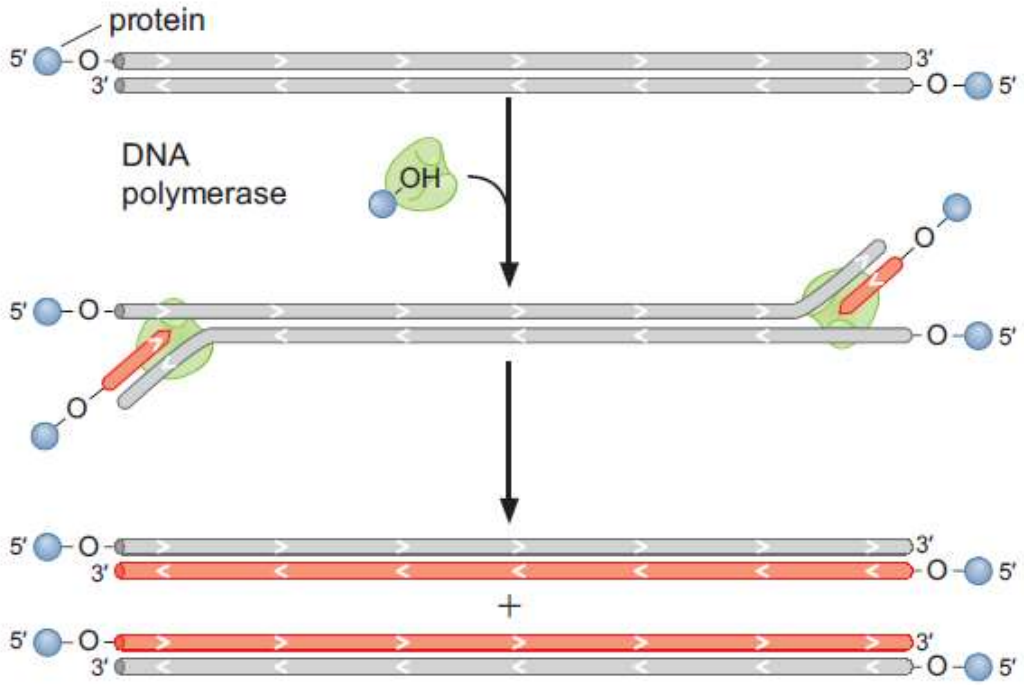


Several ways to solve the end replication problem

- One solution is to use a protein instead of an RNA as the primer for the last Okazaki fragment at each end of the chromosome. In this situation, the “priming protein” binds to the lagging-strand template and uses an amino acid to provide an OH(typically a tyrosine) that replaces the 3’-OH normally provided by an RNA primer. By priming the last lagging strand, the priming protein becomes covalently linked to the 5’ end of the chromosome. Terminally attached replication proteins of this kind are found at the end of the linear chromosomes of certain species of bacteria and at the ends of the linear chromosomes of certain bacterial and animal viruses.

1. Protein priming as a solution to the end replication problem

By binding to the DNA polymerase and to the 3’ end of the template, a protein provides the priming hydroxyl group to initiate DNA synthesis. In the example shown, the protein primes all DNA synthesis as is seen for many viruses. For longer DNA molecules, this method combines with conventional origin function to replicate the chromosomes.



2. Telomerase Solves the End Replication Problem by Extending the 3’ End of the Chromosome

- Ends of eukaryotic chromosomes are called **telomeres.**
- The sequence. For example, human telomeres consist of many head-to-tail repeats of the sequence 5’-TTAGGG-3’.Y are generally composed of head-to-tail repeats of a TG-rich DNA.
- Although many of these repeats are double-stranded, the 3’ end of each chromosome extends beyond the 5’ end as ssDNA.
- This unique structure acts as a novel origin of replication that compensates for the end replication problem.
- This origin does not interact with the same proteins as other eukaryotic origins, but it instead recruits a specialized DNA polymerase called **telomerase.**

- **Telomerase** enzyme was discovered in 1985 by Elizabeth Blackburn and Carol Greider.
- They shared the 2009 Nobel Prize in Physiology or Medicine with Jack Szostak, who, along with Blackburn, determined how the unique structures of telomeres protected them from degradation.

Telomerase Is a Novel DNA Polymerase That Does Not Require an Exogenous Template

- **Telomerase** is a remarkable enzyme that includes multiple protein subunits and an RNA component (ribonucleoprotein).
- Like all other DNA polymerases, telomerase acts to extend the 3’ end of its DNA substrate.
- But Telomerase does not need an exogenous DNA template to direct the addition of new dNTPs like DNA polymerase. Instead, the RNA component of telomerase serves as the template for adding the telomeric sequence to the 3’ terminus at the end of the chromosome
- Telomerase specifically elongates the 3’-OH of telomeric ssDNA sequences using its own RNA as a template. As a result of this unusual mechanism, the newly synthesized DNA is single-stranded.
- “**Telomerase RNA**” (TER) is the key to telomerase’s unusual functions. TER varies in size from 150 to 1300 bases.

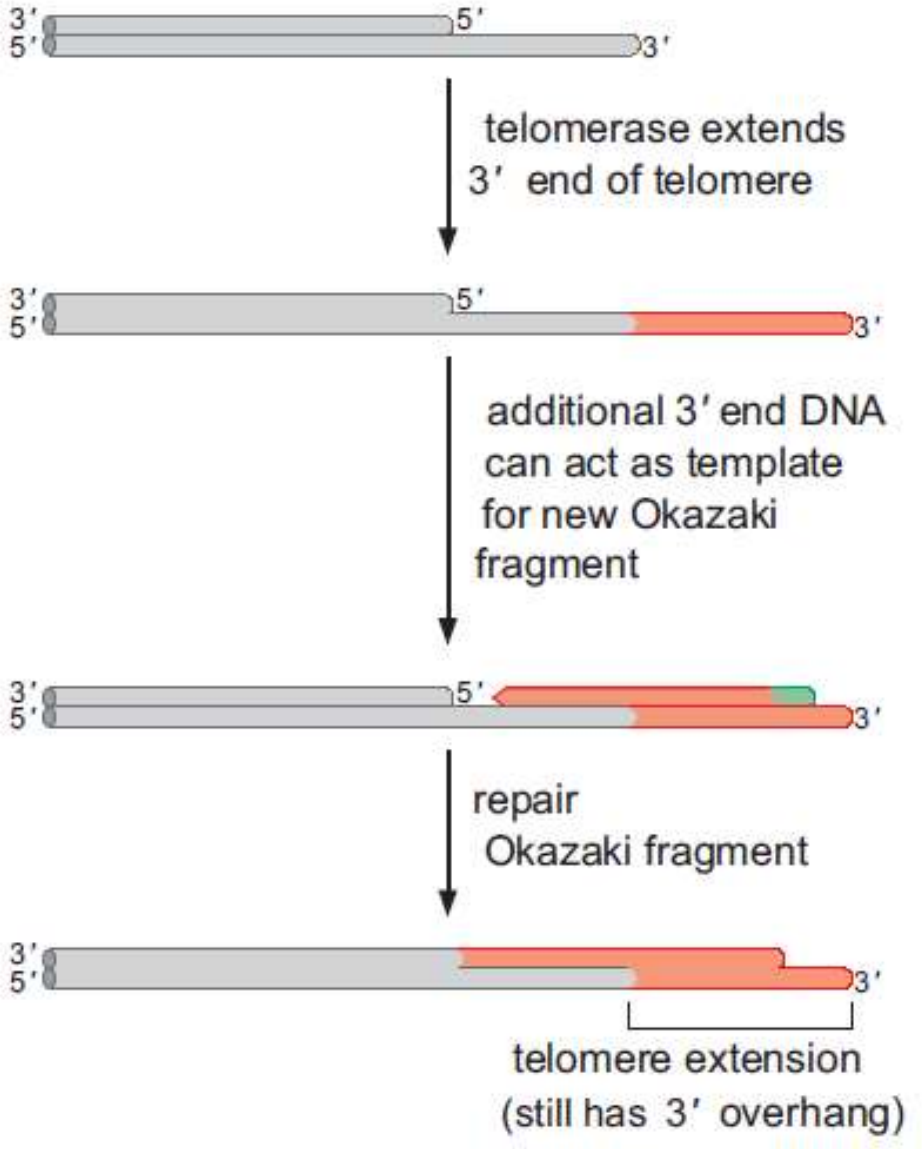
- This region of the RNA can anneal to the ssDNA at the 3’ end of the telomere.
- Annealing occurs in such away that a part of the RNA template remains single-stranded, creating a primer:template junction that can be acted on by telomerase.
- one of the protein subunits of telomerase is a member of a class of DNA polymerases that use RNA templates called “**reverse transcriptases**” (this subunit is called “**telomerase reverse transcriptase**,” or TERT).
- Using the associated RNA template, TERT synthesizes DNA to the end of the TER template region but cannot continue to copy the RNA beyond that point.
- At this point, the RNA template disengages from the DNA product, reanneals to the last four nucleotides of the telomere, and then repeats this process.

How is the 5’end extended?

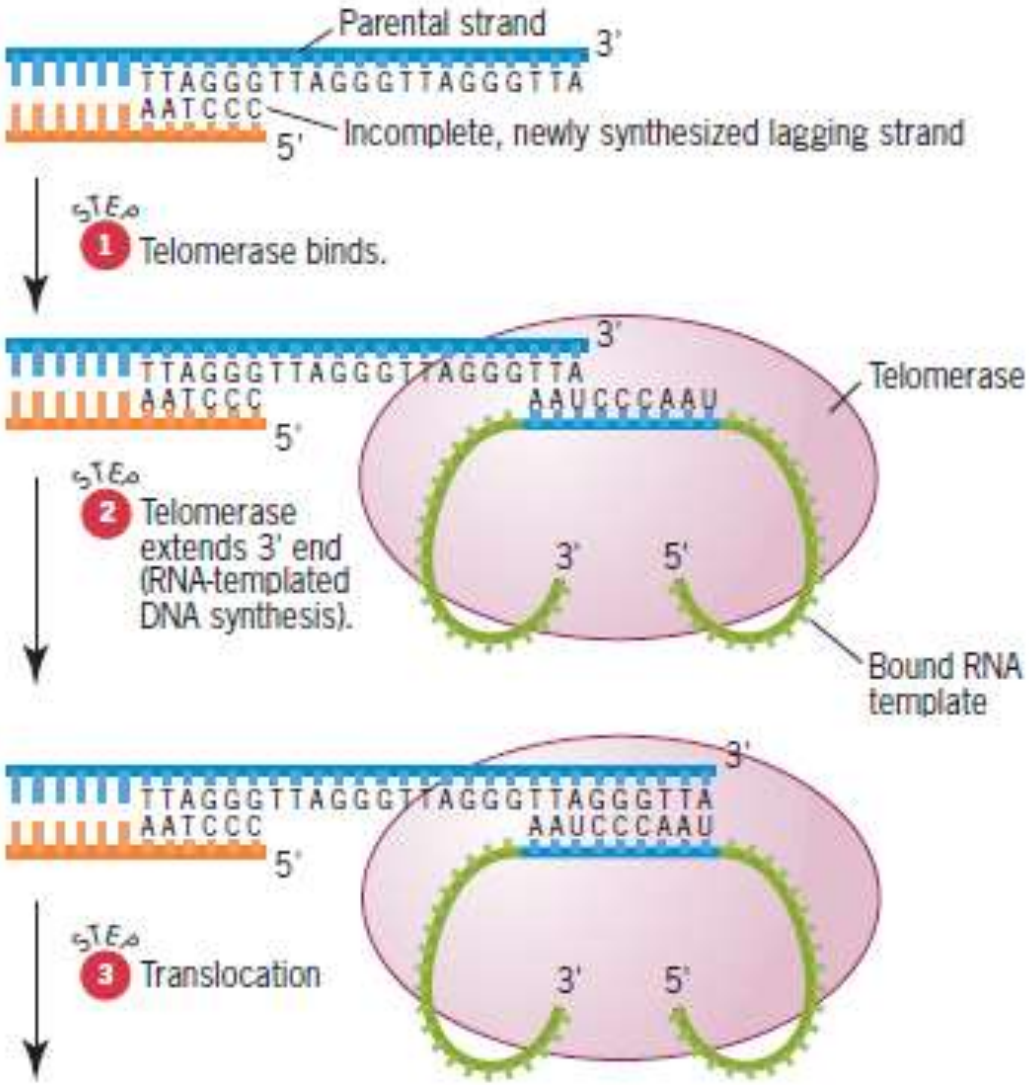
- This is accomplished by the lagging-strand DNA replication machinery.
- By providing an extended 3’ end, telomerase provides additional template for the lagging-strand replication machinery. By synthesizing and extending RNA primers using the telomerase extended 3’ end as a template, the cell can effectively increase the length of the 5’ end of the chromosome as well.

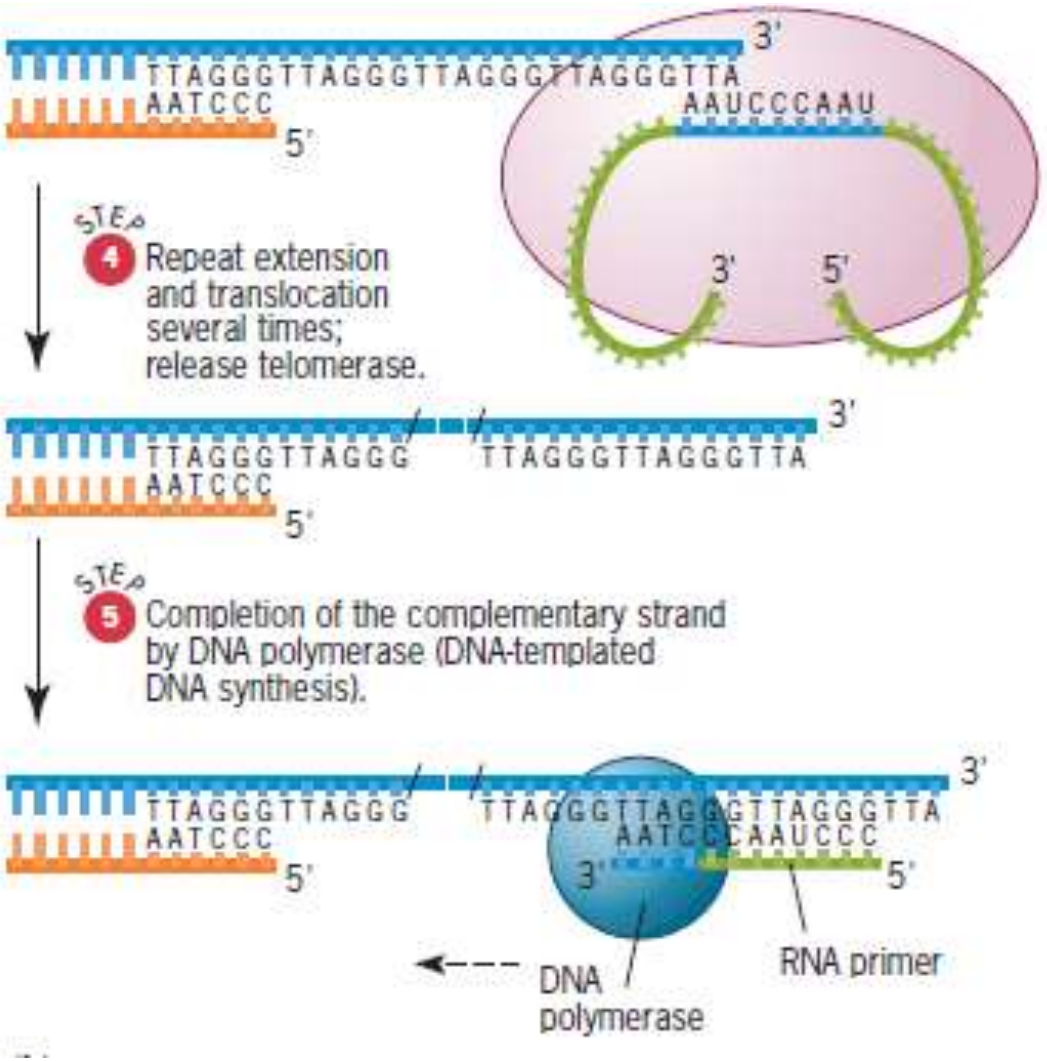
Extension of the 3’ end of the telomere by telomerase solves the end replication problem

Although telomerase only directly extends the 3’ end of the telomere, by providing an additional template for lagging-strand DNA synthesis, both ends of the chromosome are extended



Telomerase resolves the terminal primer problem.



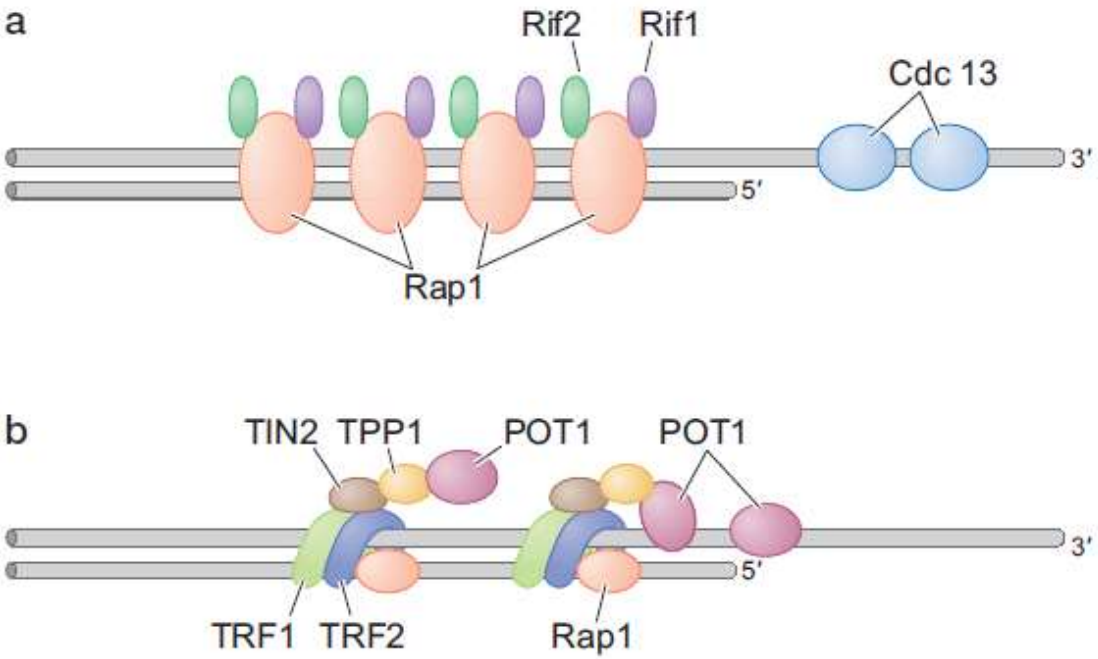


Replication of chromosome telomeres

Telomere-Binding Proteins Regulate Telomerase Activity and Telomere Length

- Proteins bound to the double-strand regions of the telomere regulate telomere length.
- In *S. cerevisiae* cells, proteins bound to the telomere act as weak inhibitors of telomerase activity.
- When there are relatively few copies of the telomere sequence repeat, few of these proteins are bound to the telomere, and telomerase can extend the 3’-OH end of the telomere.
- As the telomere becomes longer, more of the telomere-binding proteins accumulate and inhibit telomerase extension of the 3’-OH end of the telomere.
- This simple negative-feedback loop mechanism (longer telomeres inhibit telomerase) is a robust method to maintain a similar telomere length at the ends of all chromosomes

Telomere-binding proteins.



(a) In *S. cerevisiae*

- The cdc13 protein binds to single-stranded regions of the telomere and recruits telomerase to the telomeres. Thus, cdc13 is a positive activator of telomerase.
- Rap1 directly binds to the double stranded telomere repeat DNA, whereas Rif1 and Rif2 associate with the telomere indirectly by binding to Rap1. All three proteins have been implicated in the inhibition of telomerase activity.

(b) In Human cells

- TRF1 and TRF2 bind directly to the double-stranded telomere repeat DNA.
- The human homolog of Rap1 as well as TIN2, TPP1, and POT1 all associate with either TRF1 or TRF2.
- Together these proteins form a complex that is called Shelterin for its ability to “shelter” the telomeres from the action of DNA repair enzymes.
- POT1 also binds directly to the single-stranded telomere repeat DNA and inhibits telomerase activity.

Telomere-Binding Proteins Protect Chromosome Ends

- Ordinarily in a cell, the presence of a DNA end is considered as a double-stranded break in the DNA, which is targeted by the DNA repair machinery.

- The most common outcome of this repair is to initiate recombination with other DNA in the genome.

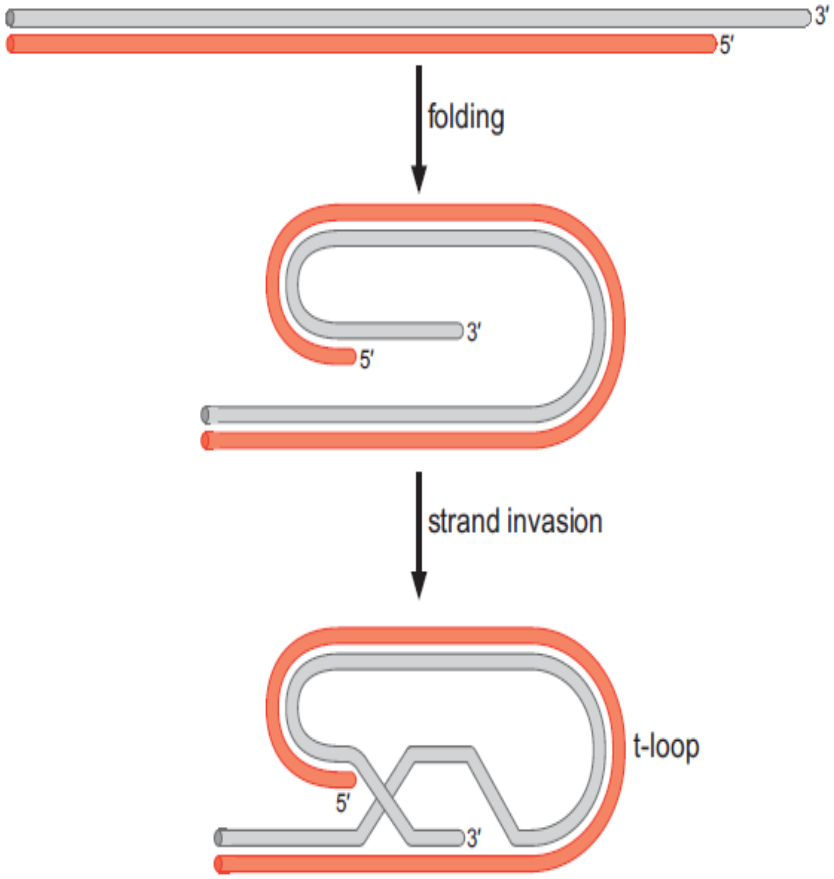
- Whereas this response is appropriate for random DNA breaks, it would be disastrous for the telomeres to participate in the same events. Attempts to repair telomeres in the same manner as double-stranded DNA breaks would lead to **chromosome fusion** events, which eventually result in random chromosome breaks.

What protects the telomeres from this fate?

- Elimination of proteins bound at the telomere leads to the recognition of the telomeres as normal DNA breaks suggesting that these proteins distinguish telomeres from other DNA ends in the cell.
- It is possible that protection is conferred simply by coating the telomere with binding proteins.
- Telomeres isolated from human cells were observed by electron microscopy and found to form a **t-loop** structure, by the 3'-ssDNA end of the telomere invading the dsDNA region of the telomere
- It has been proposed that by forming a t-loop, the end of the telomere is masked and cannot be recognized as a normal DNA end.
- Interestingly, purified TRF2 is capable of directing t-loop formation with purified telomere DNA.
- The lack of single-strand 3' end in the t-loop structure of telomere, may create recognition problem for telomerase. Thus telomere length may be controlled.
- It has been proposed that as telomeres shorten, they would have an increasingly difficult time forming the t-loop, thereby allowing increased access to the 3' end of the Telomere.

Telomeres form a looped structure in the cell – proposed mechanism

- The first step folds the telomere such that the ssDNA at the end of the telomere can access the dsDNA telomeric repeats. Once the ssDNA end is positioned properly, it can invade the dsDNA repeats and form a helix with the complementary strand, displacing the other strand of the dsDNA. This is called a **displacement loop** and is a common intermediate in homologous recombination. It is likely that telomere-binding proteins and other cellular proteins (e.g., recombination proteins) facilitate this process.



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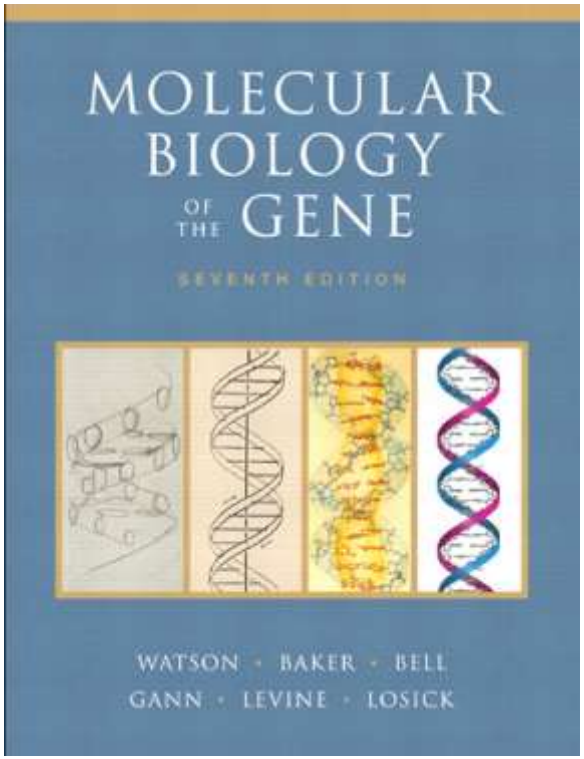
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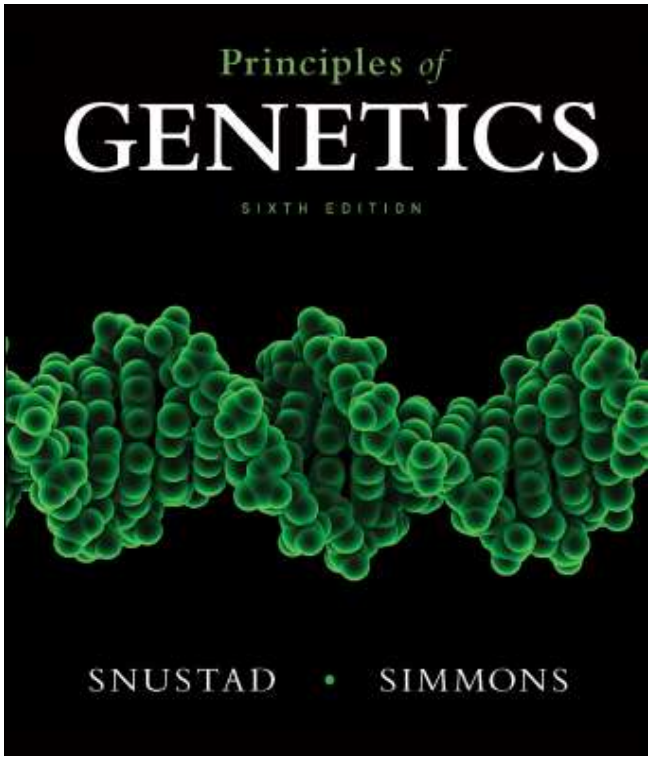
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