Chapter 1
DNA Replication

Abstract DNA carries genetic information that is transferred from one generation to the next. Various DNA polymerases perform accurate DNA replication. Only correct dNTP can be selected and incorporated opposite template base. DNA polymerization consists of several steps. DNA polymerases can be classified into different families, and their functional domains include thumb, palm, figure, and other assistant domains.

Keywords DNA polymerase · dNTP incorporation · Polymerization mechanism · Polymerase structure

1.1 The Mechanism of DNA Replication

DNA encodes the genetic information used in the development and functioning of all known living organisms and many viruses. Most DNA molecules consist of two strands in a double helix. Each DNA strand is composed of guanine (G), adenine (A), thymine (T), and cytosine (C), as well as a deoxyribose and a phosphate group. Hydrogen bonds between base pairs of A:T and C:G make double-stranded DNA. DNA stores biological information by its sequence of these four nucleobases. Both strands of the double-stranded structure store the same biological information.

Within cells, DNA is organized into chromosomes. During cell division, these chromosomes are duplicated, providing each cell its own complete set of chromosomes. Eukaryotic organisms (animals, fungi, plants, and protists) store most of their DNA inside the cell nucleus and some of their DNA in organelles, such as mitochondria or chloroplasts. Prokaryotes (archaea and bacteria) store their DNA only in the cytoplasm. Within the chromosomes, chromatin proteins compact and organize DNA. These compact structures guide the protein–DNA interactions, regulating DNA replication.

One original DNA molecule can be replicated into two identical replicas. This biological process is the basis for biological inheritance. Each strand of the original DNA molecule serves as template for the production of the complementary strand, a

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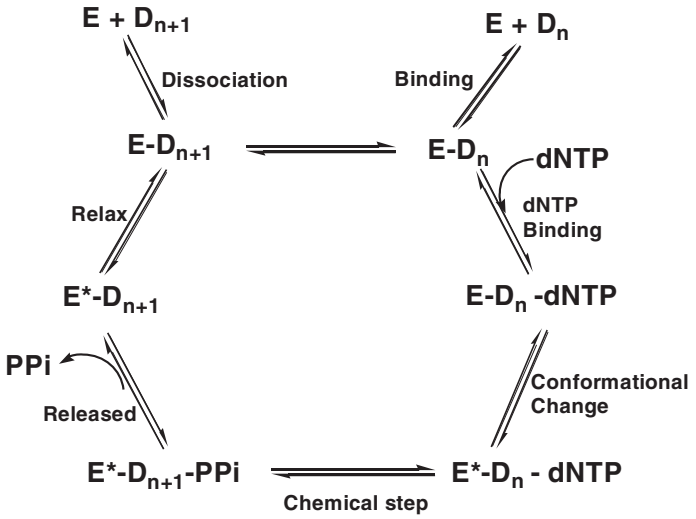

Fig. 1.1 The mechanism of single dNTP incorporation. *E* DNA polymerase, *Dn* DNA substrate, *E** conformationally changed DNA polymerase, D_{n+1} DNA substrate extended by one base (product), *PPi* pyrophosphate. The "chemical step" is also termed phosphodiester bond formation or nucleotidyl transfer

process referred to as semiconservative replication. Each strand of DNA is replicated from 5′ to 3′; therefore, both strands of DNA are replicated in opposite directions.

DNA replication is performed by DNA polymerases. For most of the DNA replication, only the correct dNTP is selected to make standard W-C base pairing with template base and is rapidly incorporated at the 3′ end of the primer. The unpaired dNTPs are repulsed outside the active site or incorporated very inefficiently. Most DNA polymerases catalyze single dNTP incorporation according to a general mechanism (Fig. 1.1). DNA polymerase binds DNA to form a binary complex. This binary complex selectively binds correct dNTP based on Watson–Crick base pairing to form a polymerase–DNA–dNTP ternary complex, followed by inducing a conformational change to facilitate the formation of the phosphodiester bond. After the chemical reaction (nucleotidyl transfer), pyrophosphate is released and the binary complex is relaxed to initiate a new cycle. Some DNA polymerases, such as T7 DNA polymerase, have revealed a nucleotide-induced conformational change. Efficient polymerization is dependent on the selection of correct dNTP, conformational change, and phosphodiester bond formation. Therefore, these three steps are considered as three checkpoints to control the fidelity of a DNA polymerase. Single-molecule FRET studies have shown that only the closed conformation is observed upon correct dNTP binding, which stabilizes the polymerase–DNA–dNTP ternary complex, whereas incorrect dNTPs destabilize the complex.

1.2 Classification and Structure of DNA Polymerases

DNA replication is performed by various DNA polymerases. At least seven families of DNA polymerases have been classified based on their sequence and structural similarities: A, B, C, D, X, Y, and reverse transcriptase. Each family has specific functions in DNA polymerization. Generally, Y-family polymerases carry out translesion DNA synthesis, and A-family DNA polymerases perform fast and accurate DNA replication.

DNA polymerases of humans, yeast, *Sulfolobus solfataricus*, *Escherichia coli*, and bacteriophage T7 have been extensively studied. Humans possess at least 19 enzymes: pols α, δ, ε, and ζ belong to B-family; mitochondrial pol γ and pols ν and θ belong to A-family; pols β, λ, and μ belong to X-family; pols η, ι, and κ and REV1 belong to Y-family; and other enzymes are pols $\sigma 1$, $\sigma 2$, φ, terminal deoxynucleotidyl transferase, and telomerase. DNA polymerases of yeast are similar but not identical to those of human. *E. coli* and some other prokaryotes have five DNA polymerases. A-family pols I and II assist replication (or repair). C-family Pol III is the major replicative polymerase for fast and accurate DNA replication. Polymerases IV and V are Y-family members for bypass of DNA damage and facilitation of adaptive mutation. The *S. solfataricus* has three B-family DNA polymerases (Dpo1, Dpo2, and Dpo3) and one Y-family DNA polymerase (Dpo4) for translesion DNA synthesis. Bacteriophage T7 has only one A-family DNA polymerase, gene 5 protein (gp5), with high fidelity in DNA replication.

DNA polymerases consist of thumb, palm, and finger domains, holding DNA in a right-hand mode. A-family T7 DNA polymerase has a tight active site, into which only standard Watson–Crick base pairs can fit. Differently, Y-family DNA polymerases have more open and flexible active sites and can accommodate bulky DNA damage. Additionally, little finger (or PAD) domain is also present in Y-family DNA polymerases. Subtle variations in the little finger domain are important for bypass of DNA damage for different Y-family members. *S. solfataricus* Y-family DNA polymerase Dpo4 is comprised of the palm domain (containing the catalytic residues), the finger domain (playing a role in nucleotide selectivity), the thumb domain (making important contacts with DNA substrate), and the little finger domain (believed to play an important role in lesion bypass and polymerase processivity). Pol κ also has an additional region, referred as N-clasp, which is comprised of two α-helical elements that are placed directly above the DNA substrate to encircle DNA.

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