DNA REPLICATION II
EUKARYOTIC DNA SYNTHESIS IS SIMILAR TO SYNTHESIS IN PROKARYOTES, BUT MORE COMPLEX

IN EUKARYOTIC CELLS:

– there is more DNA than prokaryotic cells
– the chromosomes are linear
– the DNA is complexed with proteins
MULTIPLE REPLICATION ORIGINS

Before S phase

During S phase

End of S phase

Chromosome

Origin

Origin

Origin

Origin

Centromere

Before S phase

During S phase

End of S phase

Sister chromatids

BIDIRECTIONAL DNA SYNTHESIS
EUKARYOTIC CHROMOSOMES CONTAIN MULTIPLE ORIGINS OF REPLICATION TO ALLOW THE GENOME TO BE REPLICATED IN A FEW HOURS
MULTIPLE REPLICATION ORIGINS IS ESSENTIAL FOR THE COMPLETE GENOME TO BE REPLICA TED IN A REASONABLE TIME. THERE ARE DIFFERENT NUMBER OF REPLICONS PER GENOME.

Ex: Yeast (S. cerevisiae) has 250-400 replicons. Mammalian cells have 25,000 replicons.

REPLICATION ORIGINS ARE CALLED AS AUTONOMOUSLY REPLICATING SEQUENCES (ARSs).
ALL ARSs ARE INITIALLY BOUND BY A GROUP OF SPECIFIC PROTEINS WHICH IS CALLED AS ORIGIN RECOGNITION COMPLEX (ORC). THESE COMPLEXES ARE FORMED IN G1 PHASE OF CELL CYCLE BEFORE S PHASE. CELL CYCLE CONTROL PROTEINS (KINASES) ARE ACTIVATED AND BIND TO ORCs, WHICH CAN BE ACCESSED BY DNA POLYMERASES.
Cdk activity low

- pre-RC formation allowed
- no pre-RC activation

Cdk activity high

- new pre-RC formation inhibited
- existing pre-RC activation
THE ACTIVATION INHIBITS REFORMATION OF THE PREREPLICATION COMPLEX ONCE DNA SYNTHESIS HAS BEEN COMPLETED AT EACH REPLICON

THIS MECHANISM ENSURES THAT REPLICATION ONLY OCCURS ONCE ALONG EACH STRECH OF DNA DURING CELL CYCLE
### EUKARYOTIC DNA POLYMERASES

#### Properties of Eukaryotic DNA Polymerases

<table>
<thead>
<tr>
<th></th>
<th>Polymerase α</th>
<th>Polymerase β</th>
<th>Polymerase δ</th>
<th>Polymerase ε</th>
<th>Polymerase γ</th>
<th>Polymerase ζ</th>
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<tbody>
<tr>
<td>Location</td>
<td>Nucleus</td>
<td>Nucleus</td>
<td>Nucleus</td>
<td>Nucleus</td>
<td>Mitochondrion</td>
<td>Nucleus</td>
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<td>3’–5’ Exonuclease</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Activity</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Essential to</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Nuclear Replication</td>
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3 DNA POLYMERASES ARE INVOLVED IN REPLICATION OF NUCLEAR DNA

1 INVOLVES MITOCHONDRIAL DNA REPLICATION

OTHERS ARE INVOLVED IN REPAIR PROCESSES
<table>
<thead>
<tr>
<th>Eukaryotic</th>
<th>Number of subunits</th>
<th>Function</th>
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<tbody>
<tr>
<td>Pol α</td>
<td>4</td>
<td>Primer synthesis during DNA replication</td>
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<tr>
<td>Pol β</td>
<td>1</td>
<td>Base excision repair</td>
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<tr>
<td>Pol γ</td>
<td>3</td>
<td>Mitochondrial DNA replication and repair</td>
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<tr>
<td>Pol δ</td>
<td>2–3</td>
<td>DNA replication; nucleotide and base excision repair</td>
</tr>
<tr>
<td>Pol ε</td>
<td>4</td>
<td>DNA replication; nucleotide and base excision repair</td>
</tr>
<tr>
<td>Pol θ</td>
<td>1</td>
<td>DNA repair of crosslinks</td>
</tr>
<tr>
<td>Pol ζ</td>
<td>1</td>
<td>Translesion synthesis (TLS)</td>
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<tr>
<td>Pol λ</td>
<td>1</td>
<td>Meiosis-associated DNA repair</td>
</tr>
<tr>
<td>Pol μ</td>
<td>1</td>
<td>Somatic hypermutation</td>
</tr>
<tr>
<td>Pol κ</td>
<td>1</td>
<td>TLS</td>
</tr>
<tr>
<td>Pol η</td>
<td>1</td>
<td>Relatively accurate TLS past <em>cis-syn</em> cyclobutane dimers</td>
</tr>
<tr>
<td>Pol τ</td>
<td>1</td>
<td>TLS, somatic hypermutation</td>
</tr>
<tr>
<td>Rev1</td>
<td>1</td>
<td>TLS</td>
</tr>
</tbody>
</table>

*Source: Data from Sutton and Walker, 2001 and references therein.*
Pol $\alpha$ and $\delta$

- MAJOR FORMS OF THE ENZYME INVOLVED IN INITIATION AND ELONGATION

Pol $\alpha$

- POSSESSES LOW **PROCESSIVITY**
- FUNCTIONS IN SYNTHESIS OF RNA PRIMERS DURING INITIATION ON THE LEADING AND LAGGING STRANDS

Polymerase switching **occurs**

- Pol $\alpha$ IS REPLACED BY Pol $\delta$, WHICH HAS HIGH PROCESSIVITY FOR ELONGATION
DNA Polymerase Switching
TO ACCOMODATE THE INCREASED NUMBER OF REPLICONS, EUKARYOTIC CELLS CONTAIN MANY MORE DNA POLYMERASE MOLECULES THAN BACTERIA

Ex: *E. coli* has 15 copies of DNA polymerase III per cell while, animal cells contain 50,000 copies of α-DNA polymerase
THE PRESENCE SEVERAL NUMBERS OF REPLICONS WITH SMALLER SIZES (100-150 nucleotides) COMPENSATES FOR THE SLOWER RATE OF DNA SYNTHESIS IN EUKARYOTES

Ex: *E. coli* NEEDS 20-40 MINUTES TO REPLICATE ITS CHROMOSOME WHILE *Drosophila* (with 40 times more DNA) NEEDS 3 MINUTES TO COMPLETE THIS TASK
TELOMERES PROVIDE STRUCTURAL INTEGRITY AT CHROMOSOME ENDS BUT ARE PROBLEMATIC TO REPLICATE

TELOMERES AT THE ENDS OF LINEAR CHROMOSOMES CONSIST OF LONG STRETCHES OF SHORT REPEATING SEQUENCES AND PRESERVE THE INTEGRITY AND STABILITY OF CHROMOSOMES
EUKARYOTIC CHROMOSOMES END IN DISTINCTIVE SEQUENCES CALLED AS TELEOMERES
THESE SEQUENCES CONSIST OF SHORT TANDEM REPEATING SEQUENCES “TTGGGGG”

Ex: IN HUMAN CHROMOSOMES 5’-TTAGGG-3’
SEQUENCE IS REPEATED SEVERAL TIMES AT THE ENDS “G” RICH STRANDS OF THE CHROMOSOMES

THE G- RICH SINGLE STRANDED TAILS CAN LOOP BACK IN THEMSELVES AT THE CHROMOSOME ENDS CALLED AS T-LOOPS (TELOMERE LOOPS)
Telomeres protect chromosome ends

- Telomeres have a G-strand overhang.
- They consist of 2-50 kb duplex telomeric repeats.
- The repeats include [TTAGGG]n and [CCCTAA]n.
- There is also non-telomeric DNA.
- The diagram shows a t-loop and a large duplex loop.
- The loop contains 75-200 nt ss DNA.
T-Loop in Telomeres

A

Centromere

4-12 kb of telomere repeats

TTAGGG

AATCCC

3' overhang

100-200 nt

Open Configuration

T-loop

B

Shelterin/Telesome

TRF1

RAP1

TRF2

TPP1

TIN2

POT1

Nucleosome

molekulce.com/Tuba ERTÜRK
TELOMERIC SEQUENCES HAVE HEXANUCLEOTIDE OR HEPTANUCLEOTIDE REPEATING SEQUENCES AFTER EACH REPLICATION ONE STRAND IN EACH CHROMOSOME ENDS HAVE 12-16 NUCLEOTIDES LONG OVER HANG, THIS IS ON THE ‘G’ RICH 3’ENDING
REPLICATION AT THE TELOMERE

LAGGING STRAND SYNTHESIS AT END OF CHROMOSOME IS A PROBLEM ONCE THE RNA PRIMER IS REMOVED, THERE IS NO FREE 3'-HYDROXYL GROUP FROM WHICH TO ELONGATE
DNA polymerase cannot do this

No place for a primer

5'

3'
TELOMERASE DIRECTS SYNTHESIS OF THE TELOMERE REPEAT SEQUENCE TO FILL GAP

THIS ENZYME IS A RIBONUCLEOPROTEIN WITH AN RNA MOLECULE THAT SERVES AS THE TEMPLATE FOR THE SYNTHESIS OF ITS DNA COMPLEMENT
SIMILAR ENZYMES FUNCTION FOR TELOMERIC SEQUENCES IN ALMOST ALL EUKARYOTES

TELOMERIC SEQUENCES HAVE BEEN HIGHLY CONSERVED THROUGHOUT EVOLUTION

TELOMERE SHORTENING HAS BEEN LINKED TO THE MOLECULAR MECHANISMS INVOLVED IN CELLULAR AGING

IN FACT IN MOST OF THE EUKARYOTIC SOMATIC CELLS TELOMERASE IS NOT ACTIVE
# Table 11.5
Telomeric Sequences within Selected Organisms

<table>
<thead>
<tr>
<th>Group</th>
<th>Example</th>
<th>Telomeric Repeat Sequence</th>
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</thead>
<tbody>
<tr>
<td>Mammals</td>
<td>Humans</td>
<td>TTAGGG</td>
</tr>
<tr>
<td>Slime molds</td>
<td>Physarum, Didymium</td>
<td>TTAGGG</td>
</tr>
<tr>
<td></td>
<td>Dictyostelium</td>
<td>AG&lt;sub&gt;(1–8)&lt;/sub&gt;</td>
</tr>
<tr>
<td>Filamentous fungi</td>
<td>Neurospora</td>
<td>TTAGGG</td>
</tr>
<tr>
<td>Budding yeast</td>
<td>Saccharomyces</td>
<td>TG&lt;sub&gt;(1–3)&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>cerevisiae</td>
<td></td>
</tr>
<tr>
<td>Ciliates</td>
<td>Tetrahymena</td>
<td>TTGGGG</td>
</tr>
<tr>
<td></td>
<td>Paramecium</td>
<td>TTGGG(T/G)</td>
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<td></td>
<td>Euplotes</td>
<td>TTTTGGGGGG</td>
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<tr>
<td>Higher plants</td>
<td>Arabidopsis</td>
<td>TTTAGGG</td>
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