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Review

# Terminal deoxynucleotidyl transferase: The story of a misguided DNA polymerase

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ABSTRACT

Nearly every DNA polymerase characterized to date exclusively catalyzes the incorporation of mononucleotides into a growing primer using a DNA or RNA template as a guide to direct each incorporation event. There is, however, one unique DNA polymerase designated terminal deoxynucleotidyl transferase that performs DNA synthesis using *only* single-stranded DNA as the nucleic acid substrate. In this chapter, we review the biological role of this enigmatic DNA polymerase and the biochemical mechanism for its ability to perform DNA synthesis in the absence of a templating strand. We compare and contrast the molecular events for template-independent DNA synthesis catalyzed by terminal deoxynucleotidyl transferase with other well-characterized DNA polymerases that perform template-dependent synthesis. This includes a quantitative inspection of how terminal deoxynucleotidyl transferase binds DNA and dNTP substrates, the possible involvement of a conformational change that precedes phosphoryl transfer, and kinetic steps that are associated with the release of products. These enzymatic steps are discussed within the context of the available structures of terminal deoxynucleotidyl transferase in the presence of DNA or nucleotide substrate. In addition, we discuss the ability of proteins involved in replication and recombination to regulate the activity of the terminal deoxynucleotidyl transferase. Finally, the biomedical role of this specialized DNA polymerase is discussed focusing on its involvement in cancer development and its use in biomedical applications such as labeling DNA for detecting apoptosis.



## 1. Introduction

DNA polymerases play essential roles in replication, repair, and recombination of nucleic acid. During each of these biological processes, the polymerase extends a primer using a DNA (or RNA in the case of reverse transcription) template to guide each incorporation event. Even during the bypass of lethal forms of DNA damage, the presence of a templating strand is absolutely essential for polymerase activity. However, the requirement for using a template is not universal as there exists a unique enzyme, denoted as terminal deoxynucleotidyl transferase (TdT), that possesses the unusual ability to incorporate nucleotides in a *template-independent manner using only single-stranded DNA* as the nucleic acid substrate [1,2] (Fig. 1). The unique ability of TdT to create genomic material *de novo* makes it one of the most fascinating DNA polymerases found in nature. Although TdT was one of the first DNA polymerase activities identified in mammals [3], it remains one of the most poorly understood enzymes that catalyzes DNA synthesis. Indeed, the specific physiological role for TdT remained elusive for several decades [4–11]. It is now recognized that TdT is responsible for the random addition of nucleotides to single-stranded DNA during V(D)J recombination [12,13]. By deliberately generating subtle randomization of this genetic material, TdT plays a crucial role in the evolution and adaptation of the vertebrate immune system [6,9,14,15]. The ability of TdT to randomly incorporate nucleotides increases antigen receptor diversity and aids in generating the ~$10^{14}$ different immunoglobulins and ~$10^{18}$ unique T cell antigen receptors that are required for the neutralization of potential antigens [16,17].

This review explores the cellular and molecular mechanisms accounting for the activity of this specialized DNA polymerase. Our discussion begins by examining the biological role of TdT and how synthesizing DNA without using a templating strand is important for V(D)J recombination. Attention will then focus on understanding the molecular mechanism by which TdT performs template-independent polymerization. In this section, we will compare and contrast the mechanism of TdT with template-dependent polymerases that are involved in normal and translesion DNA synthesis, i.e., replication in the absence of correct templating information. The reported structure of TdT is used to provide biophysical insight into the kinetic properties

*Abbreviations:* TdT, terminal deoxynucleotidyl transferase; CpG, cytidine guanine base pair; *RAG-1*, recombination-activating gene 1; *RAG-2*, recombination-activating gene 2; RSS, recombination signal sequences; Ig, immunoglobulin; NHEJ, non-homologous end-joining; TCR, T cell receptor; PK, protein kinase; $PP_i$, inorganic pyrophosphate; 5-NIMP, 5-nitro-indolyl-2′-deoxyriboside-5′-monophosphate; 5-AIMP, 5-amino-indolyl-2′-deoxyriboside-5′-monophosphate; 5-PhIMP, 5-phenyl-indolyl-2′-deoxyriboside-5′-monophosphate; 5-CEIMP, 5-cyclohexenyl-indolyl-2′-deoxyriboside-5′-monophosphate; TdiFs, TdTase interacting factors; PCNA, proliferating cell nuclear antigen

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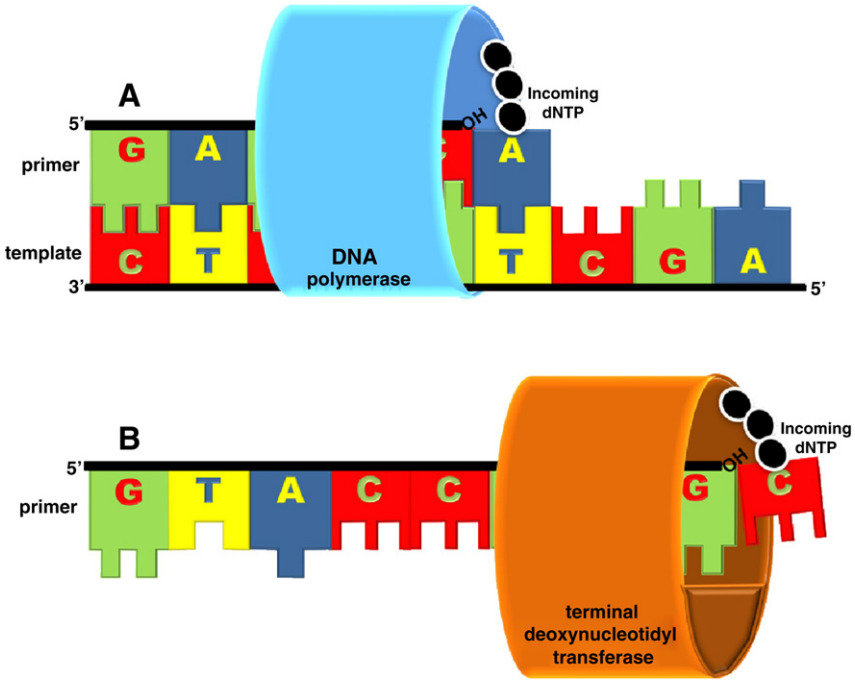


**Fig. 1.** Simplified models for template-dependent and template-independent DNA polymerase activity. (A) Most DNA polymerases require double-stranded DNA as a substrate, where the 5′ → 3′ strand is used as a primer and the complementary strand 3′ → 5′ is used as a template. (B) Terminal deoxynucleotidyl transferase is unique in its ability to catalyze phosphoryl transfer in the absence of a template that can not be accommodated in its active site.

of the polymerase that include the utilization of various metal ion cofactors, nucleic acids, and nucleotide substrates. Finally, the biomedical importance of TdT will be discussed with emphasis on its potential role in the development of certain forms of leukemia as well as its utilization as a biochemical marker for apoptosis.

## 2. The role of TdT in V(D)J recombination

Most organisms possess sophisticated defense mechanisms to protect them against the invasion of foreign agents such as viruses, bacteria, and parasites. Simple prokaryotes use a complementary system involving DNA methylation of the host genome and endonuclease degradation of foreign genomic material to differentiate self from non-self [18]. Eukaryotes have developed more sophisticated systems to thwart off the invasion of foreign substances. Indeed, the mammalian immune system is arguably one of the most intricate and ingenious methods for actively seeking out and killing a wide variety of invaders.

The vertebrate immune system is divided into two subcategories, the innate and adaptive immune systems, that differ in their specificity. The innate immune system is generally considered to be less specific due to the promiscuous ability of the immune receptors to recognize a limited number of molecules that are common features to many infectious agents including polysaccharides, peptidoglycans, non-methylated CpG DNA, and double-stranded RNA [19–22]. This promiscuous activity allows the innate immune system to act as the first line of defense against infection by rapidly recognizing and responding to pathogens. If the defensive line of the innate system is breached, then a more specific and highly specialized offense system, the adaptive immune response, is mobilized to its full potential.

Adaptive immunity came into existence in vertebrates roughly 500 million years ago [23]. The cells of the adaptive immune system, namely T- and B cells, have a diverse repertoire of antigen receptors and antibodies that can recognize any antigen encountered throughout life [24]. After the adaptive immune cell receptors bind an antigen, they mount a rapid and robust protective response by a dramatic expansion in the number of pathogen-specific T cells [25–28]. Over the course of 1 week, thousands of clones are produced that possess effector functions [29–31]. Approximately 95% of these activated T cells undergo apoptosis [30,32]. However, a stable population of long-lived T cells resides in the lymphoid and non-lymphoid tissues [33,34] and patrol for these previously encountered pathogens. The immunological memory displayed by the adaptive immune system provides the vertebrate host with long-lasting protection against subsequent infection. For example, most individuals remain immune to measles for up to 75 years once exposed to an attenuated form of measles virus [35].

At the molecular level, the cells of the immune system have developed a strategy to increase acquired immunity against subsequent biological assaults [36] (Fig. 2). This process, commonly known as V(D)J recombination, plays an essential role in abrogating these antigens. Rearrangement of the variable (V), diversity (D) and joining (J) gene segments creates versatility to a competent immune system by generating a diverse repertoire of antigen receptors with unique antibody specificities [37]. This transaction of breaking, rearranging, and rejoining of the V, D, and J regions of the germline immunoglobulin genes requires the collaborative efforts of the three distinct enzyme activities that include nucleases, polymerases, and ligases. Of the three major types of enzymatic activities, our understanding of how specific DNA polymerases function during V(D)J recombination is not yet firmly established. However, crucial information for understanding the role of specific polymerases in V(D)J recombination has started to emerge. The relative functions of the various members of the X family of DNA polymerases (TdT, pol μ, and pol λ) during the processing of DNA in V(D)J recombination are distinct [38] and non-overlapping [9] *in vivo*. Ramsden et al. have indicated that a "gradient" of weak to strong terminal deoxynucleotidyl transferase activity defines the distinct roles of pol λ, pol μ, and TdT in non-homologous end-joining (NHEJ), respectively [38]. Moreover, Bertocci et al. have shown that pol μ participates exclusively in light chain and not in heavy chain gene rearrangement [9,39]. In contrast, pol λ is reported to be recruited only in the heavy chain junctions during V(D)J recombination and precedes the action of TdT [9], which is primarily involved in the random addition of nucleotides to unpaired primer termini [38]. While pol μ