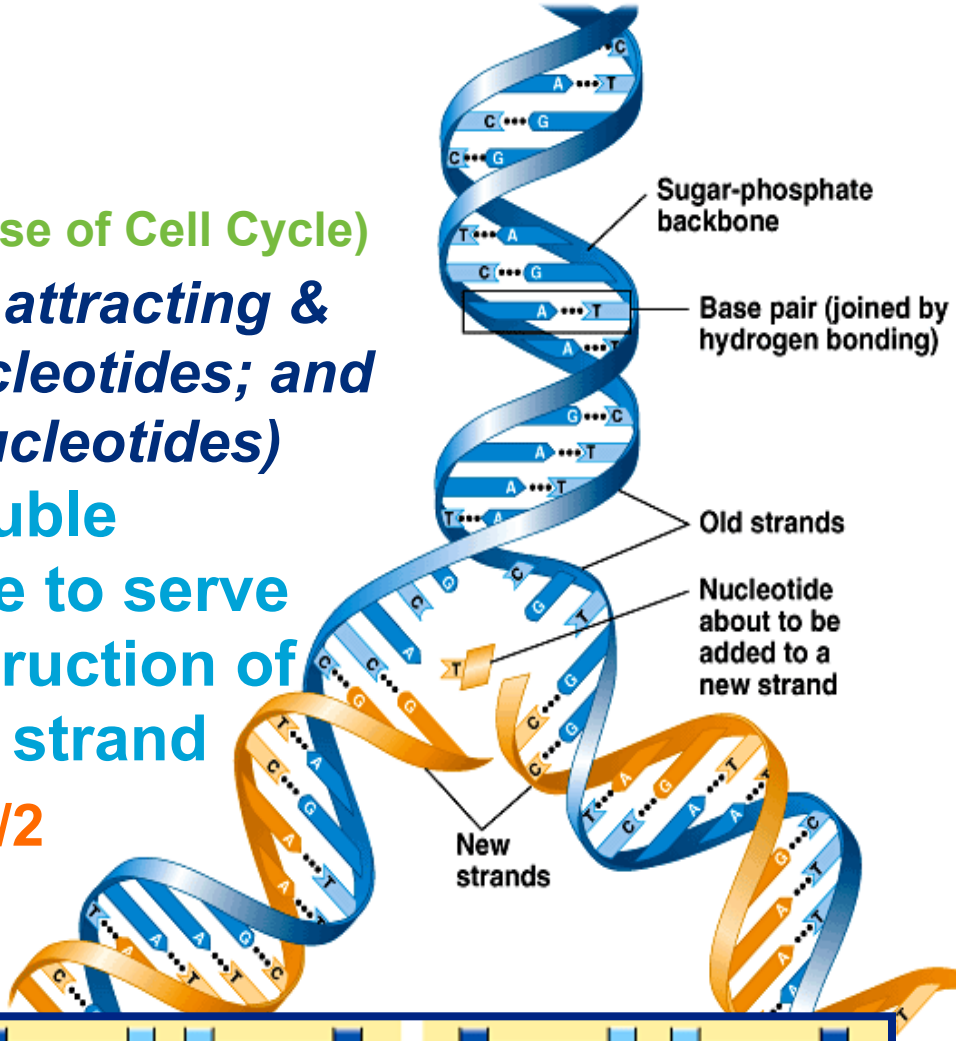
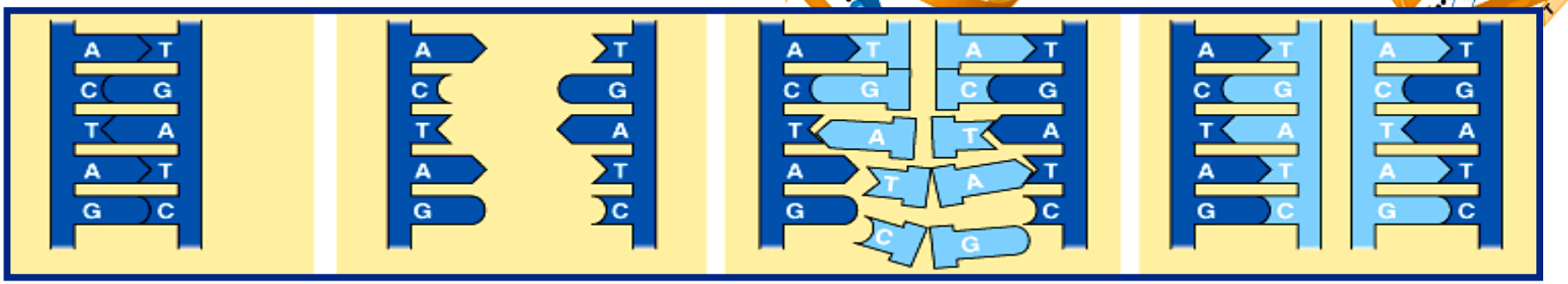
DNA Replication

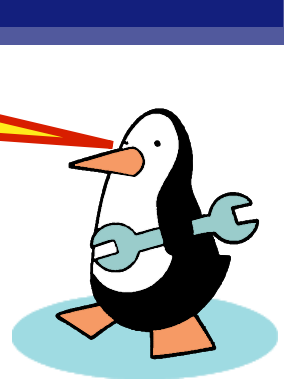
Copying DNA

- **Replication of DNA** (S Phase of Cell Cycle)
  - base pairing *(A nucleotides attracting & hydrogen bonding with T nucleotides; and G nucleotides attracting C nucleotides)* allows **each** strand in a double stranded (ds)DNA molecule to serve as a **template** for the construction of a new complimentary DNA strand
    - a new DNA molecules is 1/2 parent template & 1/2 new daughter DNA strand

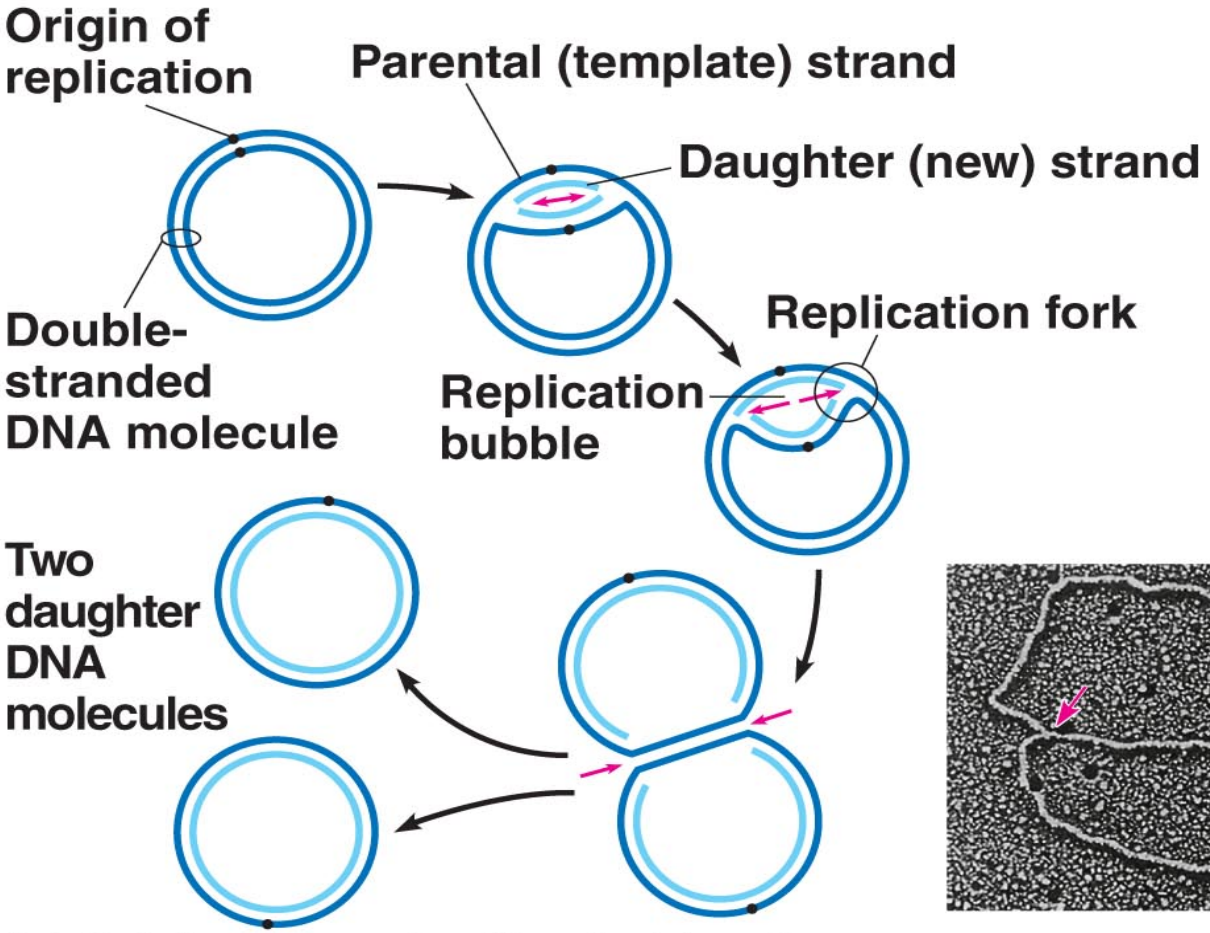




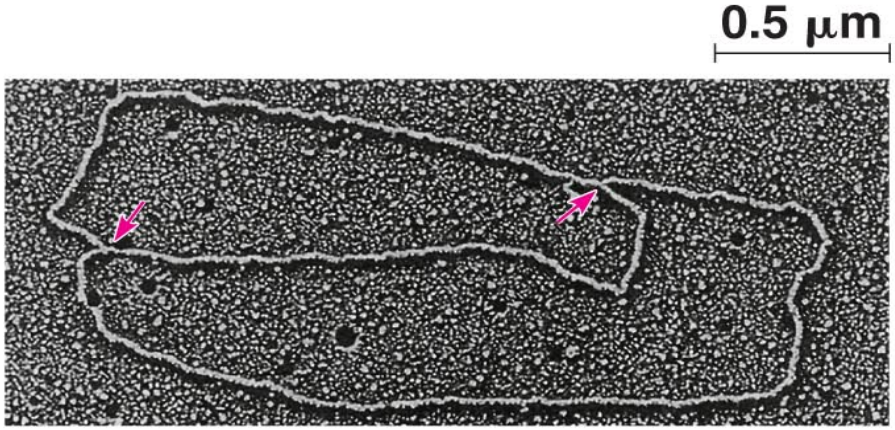
Bacterial DNA Replication



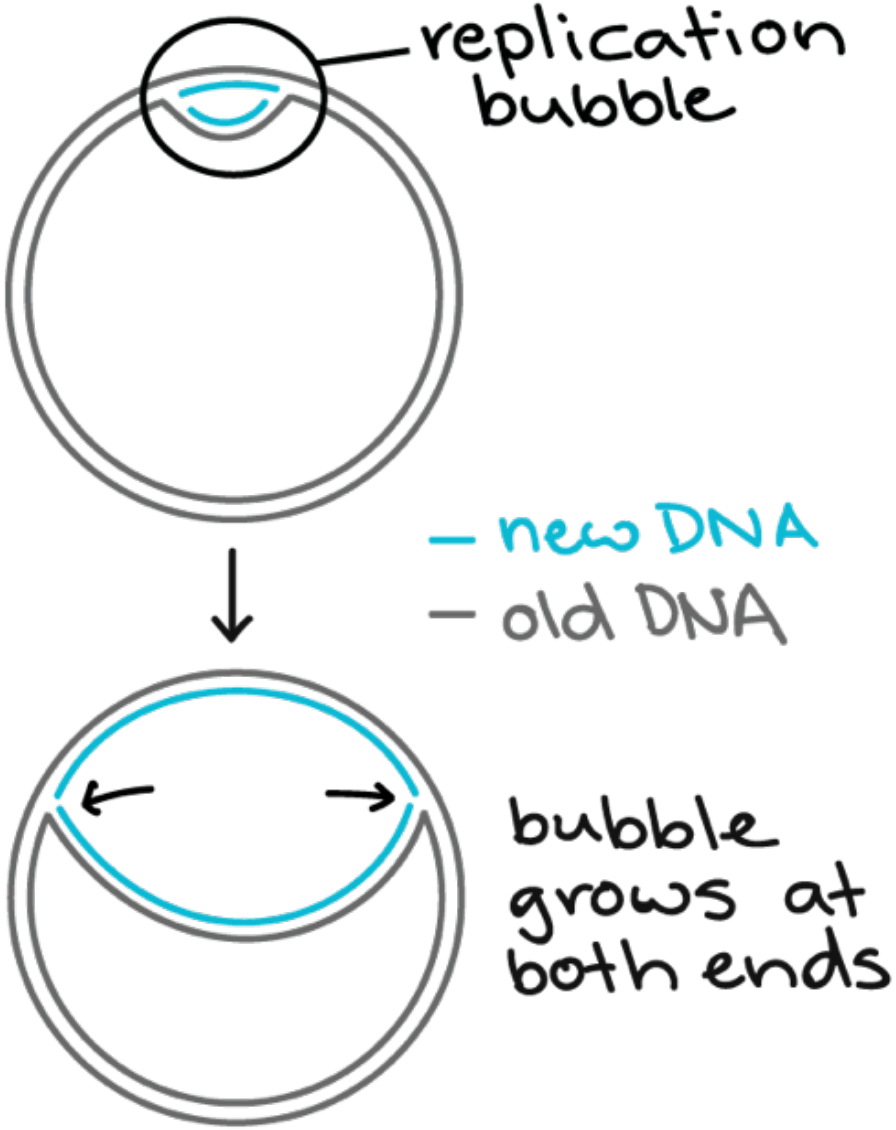
- Bacterial DNA consists of one circular double-stranded, helical molecule with one origin of replication where DNA replication always begins.



(a) Origins of replication in *E. coli*

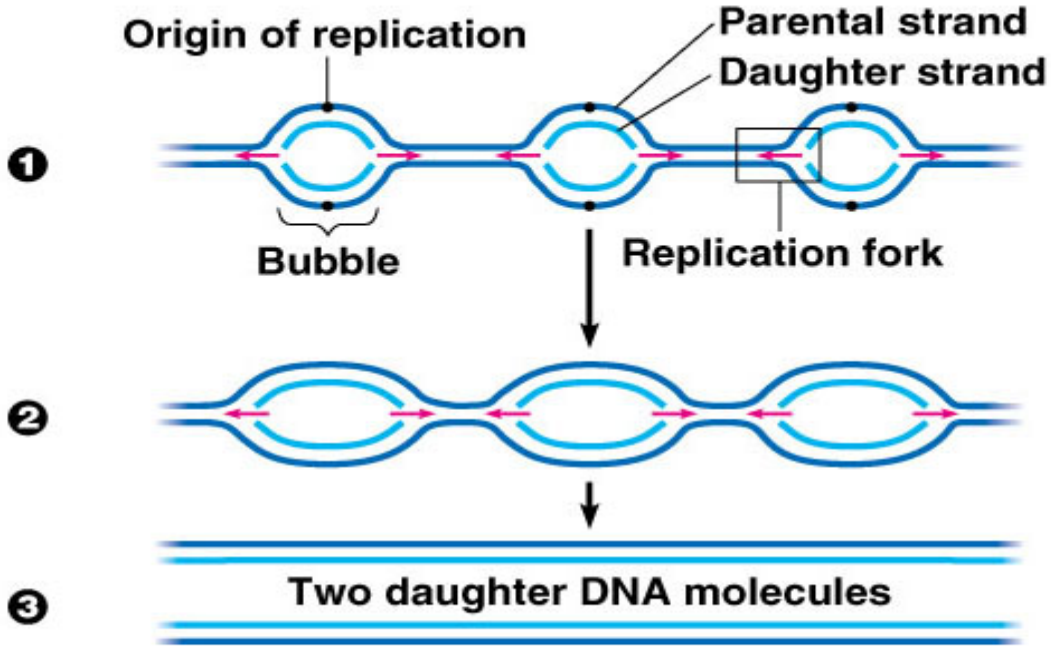


Bacterial DNA Replication

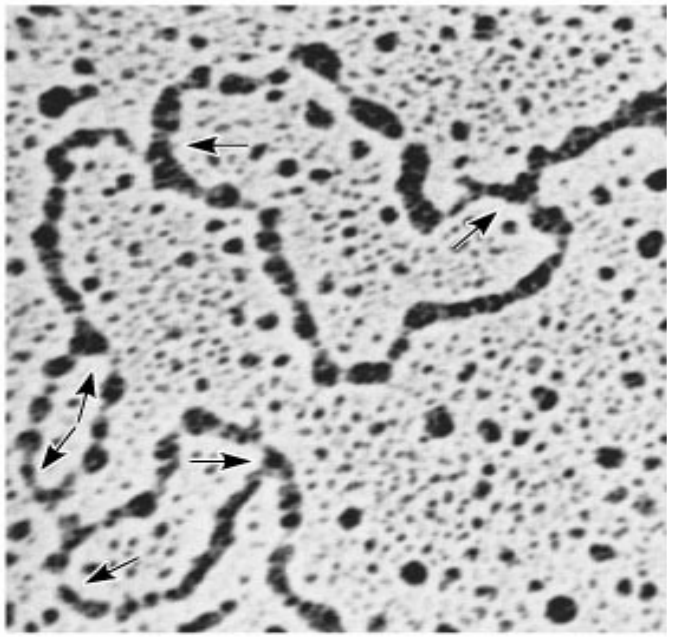


Eukaryotic DNA Replication

- Eukaryotic DNA consists of multiple linear double-stranded, helical molecule with many origins of replication
  - In Eukaryotic & Prokaryotic DNA replication, a large team of enzymes coordinates the process.



(a) In eukaryotes, DNA replication begins at many sites along the giant DNA molecule of each chromosome.

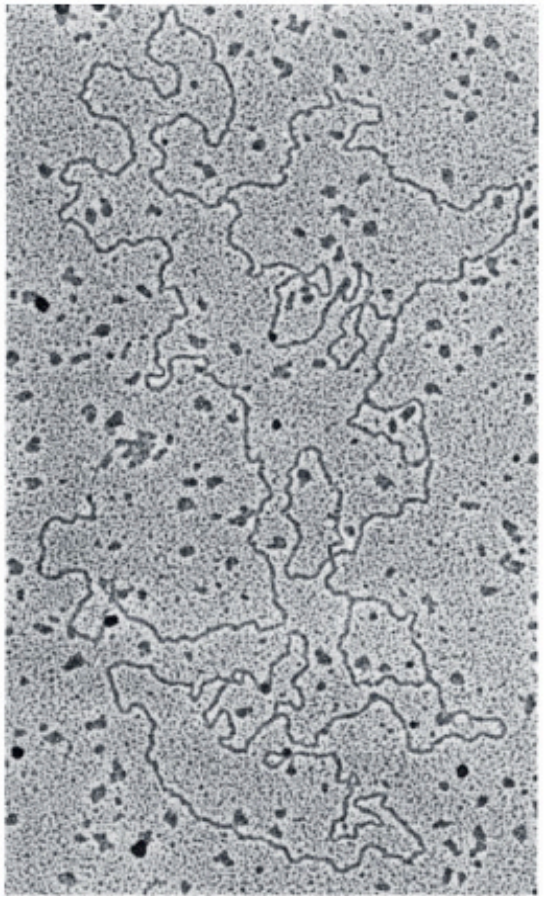


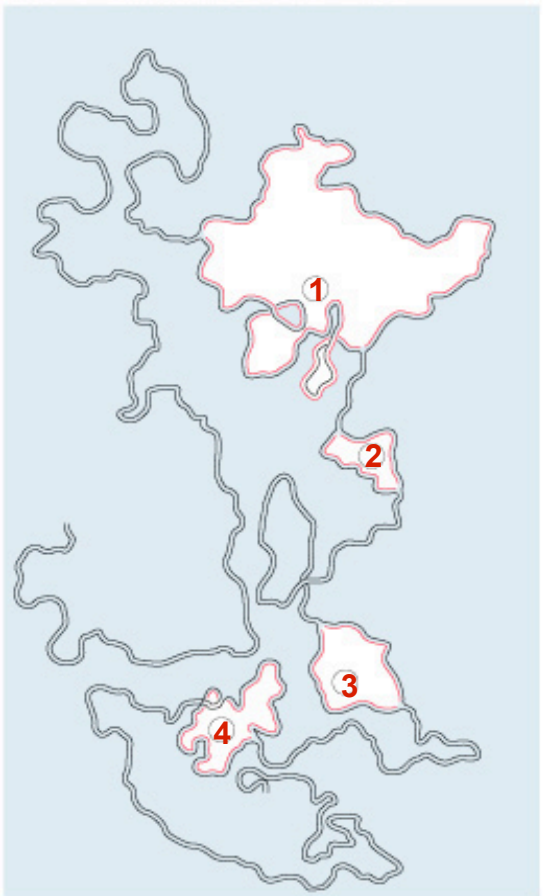
(b) In this micrograph, three replication bubbles are visible along the DNA of cultured Chinese hamster cells. The arrows indicate the direction of DNA replication at the two ends of each bubble (TEM).

AP

What does it really look like?

While prokaryotes have one origin of replication,
Eukaryotic organisms have many origins of replication
otherwise the DNA will never get copied fast enough

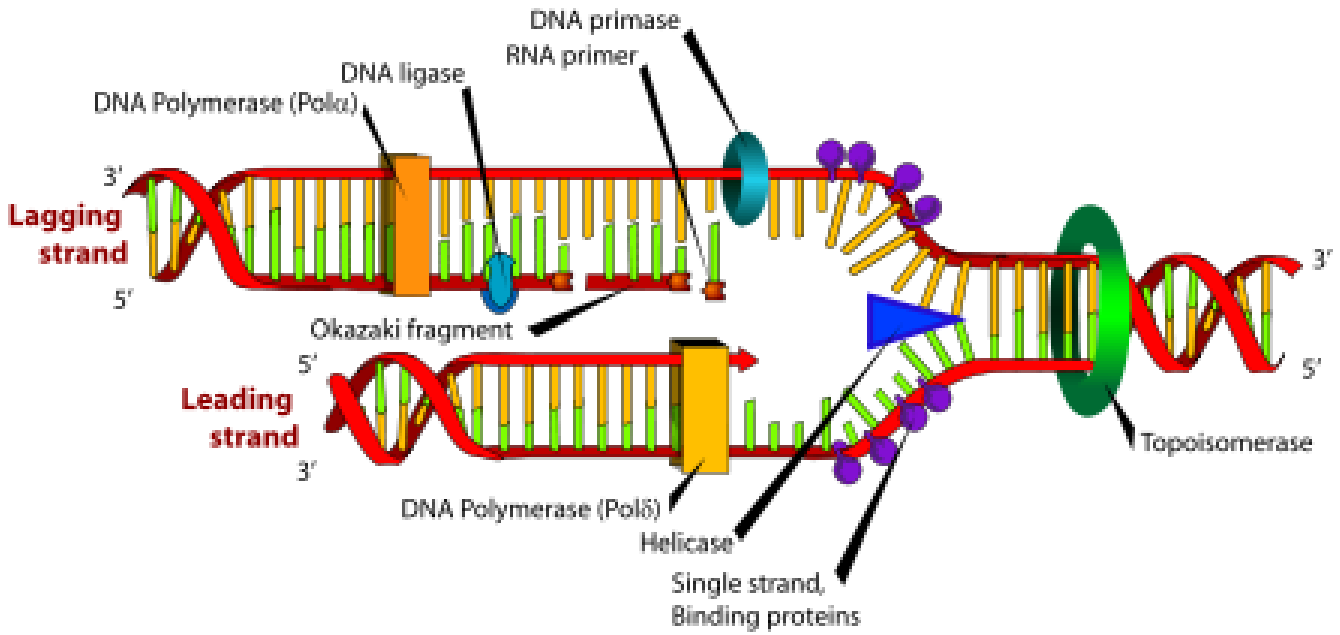




Overview of Replication process

- **Requires 3 things:**
  1. Something to copy
  2. Something to do the copying
  3. Building blocks to make the copy

- **In DNA replication:**
  1. Parental DNA molecule serves as a template
  2. Enzymes perform the action of copying the template
  3. The building blocks are <u>nucleotide triphosphates</u>



The Three phases of Replication

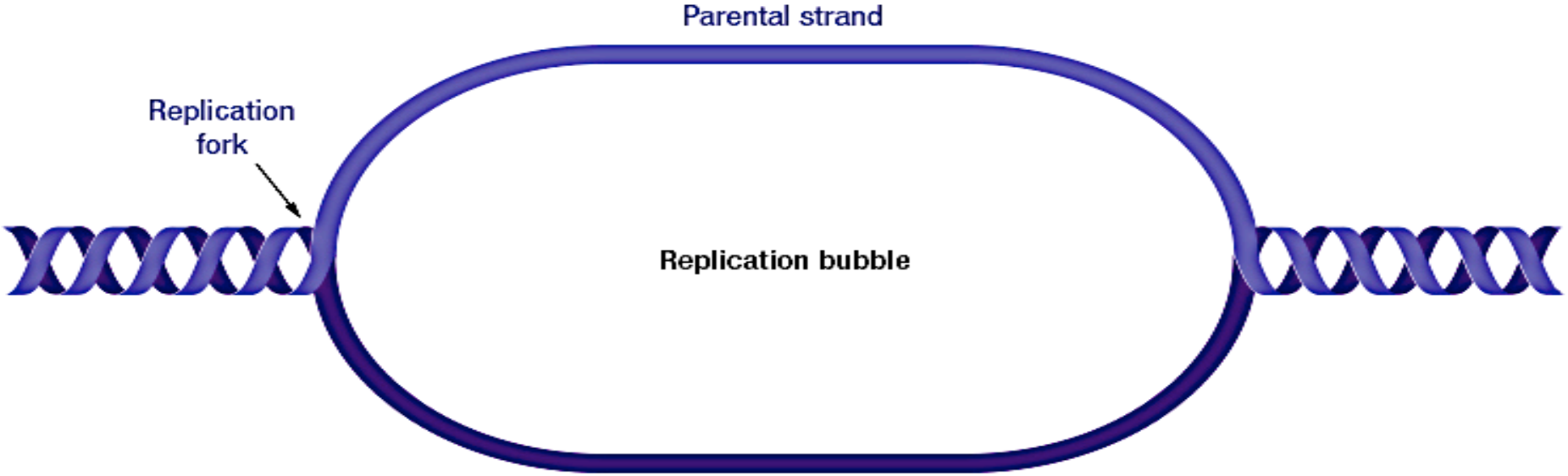
1. **INITIATION**
   a) Replication begins at specific sites called origins of replication.
   b) Initiator proteins recognize and bind to the origin, forming a complex that opens the helix to expose single-stranded templates used for the process of building a new strand

2. **ELONGATION**
   a) DNA polymerase matches the existing DNA bases with complimentary nucleotides and then links the nucleotides together to make a new strand
   b) DNA polymerases add bases to 3' ends of EXISTING strands
      - NOTE: DNA Polymerase *cannot* begin a new daughter strand by itself. It can only elongate a preexisting strand of DNA or RNA already base-paired to the template strand, unlike RNA polymerase that *can* put down the first nucleotide complimentary to a template strand.

3. **TERMINATION**
   a) In prokaryotes with circular DNA, replication ends when the process comes around to the origin again
   b) In eukaryotes, end points for each chromosome are indicated by telomeres, regions of repeated bases at the tips of chromosomes.

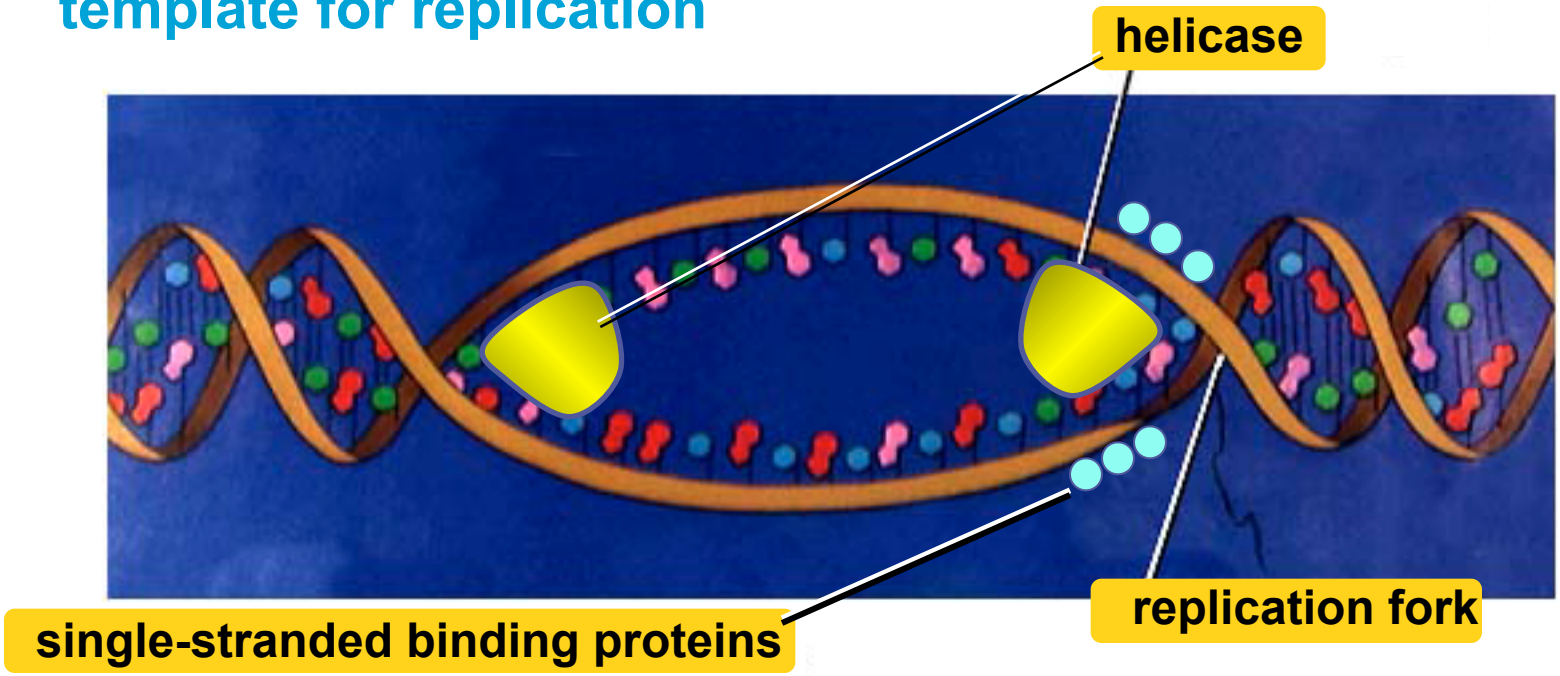
Replication 1st step = INITIATION

- **Begin unwinding DNA, starting at the Origin of Replication (a particular DNA sequence)**
  - **involves helicase enzymes, which break the hydrogen bonds between the two strands in DNA**
    - **As the DNA strands separate, a replication bubble forms (an region of single-stranded DNA strands, which we call DNA template strands or parental strands)**
      - **The replication bubble continues to grow as two helicase enzymes continue to unwind DNA by working at each of the two replication forks (regions to the left and right of the replication bubble were DNA is still double-stranded)**



Replication 1st step = INITIATION

- **Single-stranded DNA will want to hydrogen bond with the complimentary strand again**
  - **Once unwound, single-stranded DNA is, therefore, immediately covered with single-stranded binding proteins, that stabilize it *(preventing DNA annealing = hydrogen bonding)* until each single-stranded (ss)DNA strand starts getting used as a template for replication**



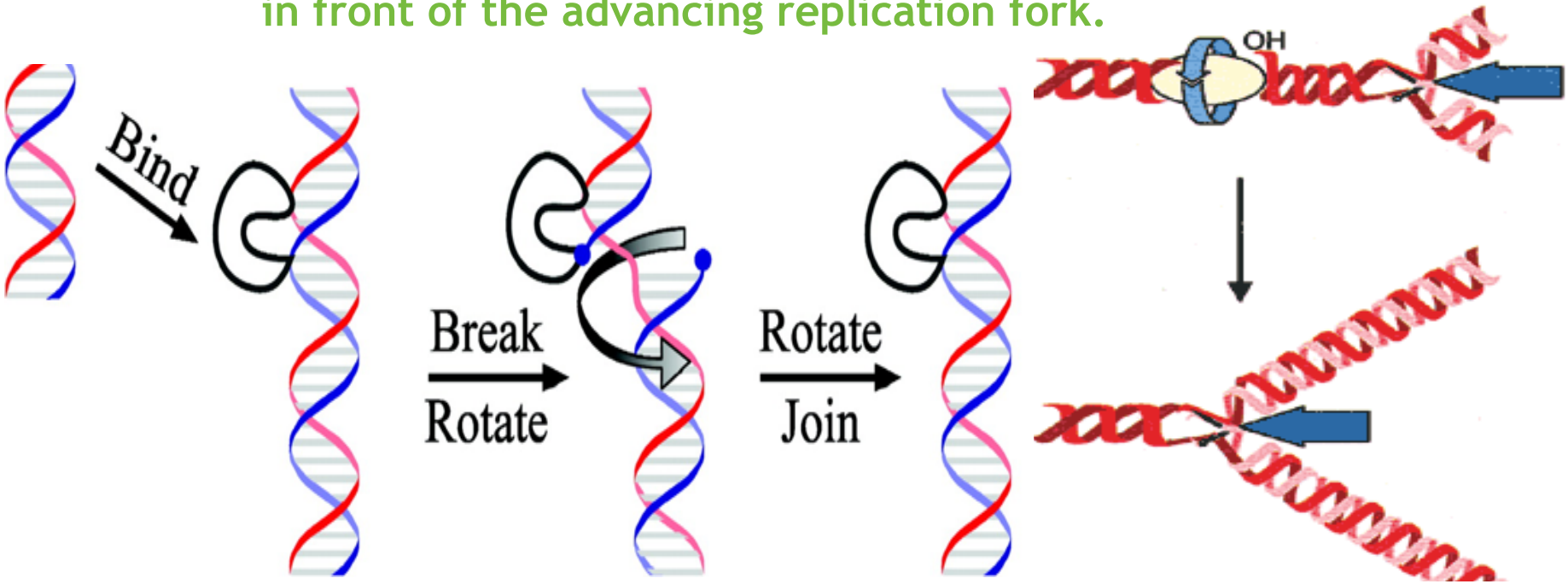
DNA Replication

- As the two helicase enzymes keep separating more of the dsDNA helix into two ssDNA strands, the dsDNA outside of the Replication bubble over rotates as rotational tension builds up.
  - If this continues, it would make it impossible for helicase to keep separating the double-stranded DNA at a certain point.

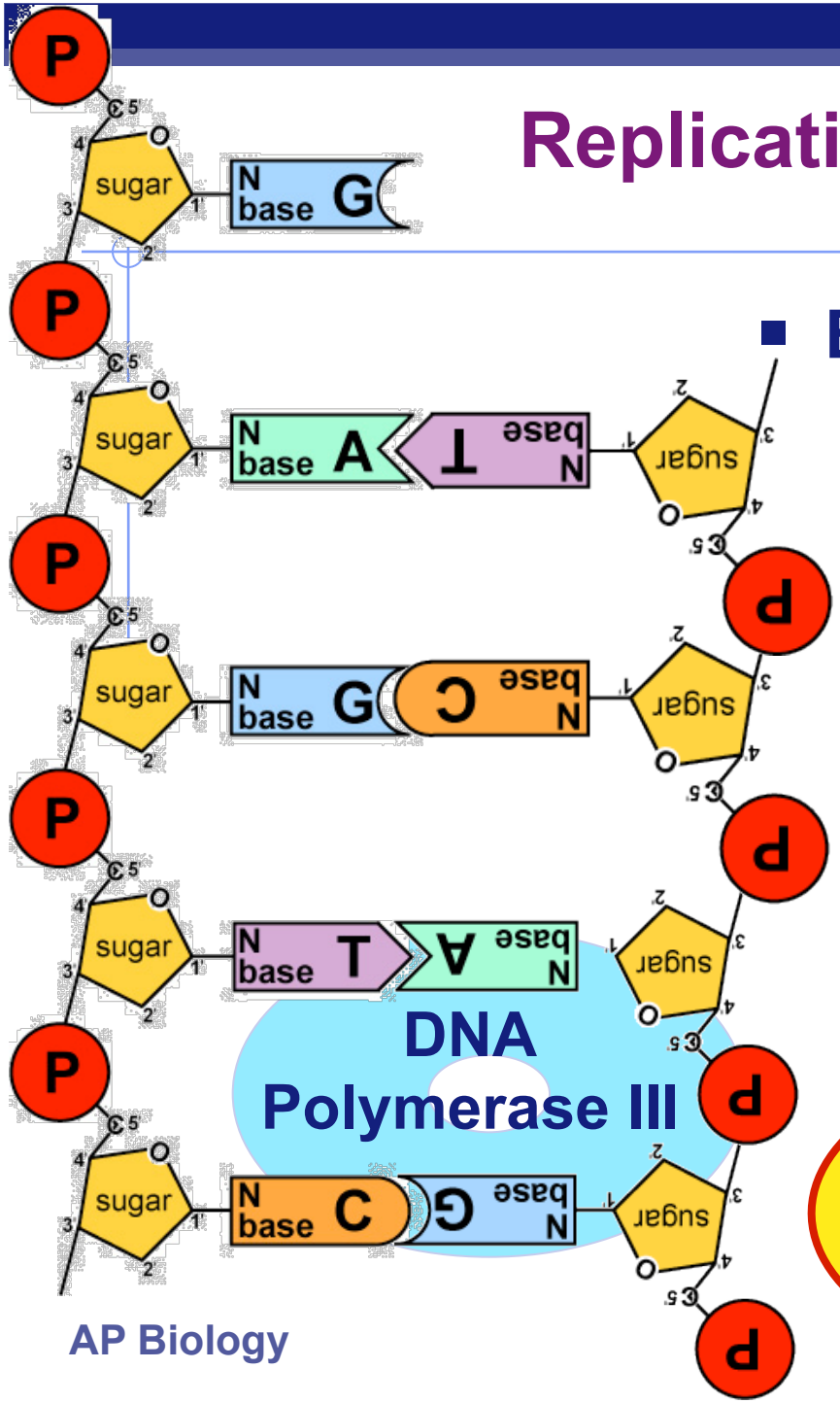
Hyperrotation of DNA outside of a DNA Replication Fork

DNA Replication

- **Topoisomerase (called DNA GYRASE IN EUKARYOTIC REPLICATION) is an enzyme used to correct the hyper-rotation of dsDNA so that each helicase can keep opening the dsDNA at the Replication Forks.**
  - This enzyme cuts the DNA backbone of one strand, allowing this one strand to rotate around the other, undoing any over-rotation. The cut strand is then repaired again.
    - This releases the torsional strain, which otherwise accumulates in front of the advancing replication fork.

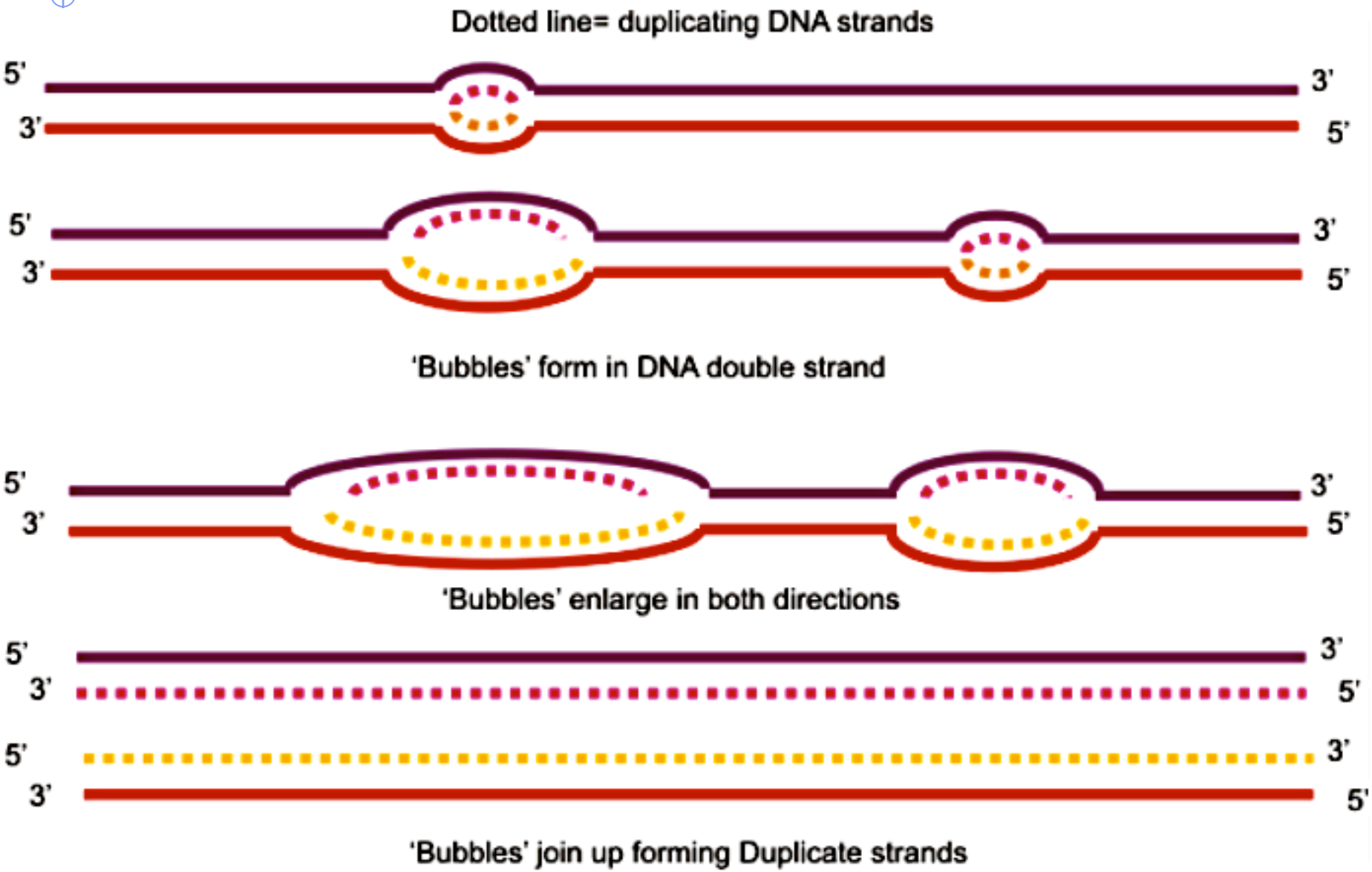


Replication 2nd Step: ELONGATION

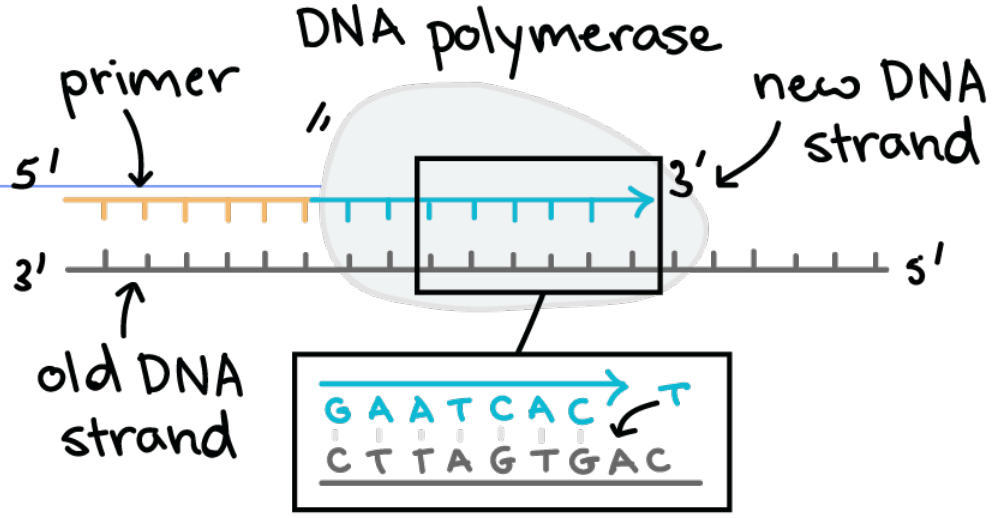


- **Build daughter DNA strand**
  - **join new bases (nucleotides), complementary to the two single-stranded template DNA strands**
  - **Main enzyme in Eukaryotes:**
    - **DNA polymerase III**

Replication 2nd Step: ELONGATION



DNA Polymerase



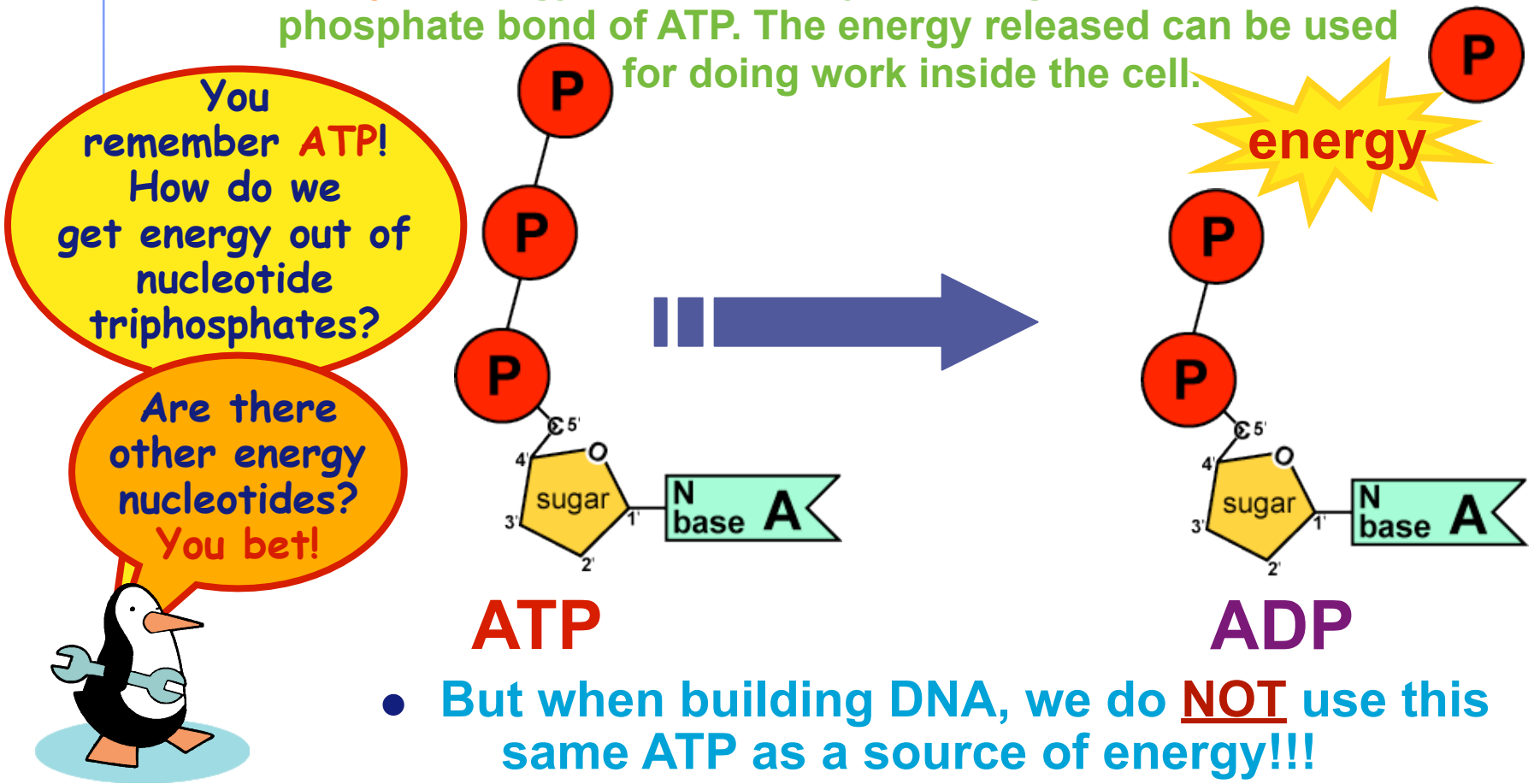
- **DNA polymerases synthesize new DNA:**
  - **They add nucleotides, one by one, to a growing DNA strand, incorporating only those bases that are complementary to the template DNA strand's bases.**
- **Key features of DNA polymerases:**
  1. **They always need a template DNA strand to build a complimentary daughter strand off of**
  2. **They can only add nucleotides to the 3' end of a growing DNA strand**
  3. **They can't start making a DNA chain from scratch, but require a short stretch of RNA nucleotides to be laid down, called a primer, which the DNA Polymerase can then elongate.**
  4. **Eukaryotic DNA Polymerases only can proofread, or check their work, removing the vast majority of "wrong" nucleotides that are accidentally added to the growing daughter DNA strand**

Building Macromolecules Requires Energy

Where does energy for cellular work (like building lipids and carbohydrates - anabolism) *usually* come from in the cell?

ATP (a phosphorylated RNA nucleotide made with ribose sugar - See Ch.5)

- **Normally** = Energy is obtained by breaking the terminal phosphate to phosphate bond of ATP. The energy released can be used for doing work inside the cell.

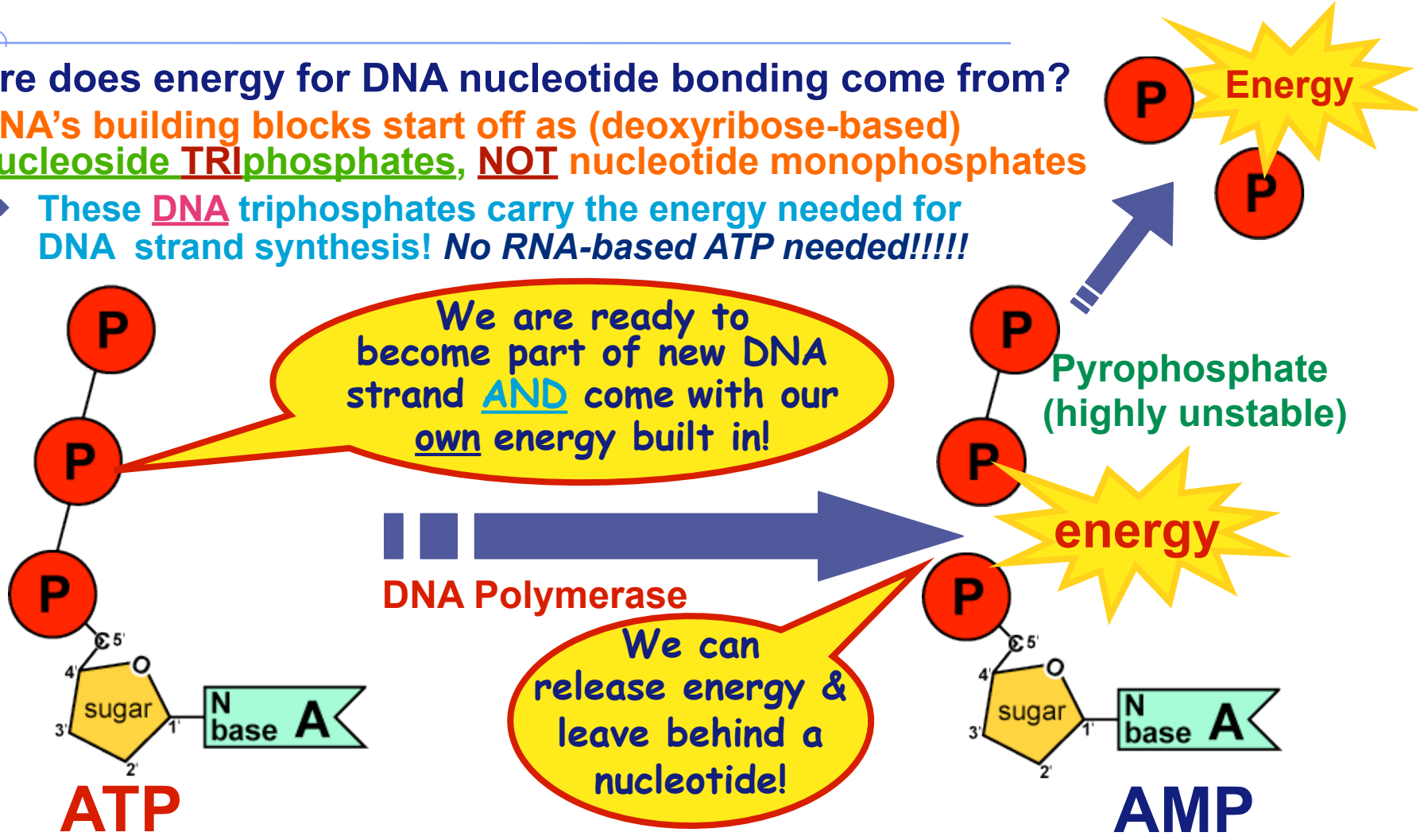


- **But when building DNA, we do NOT use this same ATP as a source of energy!!!**

Source of Energy for DNA Replication

Where does energy for DNA nucleotide bonding come from?

- **DNA's building blocks start off as (deoxyribose-based) nucleoside TRIphosphates, NOT nucleotide monophosphates**
  - **These DNA triphosphates carry the energy needed for DNA strand synthesis! *No RNA-based ATP needed!!!!!***

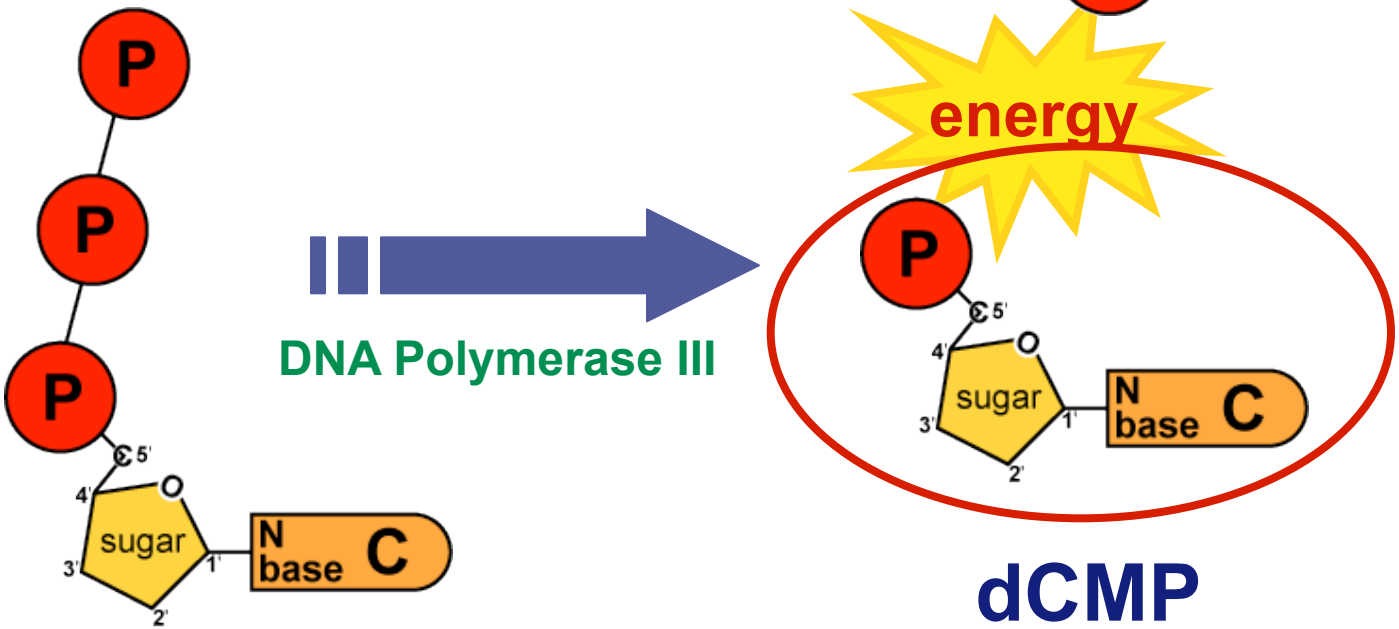


***ATP** = Containing deoxyribose not ribose like the ATP used to provide energy for other work in the cell! We sometimes refer to the DNA nucleotide as deoxyATP or dATP.*

*Hydrolysis of dATP, dGTP, dCTP, dTTP carried out by **DNA Polymerase**, which also performs the dehydration synthesis reaction that covalently bonds a new nucleotide to an existing nucleotide.*

Energy for DNA Replication

ALL nucleotides used for nucleic acids synthesis start off as deoxyribose-based nucleoside triphosphates (for DNA) - *deoxyribose sugar + base PLUS three phosphates* - or ribose-based nucleoside triphosphates (for RNA)



dCTP

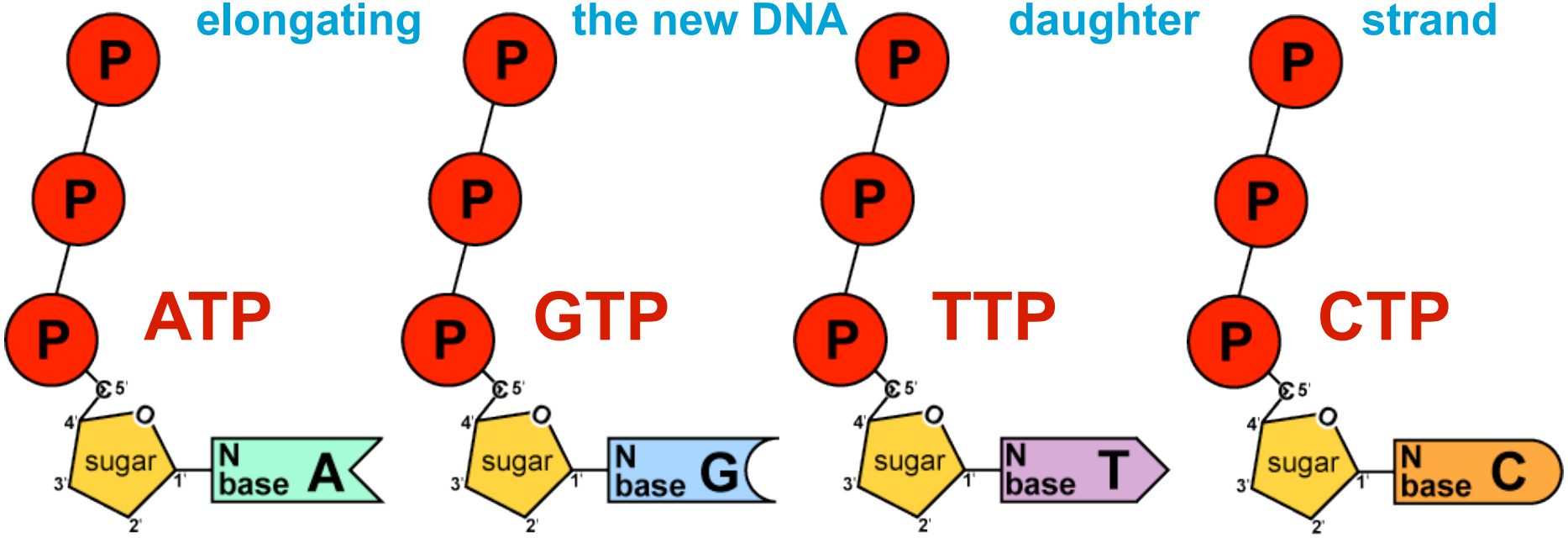
phosphorylated deoxynucleotide

dCMP

The monosphosphate left after release of the terminal phosphates will become part of the new nucleotide polymer

Energy for DNA Replication

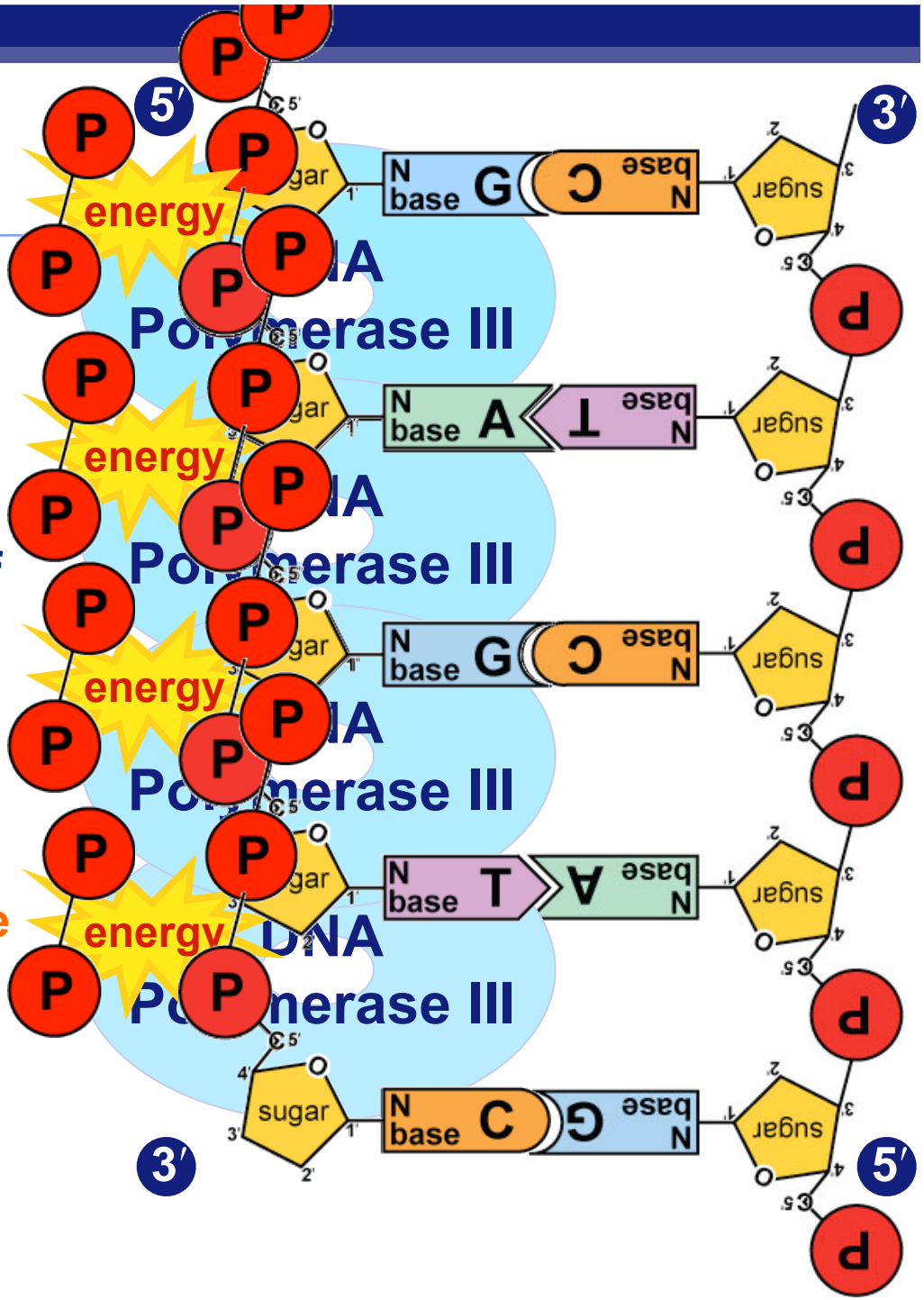
- **The nucleotides arrive in the active site of DNA Polymerase III as nucleoside triphosphates**
  - DNA bases arrive with P–P–P portion = carries the energy for bonding
  - DNA bases arrive with their OWN energy source for bonding
    - NO RNA-based ATP molecules needed for energy
  - With the energy released from each dNTP (deoxynucleotide triphosphate) DNA polymerase III covalently bonds a new nucleotide monophosphate to a previous nucleotide monophosphate, elongating the new DNA daughter strand



DNA Replication

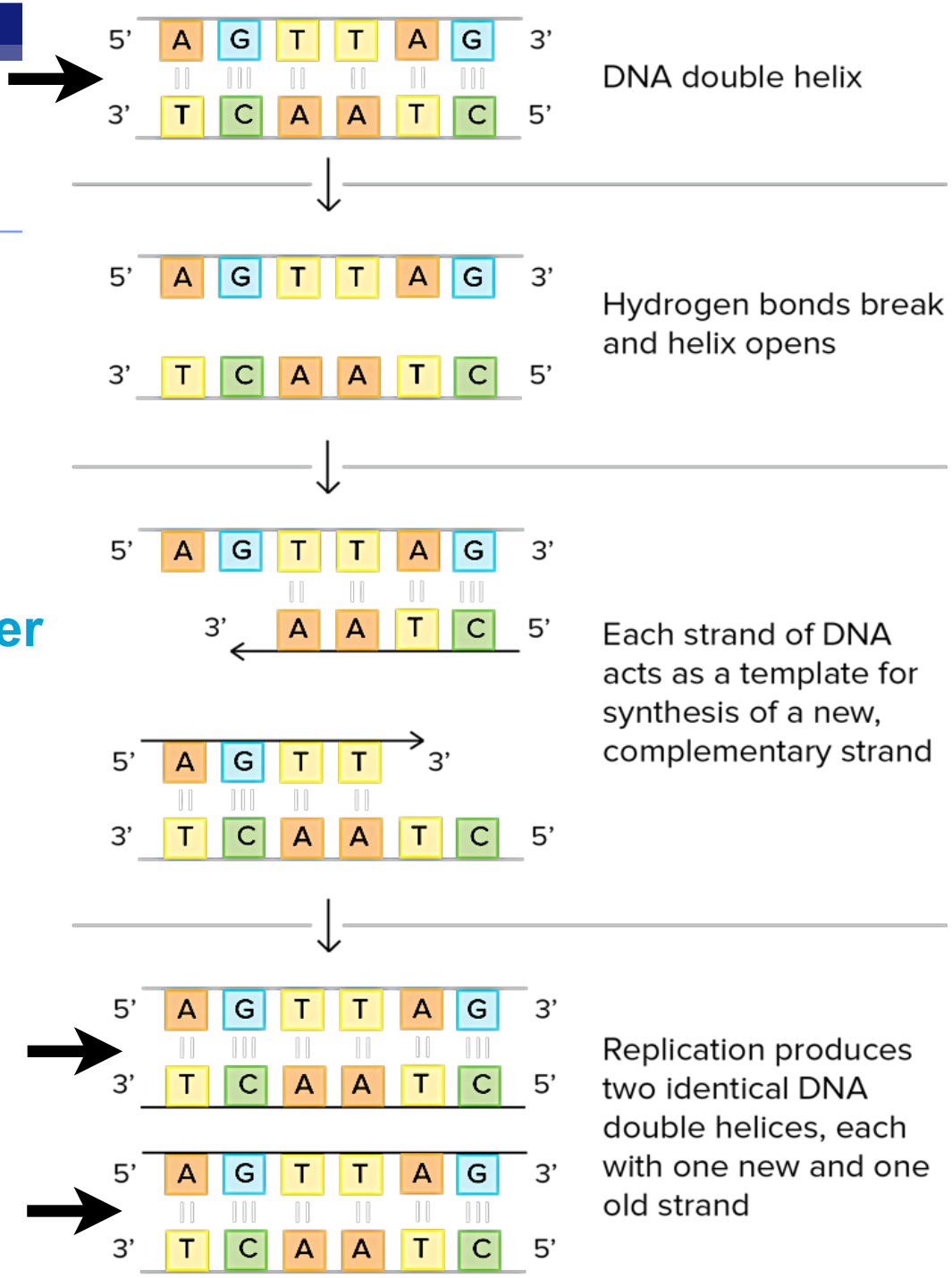
Adding bases to build the new daughter DNA strand using a template parental DNA strand:

- **DNA Pol III can only add nucleotides to the 3′ end of a GROWING *(existing)* DNA strand!!!!!!!**
  - **DNA Pol. cannot lay down the first complimentary nucleotide. It needs a "starter" nucleotide to bond more DNA nucleotides of the growing daughter strand to.**
- **strand only grows 5′→3′**



DNA Replication is Semiconservative

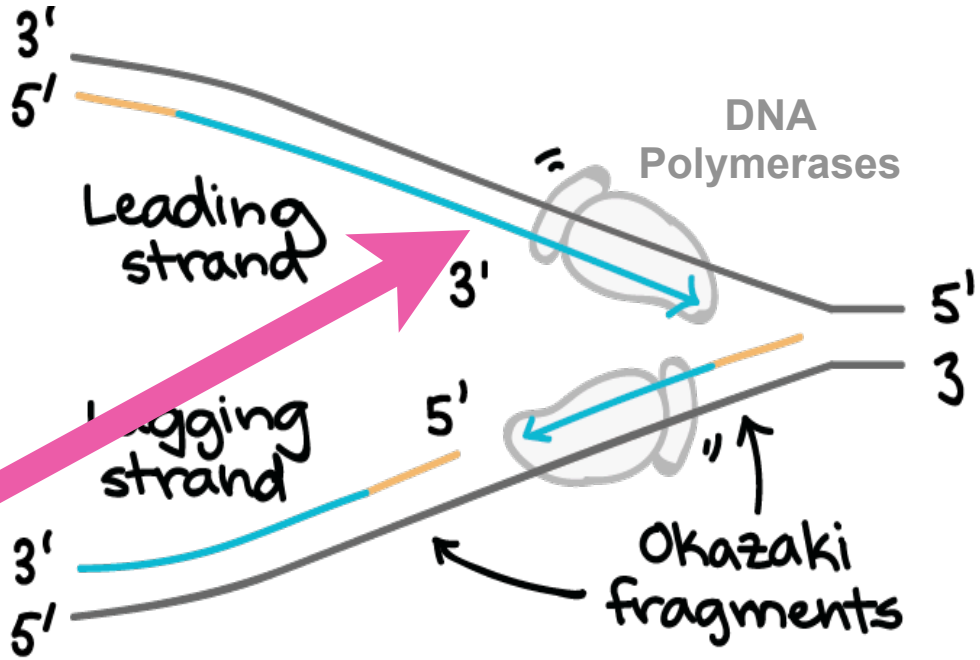
- **Semiconservative DNA Replication:**
  - **Each strand in the DNA double helix acts as a template for the synthesis of a new, complementary daughter strand.**
    - **This process takes us from one starting molecule (of double-stranded or ds DNA) to two "daughter" molecules, each of double-stranded DNA.**
      - **Each newly formed double helix contains one new and one old strand of DNA.**



Leading & Lagging Strands of a Replication Fork

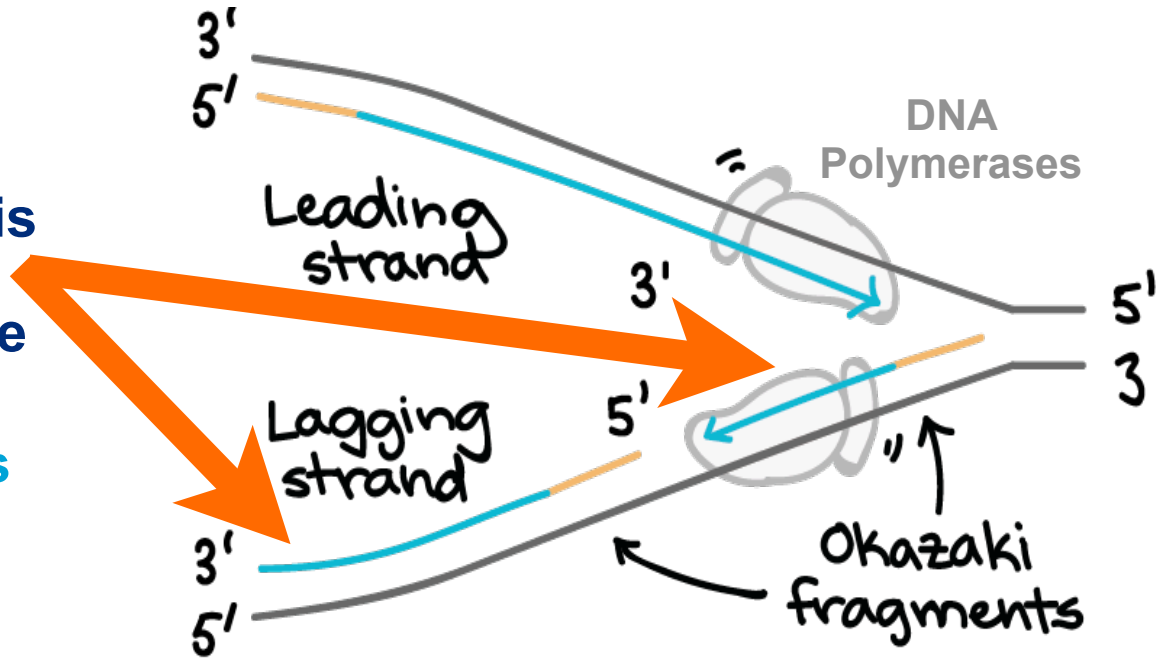
- DNA polymerases can only make DNA in the 5' to 3' direction
  - An initial DNA double helix is always anti-parallel: one strand runs in the 5' to 3' direction, while the other runs in the 3' to 5' direction.
    - This makes it necessary for the two new daughter strands, which are also antiparallel to their templates, to be made in slightly different ways.

- One new strand, being built 5' to 3' towards the replication fork, is made continuously, because the DNA polymerase is moving in the same direction as the replication fork.
  - This continuously synthesized daughter strand is called the leading strand.



Leading & Lagging Strands of a Replication Fork

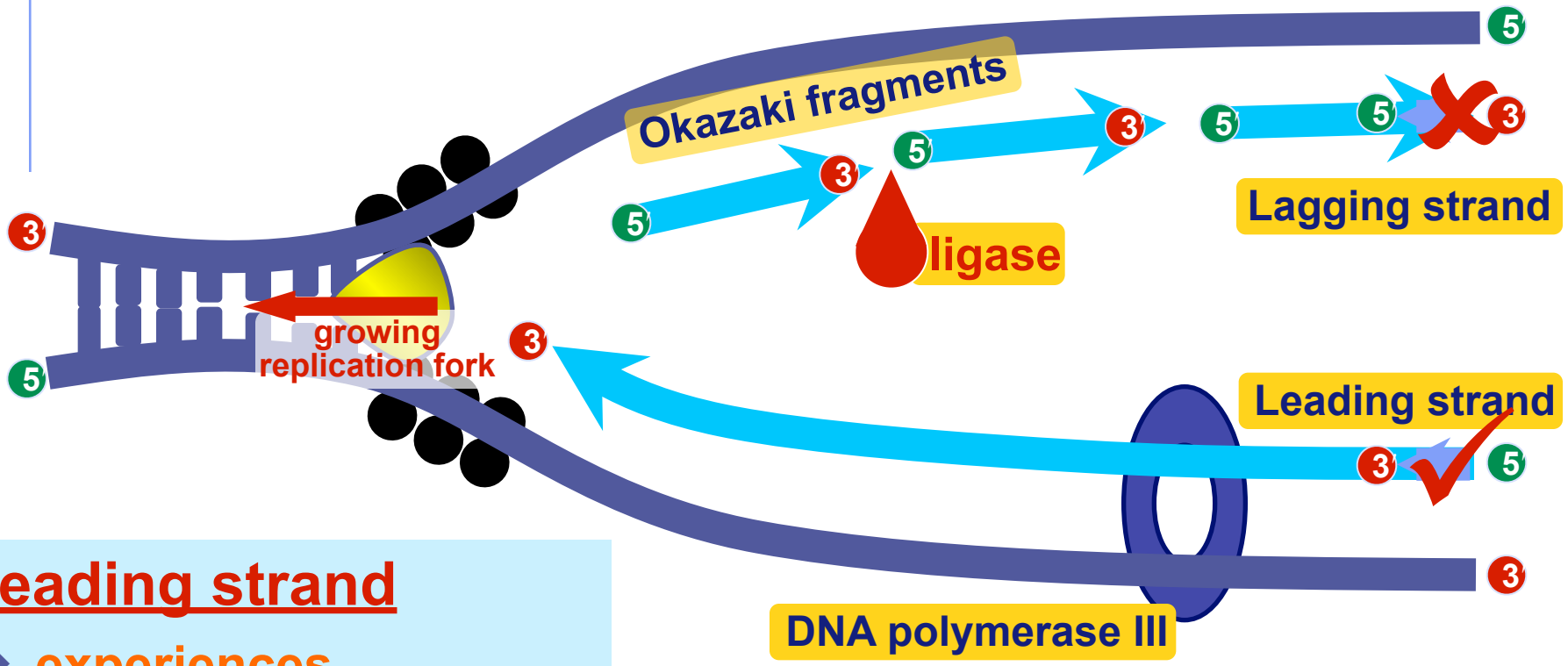
- The other new daughter strand, which is being built 5' to 3' away from the fork is made in fragments
  - As the fork moves forward (as helicase in the fork converts more dsDNA into ssDNA template to be copied), the DNA polymerase (which moves away from the fork) has to let go of the DNA template it just copied, reposition itself closer to the fork where new single-stranded DNA is found, and reattach on the newly exposed ssDNA to copy a new segment of the template strand, while again moving away from the fork.
- This DNA strand that is thus copied in small fragments is called the lagging strand.
  - The small fragments are called Okazaki fragments.



Leading & Lagging strands of a Replication Fork

Limits of DNA polymerase III

- can *only* build a stand of DNA by adding nucleotides to the 3′ end of an existing DNA polymer



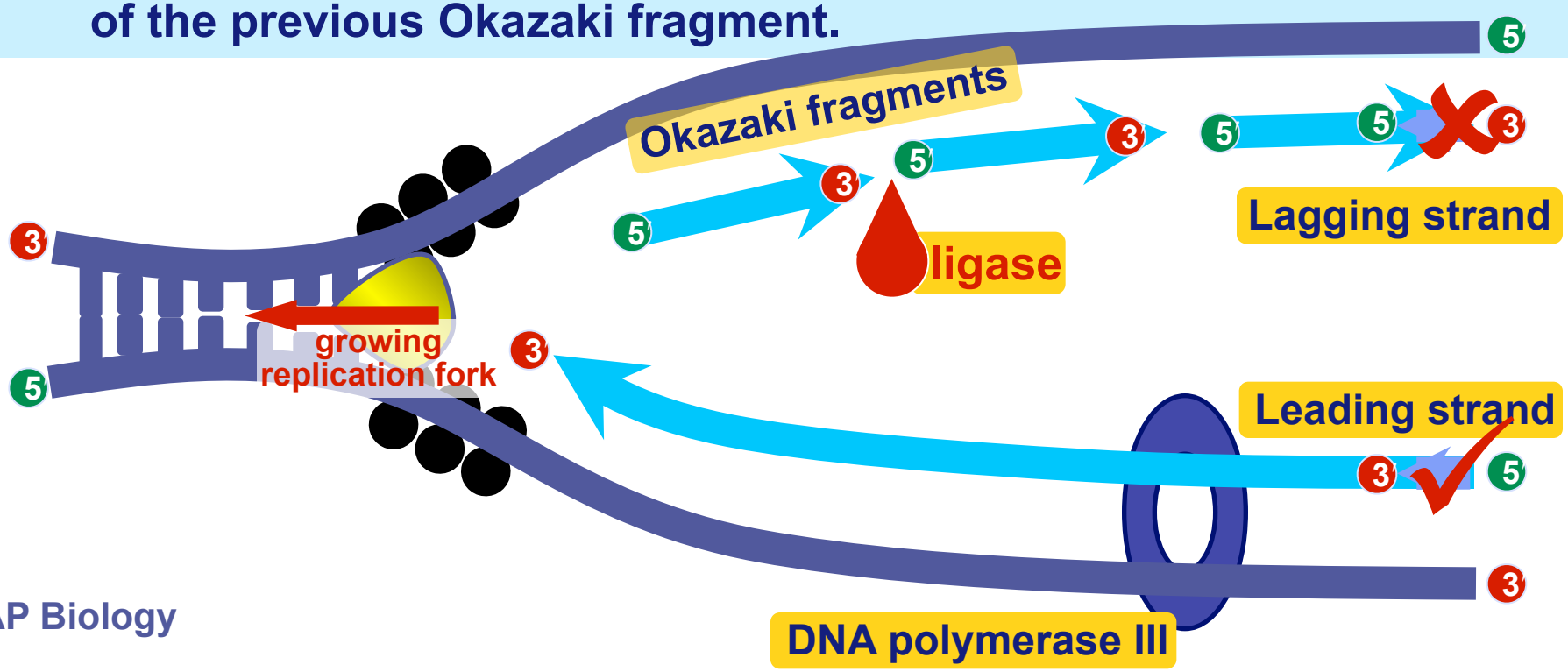
Leading strand

- experiences continuous synthesis

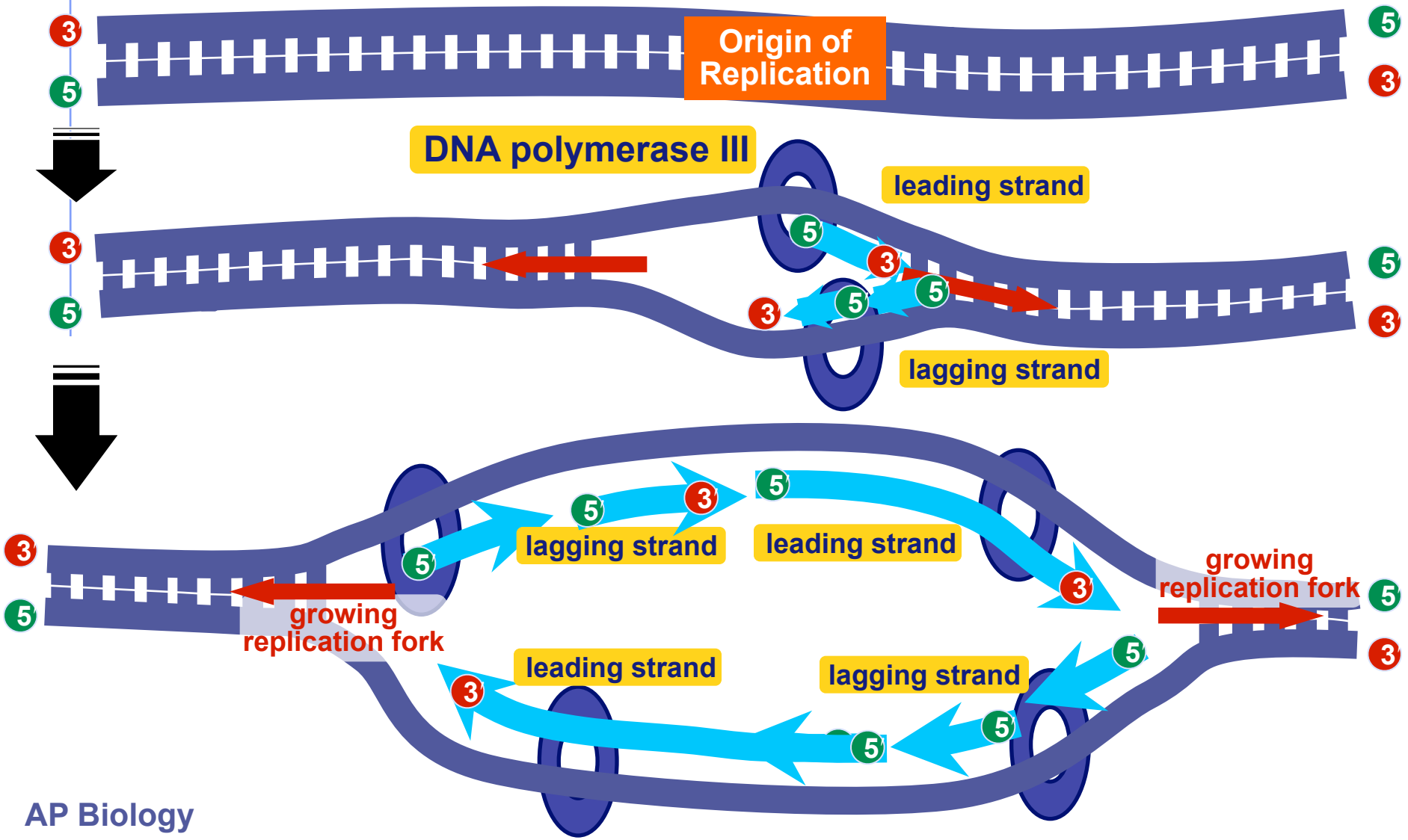
Leading & Lagging strands of a Replication Fork

Lagging strand

- composed of multiple Okazaki fragments
  - 100-200 nucleotides long
- joined together later by the enzyme ligase
  - performs dehydration synthesis reactions between the 3' hydroxyl group of the newest Okazaki fragment and the 5' phosphate group of the previous Okazaki fragment.

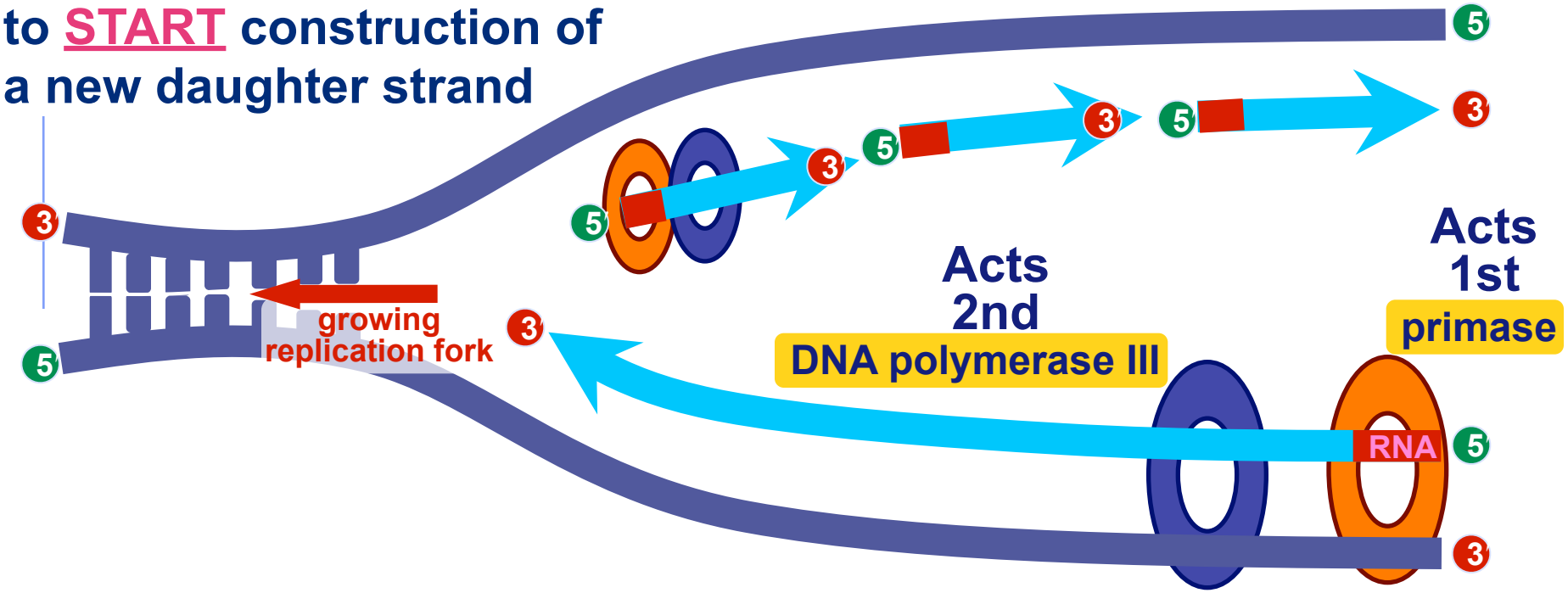


Replication forks of Replication bubble



Starting DNA synthesis: RNA primers

Unlike RNA Polymerases, DNA Polymerases CANNOT lay down the first complimentary nucleotide base to START construction of a new daughter strand



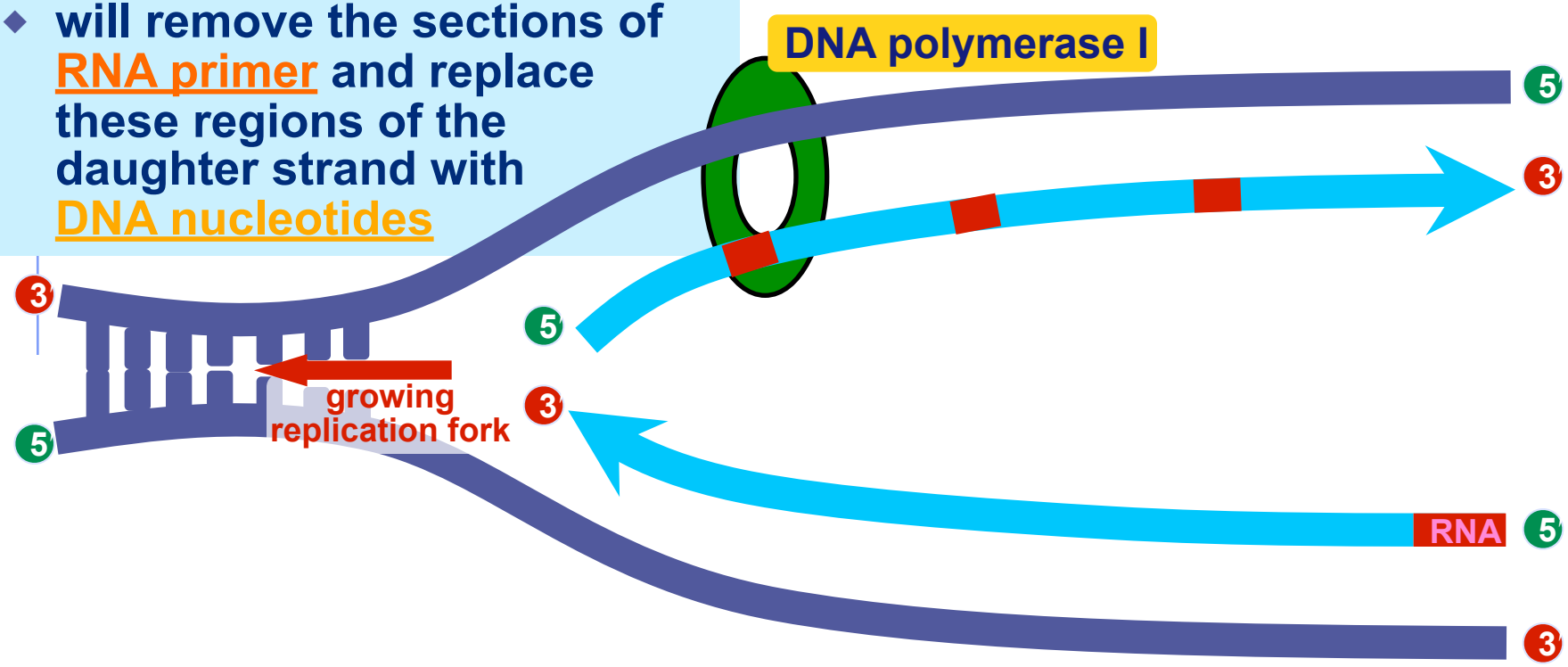
Because RNA polymerases CAN lay down the first complimentary nucleotide to start a daughter strand off a template, the cell builds a temporary RNA primer as a start to the new daughter strand

- **built by primase *(a type of RNA Polymerase)***
- **serves as starter sequence for DNA polymerase III to then elongate**

Replacing RNA primers with DNA

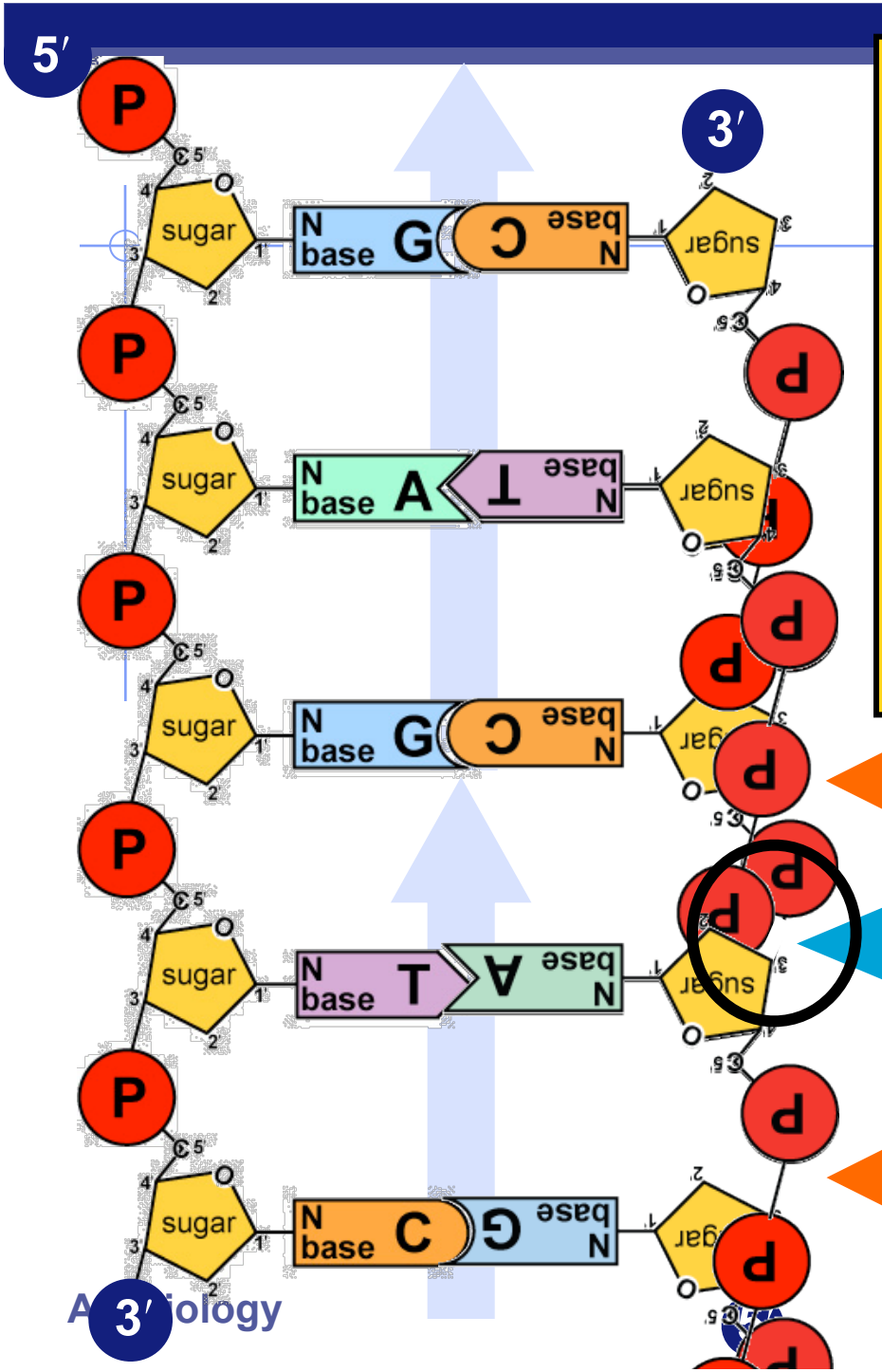
DNA polymerase I

- will remove the sections of RNA primer and replace these regions of the daughter strand with DNA nucleotides



BUT DNA Pol I still can only build onto 3′ end of an existing DNA strand.

- The DNA Pol I will extend the 3' end of DNA from one Okazaki fragment until reaching the next fragment
  - DNA Pol I thus replaces the primer from the previous Okazaki fragment with DNA nucleotides instead of the RNA nucleotides that were laid down by primase.

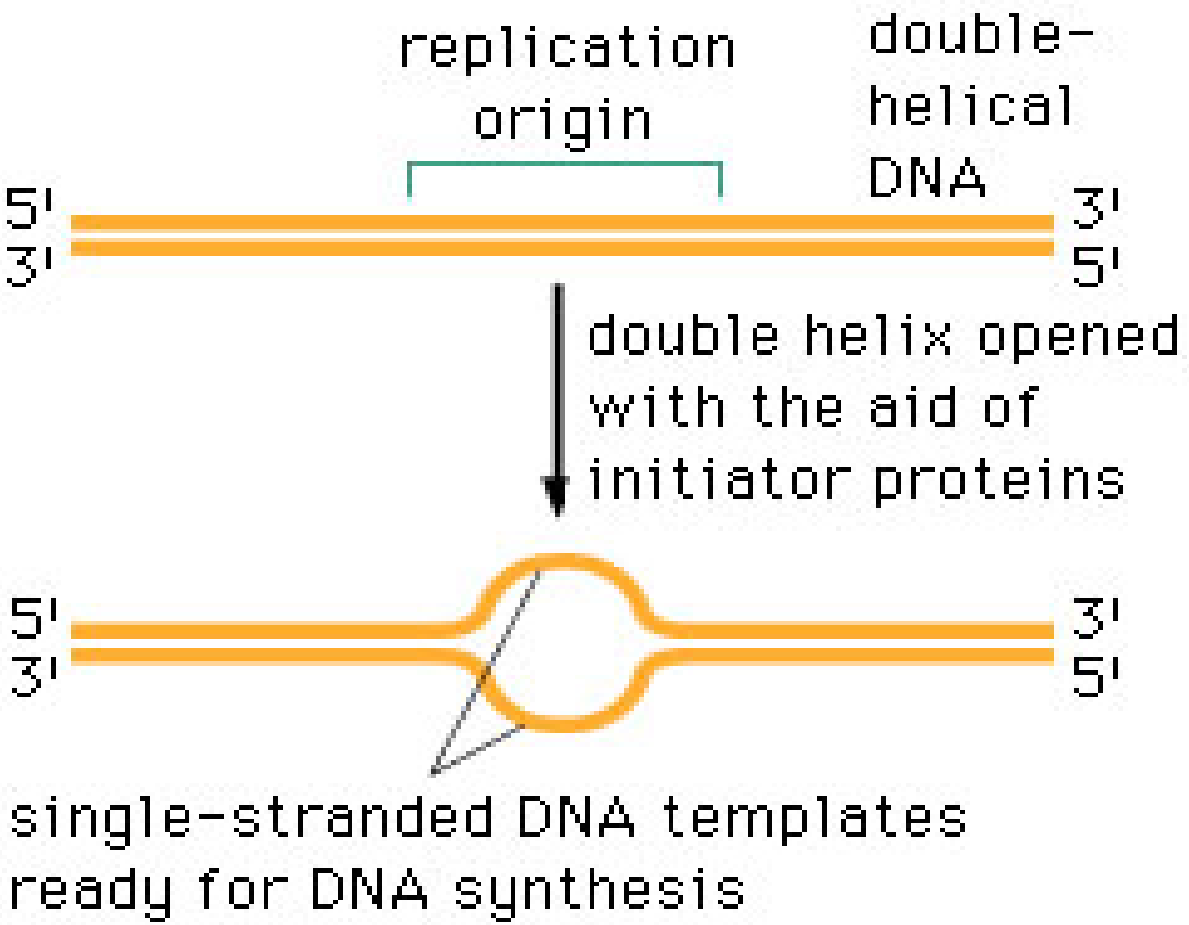


- After all the RNA primer sections in the daughter DNA are replaced by DNA Pol I with DNA nucletides, LIGASE "seals" one DNA strand fragment to the next DNA strand fragment in the lagging strand to create one continues strand of daughter DNA
  - ligase builds the phosphodieter bond to connect to DNA fragments together covalently

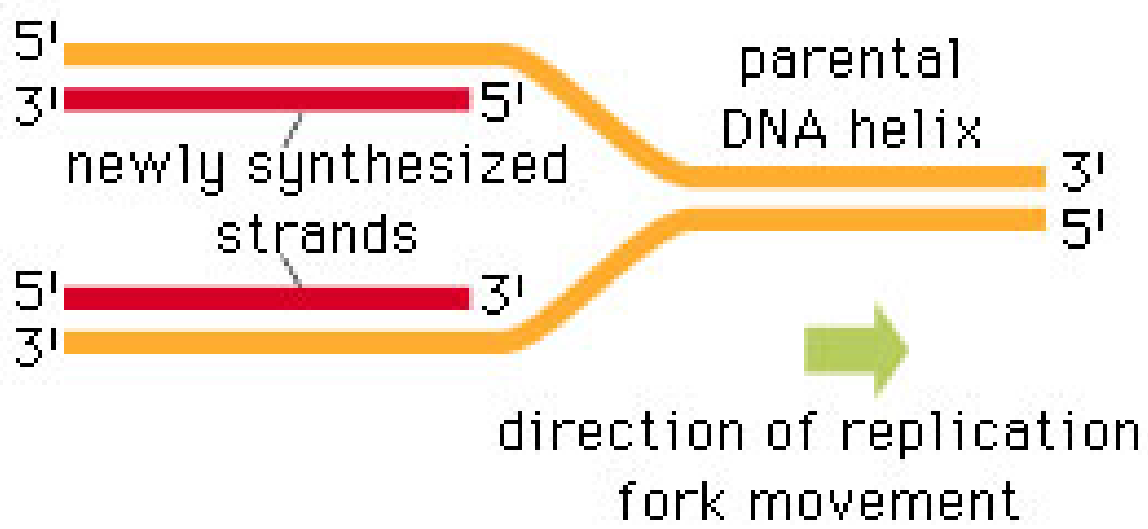
ligase

Okazaki Fragments

Quick Review - Replication

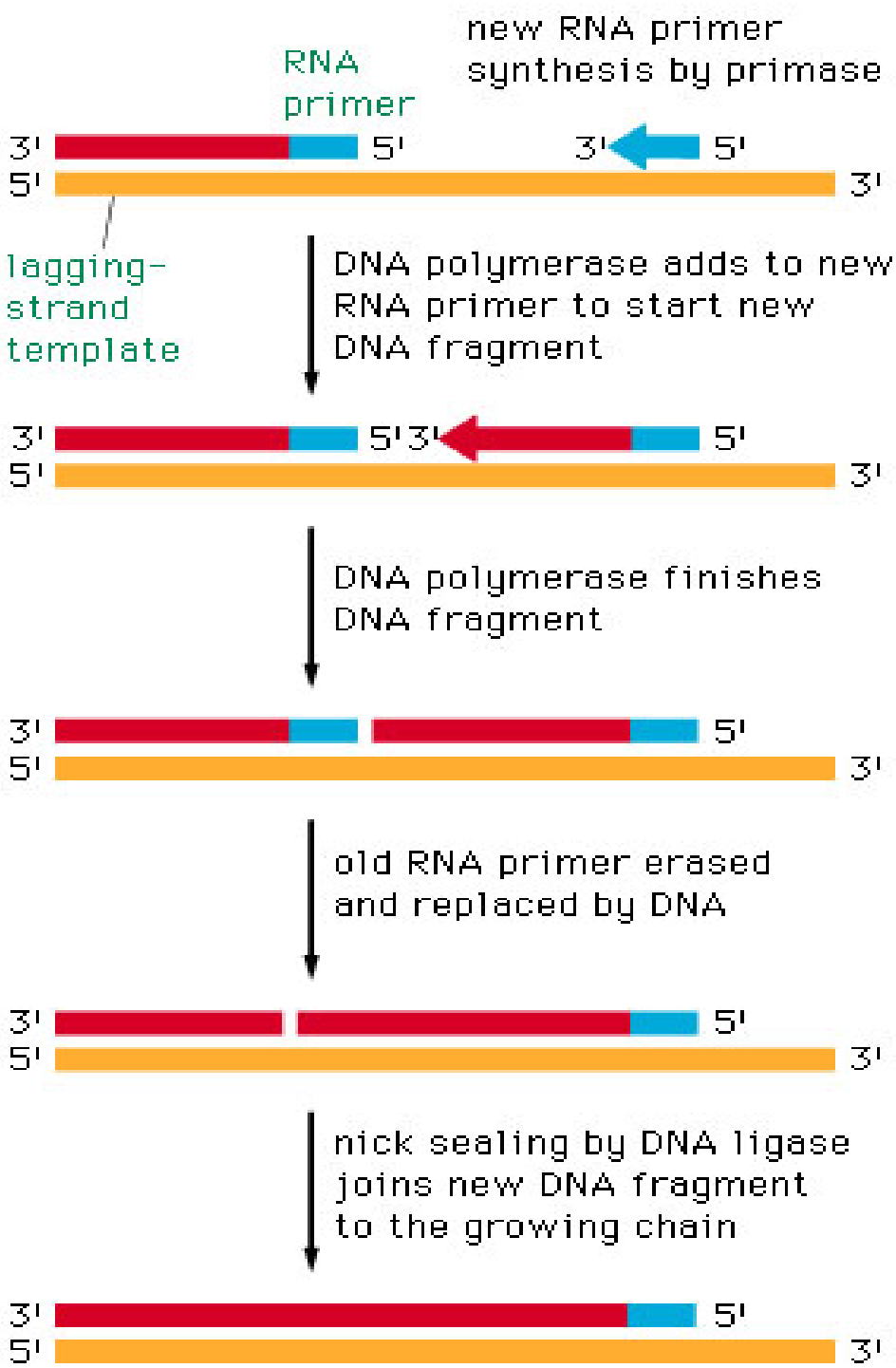


Quick Review - Replication



Replication of DNA is bidirectional!

Two Y-shaped replication forks are moving in the opposite directions during DNA replication, DNA being copied from both templates at each of the two forks!



Quick Review - Replication

Primase lays down a short RNA Polymer complimentary to the DNA Template

DNA Pol III elongates the RNA Primer by adding DNA nucleotides to it.

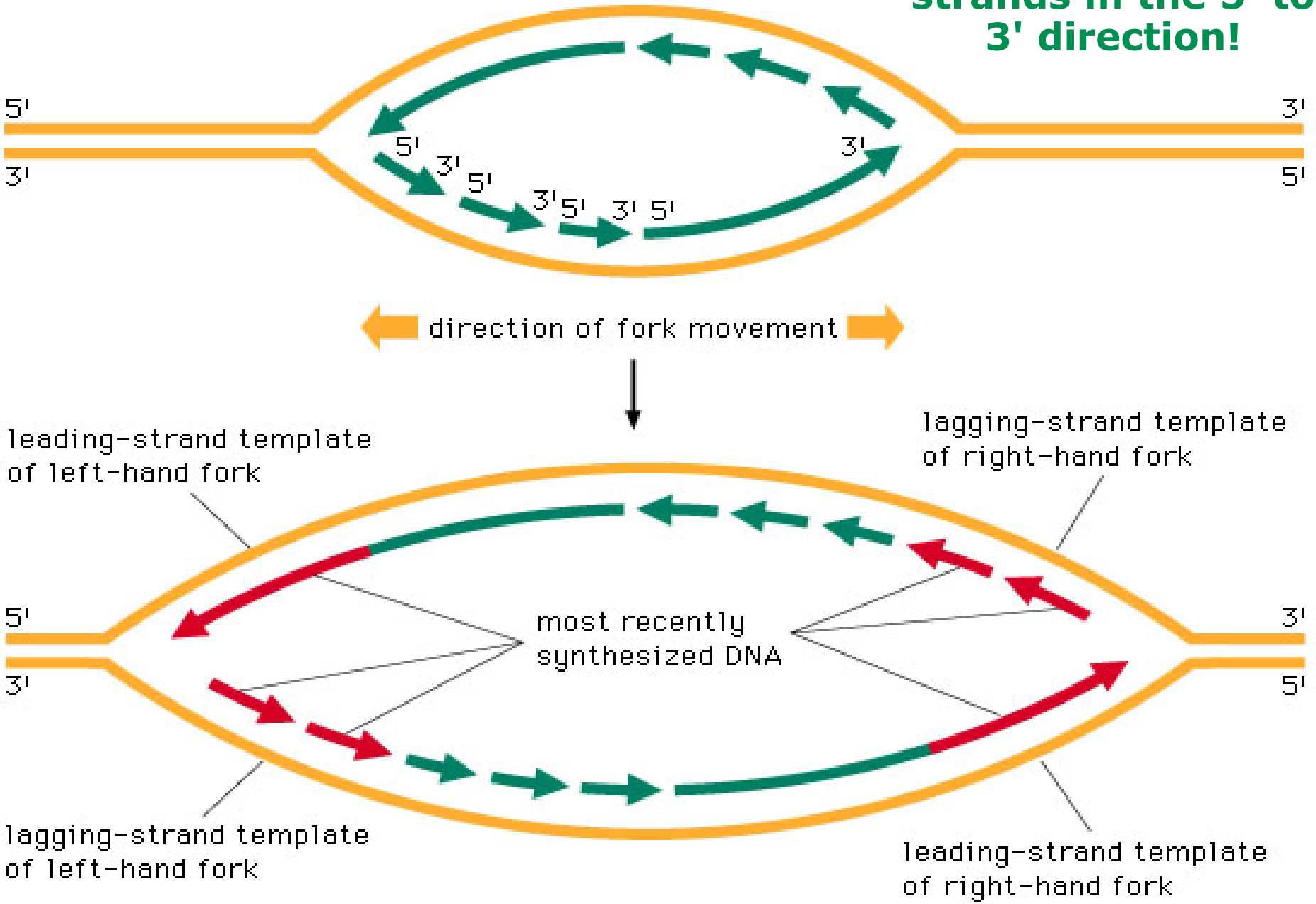
DNA Pol I later removes the RNA nucleotides of the primer.

DNA Pol I also extends the previous Okazaki fragment's 3" end by adding DNA nucleotides in order to fill in the gap left behind when the RNA primer was removed from the neighboring fragment.

Ligase covalently connects the fragments once they are made of only DNA nucleotides.

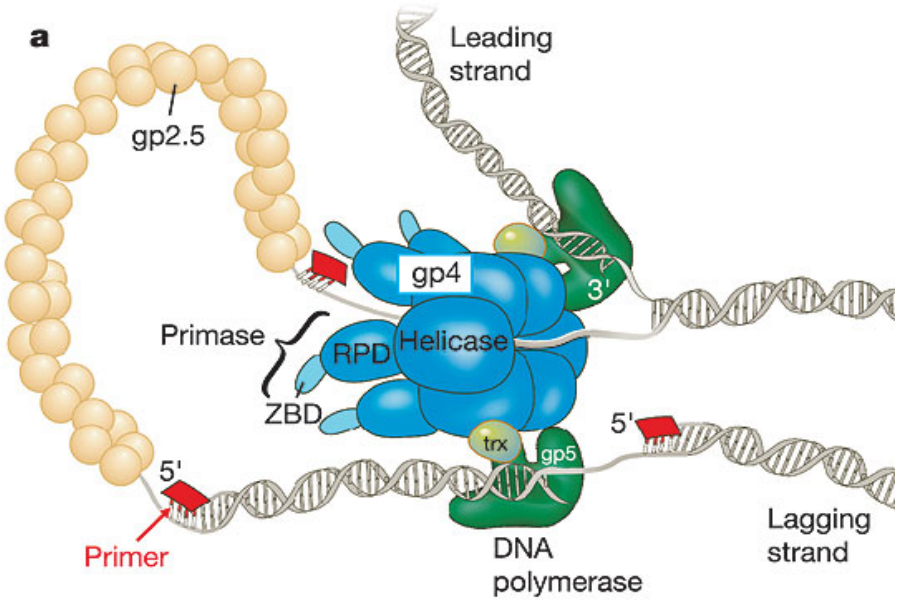
Quick Review - Replication

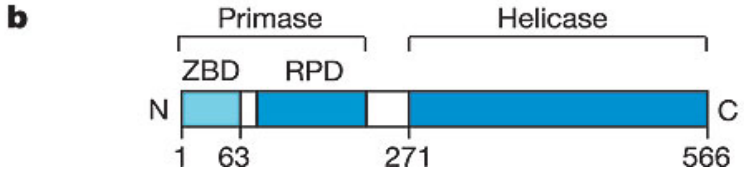
It is not trivial to replicate both DNA strands in the 5' to 3' direction!



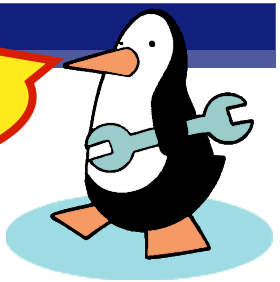
Replisome

- **The enzymes involved in DNA replication form a macromolecular assembly called the REPLISOME.**
  - **Replisomes are made up of the primosome & two DNA pol III enzymes (one for each strand)**
    - **DNA Pol III**
      - **Both are active on the leading and lagging strand simultaneously**
    - **Primosome composed**
      - **Primase**
      - **Helicase**
      - **Accessory proteins**



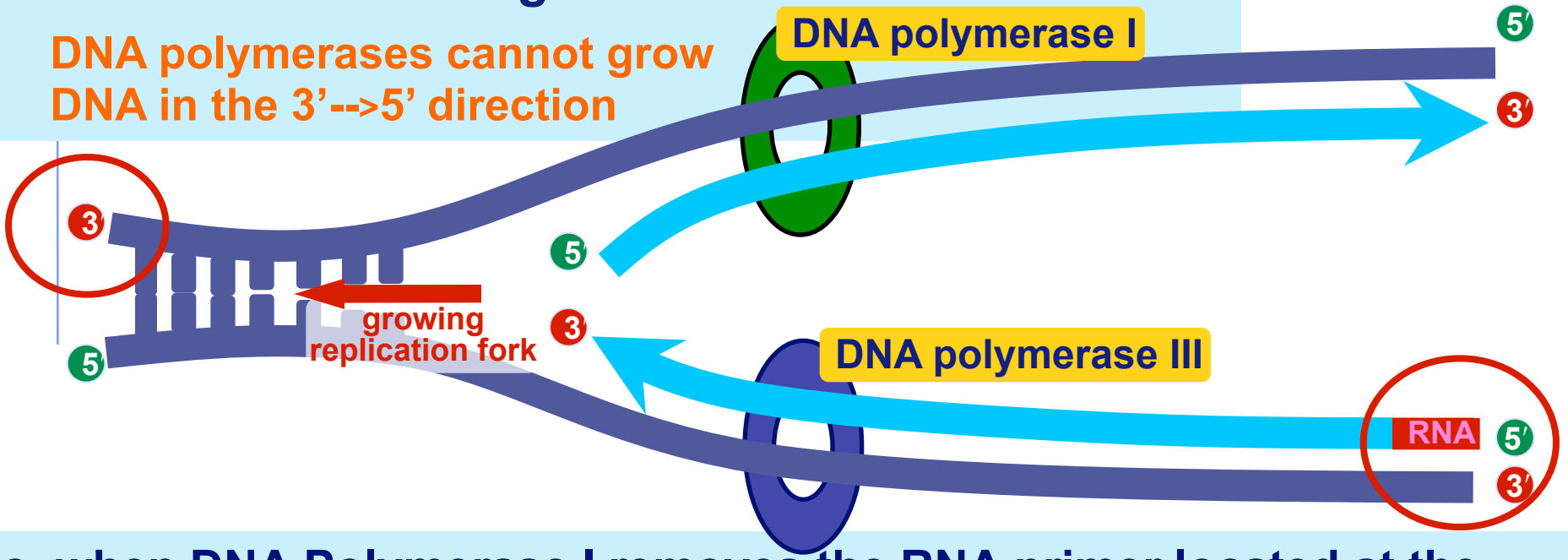


Chromosome erosion



ALL DNA polymerases can ONLY add nucleotides to 3′ end of an existing DNA strand

DNA polymerases cannot grow DNA in the 3'-->5' direction



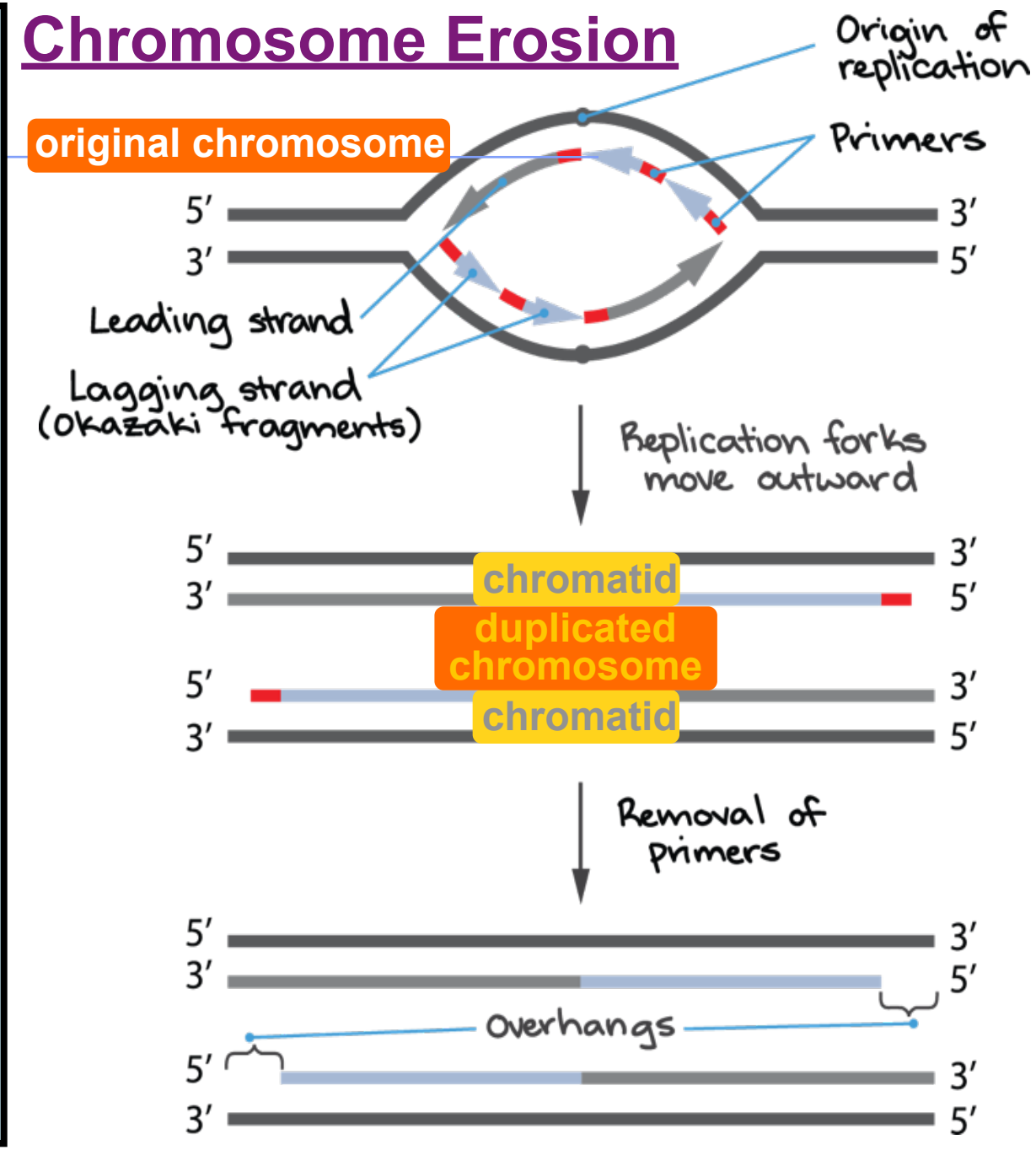
So, when DNA Polymerase I removes the RNA primer located at the very tip of the chromosome (the primer placed at the 5' end of the daughter strand), DNA Polymerase I cannot fill in the gap left behind.

- **There is, thus, a loss of bases at 5′ ends of daughter strands (compared to the parent template strand being copied) in every replication cycle**
  - **Chromosomes get shorter with each replication**
    - **Is there a limit to number of cell divisions possible?**

Chromosome Erosion

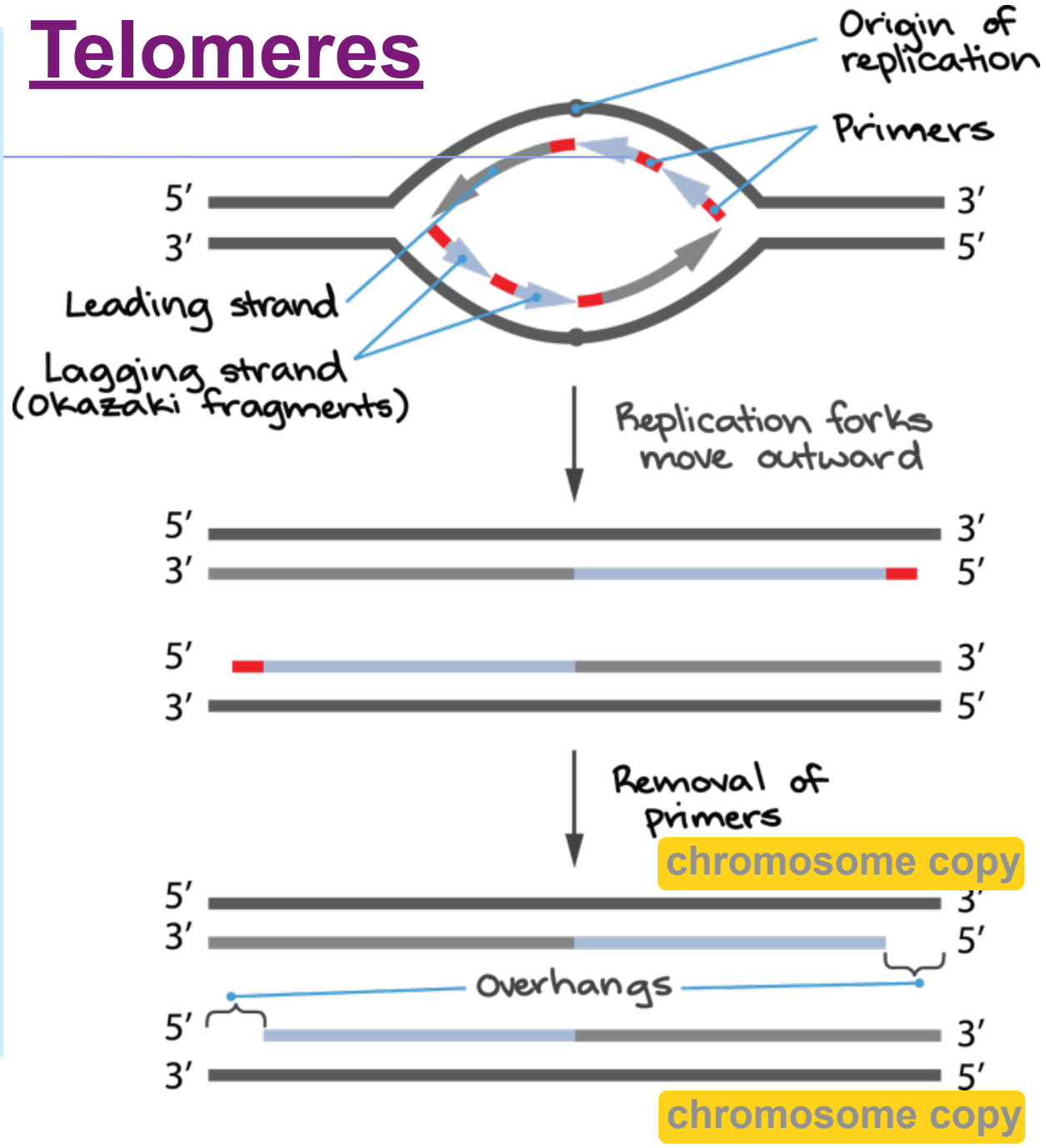
If the 3' tips of chromosome template strands are not copied into the 5' complimentary ends of daughter strand, genes located near the tips of chromosomes would not get copied into daughter strands at their 5' ends.

After DNA Replication in S phase is complete, G2 and M phase will follow, each original template strand and its complimentary new daughter strand (forming a chromatid of a duplicated chromosome) being passed down to one of the daughter cells that results after cell division.



Telomeres

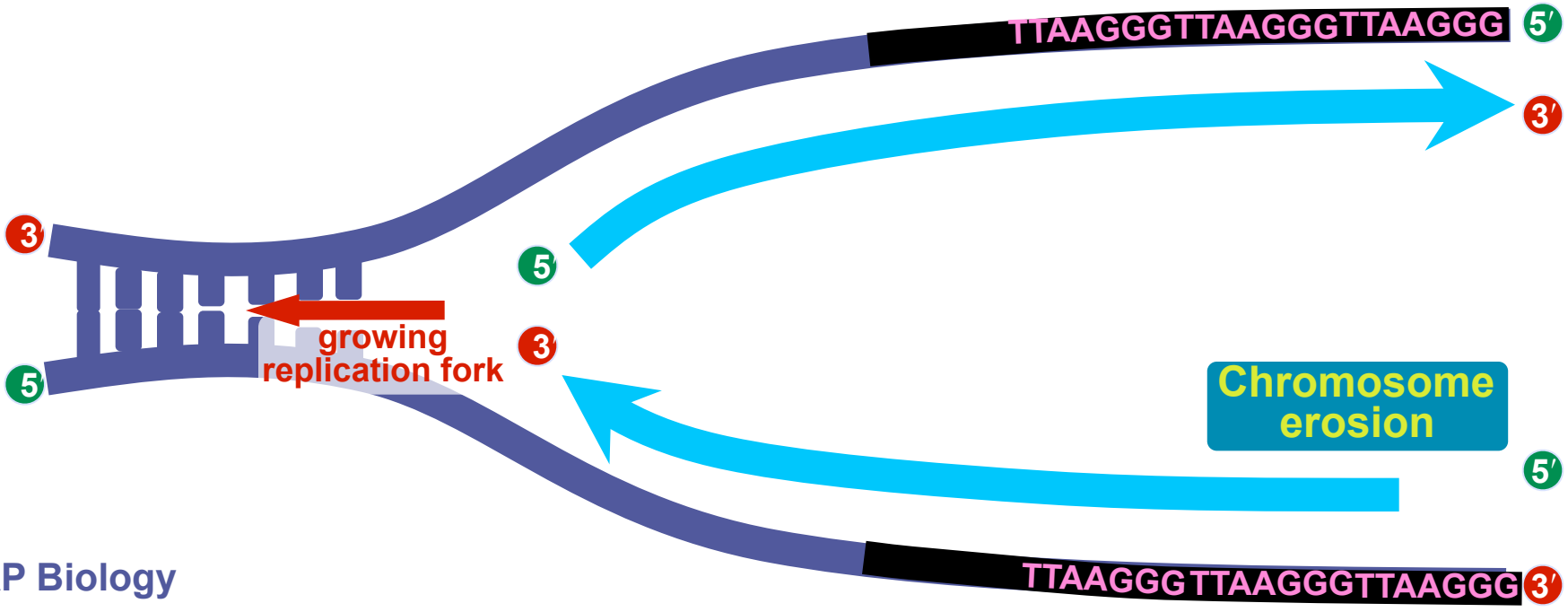
Once M phase is over, the daughter cells that form begin G1. When they get the signal to divide and enter S phase in the future, the chromosome's two strands (one strand that shorter than the other strand at its 5' end) will each become templates for making new daughter DNA strands. Again, there will be a problem copying the 3' ends of these two strands into daughter strands.



Telomeres

Chromosome erosion could become a problem:

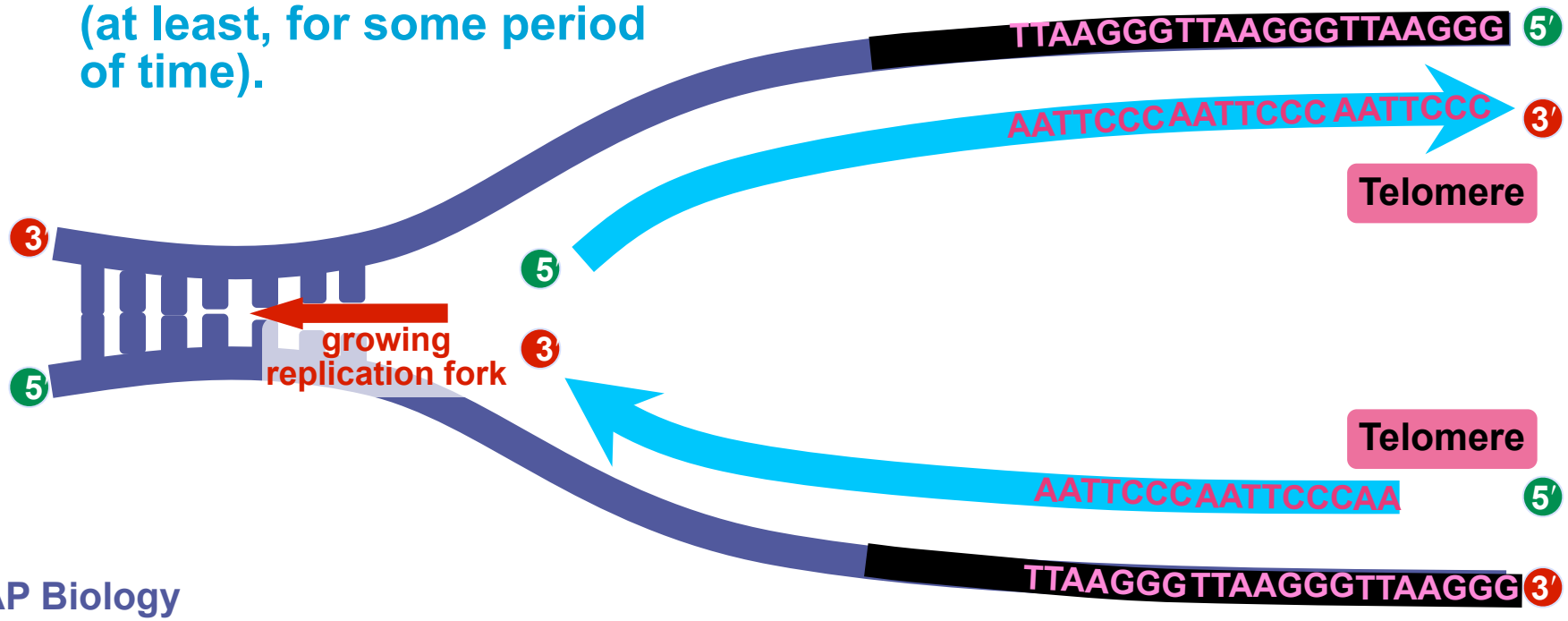
- If the tips of chromosomes are not copied into the daughter strands during every sequential S phase and, thus, the tips of chromosomes progressively become shorter during each new cell cycle, genes located near the tips of chromosomes would eventually not get copied.
  - Over time, repeat DNA sequences (that do not code for any RNA or proteins) have evolved at the tips of chromosomes



Telomeres

Telomeres = 100s or 1000s of repeating, short, non-coding DNA sequences at the end of chromosomes (telomeres are made up of repeat sequence of 5'-TTAGGG-3' in mammals).

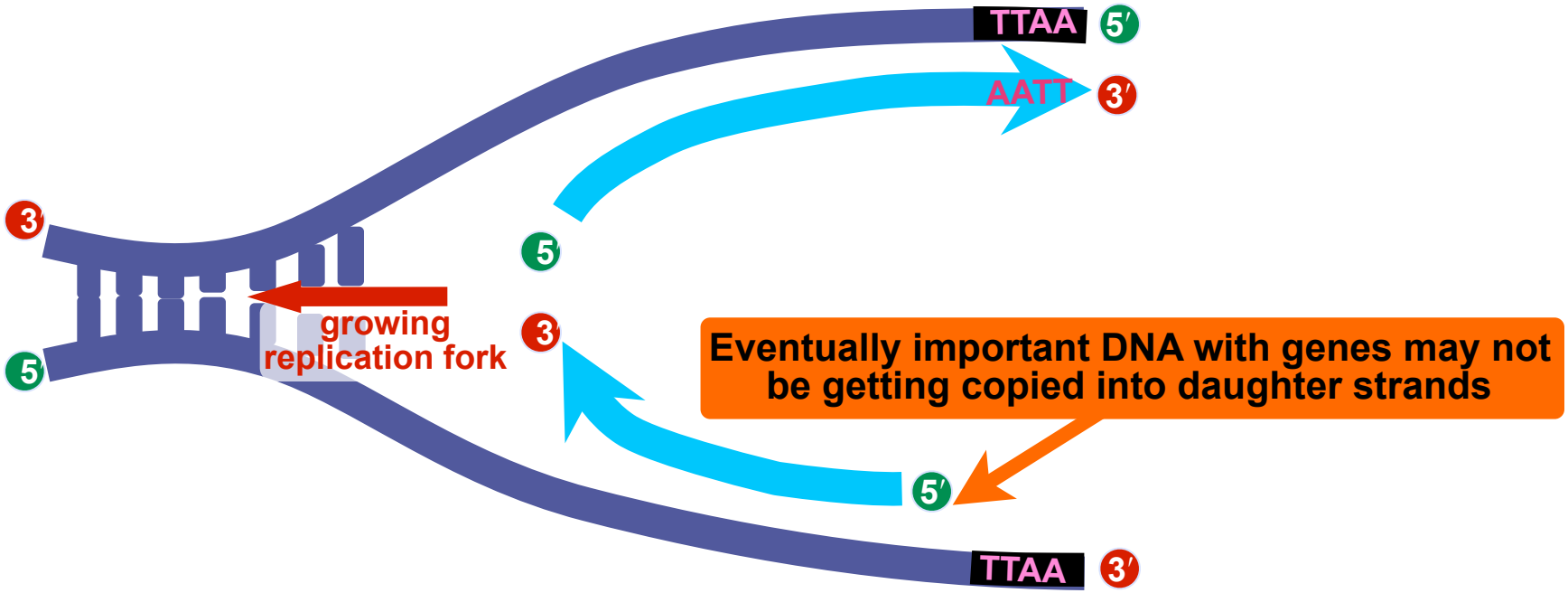
- **Telomeres act as protective caps that protect the internal regions of the chromosomes.**
- **They are worn down a small amount in each round of DNA replication, providing a buffer that protects the internal chromosome regions bearing the genes (at least, for some period of time).**



Telomeres

Telomere shortening has been connected to the aging of cells and the progressive loss of telomeres may explain why somatic (body) cells in an organism can only divide a certain number of times

- **Telomere erosion limits a cell to ~50 cell divisions**

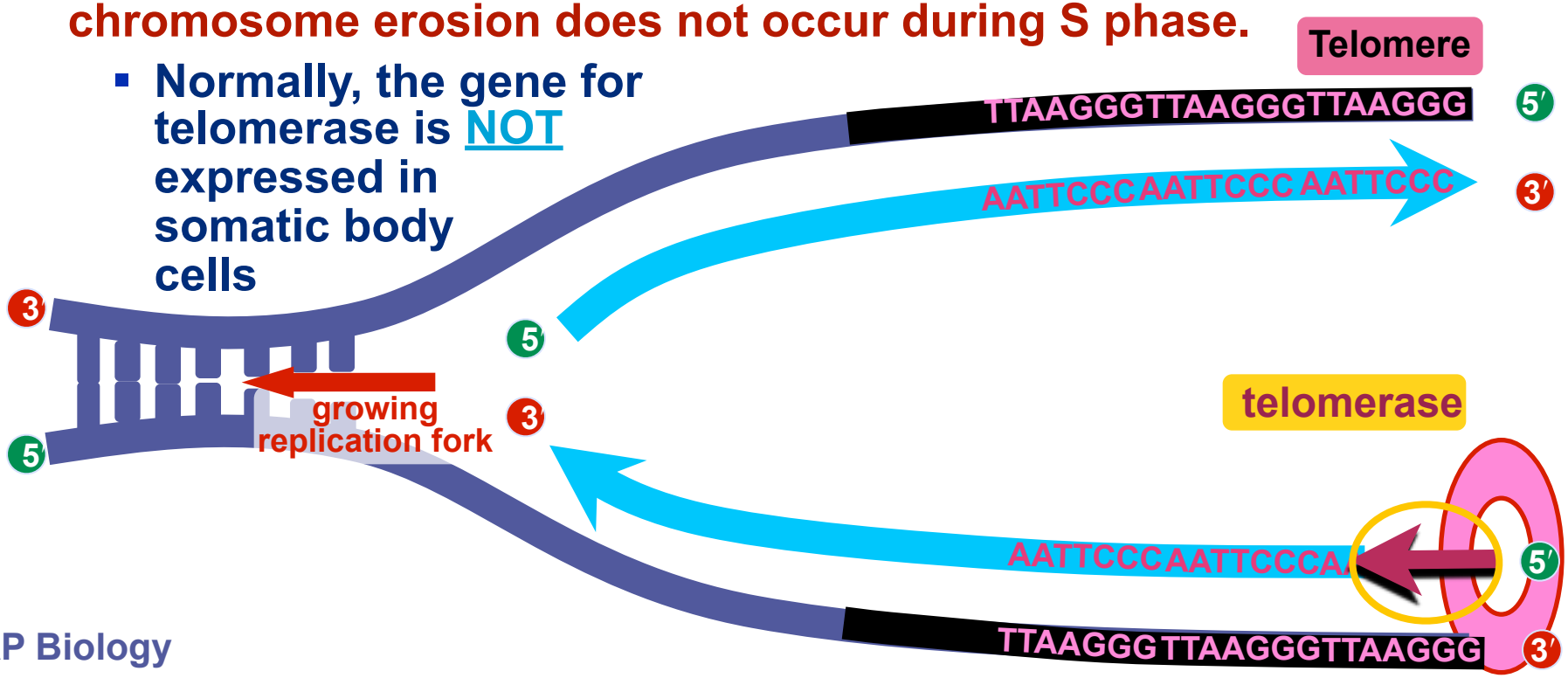


- **After DNA has been copied in many cell cycles, telomeres may get fully eroded away so that genes further inside the chromosome may be damaged by not being fully copied during S phase any more**

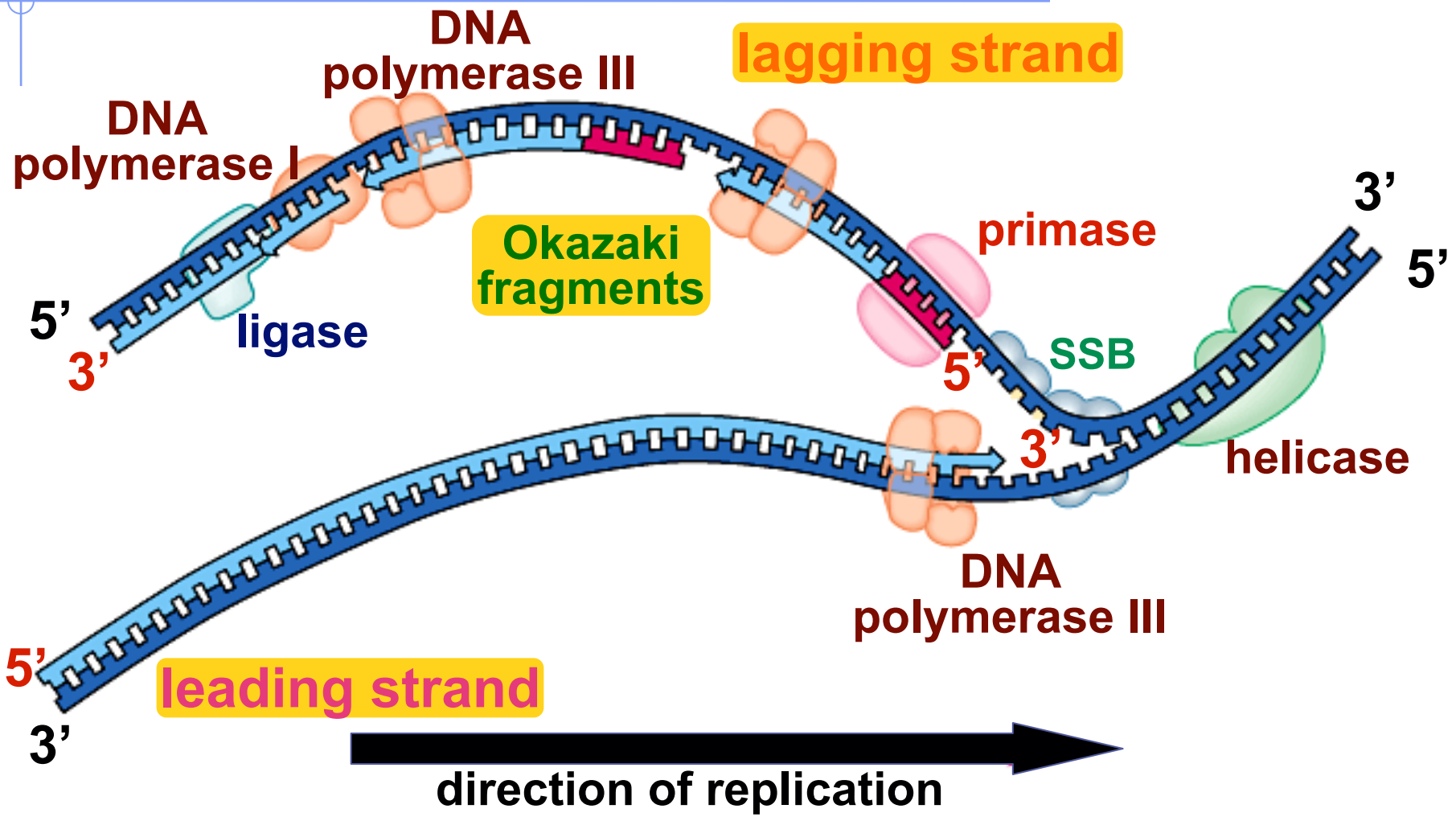
Telomerase Enzyme

Telomerase (enzyme) is found in some cells like stem cells *(= unspecialized cells that can divide to form new cells that then specialize into specific tissue cells)* **and germ line cells, which divide to form reproductive cells (gametes) which must contain chromosomes of full length to pass down to the next generation**

- **This enzyme extends telomeres of daughter strands being copied, filling in the gap at the ends of daughter strands were DNA Polymerase I cannot add missing DNA bases. This way, chromosome erosion does not occur during S phase.**
  - **Normally, the gene for telomerase is NOT expressed in somatic body cells**



Overview of Proteins Active at Replication Forks

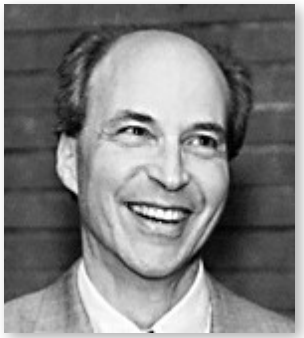


AP

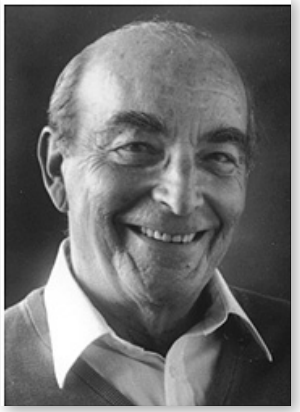
SSB = single-stranded binding proteins - stabilizes single stranded DNA and prevents re-annealing (DNA strands sticking back together)

DNA polymerases

- DNA polymerase III
  - 1000 bases/second*!*
  - main DNA builder
- DNA polymerase I
  - 20 bases/second
  - editing, repair & primer removal

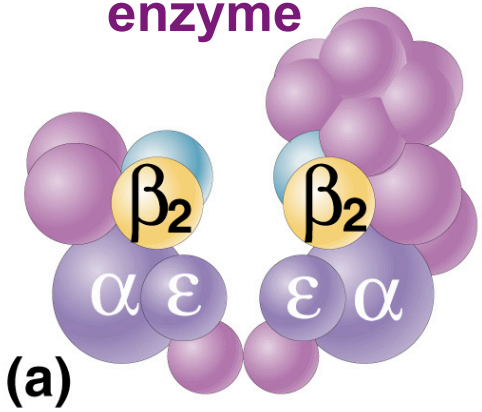


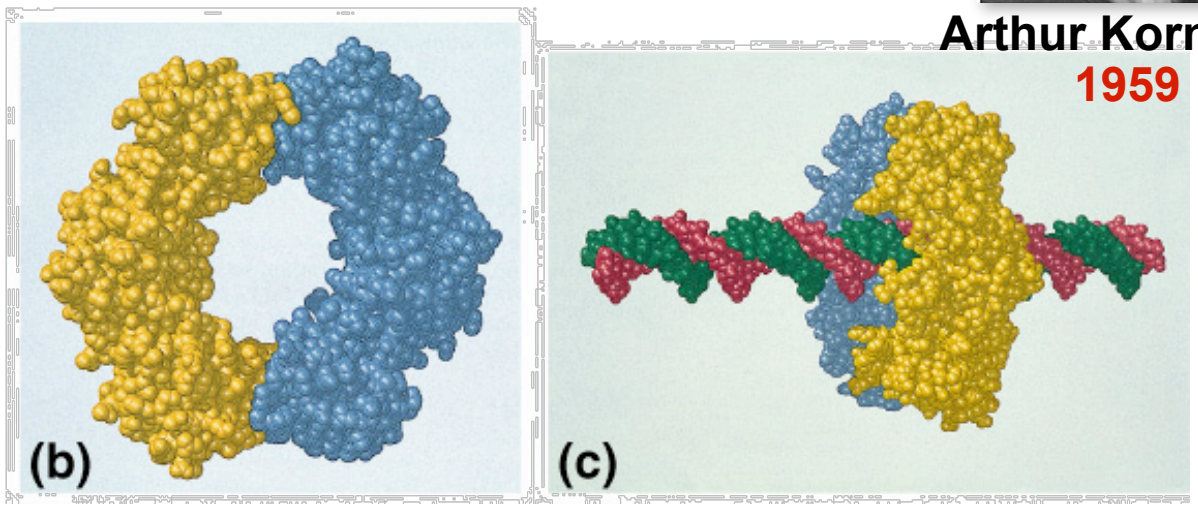
Roger Kornberg
2006



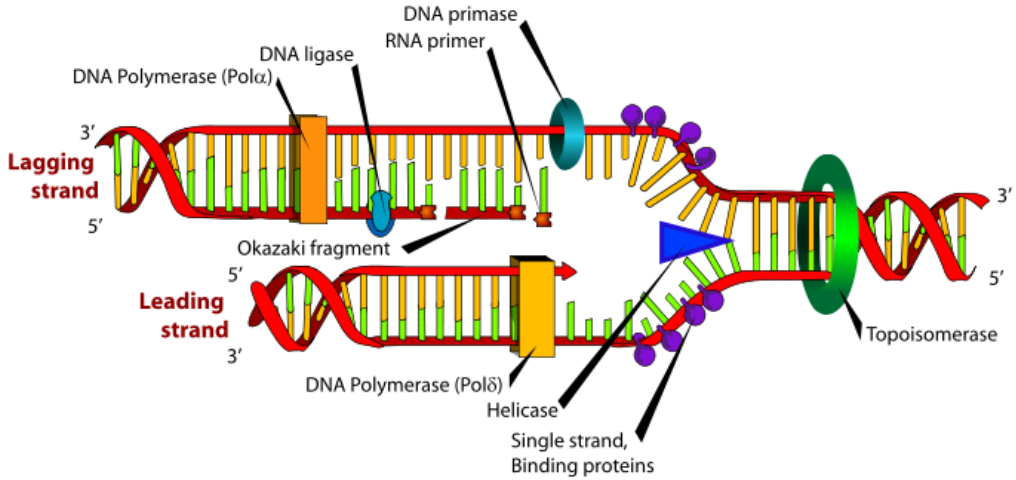
Arthur Kornberg
1959

DNA polymerase III enzyme





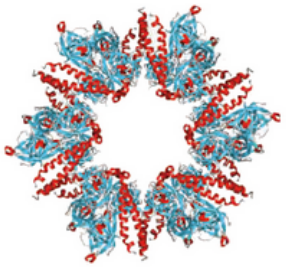
Fast & accurate!



- It takes *E. coli* <1 hour to copy 5 million base pairs in its single chromosome
  - cell divides to form 2 identical daughter cells
- Human cell copies its 6 billion bases & divide into daughter cells in only few hours
  - remarkably accurate
  - only ~1 error per 100 million + bases
    - ~30 errors per cell cycle

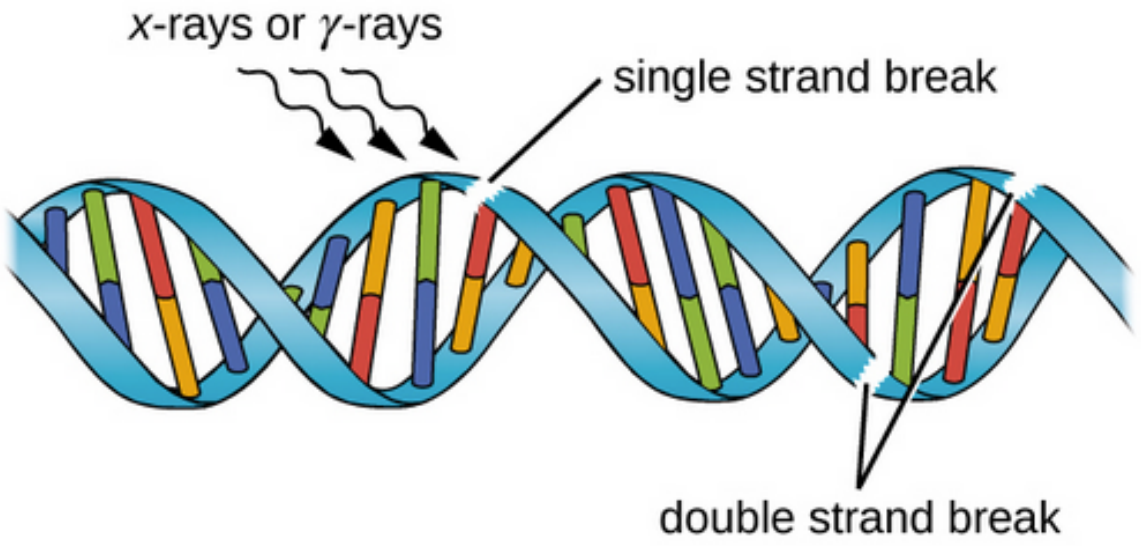
What happens when a gene's DNA sequence is changed?

- **It is important that DNA be copied correctly so each daughter cell, forming after mitosis (or meiosis), gets copies of all genes with the correct DNA sequences so they can use these gene instructions to correctly make all necessary RNAs & proteins for normal functioning.**
  - **Remember, that if the RNA nucleotide sequences or protein's primary structure (the polypeptide's amino acid sequences) change because the DNA sequence in the RNA or protein's gene is randomly changed, the RNA or protein *may* fold into a slightly or even very different 3D shape.**
    - **Changes in shape may cause changes in function!**
      - **Rarely, altering a protein's function is beneficial *(the protein working more effectively or engaging in a new function the cell couldn't perform before)***
      - **Most of the time, altering a protein's function will be harmful *(that protein will either engages in an undesirable behavior or fails to complete a needed function!)***



How can DNA get damaged?

- **Exposure to Radiation (high amount of energy)**
  - Expose to high amounts of energy can lead to the breaking of existing or forming of new covalent bonds within or between nucleotides or within or between DNA strands.
    - Gamma rays & X-rays can cause chemical damage to DNA
      - **Result**: Strong radiation can cause single- and double-stranded breaks in the DNA backbone
      - **Result**: Ionizing radiation can modify bases; for example, the deamination of cytosine into uracil
        - Broken DNA strands cannot be copied correctly into daughter DNA during replication or into mRNA during DNA transcription

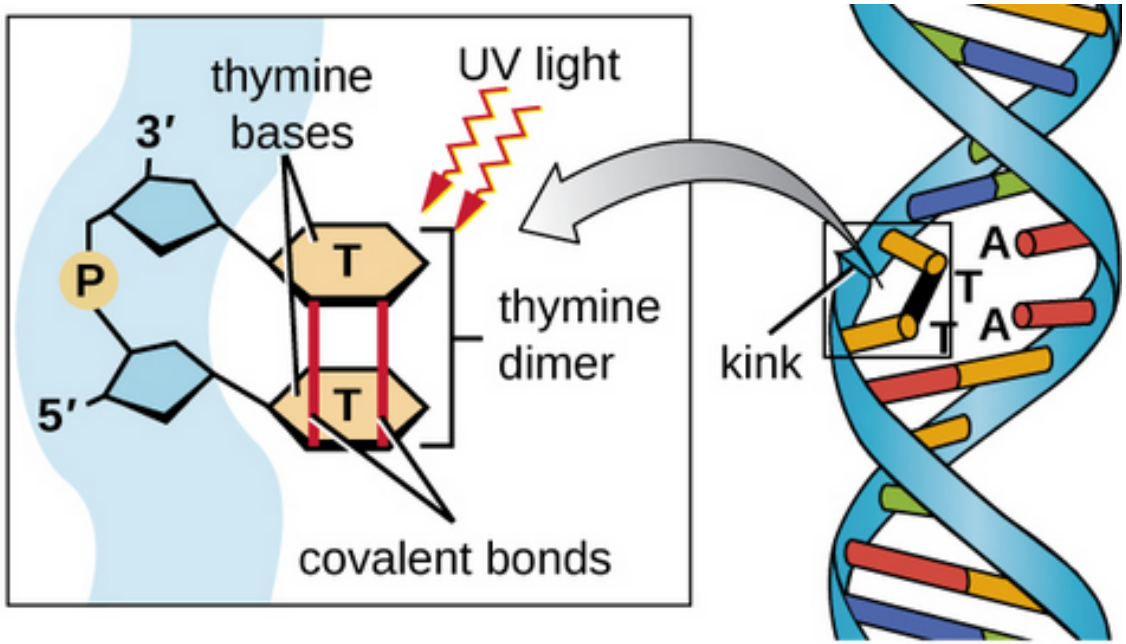


How can DNA get damaged?

- **Exposure to Radiation (high amount of energy)**
  - Expose to high amounts of energy can lead to the breaking of existing or forming of new covalent bonds within or between nucleotides or within or between DNA strands.
    - Ultra Violet radiation can cause damage to DNA
      - **Result**: The formation of thymine dimers in which the nitrogenous base portions of two neighboring T nucleotides within the same DNA strand abnormally covalently bond to one another causing the DNA strand to bulge outward

Thymine dimers can interfere with proper replication and transcription of the DNA.

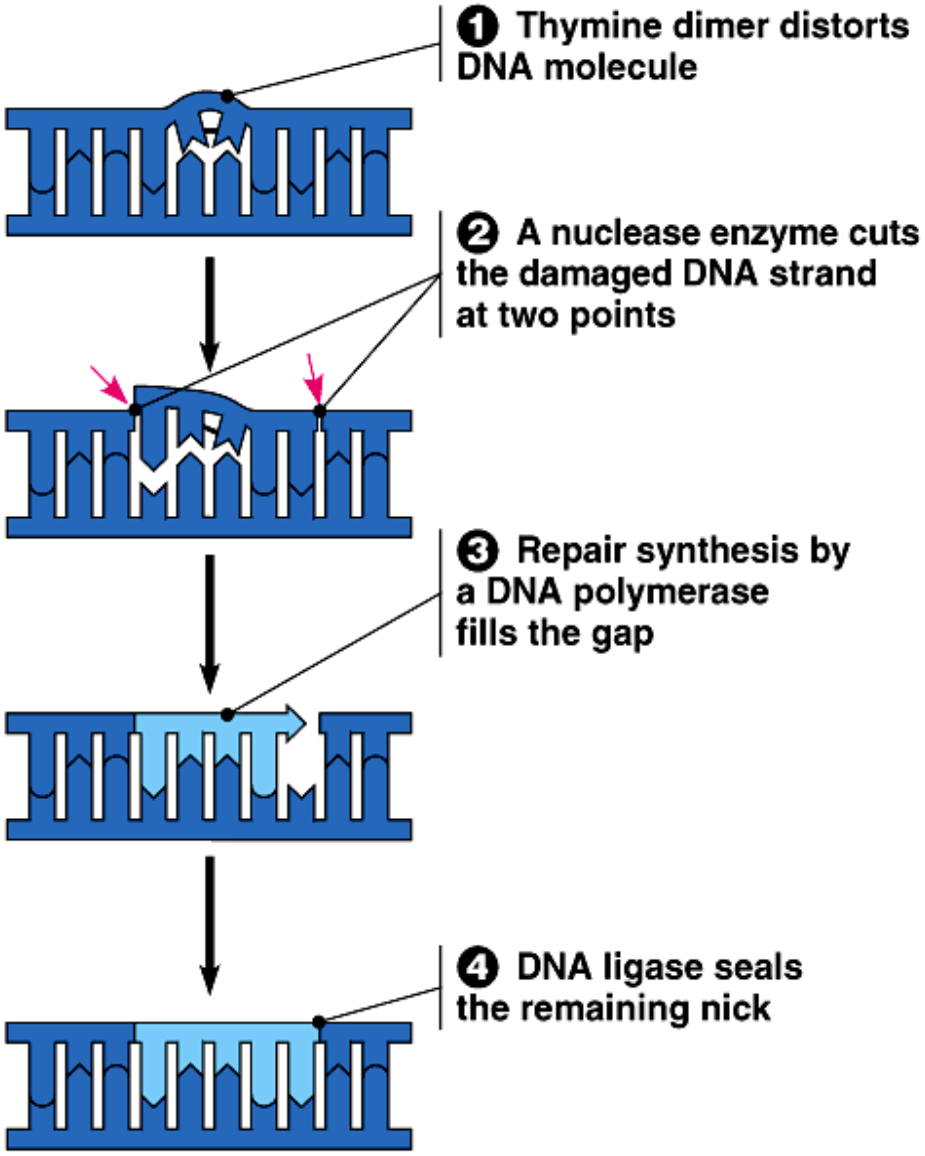
They can also lead to permanent mutations forming in the DNA since the correct sequence cannot be copied into the daughter DNA strand during DNA Replication.



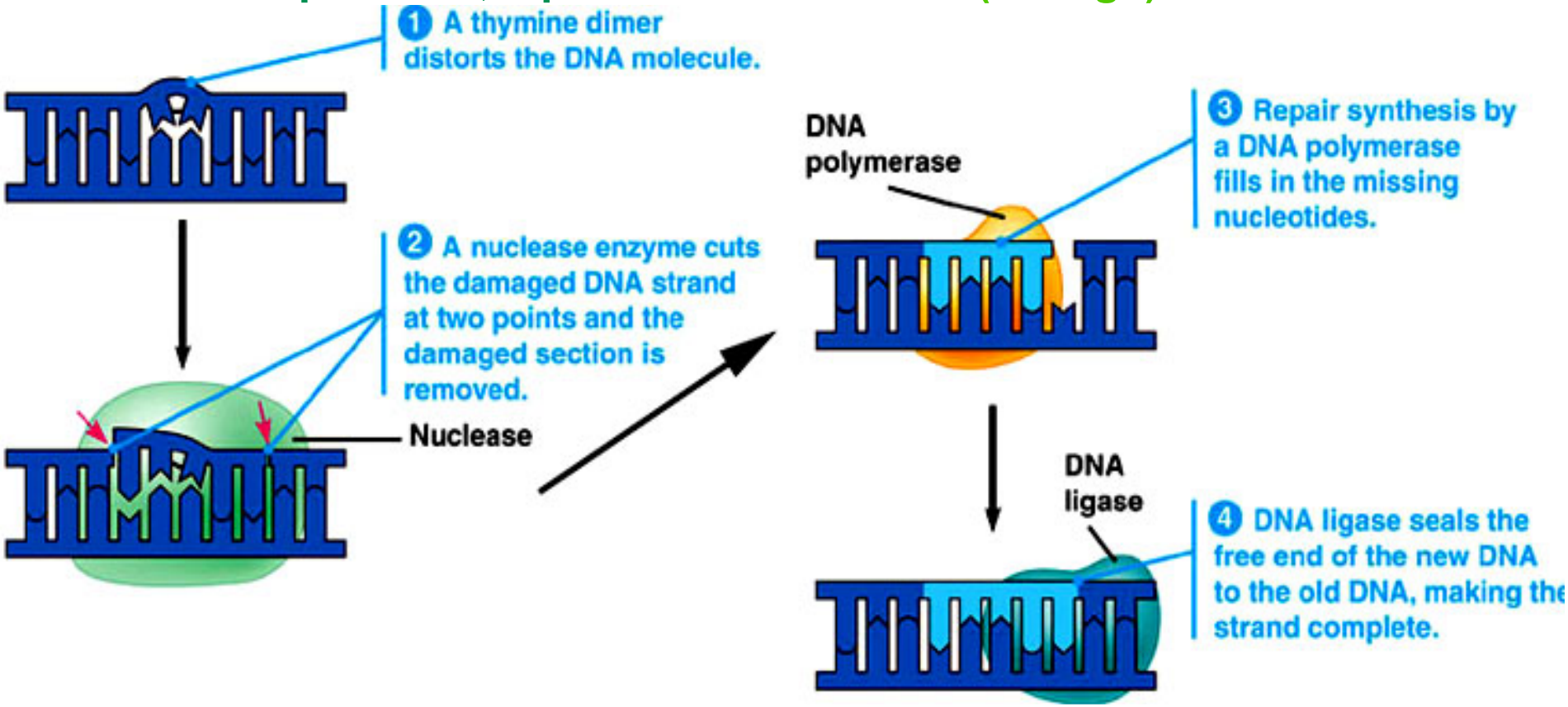
What about repairing DNA Damage?

Many genes for making DNA repair proteins have been identified. The proteins that can be made from these genes help fix damaged or mistakes in DNA.

- **Sometimes DNA is damaged because of chemical or physical mutagens in a way that the DNA double helix becomes bulkier.**
- **Repair Mechanism: NUCLEOTIDE EXCISION REPAIR**
  - **Nuclease cuts out segment of DNA showing bulky error in normal DNA base pairing.**
  - **Polymerase I or II fills gap with new correctly matched bases**
  - **Ligase joins together new DNA segment with rest of DNA strand *(builds the phosphodiester bond)***

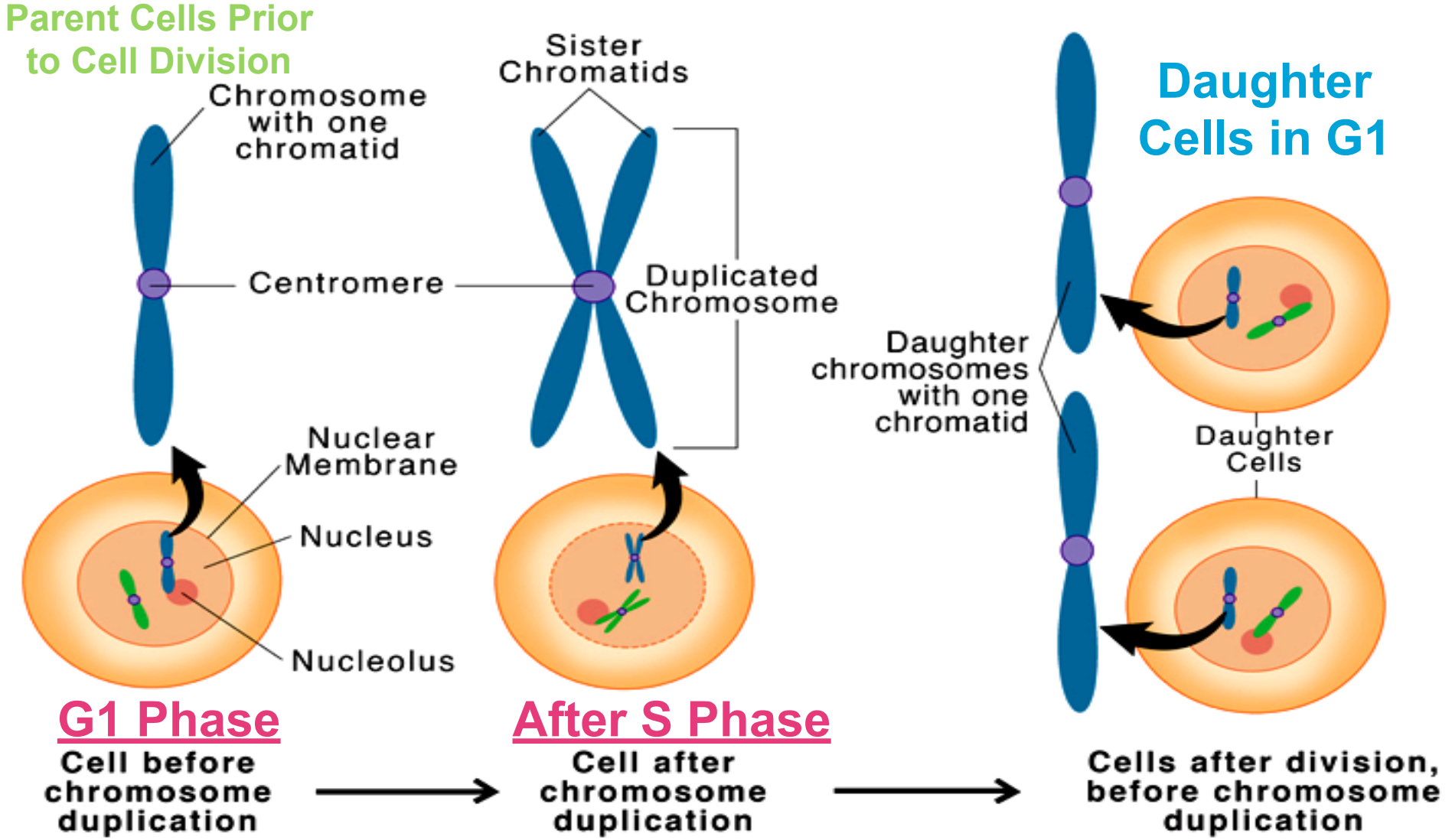


- # Thymine Dimers
  - ## Ex: UV rays can cause thymine bases in skin cells to become covalently linked = forming a ***Thymine Dimer***
    - ### Distorts the DNA (bulges out)
      - This will interfere with normal DNA replication & transcription.
      - If a DNA template is wrongly replicated, when the incorrect daughter strand is used as a template in the next round of replication, a permanent mutation (change) in the DNA results.



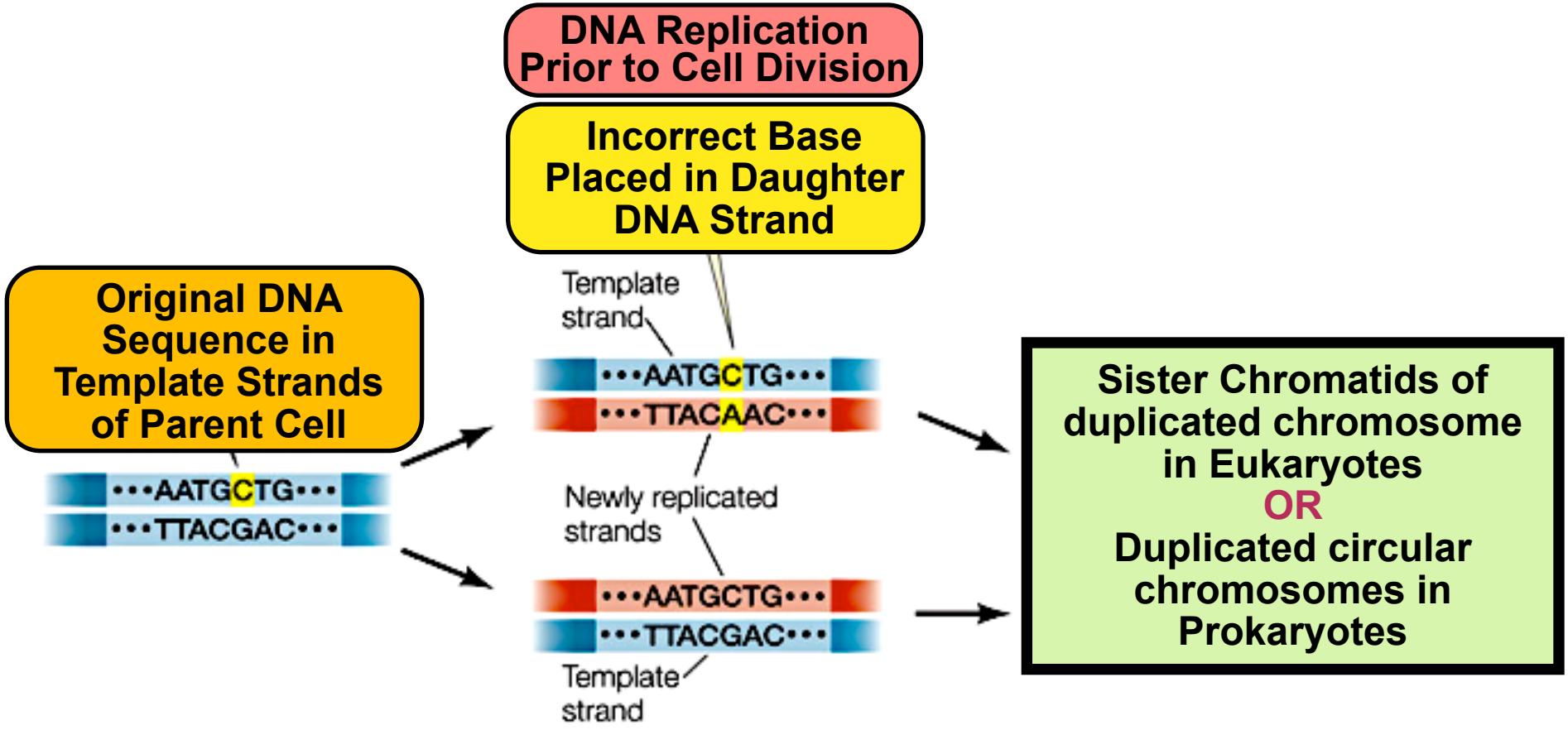
Cells Only Replicate their DNA if they must proceed through the cell cycle in order to DIVIDE!

Chromosome and Chromatid



How can changes in DNA Sequences Occur?

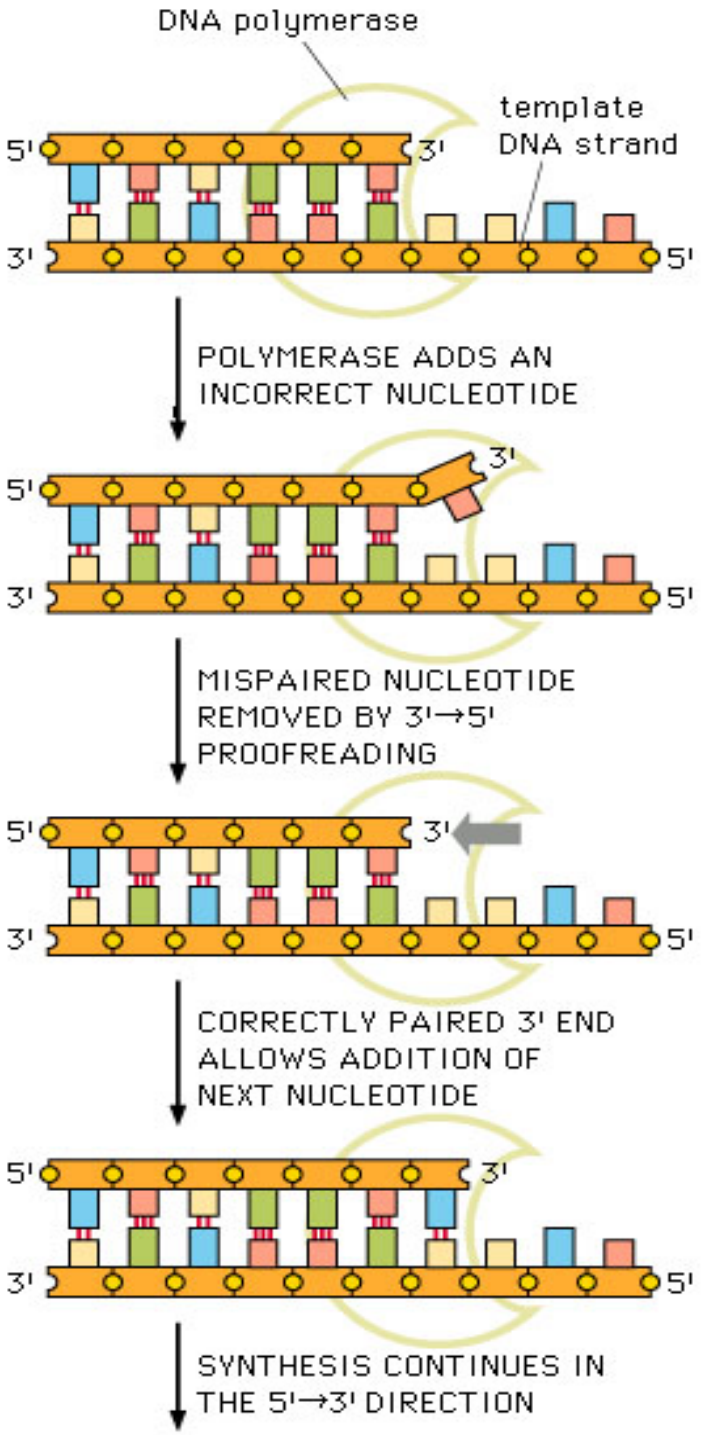
- **Errors in DNA Replication**
  - On occasions DNA polymerase incorporates an erroneous DNA base (nucleotide) into the daughter strand being built that is NOT complimentary to the template strand.



  - Incorrect base pairing is very rare in Eukaryotes since their DNA Polymerase has a self-correcting "proofreading" mechanism.

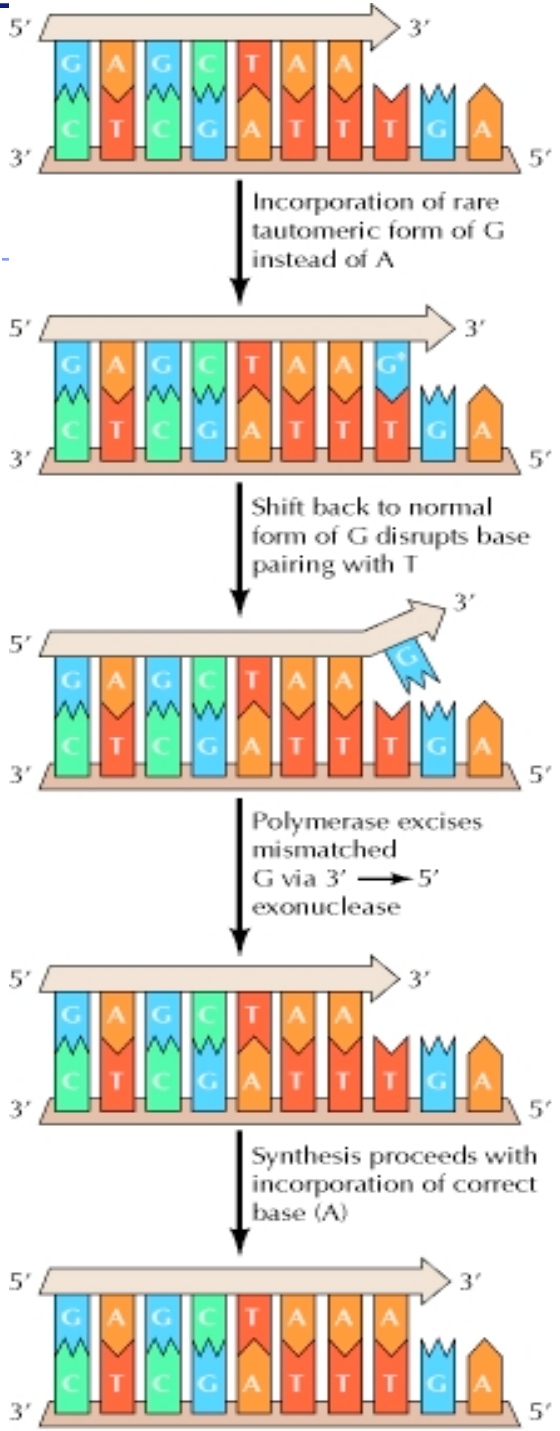
Eukaryotic DNA Polymerase III is SELF-CORRECTING!

- DNA Polymerase puts down a wrong nucleotide about 1 out of every 100,000 bases.
  - However, in reality, the error rate is about 1/10,000,000,000 in eukaryotes (unlike prokaryotes)
    - DNA polymerase III of Eukaryotes can proofread & correct its own work!

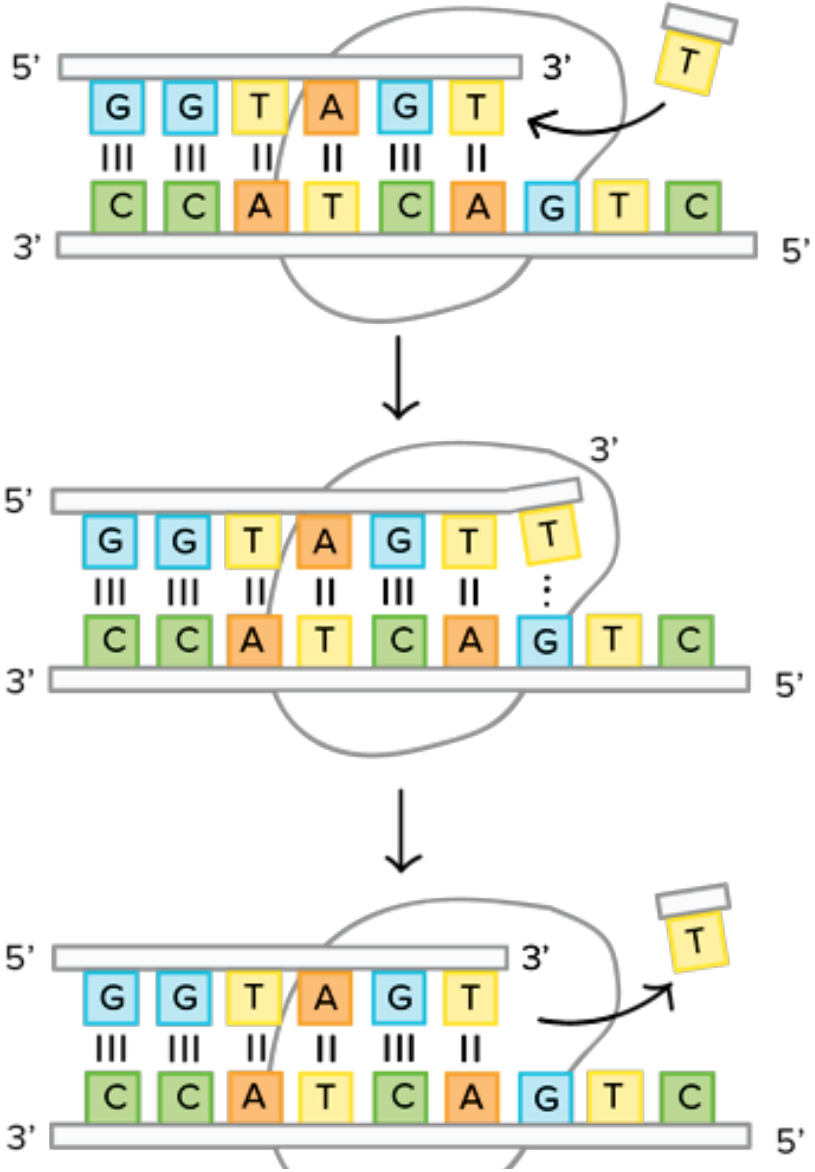


EUKARYOTIC DNA POLYMERASE PROOFREADING ACTIVITY

- **RECALL:** DNA polymerase can add free nucleotides to only the 3' end (pre-existing -OH) of the newly-forming strand.
- **PROOFREADING:** Corrects mistakes in newly-synthesized DNA.
  - When an incorrect base pair is recognized, eukaryotic DNA polymerase reverses its direction of movement by one base pair of DNA to correct the error it just made in the last base added.
    - The 3'->5' EXONUCLEASE ACTIVITY of the enzyme allows the incorrect base pair to be excised (cut out) from the growing daughter strand and the correct base re-inserted before replication continues.

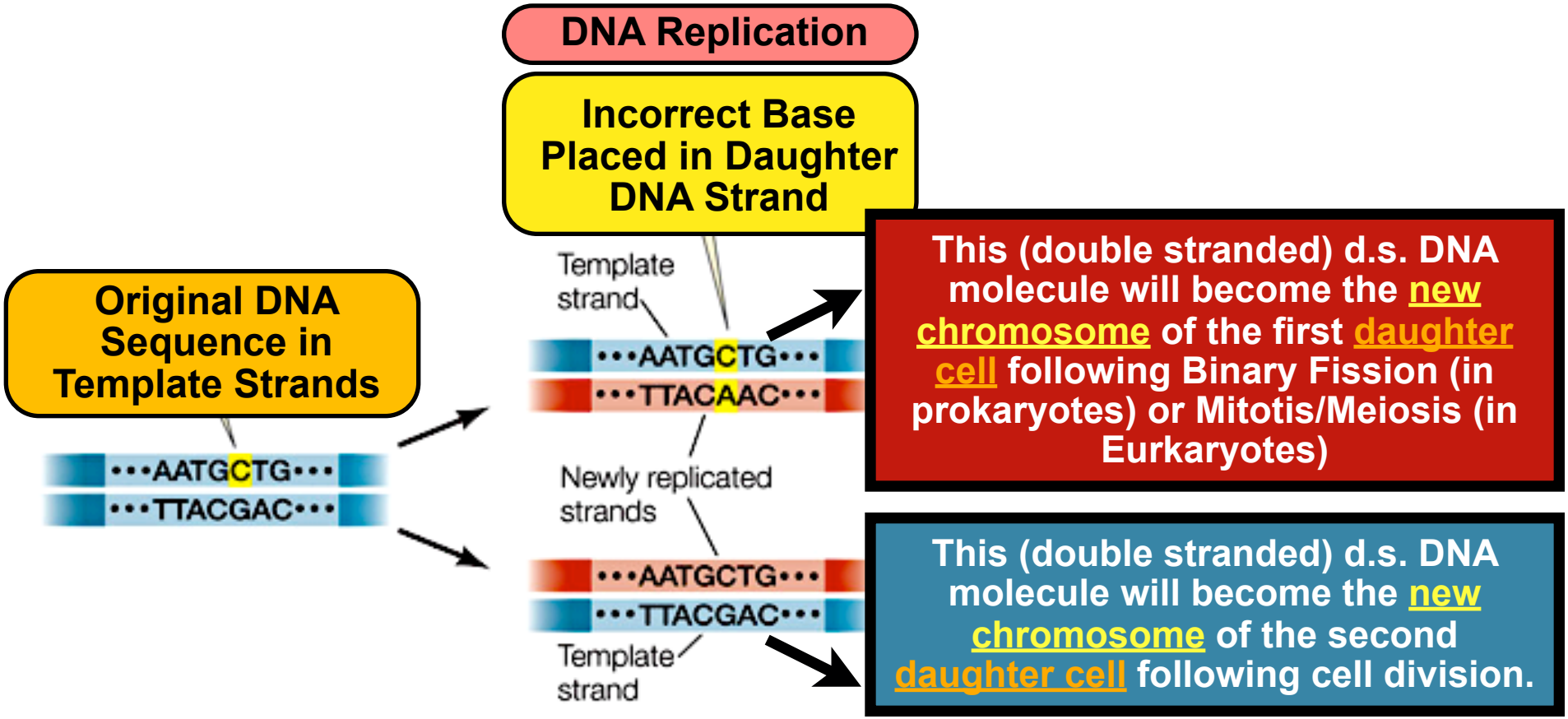


EUKARYOTIC DNA POLYMERASE PROOFREADING ACTIVITY



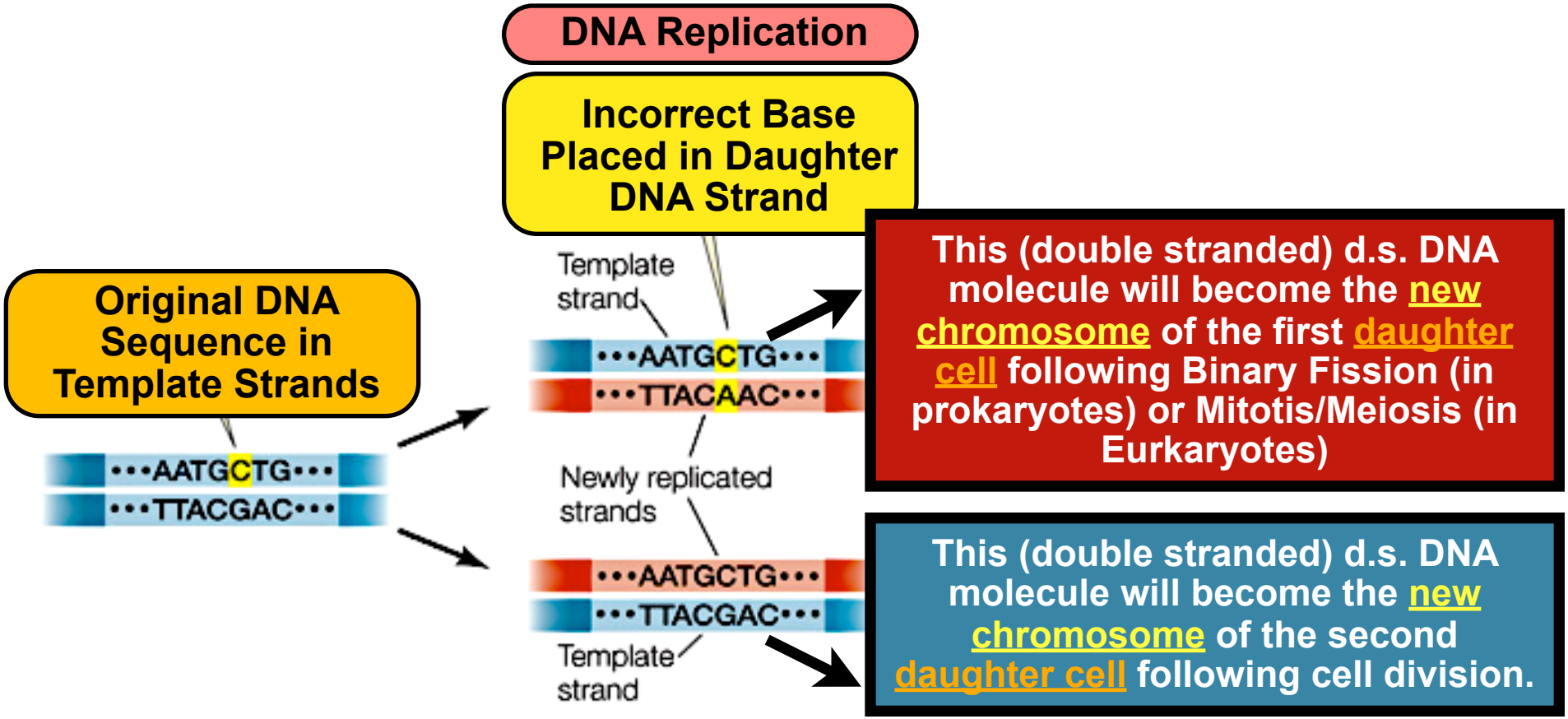
How can changes in DNA Sequences Occur?

- After DNA is replicated (duplicated), the duplicated DNA will get divided into the two new daughter cells that will form following cell division.
  - In this scenario, the first daughter cell gets a copy of the original parent cell DNA but one of the two strands of the double helix has a base-pair mismatch error in it.



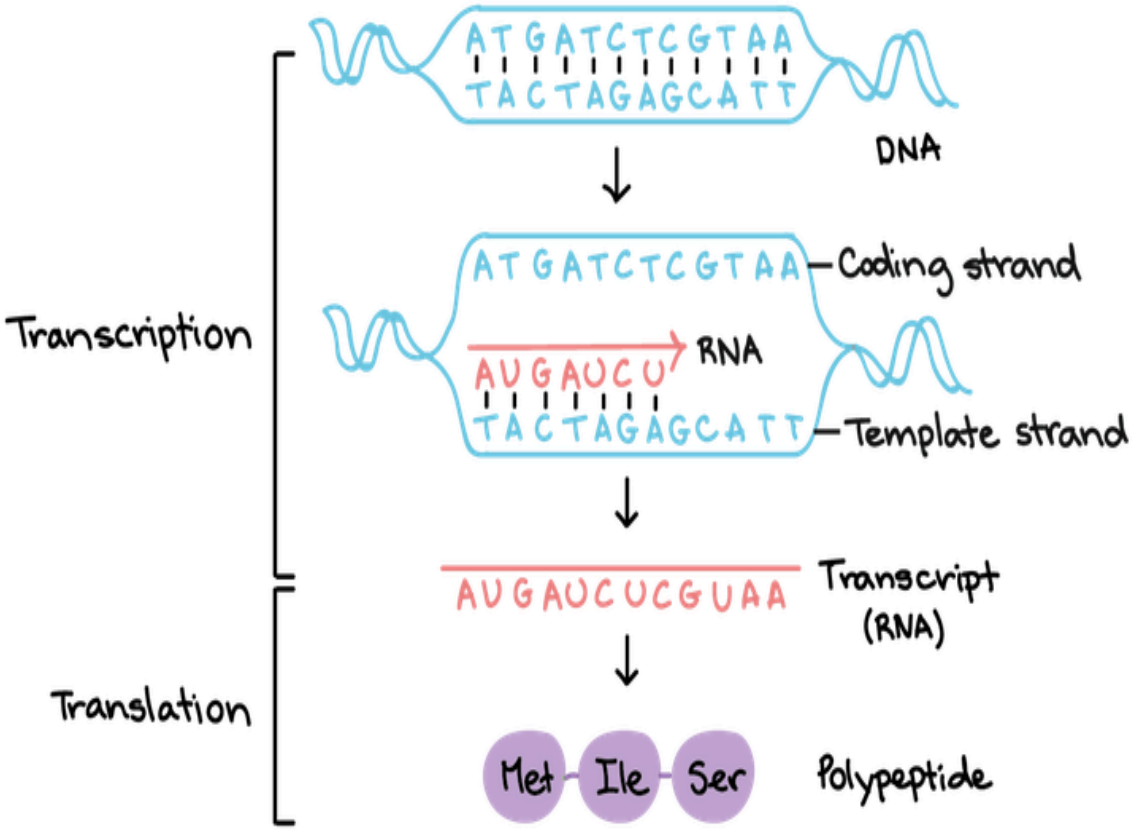
How can changes in DNA Sequences Occur?

- After DNA is replicated (duplicated), the duplicated DNA will get divided into the two new daughter cells that will form following cell division.
  - In this scenario, the second daughter cell gets a "perfect" copy of the original parent cell DNA with no nucleotides paired incorrectly.



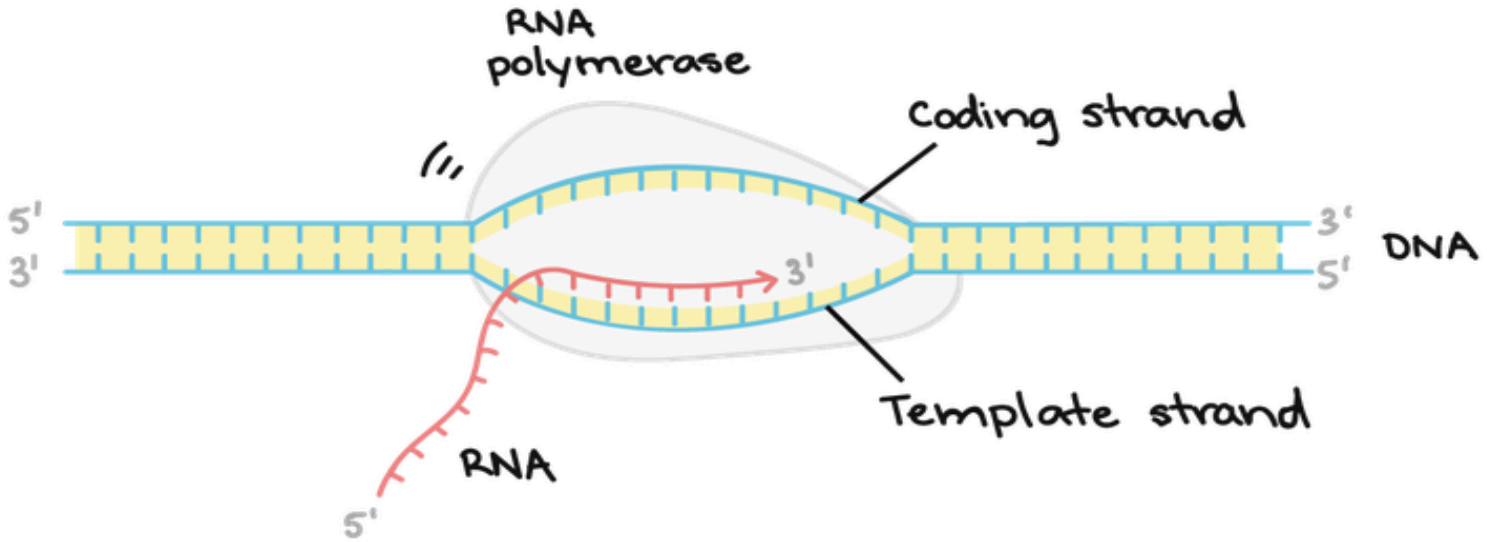
What affect does a daughter DNA strand with an error in nucleotide sequence have on the cell?

- Organisms have evolved several mechanisms for detecting and fixing errors in DNA, but what happens on the rare occasion when an error fails to get fixed and apoptosis isn't triggered?
- Let's review the basics of DNA transcription, a process that must occur to make RNA molecules or Proteins from the instructions in genes.
  - In a gene, one of the two DNA strands is always the template that RNA Polymerases copy in order to make RNA molecules, including messenger RNA, which is taken to a ribosome that uses the information encoded in the mRNA to construct the polypeptides of proteins



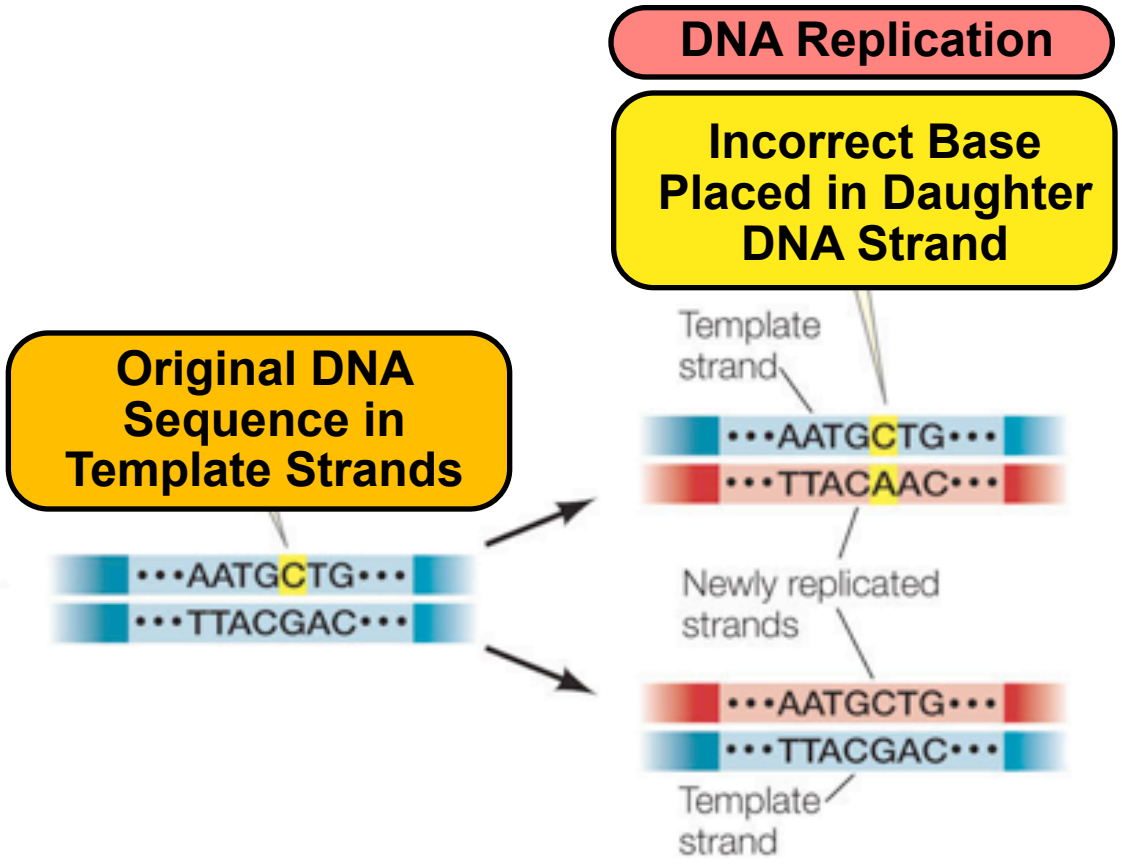
What affect does a daughter DNA strand with an error in nucleotide sequence have on the cell?

- If the new daughter strand ***is*** the template strand that gets copied into mRNA by RNA Polymerase when that gene is transcribed, the daughter cell will now place one or more incorrect nucleotides in any RNA transcribed from the DNA template strand.
  - Copying the wrong nucleotide into the RNA built during transcription of the gene can affect the functioning of the final RNA or, if that RNA is mRNA, the shape and thus function of the protein built from this gene's information.
    - Of course, if the template strand of the gene that is copied into mRNA is the original parental DNA strand (without the error), then the mRNA formed remains correct and no effect is seen in the daughter cell.



How might an error by DNA Polymerase cause a permanent Mutation in DNA?

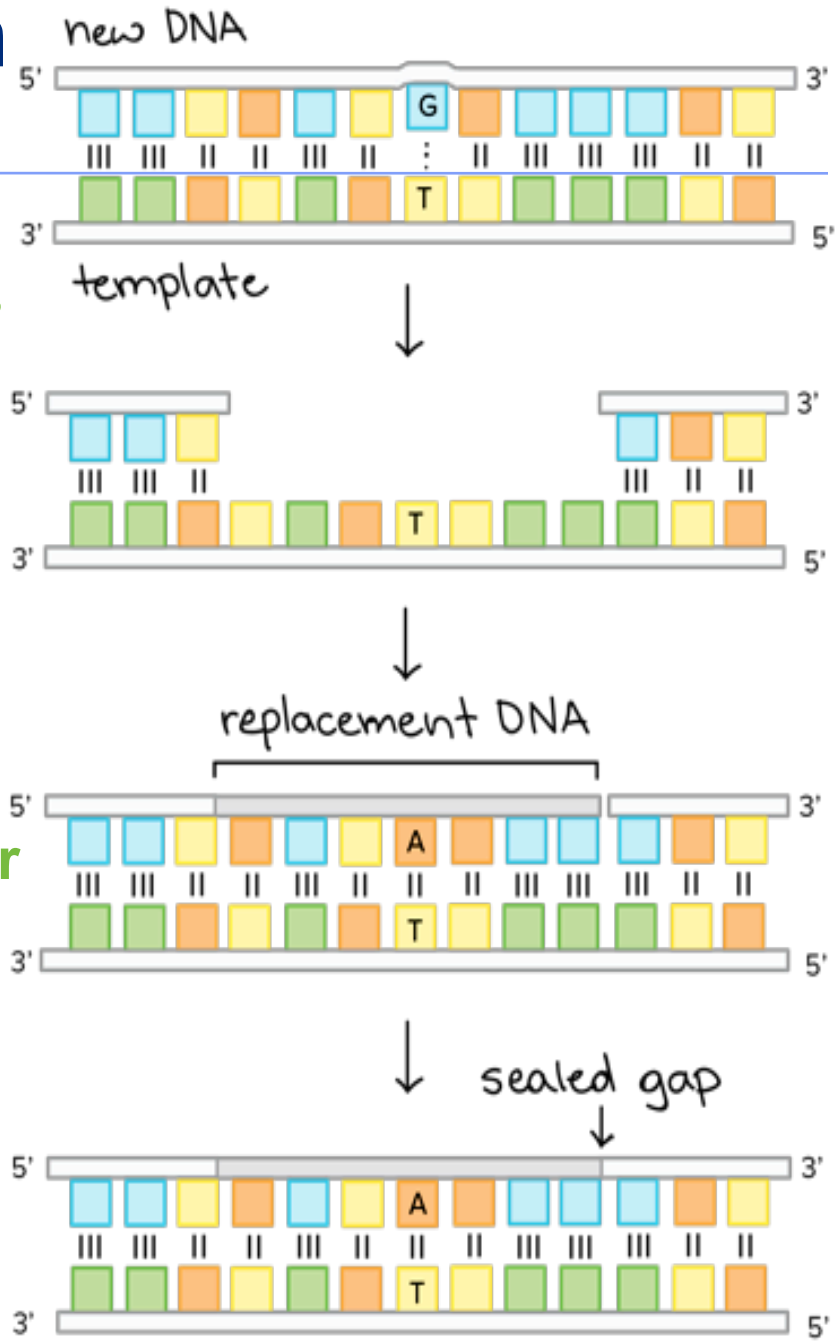
- If during DNA Replication, DNA Polymerase leaves behind a mismatched base pair, correcting the mismatch is still possible.
  - A mechanism called Mismatch Repair happens right after new DNA has been made,



1. Mismatch replaces mis-paired bases.
2. Mismatch repair can also correct mistakes involving the incorrect insertion of an extra or deletion of a nucleotides in the daughter DNA strand that happen when the polymerases "slips" on the template.

Mismatch Repair

- The enzymes involved in this mechanism recognize the incorrectly added nucleotide, excise it along with a few nucleotides before and after it, and replace the missing segment of the Daughter DNA strand with the correct bases.



A mismatch is detected in newly synthesized DNA.

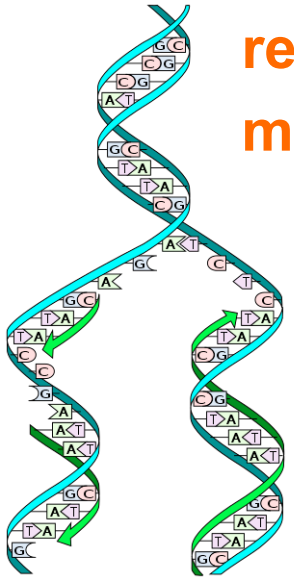
The new DNA strand is cut, and the mispaired nucleotide and its neighbors are removed.

The missing patch is replaced with correct nucleotides by a DNA polymerase.

A DNA ligase seals the gap in the DNA backbone.

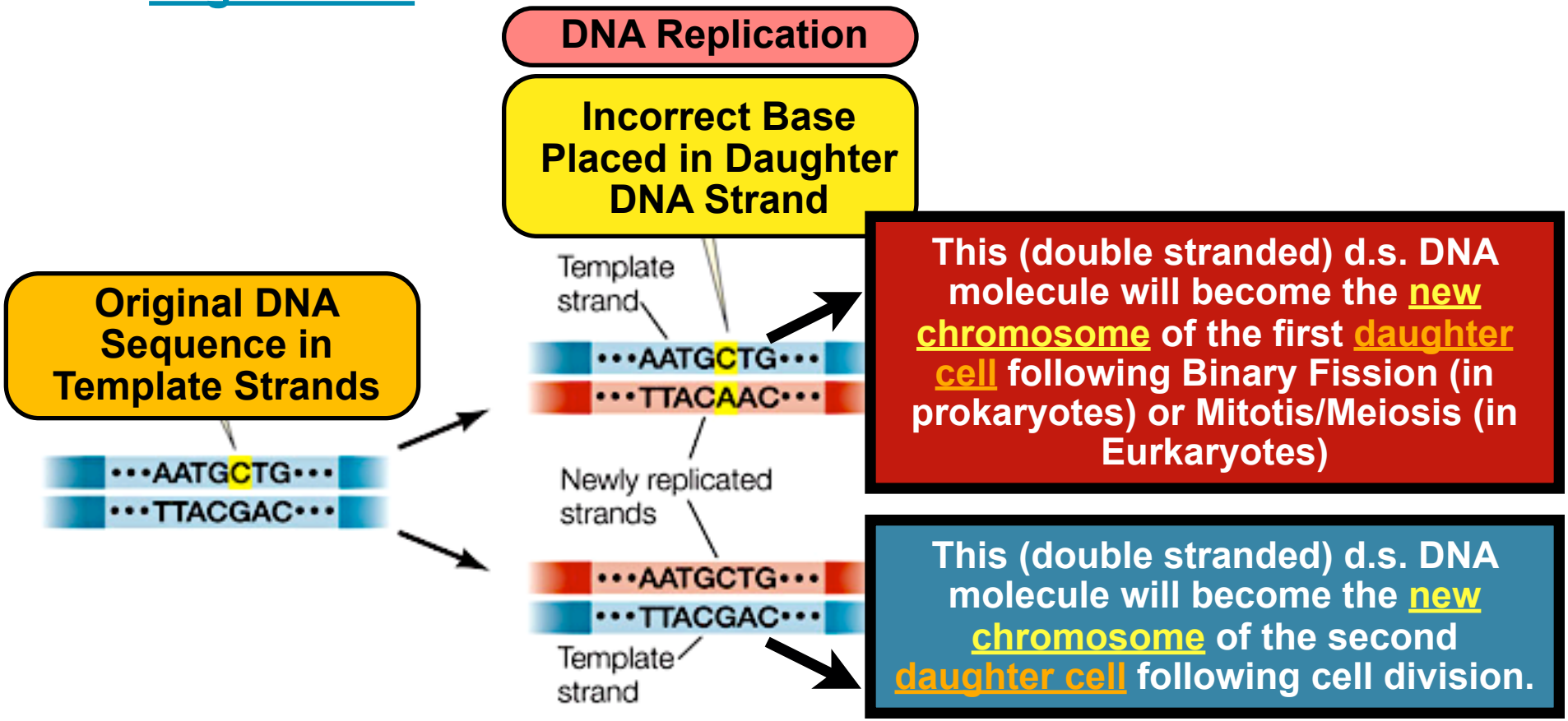
How might an error by DNA Polymerase cause a permanent Mutation in DNA?

- While most DNA replicates with fairly high fidelity, mistakes do happen.
  - DNA polymerase enzymes sometimes insert the wrong nucleotide or too many or too few nucleotides into a daughter stand's DNA sequence.
    - How can these changes in one of the two strands of the replication DNA Molecule end up becoming a permanent mutation in the DNA?
      - These mistakes, if they are not fixed, could lead to permanent changes in DNA sequences, mutations, when the daughter cell that inherited a double helix with mismatching strands in a double helix itself undergoes mitosis (or meiosis) to make another set of daughter cells.



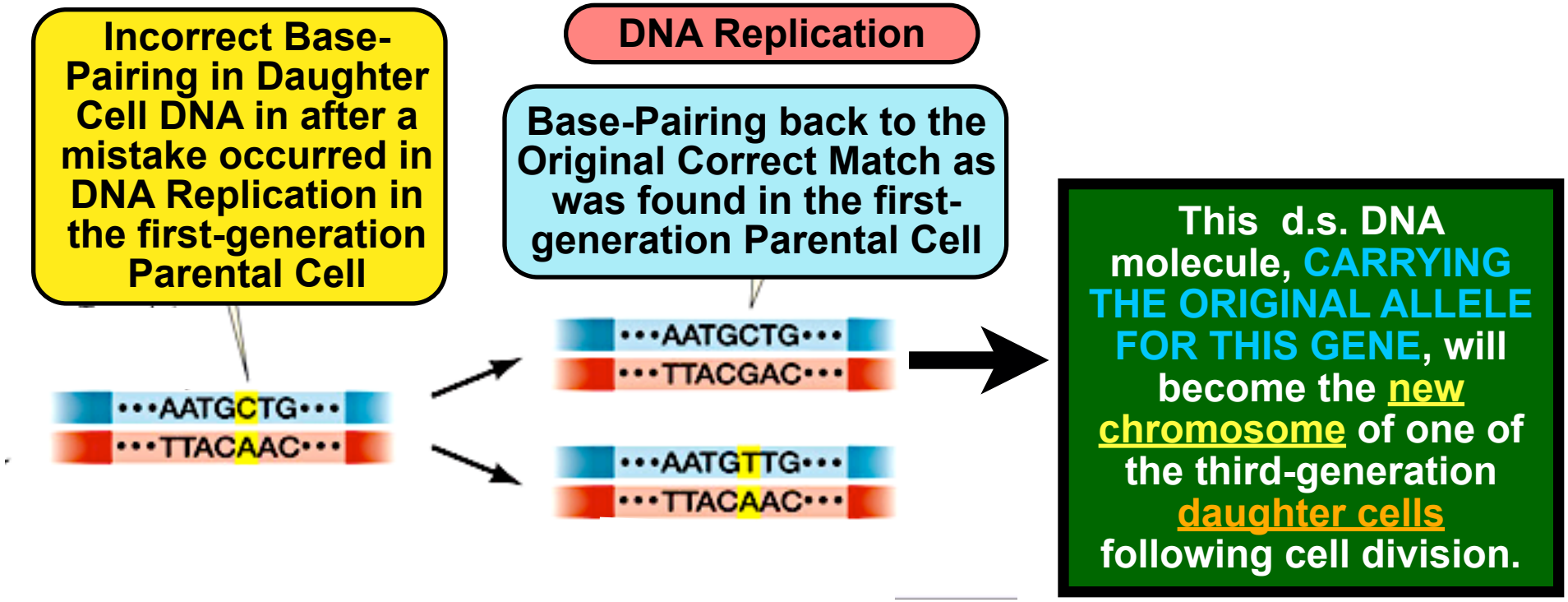
What affect does a daughter DNA strand with an error in nucleotide sequence have on future generations of cells?

- Going back to this earlier scenario, a parental cell duplicated its DNA, but one copy contains a base-pair C-A mismatch, while the other copy of DNA contains the correct base pair C-G match.
  - Therefore, one daughter cell that forms will have an error in its DNA while the other daughter cell that forms has a perfect copy of the original DNA.



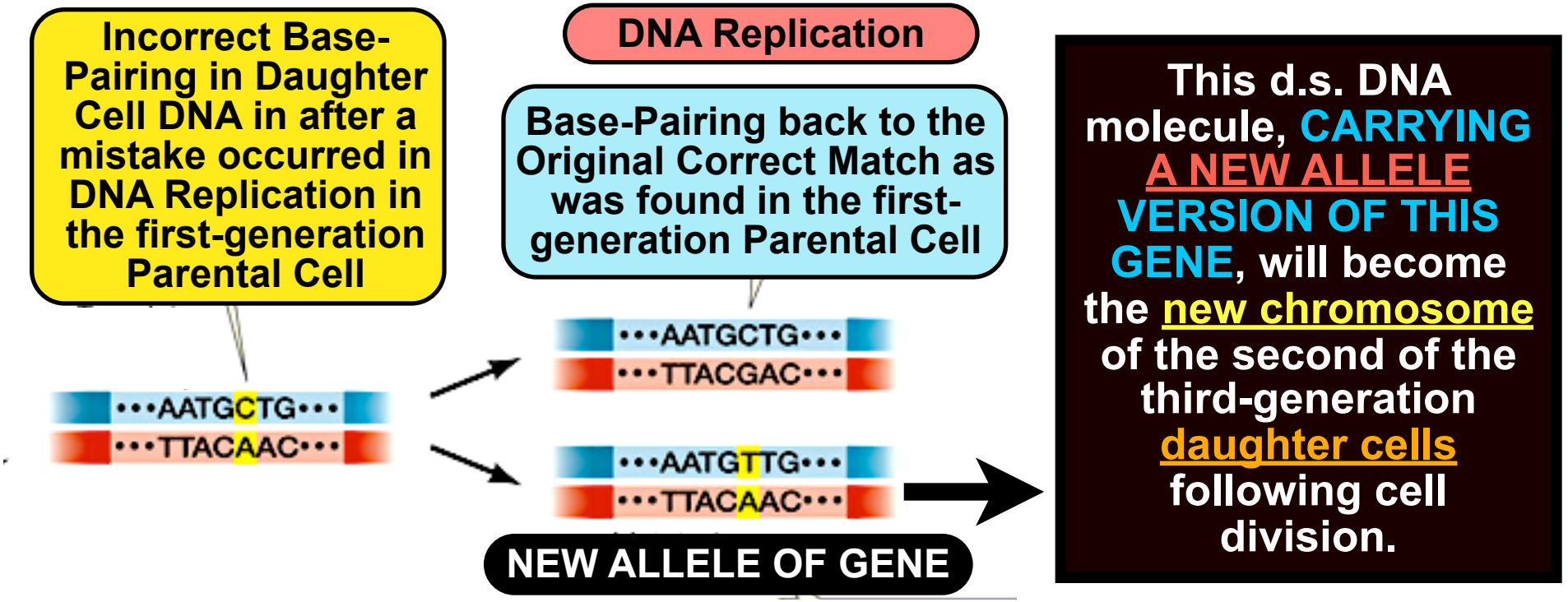
What affect does a daughter DNA strand with an error in nucleotide sequence have on future generations of cells?

- When the daughter cell containing the DNA molecule with a base C-A mismatch itself decides to divide to form a third generation of cells, each of the two strands of the chromosome with the error will themselves become templates during DNA replication.
  - The template strand that contains the original, and good C base, will be copied into a daughter strand with a G.
    - This duplicated double helix will contain the proper C-G base pairing just like the original parental cell once had.

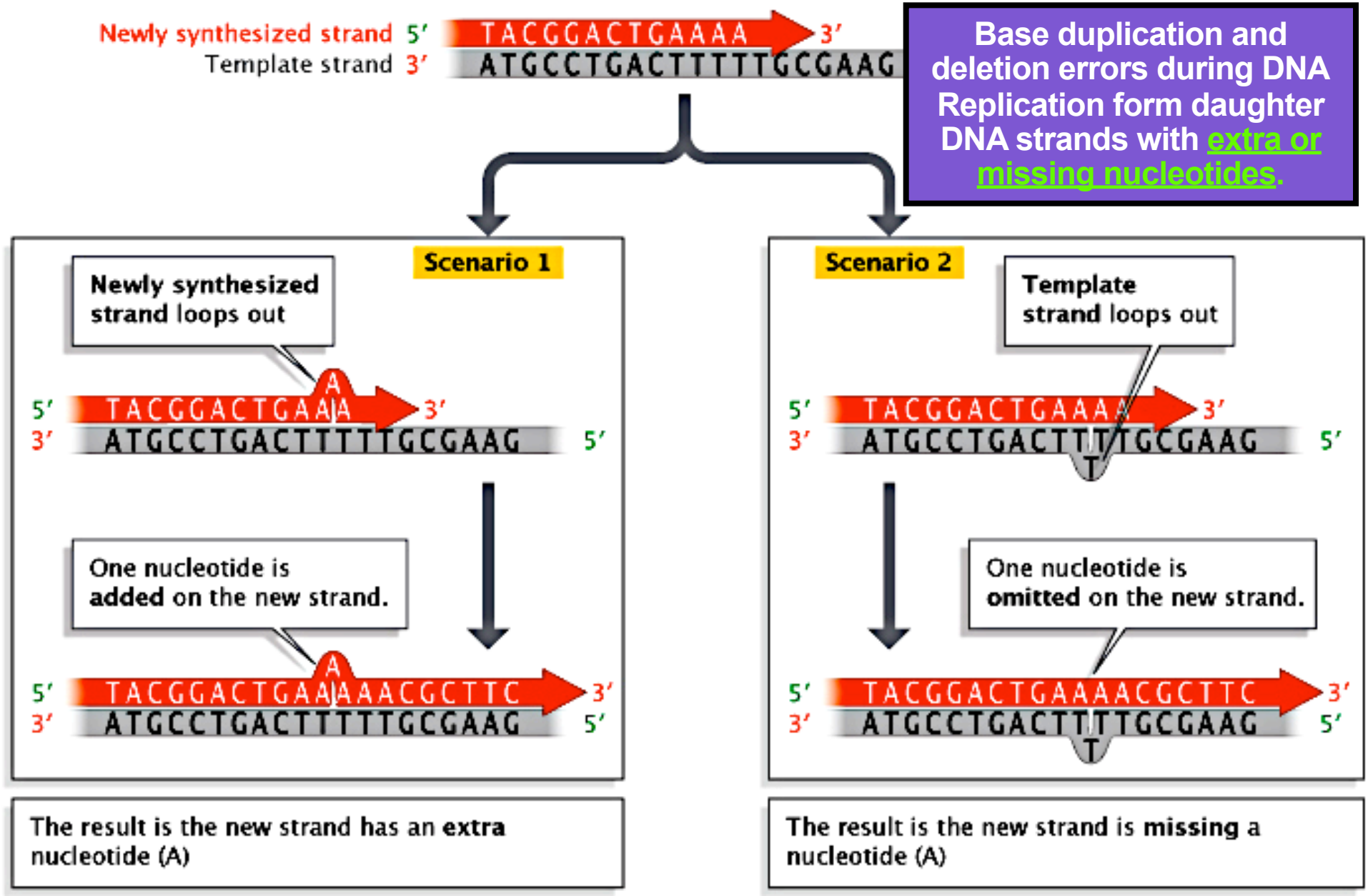


What affect does a daughter DNA strand with an error in nucleotide sequence have on future generations of cells?

- The other DNA template strand, in the daughter cell that is copying its DNA in order to divide, contains the incorrect A base, which will be copied into a daughter DNA strand with a complimentary T base.
  - This duplicated double helix will contain the incorrect A-T pairing.
    - However, the third-generation cell that inherits this DNA molecule will not think the A-T is incorrect as the base pairing is CORRECT.
      - DNA Repair mechanisms will, thus, NOT try to correct an A-T pairing: a new, permanent allele for this gene has formed and will get passed down to future daughter cells of this cell.



Mistakes that lead to future permanent mutations in a DNA Sequence do not only include base-pair mismatches by DNA Polymerases



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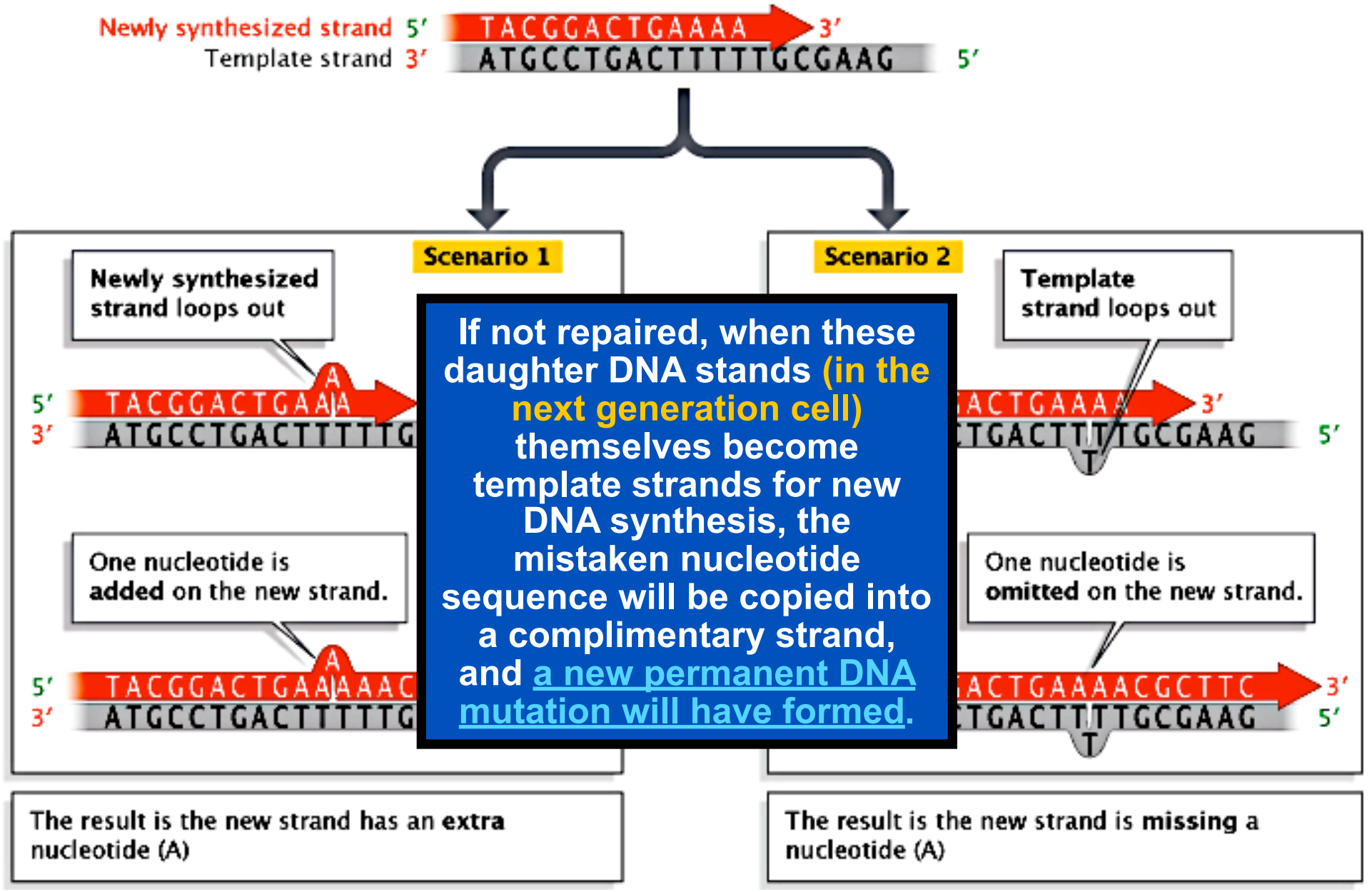

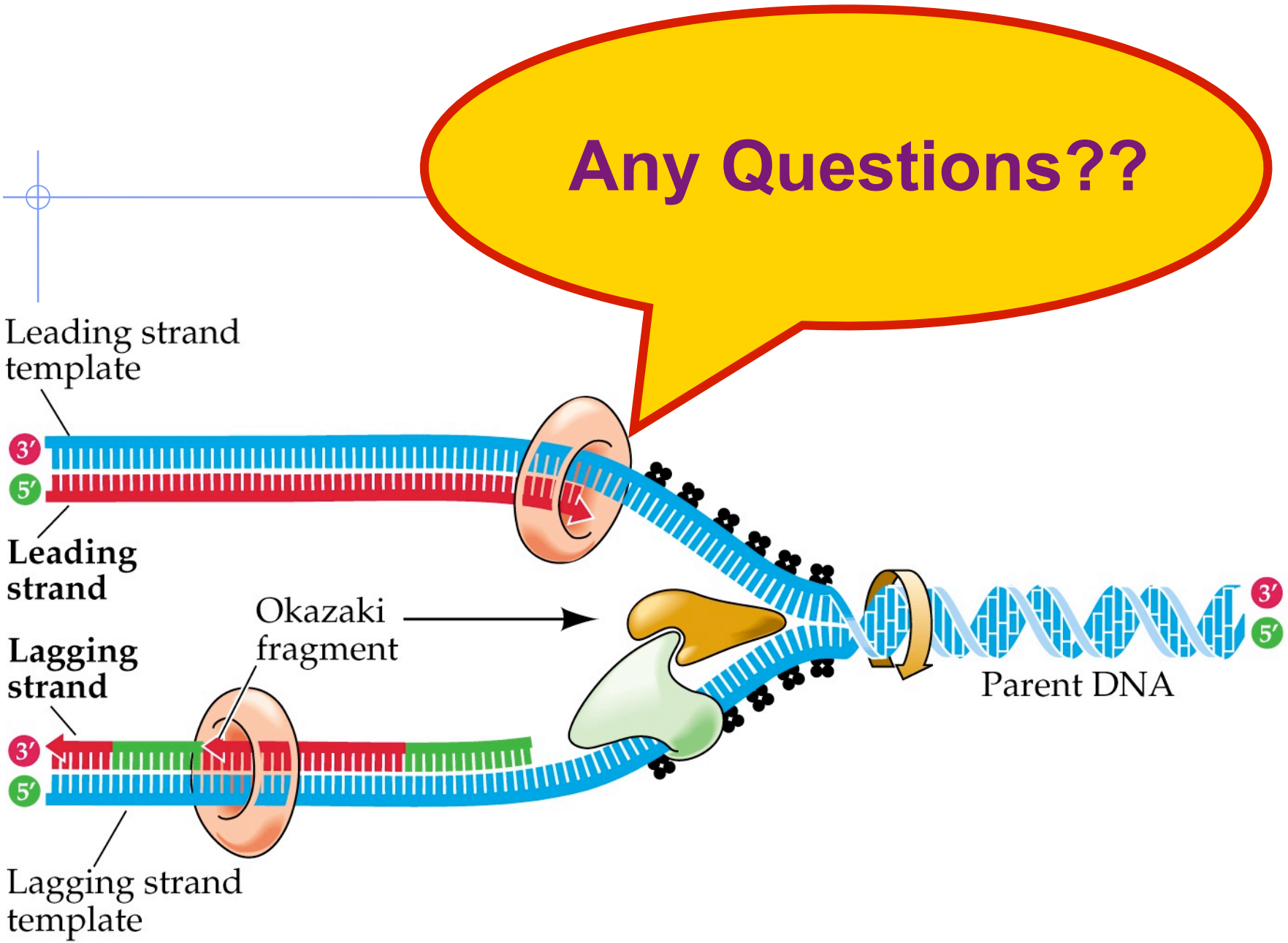
Any Questions??
Leading strand template
3′
5′
Leading strand
Lagging strand
Okazaki fragment
3′
5′
Parent DNA
3′
5′
Lagging strand template