DNA Organization,Replication& Repair

DNA Organization

CHROMATIN

- Chromatin consists of:

-Double stranded DNA molecules.

-Histones(small basic proteins).

-Nonhistone proteins.

-RNA (small amount).

- HISTONES:

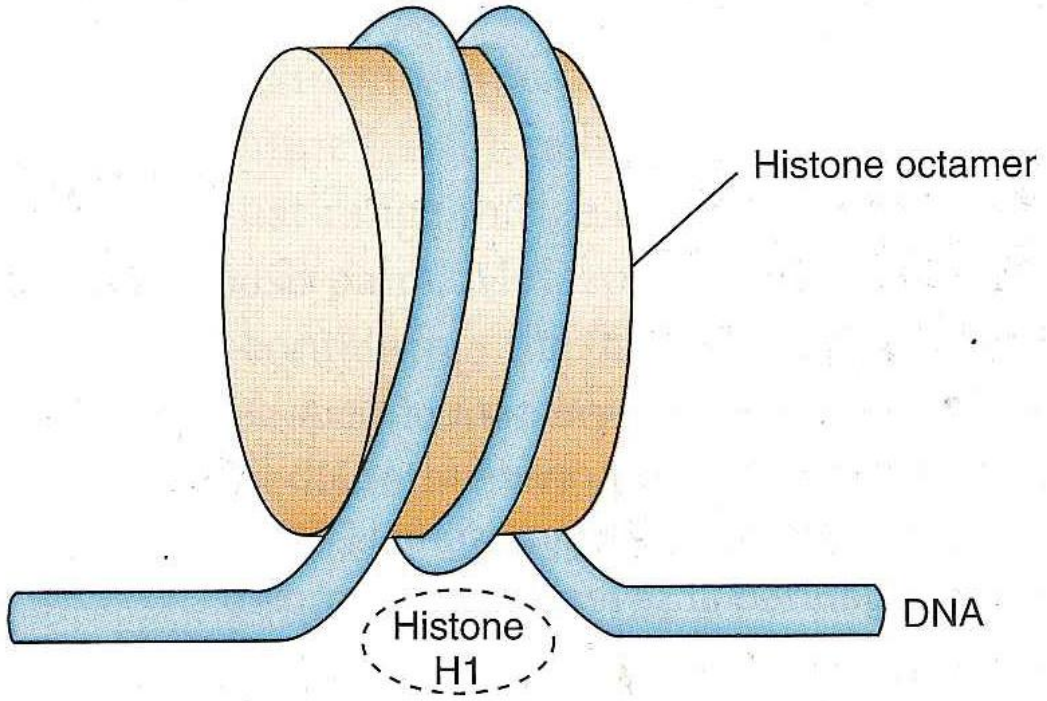
H1 histone:The least tightly bound to chromatine.

H2A-H2B-H3-H4 in the form of an octamer (H3/H4)2-(H2A-H2B)2

Nucleosome:

The Histone octamer+DNA

A Nucleosome



Compaction of Chromatin

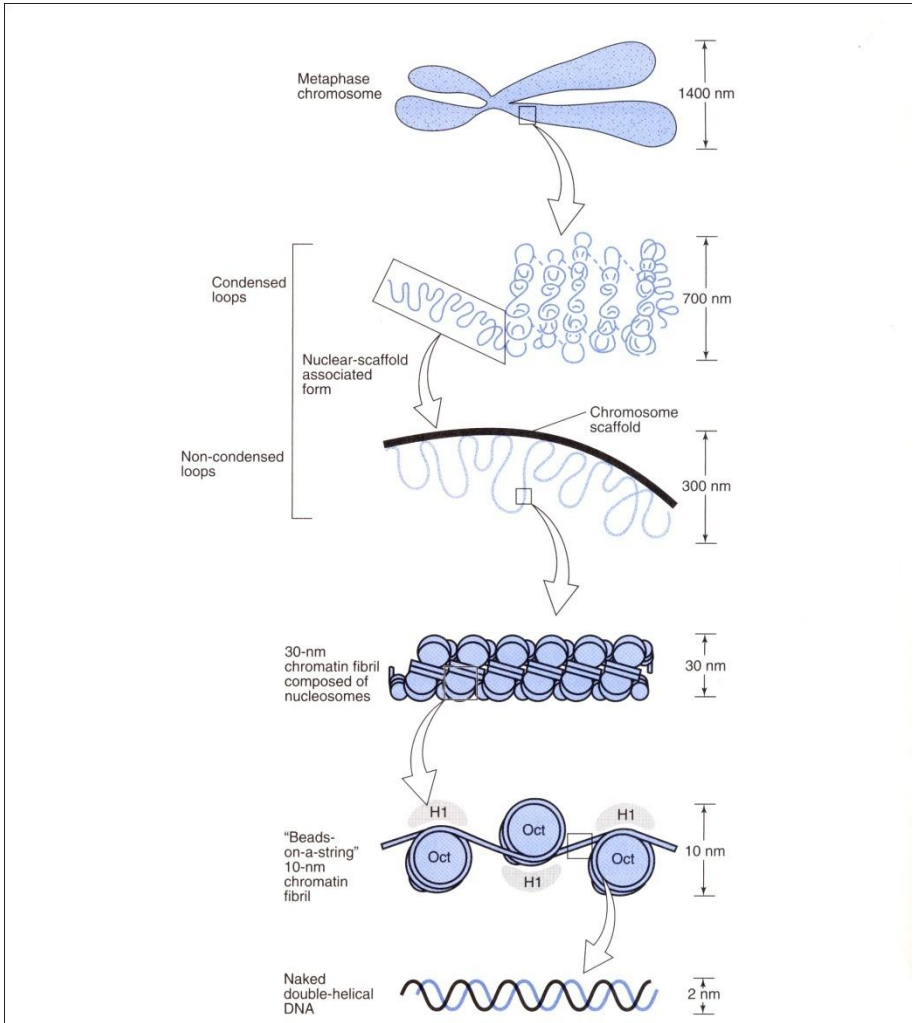


Figure 36–3. Shown is the extent of DNA packaging in metaphase chromosomes (*top*) to noted duplex DNA (*bottom*). Chromosomal DNA is packaged and organized at several levels as shown (see Table 36–2). Each phase of condensation or compaction and organization (*bottom to top*) decreases overall DNA accessibility to an extent that the DNA sequences in metaphase chromosomes are almost totally transcriptionally inert. In toto, these five levels of

Some regions of Chromatin are Active and others are Inactive

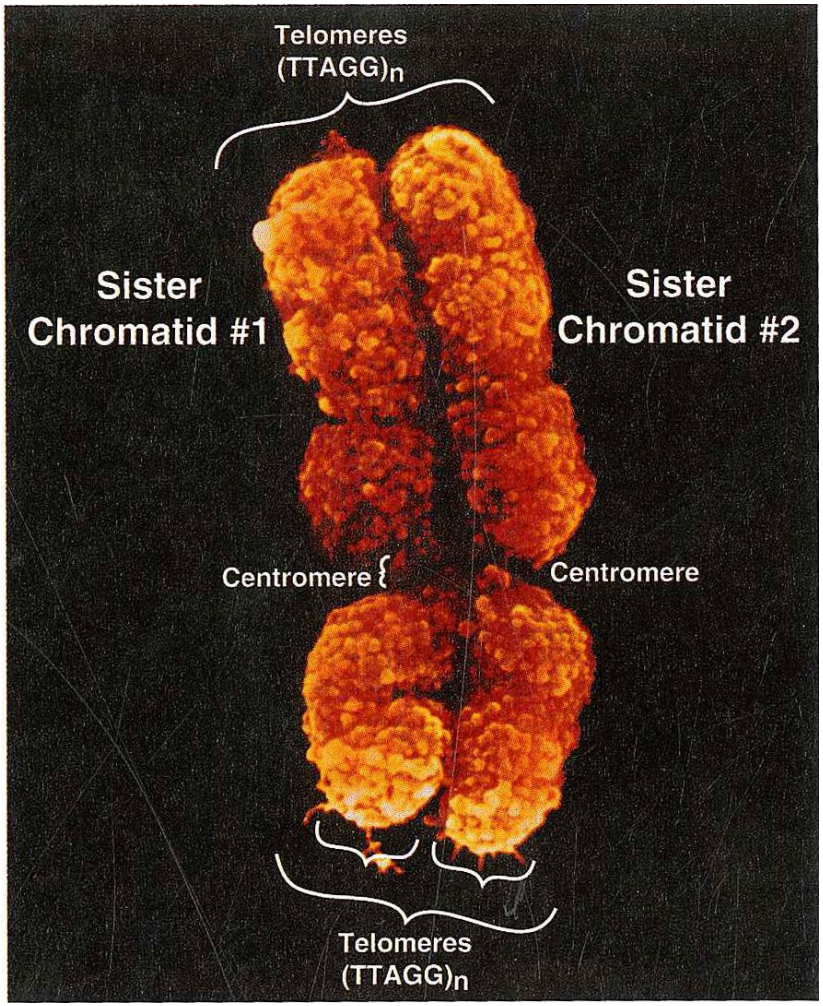
- Active Chromatine (euchromatin):
  DNA contains large regions (100,000 base) *sensitive* to digestion by a nuclease.

  Within these sensitive regions there exists *Hypersensitive* sites (100-300 base) located upstream from the active genes.
- InActive Chromatine (heterochromatin):

  Constitutive heterochromatin:always condensed and thus inactive (found near centromers and telomers).

  Faculative heterochromatin:at times condensed and at others uncondensed and actively transcribed appearing as euchromatin (X chromosome).

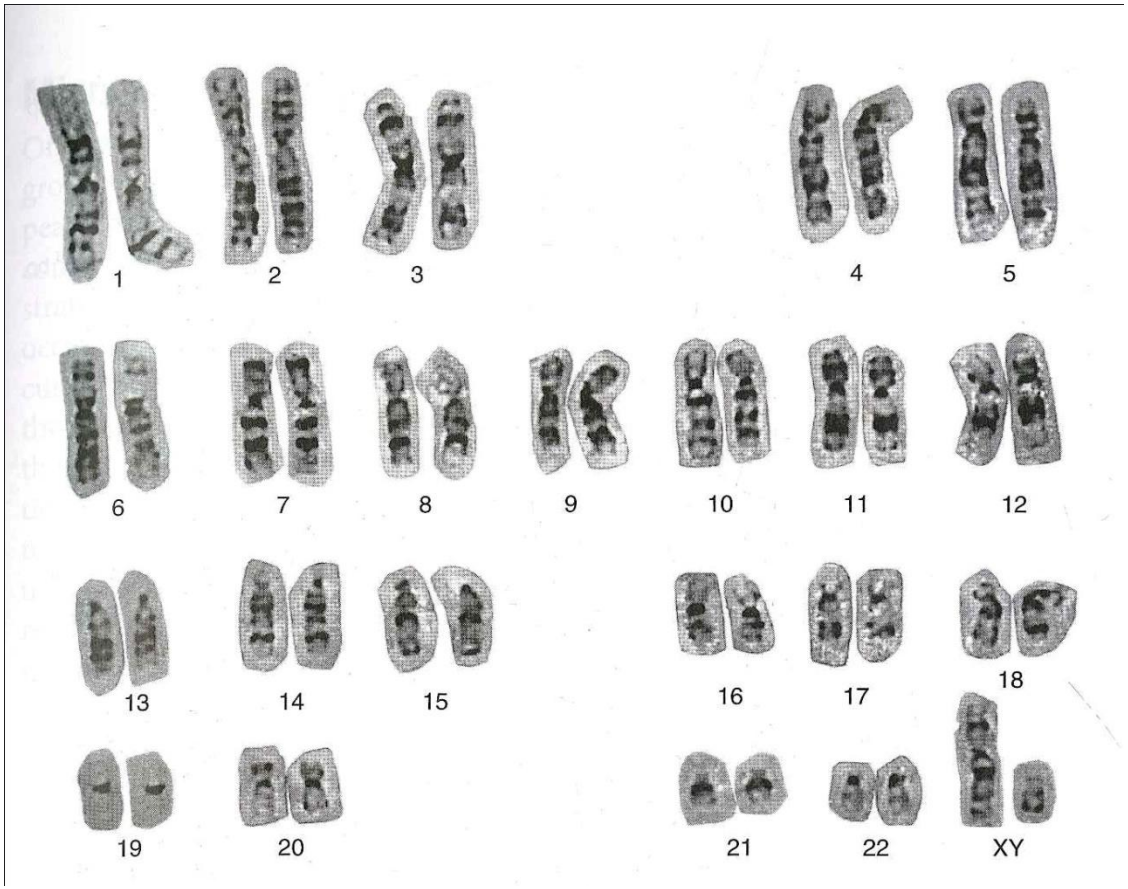
The two sister Chromatoids of Human Chromosome 12



DNA is Organized into Chromosomes

- Centromere:
  The position where the identical duplicated sister chromatides are connected.It bindes several proteins forming a complex called *Kinetochore*
- Telomeres:
  The ends of each chromosome (short TG-rich repeats). *Telomerase* is the enzyme responsible for telomere synthesis and for maintaining the length of the telomere.

A Human Karyotype of a Man



Coding regions are often interrupted by intervening sequences

- Protein coding regions:
  Transcripts appear in cytoplasm as mRNA molecules.
- Nonprotein coding regions:Transcripts appear in nucleus as hnRNA.
- The noncoding intervening sequences in the gene are called INTRONES the coding sequences are called EXONS.
- The primary transcripts must be processed by a procedure called splicing in which Introns are removed and Exons are spliced together.

The relationship between chromosomal DNA and mRNA

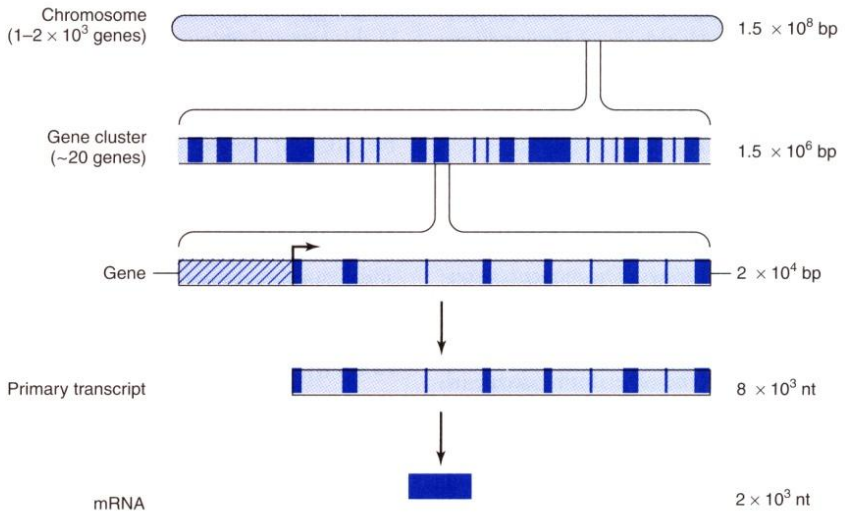


Figure 36–7. The relationship between chromosomal DNA and mRNA. The human haploid DNA complement of 3×10^9 base pairs (bp) is distributed between 23 chromosomes. Genes are clustered on these chromosomes. An average gene is 27,000 bp in length, including the regulatory region (hatched area), which is usually located at the 5′ end of the gene. The regulatory region is shown here as being adjacent to the transcription initiation site (arrow). Most eukaryotic genes have alternating exons and introns. In this example, there are nine exons (dark blue areas) and eight introns (light blue areas). The introns are removed from the primary transcript by the processing reaction, and the exons are ligated together in sequence to form the mature mRNA. (nt, nucleotides.)

of course, and how this is accomplished is one of the

Depending on their length, moderately repetitive

Much of the Mammalian genome is Redundant & much is not Transcribed

- The entire genome contains DNA to code for nearly 1.5 million gene.There is only around 100,000 protein coded by 1.1% of genome.So most of the DNA is noncoding.
- The excess DNA sequences can be:

-Regulatory: they function to regulate gene expression during development, differentiation and adaptation
-Intervening sequences (Introns) they form 24% of genome.

-Families of repeated sequences (function not clearly defined)

●DNA can be divided into sequence classes:

-Unique-sequence (nonrepetitive DNA):single copy genes that code for proteins.

-Repetitive sequences DNA:vary in copy from 2 to 10^7 copies per cell.

DNA synthesis & Replication are rigidly controlled

- The replication of DNA must be complete and carried out in such a way to maintain genetic stability within the organism and species.
- Replication occurs from a single stranded DNA(ssDNA).
- Mechanism involves:
  -target the site of initiation of replication
  -unwind double stranded DNA in that region
  -formation of replicating complex

DNA REPLICATION

Steps involved in DNA Replication

- 1.Identification of the origin of Replication.
- 2.Unwinding (Denaturation) of dsDNA to provide ssDNA template.
- 3.Formation of Replication Fork.
- 4.Initiation of DNA Synthesis and Elongation.
- 5.Formation of Replication bubbles with ligation of the newly synthesized DNA segments.
- 6.Reconstitution of chromatin structure.

Origin of Replication(ori)

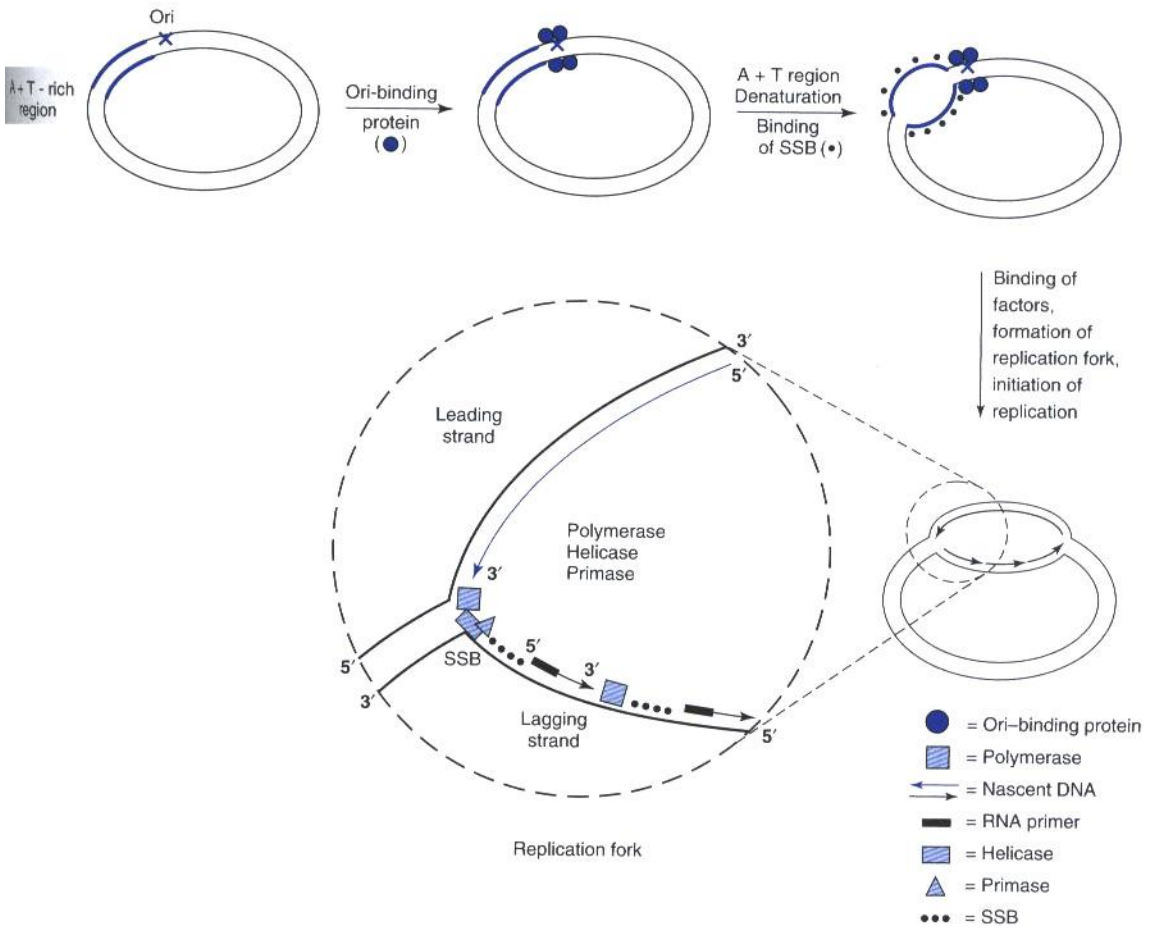
- In ori there exists an association of a sequence specifi double-stranded binding proteins with a direct repeat DNA sequences.

ori= Proteins(specific for ds-DNA

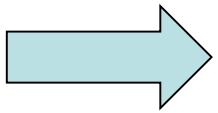
sequence)+

Direct repeats of DNA

Steps involved in DNA Replication



Unwinding of DNA

- 1-Interaction of proteins with ori 

ssDNA

Helicase: function is unwinding of DNA.

SSB(single stranded binding proteins):Stabilize ori and Helicase complex leading to DNA unwinding and active replication.

Formation of Replication Fork

- Replication fork:

1-DNA helicase unwinds a short segment of DNA.

2-Primase:initiates synthesis of an RNA essential for priming DNA synthesis.

3-DNA Polymerase:initiates nascent daughter strand synthesis.

4-SSBs:bind to ssDNA preventing reannealing of ssDNA to dsDNA.

DNA Polymerase only synthesizes DNA in the 5`to 3`direction. DNA strands are anti parallel

DNA Polymerase acts assymetrically on both strands:

1- Leading strand:DNA is synthesized continuously.

2-Lagging strand:DNA is synthesized in short segments called Okazaki fragments.

For each Replication fork many Okazaki fragments are made(~250)

In order to perform this there is an association between Helicase and Primase which will move from one fragment to the next called Primosome

DNA Polymerase complex

- Role of DNAPolymerase in Replication:

1-Chain Elongation:Rate of nucleotides per second added.

2-Processivity:Number of nucleotides added to nascent chain before polymerase disengages from the template.

3-Proofreading:Identifies copying errors and corrects them.

E *coli* polymerases

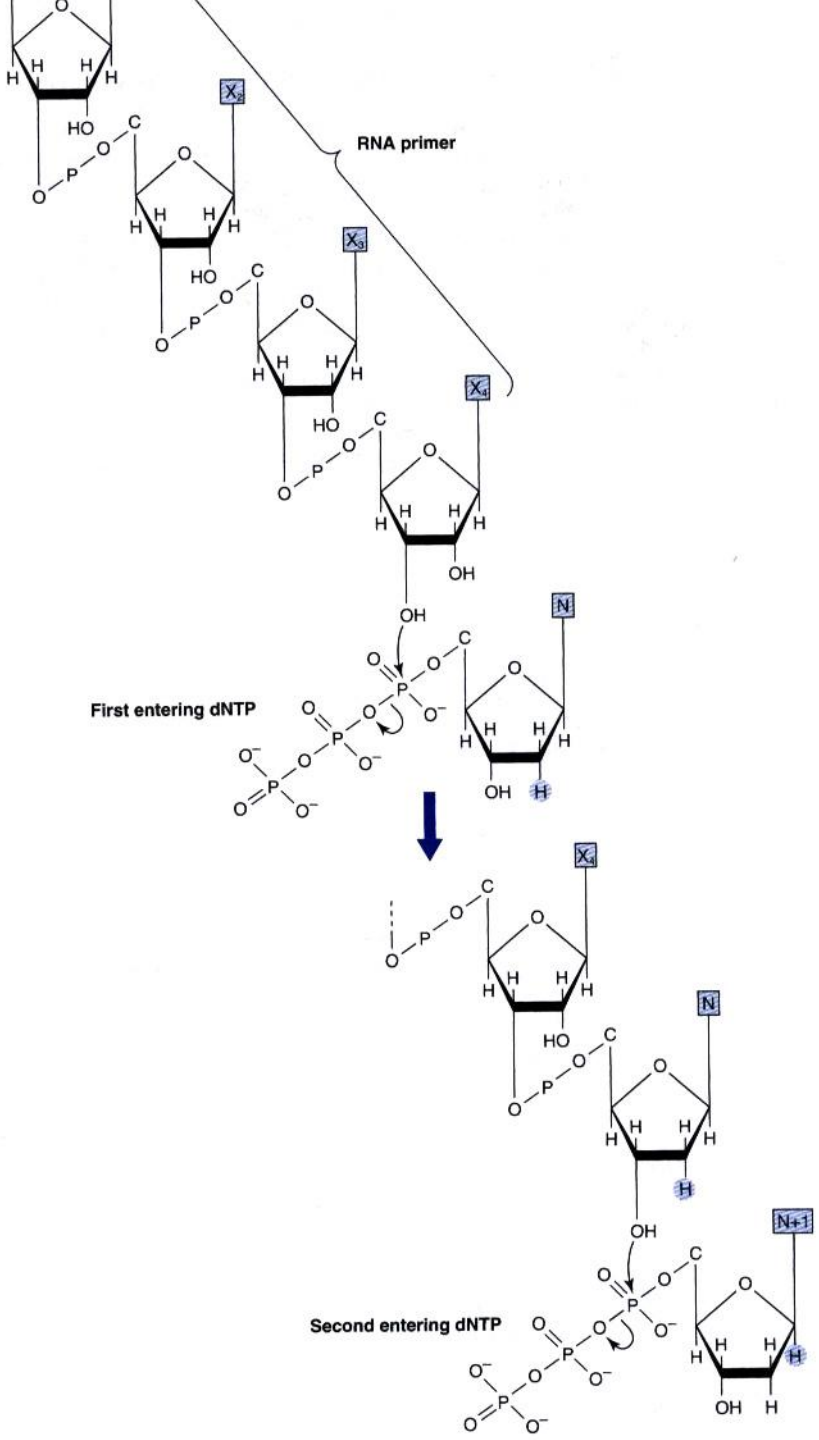
- PolymeraseIII (polII):highest rate for elongation and procesivity.
- Polymerase II(poll) :Proofreading and Repair.
- PolymeraseI(pol): completes chain synthesis between Okazaki fragments on lagging strand.

In Mammalian cells rate of polymerization is 100 nucleotide per second(10 times slower than Bacteria DNA polymerase).

Initiation & Elongation of DNA synthesis

Reaction occurs between 3`-hydroxyl group of RNA primer +α phosphate of first entering deoxy ribonucleoside tri-phosphate.

Selection of the incomming nucleotide depends on the sequence on of the replicating strand.


RNA primer
First entering dNTP
Second entering dNTP

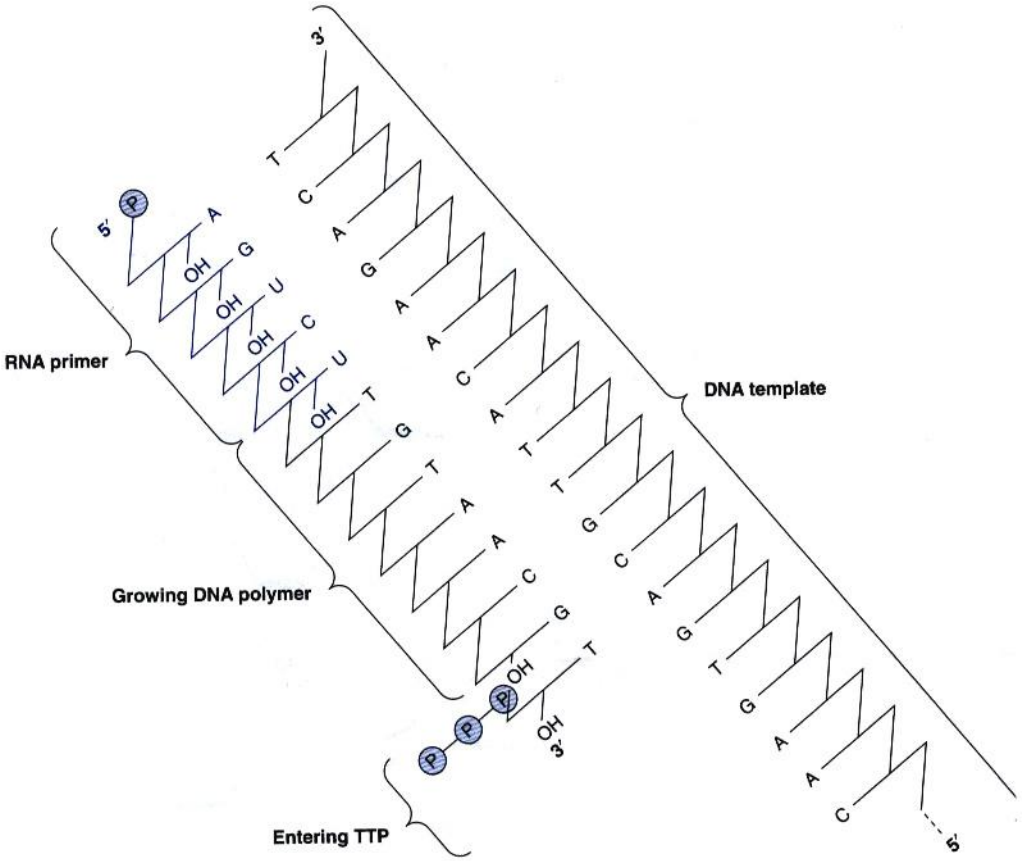
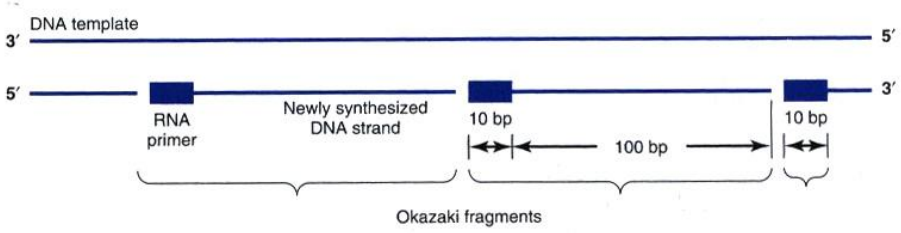


Figure 36–15. The RNA-primed synthesis of DNA demonstrating the template function of the complementary strand of parental DNA.

- Okazaki fragments



- Replication bubbles

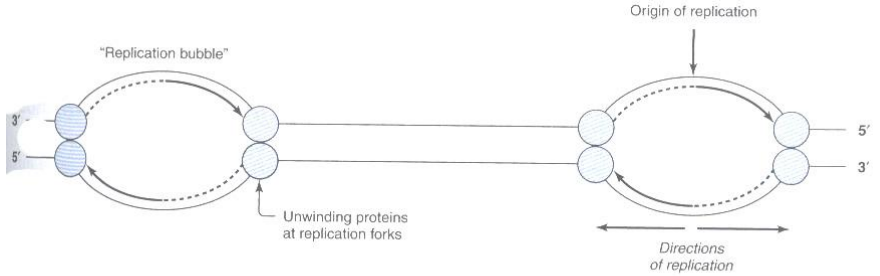


Figure 36–17. The generation of "replication bubbles" during the process of DNA synthesis. The bidirectional replication and the proposed positions of unwinding proteins at the replication forks are depicted.

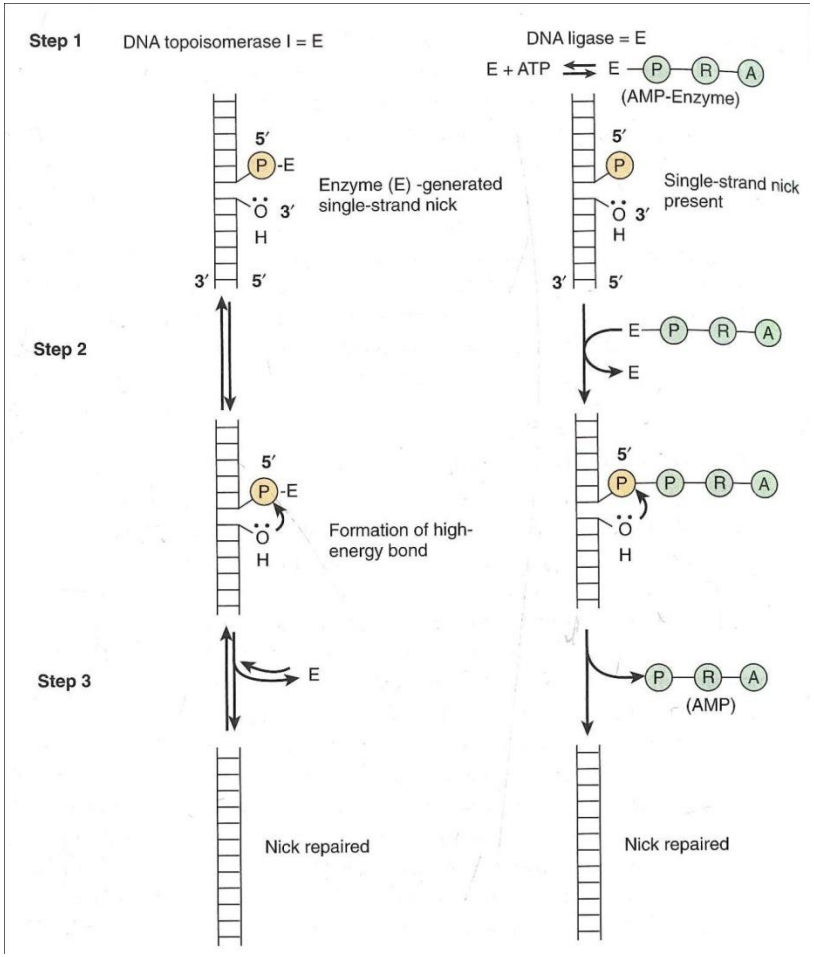
Replication exhibits polarity

- DNA strands are antiparallel,and are replicated simultaneously in 5`→3`direction.
- There exists one enzyme responsible for polymerization.
- It polymerizes new DNA strands in5`→3` (there does not exist a polymerase capable of polymerizing DNA in 3`→5`direction).
- The same complex polymerizes the leading strand continuously in the direction5`→3`the proceeds in the opposite direction for the lagging strand discontinuously again 5`→3`.
- In mammalian genome most RNA primers are removed.

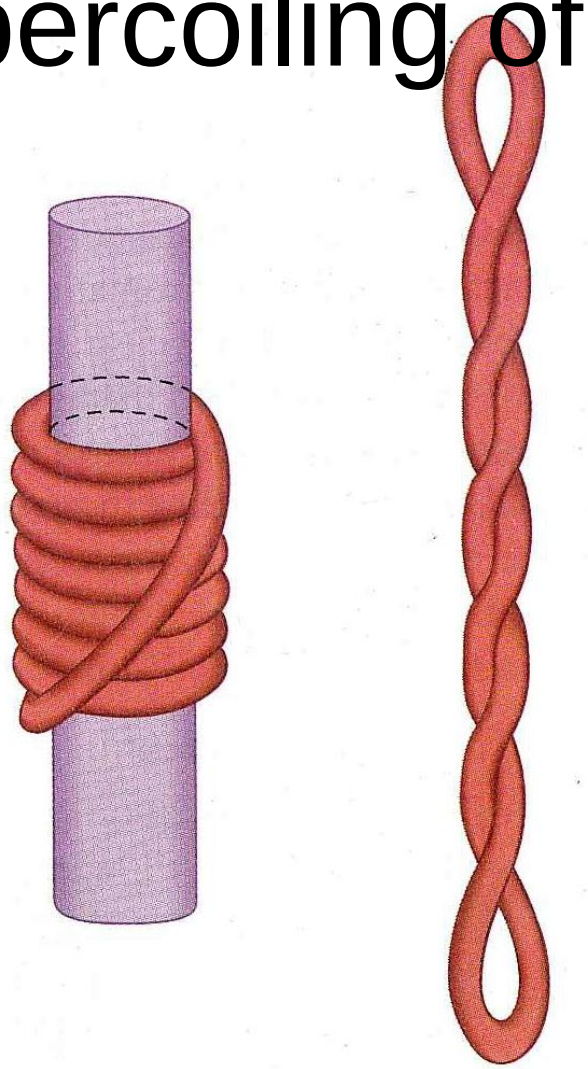
Formation of Replication bubbles

- SSBs: Stabilize the single stranded structure as the replication fork progresses.
- There is a need to unwind DNA this is provided by enzymes which introduce ***nicks*** in single strand of DNA allowing unwinding.
- After replication nicks are rapidly resealed by same enzymes.
- The nicking-resealing enzymes are called ***TOPOISOMERASEs*** (an ATP independent process).
- ***DNA Ligase*** seals Okazaki fragments ,they areATP-dependent.

Comparison of two types of nick-sealing reactions on DNA



Supercoiling of DNA



DNA Synthesis occurs During the S Phase of the Cell Cycle