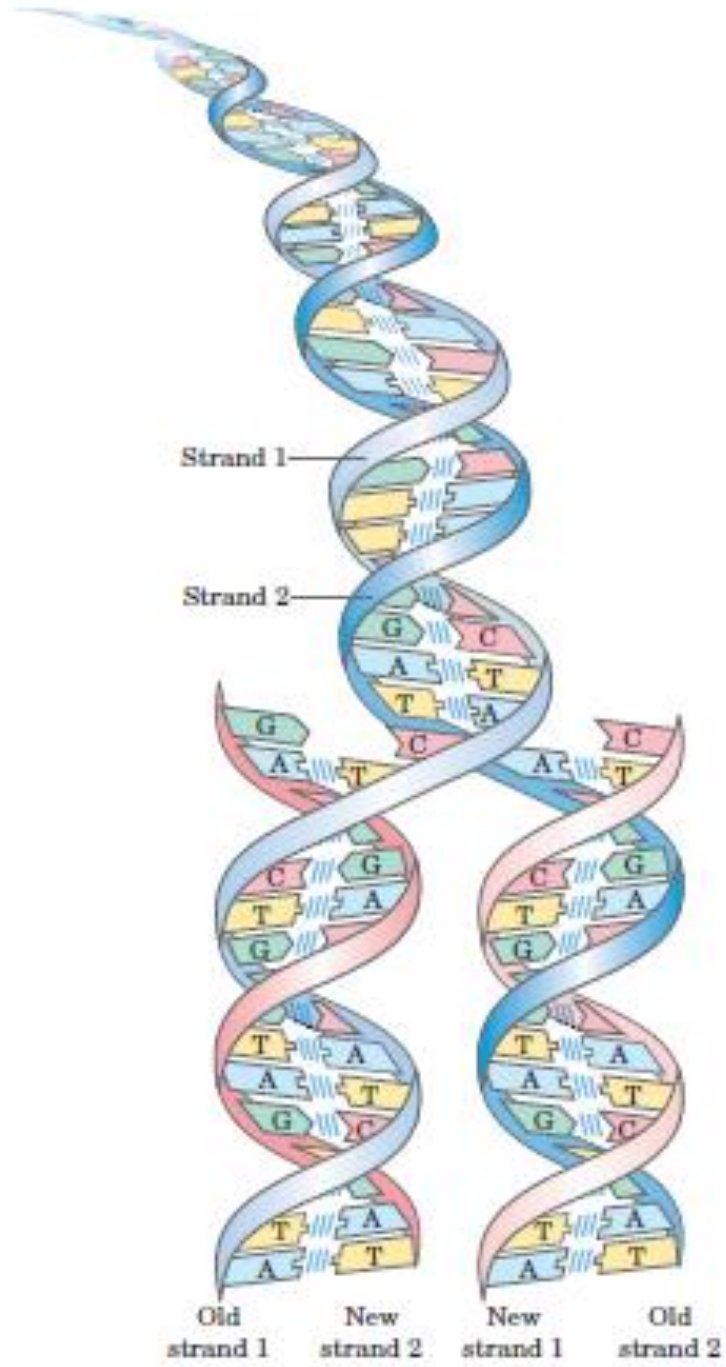
LECTURE 18


Strand 1
Strand 2
Old strand 1
New strand 2
New strand 1
Old strand 2

Stages of Replication

The synthesis of a DNA molecule can be divided into three stages:

initiation
elongation, and
termination

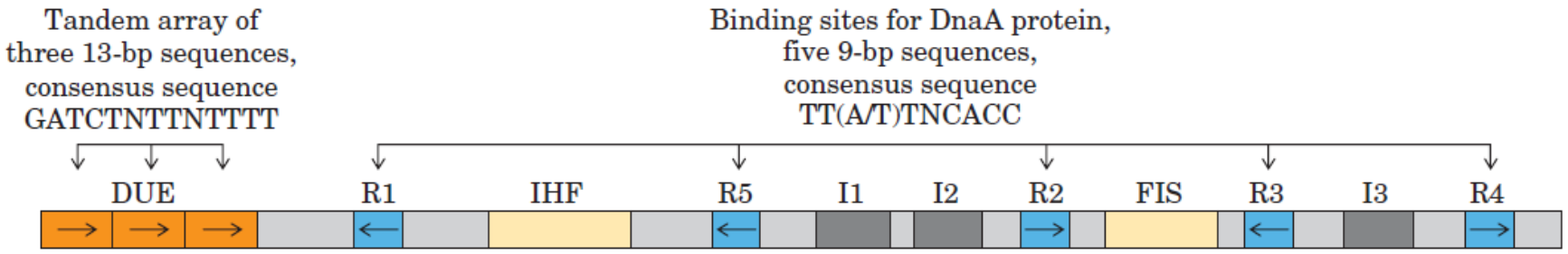
Distinguished both by the reactions taking place and by the enzymes required

Stages of Replication

Initiation
The *E. coli* replication origin, *oriC*, consists of 245 bp and contains DNA sequence elements that are highly conserved among bacterial replication origins.

Two types of sequences are of special interest:

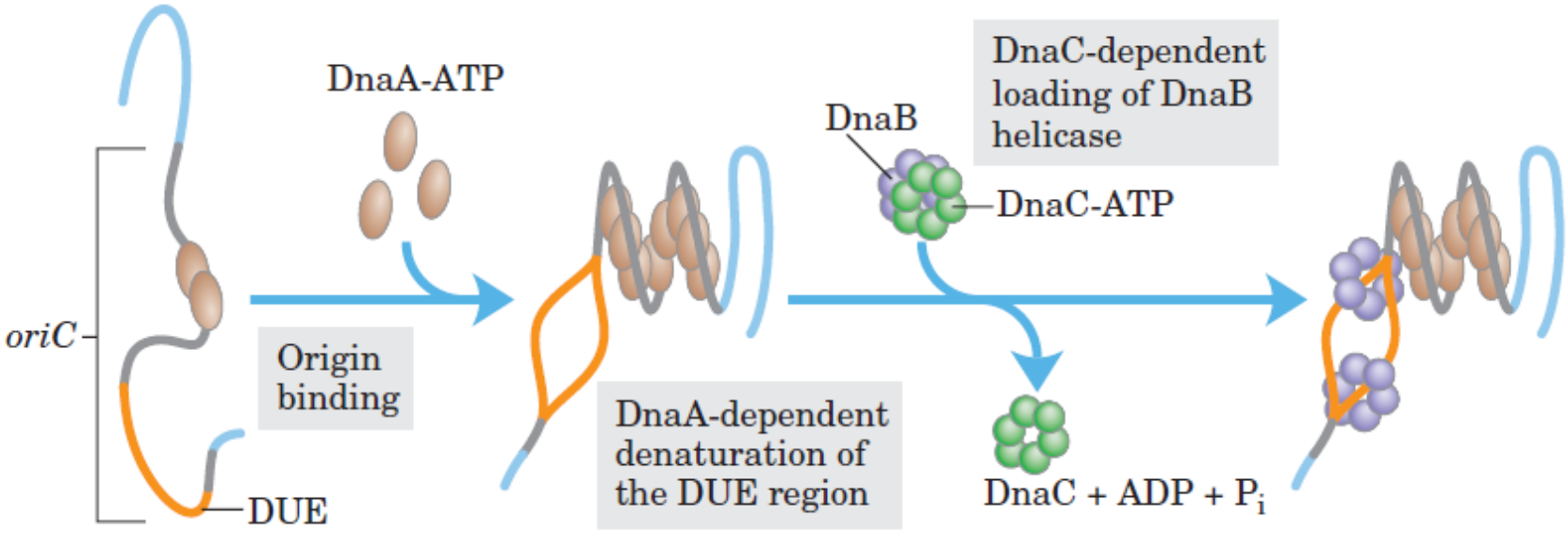
five repeats of a 9 bp sequence (R sites) that serve as binding sites for the key initiator protein DnaA, and a region rich in AT base pairs called the **DNA unwinding element (DUE)**.



Initiation

At least 10 different enzymes or proteins participate in the initiation phase of replication.

Most important are
DnaA
DnaB (helicase)
DnaC



Model for initiation of replication at the *E. coli* origin, *oriC.*

Eight DnaA protein molecules, all in the ATP-bound state, assemble to form a helical complex encompassing the R and I sites in *oriC*

Initiation

The tight right-handed wrapping of the DNA around this complex introduces an effective positive supercoil.

The associated strain in the nearby DNA leads to denaturation in the A=T-rich DUE region.

The DnaC protein, another AAA ATPase, then loads the DnaB protein onto the separated DNA strands in the denatured region.

A hexamer of DnaC, each subunit bound to ATP, forms a tight complex with the hexameric, ring-shaped DnaB helicase

The ATP bound to DnaC is hydrolyzed, releasing the DnaC and leaving the DnaB bound to the DNA

Loading of the DnaB helicase is the key step in replication initiation.

As a replicative helicase, DnaB migrates along the single-stranded DNA in the 5’-3’ direction, unwinding the DNA as it travels.

The DnaB helicases loaded onto the two DNA strands thus travel in opposite directions, creating two potential replication forks.

All other proteins at the replication fork are linked directly or indirectly to DnaB.

The *oriC* DNA is **methylated** by the Dam methylase, which methylates the N_6 position of adenine within the palindromic sequence GATC.

The *oriC* region of *E. coli* is highly enriched in GATC sequences—it has 11 of them in its 245 bp, whereas the average frequency of GATC in the *E. coli* chromosome as a whole is 1 in 256 bp.

Immediately after replication, the DNA is hemimethylated: the parent strands have methylated *oriC* sequences but the newly synthesized strands do not

Elongation

The elongation phase of replication includes two distinct but related operations:

Leading strand synthesis and lagging strand synthesis.

Several Enzymes at the replication fork are important to the synthesis of both strands.

Parent DNA is first unwound by DNA helicases, and the resulting topological stress is relieved by topoisomerases.

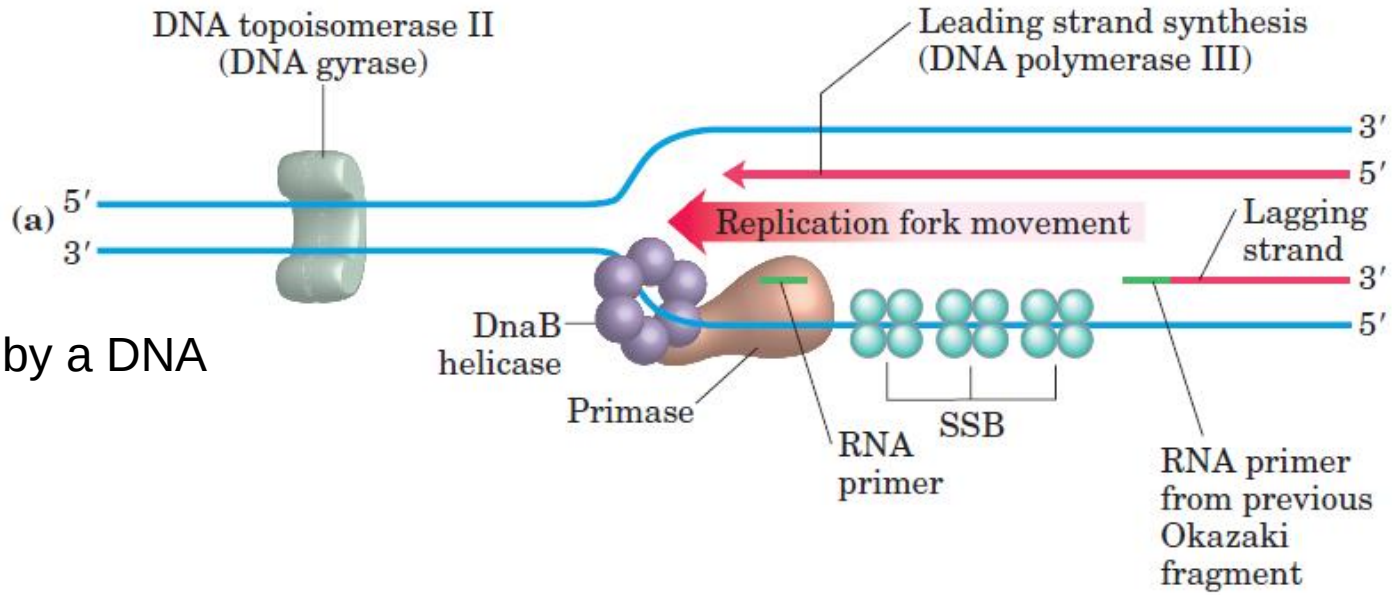
Each separated strand is then stabilized by SSB.

From this point, synthesis of leading and lagging strands is sharply different.

Leading strand synthesis

Begins with the synthesis by primase (DnaG protein) of a short (10 to 60 nucleotide) RNA primer at the replication origin.

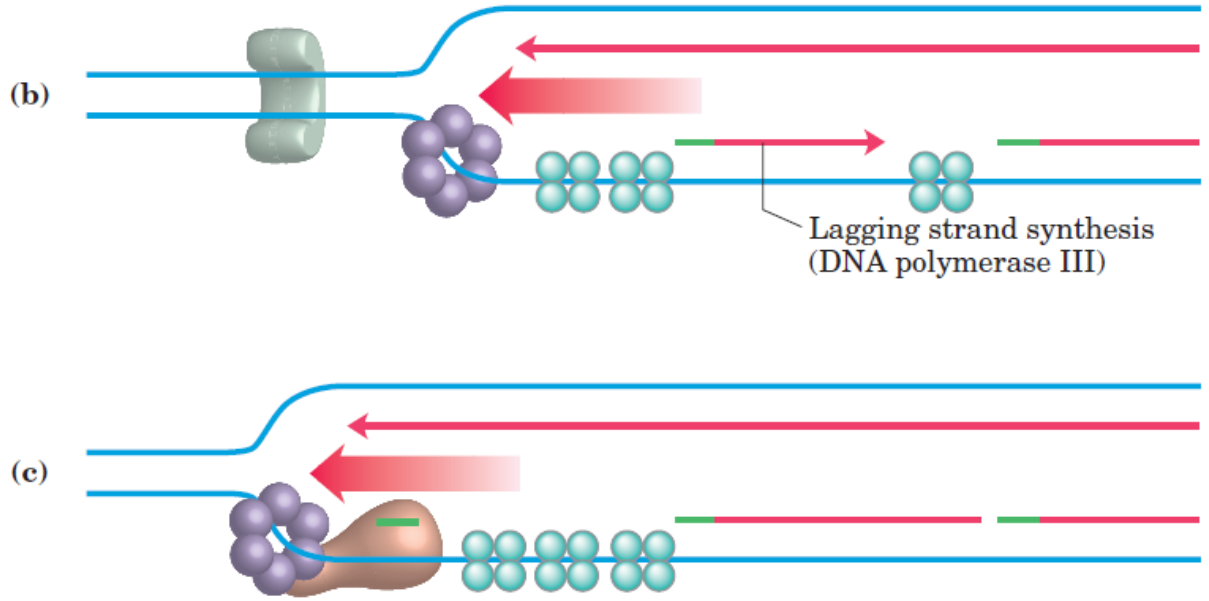
Deoxyribonucleotides are added to this primer by a DNA polymerase III complex



Lagging strand

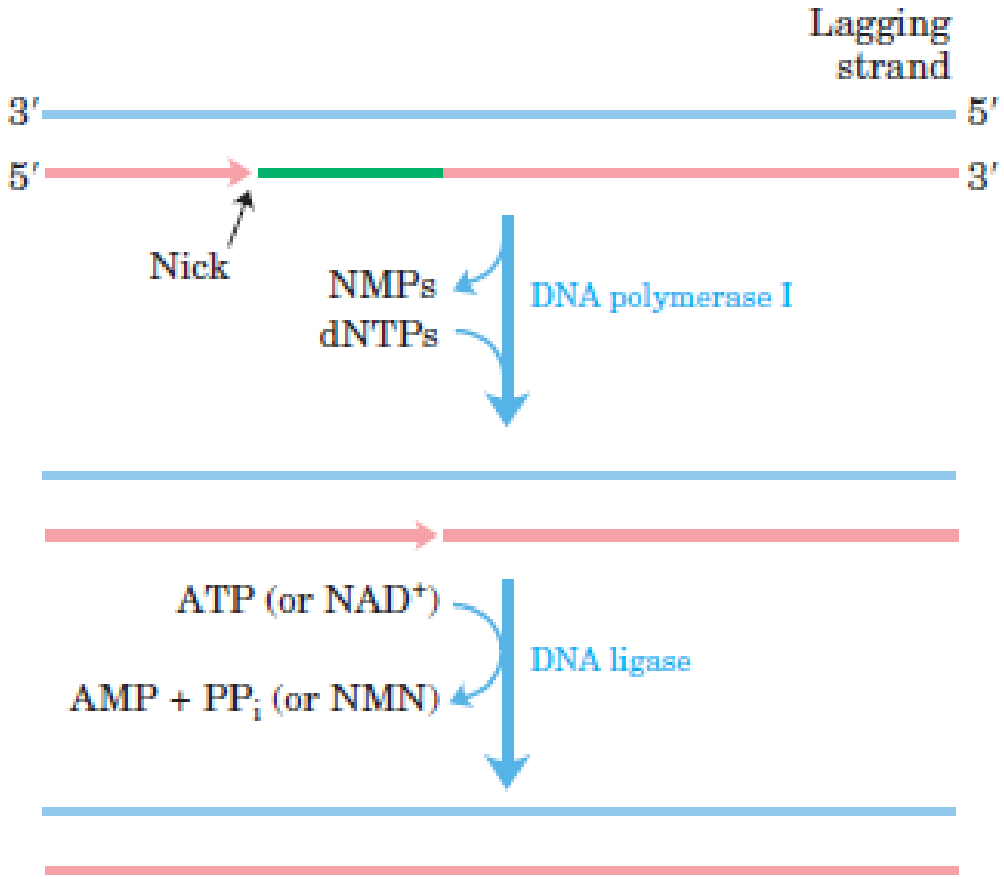
Lagging strand synthesis, as we have noted, is accomplished in short Okazaki fragments

First, an RNA primer is synthesized by primase and, as in leading strand synthesis, DNA polymerase III binds to the RNA primer and adds deoxyribonucleotides



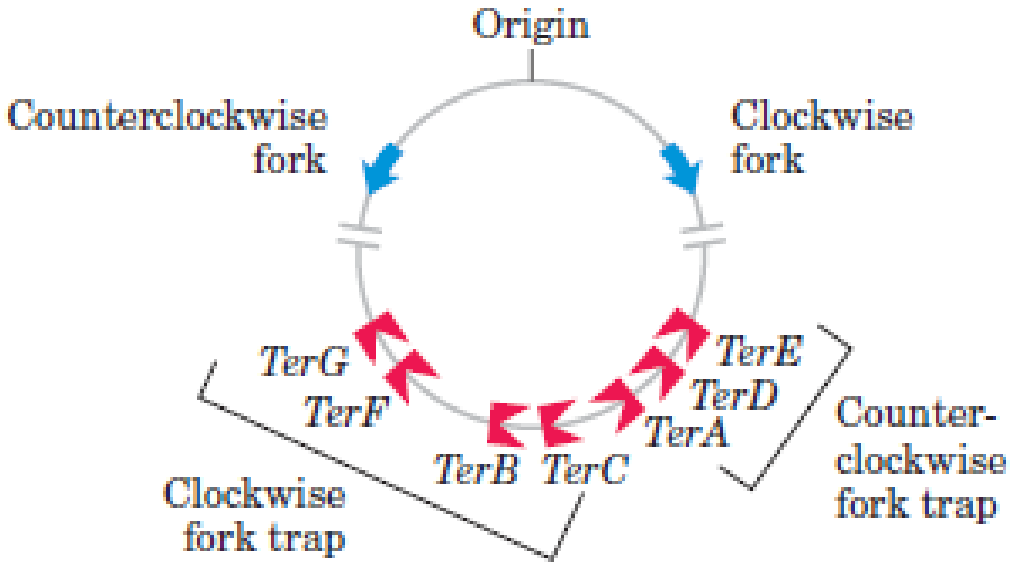
RNA primer removal and is replaced by DNA by DNA polymerase I

Nick sealed by ligase



Termination

Eventually, the two replication forks of the circular *E. coli* chromosome meet at a terminus region containing multiple copies of a 20 bp sequence called Ter



The Ter sequences are arranged on the chromosome to create a trap that a replication fork can enter but cannot leave.

The Ter sequences function as binding sites for the protein Tus (terminus utilization substance

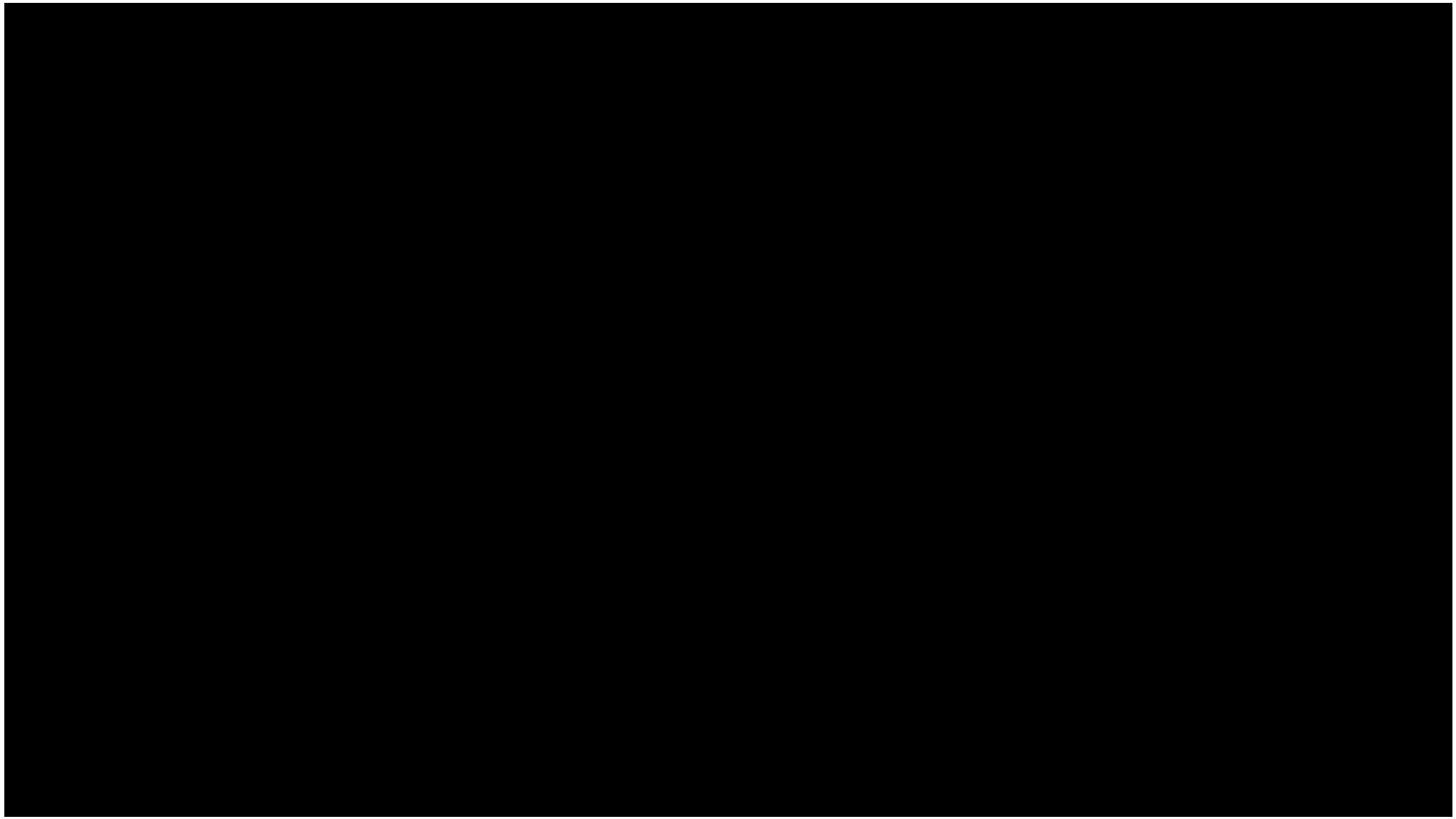
So, when either replication fork encounters a functional Tus-Ter complex, it halts; the other fork halts it meets the first (arrested) fork.

The final few hundred base pairs of DNA between these large protein complexes are then replicated (by an as yet unknown mechanism), completing two topologically interlinked (catenated) circular chromosomes.

DNA circles linked in this way are known as **catenanes**.

Separation of the catenated circles in *E. coli* requires topoisomerase IV (a type II topoisomerase).

The separated chromosomes then segregate into daughter cells at cell division.



The essential features of DNA replication are the same in eukaryotes and bacteria, and many of the protein complexes are functionally and structurally conserved.

However, eukaryotic replication is regulated and coordinated with the cell cycle, introducing some additional complexities.

- ORIGIN
- DNA POLMERASES
- TELOMERES

Origins of replication have a well-characterized structure in some lower eukaryotes, but they are much less defined in higher eukaryotes.

In vertebrates, a variety of A=T-rich sequences may be used for replication initiation, and the sites may vary from one cell division to the next.

Yeast (*Saccharomyces cerevisiae*) has defined replication origins called autonomously replicating sequences (ARS), or **replicators**.

Yeast replicators span 150 bp and contain several essential, conserved sequences.

About 400 replicators are distributed among the 16 chromosomes of the haploid yeast genome.

The rate of movement of the replication fork in eukaryotes (50 nucleotides/s) is only one-twentieth that observed in *E. coli.*

At this rate, replication of an average human chromosome proceeding from a single origin would take more than 500 hours.

Replication of human chromosomes in fact proceeds bidirectionally from many origins, spaced 30 to 300 kbp apart.

Eukaryotic chromosomes are almost always much larger than bacterial chromosomes, so multiple origins are probably a universal feature of eukaryotic cells.

Like bacteria, eukaryotes have several types of DNA polymerases.

Some have been linked to particular functions, such as the replication of mitochondrial DNA.

DNA polymerase α is typically a multisubunit enzyme with similar structure and properties in all eukaryotic cells.

One subunit has a primase activity, and the largest subunit (M_r 180,000) contains the polymerization activity.

However, this polymerase has no proofreading 3’-5’ exonuclease activity, making it unsuitable for highfidelity DNA replication.

DNA polymerase is believed to function only in the synthesis of short primers (either RNA or DNA) for Okazaki fragments on the lagging strand.

These primers are then extended by the multisubunit **DNA polymerase δ** .

The termination of replication on linear eukaryotic chromosomes involves the synthesis of special structures called **telomeres** at the ends of each chromosome