

CSIR NET Life Science Unit 3

Eukaryotic Polymerases VS Prokaryotic Polymerases (RNA Polymerase + DNA Polymerase)

DNA Polymerase: DNA Polymerase is the main enzyme required during the replication of DNA in the S phase of the cell cycle. Its precise name is DNA-dependent DNA polymerase, as it is dependent on the presence of DNA template for its working, hence, the name DNA-dependent DNA polymerase. DNA-dependent DNA polymerase present in prokaryotes differs from that present in eukaryotes.

In Prokaryotes (Bacteria) such as *Escherichia coli*, 5 different DNA-dependent DNA polymerases are present, namely DNA polymerase I, II, III, IV, and V.

1. **DNA polymerase I** enzyme provides the major part of the activity in *E. coli*. It is chiefly a DNA repair enzyme and is used for in vitro DNA replication. DNA polymerase I is encoded by gene *polA* has a single polypeptide and can initiate replication in vitro at a nick in a DNA duplex. It can be cleaved by proteolytic treatments into large and small fragments. This large fragment, called the Klenow fragment, lacks 5' → 3' exonuclease activity and is used for in vitro DNA replication. The 5' → 3' polymerase activity is responsible for primer extension or DNA synthesis. The 5' → 3' exonuclease activity is involved in the excision of DNA strands during DNA repair; it removes ~ 10 bases at a time. An exonuclease digests nucleic acids (here DNA) from one end, and it does not cut DNA internally. The 3' → 5' exonuclease activity is responsible for proof-reading.
2. **DNA polymerase II** enzyme functions in DNA repair. It has 5' → 3' polymerase and 3' → 5' exonuclease activities and uses as a template only such DNA duplexes that have short gaps.
3. **DNA polymerase III** enzymes are responsible for DNA replication in vivo. It has 5' → 3' polymerase and 3' → 5' exonuclease activities. It catalyzes DNA synthesis at very high rates, e.g., 15,000 bases/min at 37°C. It is composed of several subunits. A DNA polymerase molecule has the following 4 functional sites involved in polymerase activity. The template site binds the strand serving as a template during replication. The primer site binds to the primer used for DNA replication. Primer terminus site binds only to such primers that have free 3' -OH. The nucleotide triphosphate site binds to the deoxynucleotide 5'-triphosphate that is complementary to the corresponding nucleotide of the template. It also catalyzes a phosphodiester bond between the 5' phosphate of this nucleotide and the 3' -OH of the terminal primer nucleotide.
4. **DNA Polymerase IV and V** are Y-family DNA polymerases. Members of Y-family polymerases do not contain 3'-5' exonuclease activity. These polymerases are characterized by low catalytic efficiency, low processivity, and low fidelity. They

are involved in the translesion synthesis and replicate damaged DNA by bypassing damaged nucleotides.

In Eukaryotes such as yeast, 3 major DNA-dependent DNA polymerases are present, namely DNA pol α , DNA pol δ , DNA pol ϵ . Apart from these 3, DNA pol β and DNA pol γ are also present.

1. **Polymerase α** is present in the nuclei of the cell. DNA polymerase shows optimal activity with a gapped DNA template but shows a remarkable ability to use single-stranded DNA by forming transient hairpins. It will not bind to duplex DNA. The native, undegraded enzyme consists of a 180 K Da polymerase together with three sub-units—the 60 and 50 K Da sub-units of about 70, 60, and 50 K Da. The Association of the 180 K Da polymerase with the 70KDa protein makes the 3' \rightarrow 5' exonuclease activity of the larger sub-units comprise a primase activity which allows the enzyme to initiate replication on unprimed single-stranded cyclic DNAs. Therefore, polymerase α have dual activity, i.e., both the polymerase and primase activity. The association of primase with DNA polymerase α is restricted to the DNA synthetic phase.
2. **Polymerase δ** is present in the dividing cell and has got similar properties polymerase α , but having 3' \rightarrow 5' exonuclease activity. The activity of polymerase δ is dependent on activity on two auxiliary proteins: cyclin and activator I. Due to the presence of approximately equal activities of DNA polymerase α and δ , it has been proposed that they act as a dimer at the replication fork with the highly processive polymerase δ acting on the leading strand and the primase-associated polymerase acting on the lagging strand.
3. Cyclin or PCNA (proliferating cell nuclear antigen), independent form of DNA polymerase δ , is known as **polymerase ϵ** , which has two active polymerase sub-units of 220 and 145 K Da. DNA polymerase ϵ is also probably involved in replication, and it has been proposed that it takes over from DNA polymerase α in the synthesis of Okazaki fragments.
4. Polymerase β is a repair enzyme, whereas polymerase γ helps in mitochondrial DNA replication.

Prokaryotes	Eukaryotes
Five polymerases (I, II, III, IV, V)	Five polymerases (α , β , γ , δ , ϵ)
Functions of polymerase:	Functions of polymerases:
I is involved in synthesis, proofreading, repair, and removal of RNA primers	α : a polymerizing enzyme
II is also a repair enzyme	β : is a repair enzyme
III is main polymerizing enzyme	γ : mitochondrial DNA synthesis
IV, V are repair enzymes under unusual conditions	δ : main polymerizing enzyme
	ϵ : function unknown

RNA polymerase: RNA polymerase is the main enzyme used during the transcriptional process taking place in the cell. During transcription, a DNA code for an mRNA. The formation of mRNA is facilitated by DNA-dependent RNA polymerase. In prokaryotes, only one type of RNA polymerase is present, whereas, in eukaryotes, 5 different types of RNA polymerase are present.

1. **RNA polymerase** in *E. coli* is composed of five polypeptide subunits, two of which are identical. Four of these subunits, denoted α , α , β , and β' , comprise the polymerase core enzyme. Each subunit has a unique role; the two α -subunits are necessary to assemble the polymerase on the DNA; the β -subunit binds to the ribonucleoside triphosphate that will become part of the nascent “recently born” mRNA molecule, and the β' binds the DNA template strand. The fifth subunit, σ , is involved only in transcription initiation. It confers transcriptional specificity such that the polymerase begins to synthesize mRNA from an appropriate initiation site. Without σ , the core enzyme would transcribe from random sites and produce mRNA molecules that specified protein gibberish. The polymerase comprises all five subunits called the holoenzyme (a holoenzyme is a biochemically active compound comprised of an enzyme and its coenzyme).
2. RNA polymerase in yeast comprises 5 different types, namely RNA polymerase I, II, III, IV, and V.
 - **RNA polymerase I** synthesizes a pre-rRNA 45S (35S in yeast), which matures and will form the major RNA sections of the ribosome.
 - **RNA polymerase II** synthesizes precursors of mRNAs and most sRNA and microRNAs.
 - **RNA polymerase III** synthesizes tRNAs, rRNA 5S, and other small RNAs found in the nucleus and cytosol.
 - **RNA polymerase IV and V** found in plants are less understood; they make siRNA. In addition to the ssRNAPs, chloroplasts also encode and use a bacteria-like RNAP.

Prokaryotic RNA Polymerase

The single RNA polymerase consists of 4 subunits and an additional transcription initiation sigma factor.

The core enzyme contains 5 sub-units

They catalyze the synthesis of Polycistronic RNA

Eukaryotic RNA Polymerase

Eukaryotes consist of 5 different types of RNA polymerases

They contain 10-20 sub-units

They catalyze the synthesis of Monocistronic RNA

The size varies around to 400 KDa

Sigma factor is required for Transcription initiation

The size varies around to 500 KDa

Transcription factors are required for Transcription initiation



Mentor Guru