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The highly efficient T7 RNA polymerase: A wonder macromolecule in biological realm

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ABSTRACT

The study of bacteriophage has always been of keen interest for biologists to understand the fundamentals of biology. Bacteriophage T7 was first isolated in 1945 and its first comprehensive genetic map of was published in 1969. Since then, it gained immense attention of researchers and became a prime model system for experimental biologists. The major gene product of T7 phage, T7 RNA polymerase (T7RNAP), continues to attract researchers since a long time due to its high and specific processivity with a single subunit structure and its capability of transcribing a complete gene without additional proteins. Since the first review article in 1993 there has been around nine reviews on this polymerase till year 2009, most of which focussed on particular aspects of T7RNAP such as structure and function. However, this review encapsulates a broad view on T7RNAP, one of the simplest macromolecule catalyzing RNA synthesis including recent updates on its applications, structure, activators and inhibitors. Thus this brief review bridges the huge gap on the recent updates on this polymerase and will help the biologists in their endeavours that include the use of T7RNAP.

Keywords: Bacteriophage T7, RNA polymerase, Lysozyme, Transcription.

1. Introduction

Bacteriophage T7 RNA polymerase (T7RNAP), first isolated from bacteriophage T7-infected *Escherichia coli* cells in 1970 [1], is one of the simplest enzymes catalyzing RNA synthesis. The essential genes of the 39,937-bp T7 linear genome can be classified into three different classes and they express themselves at different phases of T7 infection cycle: Class I genes establish favorable conditions for phage growth by expressing early in infection, followed by the class II genes which are involved mainly in encoding DNA replication proteins, and class III genes are expressed during the later stages of phage growth and mainly encode structural gene products [2]. The icosahedral head of T7 phage is attached to a tail and the tail fiber proteins help in the initial attachment of the phage to the outer membrane's lipopolysaccharide (LPS) of the host cell. During the infection, about 2.5 % of the genome was reported to be ejected into the cell, while the remaining part of the DNA enters as a transcription of the genome [3]. During the early stage of infection, the class I genes are transcribed by the host (*E. coli*) RNA polymerase; whereas Class II and class III genes are both transcribed by T7RNAP. The transcription of early promoters A1, A2, and A3 (in class I) located in the first 2.5 % of the genome helps in pulling out the template DNA from the phage head to the host cell. Transcription by host RNA polymerase halts at the terminator (Te, a rho-independent terminator) which is present in the leftmost part (~19%) of the genome. Since class I genes lay within this 19 % of the genome, the transcription of class II (15.04 to 46.53 %) and class III genes (46.58 and 99.00 %) are taken over by T7RNAP. This further transcription mediates the entry of the remaining genome into the cell [3]. The detailed information on the promoters and transcription process of T7RNAP was reviewed by Rui Sousa [4] which primarily dealt with the structure, structure-function relationships and transcription process including few of its applications. However, the current review encapsulates the recent updates on T7RNAP structure and function with its emphasised role in biological realm.

The 98 kDa polypeptide, T7RNAP, has a number of interesting properties: (a) It is a single-subunit enzyme compared to multi-subunit prokaryotic and eukaryotic RNA polymerases [5], (b) It has a high specificity towards the T7 promoter; no affinity to unrelated DNAs even to closely related T3 promoters [6] , (c) It does not need any additional protein factors to perform the complete transcriptional cycle [7], (d) T7RNAP exhibits an efficient elongation. It elongates about five times faster than *E. coli* RNA polymerase and produce very long transcripts [8], (f) Transcription termination can be executed only by class I and class II termination signals and it is independent of the transcription termination factors of *E. coli* RNA polymerase [9, 10]. These properties add advantage to this enzyme and hence widely employed to express heterologous genes, under the control of the T7 promoter, for *in vivo* and *in vitro* experiments.

2. Role of T7RNAP in biological realm

Genetic engineering has been used extensively to express eukaryotic genes in prokaryotes and vice versa. Over the years T7RNAP has been used in different hosts for high level gene expression both in prokaryotes and eukaryotes and this in turn led to expansion of its use in various areas of biology (Fig. 1). Few notable studies were discussed below.

2.1 Industrial biotechnology

Corynebacterium glutamicum, a Gram-positive soil bacterium, is a model organism in industrial biotechnology due to its robustness in large-scale production processes and broad spectrum of products including organic acids, diamines, alcohols and amino acids such as: L-glutamate, L-lysine, L-leucine, L-serine etc. [11, 12] . Although, endogenous RNA polymerase based system was available for controlling gene expression in *C. glutamicum*, a T7RNAP-based IPTG (isopropyl-β-D-1-thiogalactopyranosid) inducible expression system was developed for *C. glutamicum* [12] to test an efficient and controllable protein overproduction system for the bacterium. The T7RNAP-based expression system was developed on strain MB001 (DE3), which includes prophage-free strain *C. glutamicum* MB001 and part of the DE3 region of Escherichia coli BL21(DE3) including the T7 RNA polymerase gene1 under control of the lacUV5 promoter. The expression vector pMKEx2 was constructed allowing cloning of target genes under the control of the T7 lac promoter. Thus, both gene 1 and the target gene are repressed by LacI. The system was evaluated with a target gene, eyfp, for enhanced yellow fluorescent protein. This new system allowed tightly IPTG-regulatable gene expression to levels that were about four times higher than those obtained with other available systems such as Ptac-based expression vectors using the tac promoter and the endogenous RNA polymerase.

2.2 Expression in eukaryotic environment

As T7RNAP has a number of useful properties, researchers developed interest in using T7RNAP in eukaryotic systems as well. Since T7 RNA polymerase is produced in a prokaryotic environment, expressing it in an eukaryotic environment was a tricky task due to the following requirements: i) mRNA processing, ii) capping, iii) methylation, and iv) polyadenylylation in a eukaryotic environment. Few of the examples of gene expression in eukaryotic environment are discussed below.

2.2.1 Vaccinia/T7 hybrid system

Vaccinia virus, a linear double-stranded DNA virus, was advantageous for developing it as eukaryotic expression vector. The advantages of T7RNAP include its ability to encode an entire transcription system including RNA polymerase, capping/methylating enzymes and poly (A) polymerase, large capacity for foreign DNA, genome stability, and wide vertebrate host range. To test whether T7 RNA polymerase made under control of vaccinia virus can function in mammalian cells, Fuerst and colleagues [13] constructed vaccinia/T7 hybrid systems containing target genes such as *E. coli* β-galactosidase gene (lacZ) and the CAT gene (cat) derived from the Tn9 transposon flanked by T7 promoter and termination regulatory elements. When compared, the synthesis of β -galactosidase and CAT by the hybrid system was found to be 15 to 20 fold more efficient than the straight vaccinia system and 400 to 600 fold more efficient than the conventional transient-expression system. Recombinant vaccinia virus expressing bacteriophage T7 RNA polymerase has also been used for studies on processing of i) HCV polyproteins [14], ii) editing mRNA of ebola virus [15] and iii) recovering infectious viruses from their full-size cDNA clones of negative-strand RNA viruses like vesicular stomatitis virus, rabies virus, bunya virus, respiratory syncytial virus, and sendai virus [16].

2.2.2 Expression in cell nucleus

Although vaccinia virus-based systems are successfully used but as T7RNAP, being prokaryotic, has cytoplasmic preference and vaccinia DNA also remains in cytoplasm. But to successfully utilize T7RNAP in nucleus would expand its utilization in broad range of eukaryotic cells or whole animals. A major setback in this endeavour is that T7RNAP does not contain signals for nuclear localization. One of the attempts to overcome this barrier was made by John J. Dunn and colleagues [17] by inserting a nuclear signal into T7 gene I, thereby creating T7 RNA polymerase fusion protein. They inserted a 36-bp synthetic nucleotide sequence encoding the SV40 T antigen nuclear location signal into unique restriction sites created at two locations within the cloned T7 gene 1. A minimal effect on transcription activity in *E. coli* was reported due to insertion of the nuclear location signal between codons 10 and 11 of T7 RNA polymerase.

2.2.3 Expression in Human Liver Cell Line

A T7-adenovirus system for the *in vivo* synthesis of Hepatitis C virus (HCV) minigenes in human liver cells was developed to study the molecular mechanisms of HCV translation in human liver cells. For this study, Aoki *et al* [18] created a replication-deficient recombinant adenovirus (AdexCAT7) expressing T7 RNA polymerase under the control of the CAG promoter and various mammalian cells were infected with it. Next, by the incorporation of [^{3}H] UTP using a DNA

template containing T7 promoter and a luciferase gene, cell lysates were examined for the polymerase activity. Efficient expression of HCV minigenes RNA was supported by one cell line, FLC4, derived from a human hepatocellular carcinoma.

2.3 Synthetic gene circuits

Development of synthetic gene circuits (SGC) is one of the emerging research interests in the field of biology. The transcription rate by T7RNAP needs regulation at different levels since the high rate can quickly exhaust cellular resources and accumulate toxic by-products leading to cell death. Such interesting properties made T7RNAP a new target for design of SGCs for studying the principles of native gene networks [19]. For development of SGCs, split versions of T7RNAP were used. The initial split version was created by making two halves of T7RNAP, C-terminal and N-terminal, and placing them behind two different inducible promoters. To form a successfully activated downstream gene, both halves are necessary to be active as they depend on each other in order to form a functional enzyme. This design helped to create library of transcriptional AND gates [20]. Similarly, a 3-input AND gate was also created by using a tripartite T7RNAP using a transposon based method to identify five regions where the polymerase can be bisected without loss of function [21]. By expressing T7RNAP split at two different sites, a tripartite T7RNAP was created that requires all three fragments *viz.* alpha (aa 1-67), β core (aa 67-601) and sigma (aa 601-883) for activity. Regulation of the transcriptional activity of the split polymerase was suggested to be achieved by limiting the availability of core and/or alpha fragment, or by expressing additional sigma fragments similar to multi-subunit RNA polymerases. A four-fragment enzyme can also be created by splitting the tripartite T7RNAP at the previously discovered split site [21] for different degree of regulations.

2.4 RNA editing

RNA editing is a molecular process for modifying the nucleotide sequences of an RNA molecule after it has been transcribed from DNA but before being translated into protein. It is achieved by two distinct mechanisms a) substitution and b) insertion/deletion (InDel) editing. T7RNAP displayed a relatively high level of template-dependent transcriptional infidelity. Wons *et al* [22] suggested that this feature of T7RNAP could be used for restoration of frame-shift mutation and increasing the range of phenotypic variants, which in turn has a high evolutionary potential. T7 RNAP infidelity can rescue such mutations through a transcriptional slippage mechanism in homopolymer A- and T-rich stretches, enabling the production of a heterogeneous population of the full-length protein.

2.5 RNA interference

RNA interference (RNAi), also known as gene silencing, is an evolutionarily conserved defense mechanism occurring naturally against double-stranded RNA (dsRNA) that can target cellular and viral mRNAs. It regulates the expression of protein-coding genes and also mediates resistance to both endogenous parasitic and exogenous pathogenic nucleic acids. In this process, small RNA suppresses the gene expression by interfering with the translation of target mRNA transcript [23, 24]. Recently, an RNAi system based on TRNAP was designed and examined in *Aspergillus fumigatus*, one of the most important airborne fungal pathogen [25]. This system was constructed by a T7RNAP expression cassette containing the N-terminal localization signal sequence (NLS) and an autonomously replicating plasmid (AMA1) based episomal RNAi plasmid integrated into *A. fumigatus* protoplasts. The efficiency of this system was studied using alb1 gene as a model.

3. T7RNAP structure

The primary structure of 883 amino acid polymerase, T7RNAP, was determined during the early 1980s [26]. This polypeptide is structurally related to the members of a superfamily of nucleotide polymerases that includes single-subunit DNAPs and RNAPs such as *E. coli* DNAP I and reverse transcriptases [5, 27]. A comparison by superimposing polymerization domains revealed that T7RNAP and Klenow fragment of *E. coli* DNAP I shows a strong structural similarity. TRNAP consists of an N-terminal domain (residues 1–325) and a polymerase domain (residues 326–883). The polymerase domain can be divided into sub-domains denoted as thumb, fingers and palm (Fig. 2). A deep cleft formed by the sub-domains is the binding site for the DNA template [28]. Following are the descriptions of few critical residues in each domain along with their importance (Fig. 3); however authors are encouraged to follow the review article by R. Sousa [4] to get a detailed description on the structure of T7RNAP.

3.1 N-terminal Domain

The N-terminal domain (1-325) plays a role in sequence specific promoter binding and in opening the duplex DNA. From the crystal structure of a promoter and polymerase complex, two major regions of the N-terminal were reported which contributes to its function. The region formed by the residues 93–101 bind the AT-rich -13 to -17 element of the promoter. While, an intercalating hairpin formed by residues 232–242 inserts between the template and non template strand to open the promoter [4].

The residue Lys 172 is also suggested to be involved in RNA binding through proteolytic cleavage and mutagenesis studies [4, 5]. A proteolytic nick at this residue reduced the polymerase's

processivity during transcript elongation and the affinity of the polymerase for single-stranded DNA, without affecting its other catalytic properties. However, mutational effect on the enzyme activity depends on the type of substitution such as: the Lys172Gly substitution resulted in constant binding rate with promoter with a significant decrease in activity (80%) and RNA synthesis rate (27 nucleotide/sec) as compared with the wild type of the polymerase (100% and 97 nucleotide/sec), while Lys172Leu substitution and Lys172Arg173 deletion mutation resulted in weaker binding of promoter with increased activity (120% and 110% respectively) and increased RNA synthesis rate (107 and 126 nucleotide/sec).

3.2 *Polymerase Domain*

3.2.1 Thumb Sub-domain

The thumb sub-domain (residues 326–411) forms a long α-helical projection on one side of the template-binding cleft. Comparison crystal structures of a T7RNAP: T7 lysozyme complex [28], structure of a promoter and polymerase complex [29] and one of the crystal structures of the apoenzyme [30], reveal that the thumb sub-domain is a flexible element and it might form a clamp upon binding with DNA, which would prevent complex dissociation while still allowing the complex to slide along the DNA.

3.2.2 Palm sub-domain

The palm sub-domain, containing residues 412–553 and 785–879, positions itself at deep cleft of the polymerase. It contains the most catalytically critical residues: Asp 537 and Asp 812. These conserved aspartate residues, present on a trio of β-strands, proposed to orient two metal ions (Mg^{2+}) for catalysis of the polymerase reaction [4, 28]. Magnesium ions (Mg^{2+} A and Mg^{2+} B) have important roles in nucleotide addition cycles. The pyrophosphate (PPi) is proposed to form a weaker contact with the Mg^{2+} A ion than T7 RNAP, while the Mg^{2+} B is known to form a strong contact with the PPi than with the whole protein. Hence, Mg^{2+} A ion stays permanently in active site and facilitates nucleophilic attack of the 3′-oxygen from RNA terminal nucleotide on the α-phosphate of the substrate NTP, whereas Mg^{2+} B ion dissociates from the active site with PPi release [31]. The C-terminal carboxylate (Ala-OH^{883}) is positioned near the active site with a distance of ∼6.3 Å between the Cα atoms of Asp 537 and Ala 883 is and a distance of ∼10 Å from the metal ions [32]. The palm sub-domain also harbours another important residue Lys 472 located on a flexible loop (residue 469 to 475). In a recent molecular dynamics study, it was suggested that the side chain swing of Lys 472 greatly promotes the PPi release [33].

3.2.3 The fingers sub-domain

The fingers sub-domain (aa 554–784) has also an important role in T7RNAP functional mechanism. The 563-571 region of T7RNAP is involved, directly or indirectly, in the interaction with a promoter. An indirect contribution of Pro 563 can be comprehended by the fact that the dissociation constant of the enzyme–promoter complex for mutants with Pro 563 substitutions did not differ from that for the wild type enzyme. However, the presence of proline at position 563 forms the local structure of this protein region and determines the correct interaction with the promoter of adjacent residues Asp 569 and Tyr 571. On the other hand, a direct role can be deduced by the Tyr571Ser substitution that caused the complete loss of specific binding and activity with the promoter containing template [5]. Another important region is aa 626–639. The residues Lys 627 and Lys 631 appear to contribute to the affinity of NTP binding through interactions with phosphate groups, while Met 635 makes interactions with the ribose moiety [4]. The residue Tyr 639 of the finger sub-domain provides discrimination of ribose versus deoxyribose substrates via the coordinated bond interaction mediated by a magnesium ion located between the Tyr 639 hydroxyl group and 2′-OH of the rNTP ribose [34]. Tyr 639 is stabilized by the small free energy bias at the active site upon post-translocation and is not displaced until full nucleotide insertion. To allow the nucleotide insertion, Tyr 639 has to move out of the active site during the insertion process. At the same time, the O-helix along with the finger domain rotates from open to closed form to recruit an incoming NTP from a solvent accessible pre-insertion binding site into the more buried active site in palm subdomain [34]. The positively charged patch formed by three lysine residues (K711, K713 and K714) makes interactions with the downstream DNA during transition to elongation phase. It has been found that these interactions contribute to inducing and stabilizing a bend in the downstream DNA which is important for promoter opening [35]. The residues 740-769 form a specificity loop that is involved in promoter recognition [28].

4. T7RNAP activators

As T7RNAP is one of the most important enzymes widely used in *in-vivo* and *in-vitro* methods, knowledge of activators those aid efficient transcription with T7RNAP helps one to design successful experiments. T7RNAP activators can be classified into two groups: chemical and peptide based.

4.1 Chemical activators

The natural polyamines such as spermine, spermidine and putrescine and their synthetic derivatives

can stimulate *in vivo* and *in vitro* transcription by T7RNAP [36-39]. Polyamines have the ability to interact with, or to match, scattered ionic sites on nucleic acids. This ability of polyamines helps the transcription by promoting condensation and aggregation of DNA [37].

Other potential stimulator of T7RNAP mediated transcription is dimethyl sulfoxide (DMSO) [40]. DMSO is known to increase the rate of transcription by ~1.4-folds and production of full length transcript at ~2-folds. It was also found that DMSO mediated stimulation of *in vitro* transcription from the linear DNA by T7RNAP is through mechanisms other than altering DNA structure. However, low concentrations (10%) of DMSO can alter T7RNAP structure to a compact form that can exhibit an enhanced activity in catalyzing nucleotide addition during transcription initiation [41].

4.2 Peptide based activators

In addition to the chemical activators, recently a peptide factor (1-2 kDa) was reported to activate T7RNAP [40]. This thermostable, ethanol-and acetonitrile-resistant peptide factor is present in the cytoplasm of oocytes and eggs from African clawed frog *Xenopus laevis*. This activation factor interacts with T7RNAP, but not with DNA. The study revealed that the activator binds rapidly to T7RNAP in the absence of DNA template, which was established from the fact that addition of the template DNA to the *Xenopus laevis* oocyte extracts significantly decreased magnitude of T7RNAP activation, indicating that DNA competes with the activation factor for T7RNAP binding.

5. T7RNAP inhibitors

Inhibition of T7RNAP can be divided into two major classes: A. Proteo-chemical inhibition and B. Physiological inhibition. Details of these classes are given below.

5.1 Proteo-chemical inhibition

T7RNAP can be inhibited by three ways: (i) by binding of inhibitor at the catalytic site of the enzyme, (ii) by the binding of T7 lysozyme that binds at an alternate site other than the active site or (iii) through binding to the DNA template [42].

i) At the active site of T7RNAP, heparin is reported as most efficient inhibitor. This observation was achieved by a study [43] where few bacterial RNA polymerase specific inhibitors (rifampicin, streptolydigin, streptovaricin complex) and few polyanionic compounds such as polyribonucleotides (Poly[r(U)]) and heparin were compared for their binding efficiency at the functional site of the polymerase. Heparin, was identified as a potent inhibitor of both *Escherichia coli* RNA polymerase and T7 RNA polymerase. In contrast, the antibiotics

(rifampicin, streptovaricin, and streptolydigin) have no effect on T7 RNA polymerase even at higher concentrations. In contrast to *E.Coli* RNA polymerase, heparin inhibits T7RNAP equally whether added before or after RNA synthesis has begun. In a recent study it was revealed that heparin inhibits T7RNAP by interactions with palm sub-domain and finger sub-domain [42]. Heparin binds to the important residues involved in both T7RNAP transcription initiation and elongation. This broad range of interactions by heparin also explains its effectiveness in inhibiting T7RNAP whether added before or after the beginning of RNA synthesis. This *in silico* analysis proposed that heparin inhibits T7RNAP possibly by interfering a) catalysis the phosphodiester bond synthesis, b) promoter recognition and binding by finger sub-domain, c) interactions with the ribose moiety or d) hampering the release of PPi during transcription elongation process by palm insertion module (450-527). Enoxaparin, a low molecular derivative of heparin was also proposed to be a possible alternative inhibitor of T7RNAP [42].

Other recently reported inhibitors of T7 RNA polymerase transcription mechanism are the cage metal complexes, Iron (II) clathrochelates [44]. The study found that the inhibitor molecule is located in the transcriptional bubble and thus involved in intermolecular contacts with protein residues as well as with DNA and RNA. The *in silico* blind docking results revealed that the residues from the N-terminal domain (aa 165-172) and finger sub-domain (aa 645-655) interact with the hydrophobic phenyl substituents of these inhibitors. The inhibition of transcription by these compounds can be achieved either by blocking of further strand separation by the bound inhibitor in the transcription fork; or by interfering with the translocation step of the reaction by the formation of the quaternary complex.

ii) The lysozyme of bacteriophage T7 is a multifunctional zinc amidase protein that can perform cell lysis during phage infection by cutting amide bonds in the bacterial cell wall, as a repressor of T7 RNA polymerase as well as stimulator of both replication and packaging [45]. The zinc atom located in its cleft is essential for amidase activity but its role in inhibition of T7 RNA polymerase is not reported [46]. T7 lysozyme (T7L), expressed from a class II promoter, inhibits transcription initiation and the transition from initiation to elongation by T7RNAP. The inhibition of T7RNAP by lysozyme can be termed as allosteric, as T7L does not bind to the active site rather it binds at a site distant from the active site (interacts with parts of the palm, finger, and the N-terminal domain of T7RNAP). It is believed that the C-terminal region of the T7RNAP plays an important role in lysozyme mediated inhibition along with conformational changes in the extreme C terminus rather than the overall T7RNAP structure [28, 47]. However, a stopped flow kinetics and equilibrium DNA binding study [47] in the presence or the absence of T7L revealed four interesting facts about

T7 lysozyme mediated inhibition :

- T7L does not affect promoter binding and has very little effect on the rate of pre-initiation open complex formation.
- T7L decreases the pre-steady state rate of RNA synthesis but does not significantly affect the K_d (dissociation constant) of the initiating nucleotide.
- T7L does not inhibit the rate of initial RNA synthesis on a premelted promoter.
- In the presence of the initiating nucleotide, T7L affects the formation of the open complex.

These observations indicate that T7L represses transcription either by interfering with the formation of a stable and a fully open initiation bubble or by altering the structure of the DNA in the initiation complex. The interaction of T7L with T7RNAP increases the production of abortive transcripts from 2- to 5-mer due to unstable initiation bubble and/or the inhibition of conformational changes of the N-terminal domain that are necessary to make RNA products longer than 3-mer. This in turn delays the transition from the initiation to the elongation phase. It has been observed that pH has a role in dynamics of T7L structure [48]. At its native pH (at pH 7) T7L structure is highly dynamic, and any change in pH toward the acidic side will lead to enhanced dynamics with a partially unfolded structure. The partially unfolded structure at low pH (pH <6.0) results in the loss of lysis activity. It was proposed that though T7L has poor amidase activity at low pH, it does not hamper the T7RNAP interaction ability [49]. This observation depends on the conformational changes in both T7L and T7RNAP at individual pH.

iii) Another inhibitor, Actinomycin D or D-actinomycin, a compound isolated from *Streptomyces* spp. is known to inhibit both *Escherichia coli* RNA polymerase and T7 RNA polymerase. This inhibitor is known to bind to the DNA [43]. Actinomycin, which is an older chemotherapy drug, comprises two cyclic peptides linked together by a phenoxazine derivative. In eukaryotic transcription process, the phenoxazine ring of actinomycin intercalates into GC rich sequences and stabilizes topoisomerase DNA covalent complexes that prevent RNA polymerase progression [50]

5.2 Physiological inhibition

Apart from these, T7RNAP can be inhibited by changing physiological conditions, such as i) pH, ii) salt concentration and iii) temperature and iv) urea concentration.

i) Usually pH ~8-9 is considered to be an optimum value for T7RNAP enzyme activity [5], and many of *in vitro* T7RNAP experiments are conducted near pH 7.9. A near-ultraviolet (UV) CD spectra of the polymerase reported that no major change in tertiary structure of the polymerase occur between pH 7.8 and 5.0 [51]. However, a recent study [52] reveals few possible minor

structural changes which make this enzyme less functional in lower pH (pH 5) : a) the reduced proximity of C-terminal to the catalytic core which drew analogy to the T7 lysozyme bound T7RNAP crystal structure, b) conformational changes at pH 5 in N-terminal domain (93–101 and 232–242), which led to a reduced spacing (13.08 Å) between two loops [β-hairpin loop (232–242) and specificity loop (740–769)] known to interact with the T7 promoter compared to pH 7.9 structure (17.47 Å).

ii) T7RNAP is very sensitive to salt and showed a decreasing pattern of activity as opposed to normal RNA polymerase [43]. A steady decrease in enzymatic activity was reported with increase in salt concentration (0 and 0.15 M KCl) and an essentially no activity above 0.2 M KCl. This pattern was also observed when NH_4Cl or NaCl replaced KCl. This similar response of enzyme activity with different types of cations demonstrates that the effect of salt on T7RNAP is a general effect rather than cation specific [43]. The overall salt concentration less than 0.05 M is usually recommended [53] for an optimal enzyme activity.

iii) A circular dichroism and fluorescence study [51] revealed that T7RNAP undergoes a major loss of tertiary structure on thermal unfolding. These studies showed that thermal unfolding leads to an intermediate state that has increased β-sheet and reduced α-helix content relative to the native state. According to a recent claim [54], amino acid substitutions at the positions Val 426, Ser 633, Val 650, Thr 654, Ala 702, Val 795, and combinations thereof helps T7 RNA polymerase variants with enhanced thermostability to cope with temperature dependent inhibition.

iv) T7RNAP also shows some degree of unfolding induced by urea [51]. This revelation came after the comparison of the ellipticity in the near UV region at 290 nm that reflect a loss of tertiary structure of the protein in the range of urea concentration 2-5 M at 25 °C. T7RNAP is able to exist in a stable intermediate state but after its denaturation by increasing urea concentration to 4 M, the enzyme loses its ability to fold to a unique native conformation. At 5 M urea, no positive band (near 290 nm) was detectable. A CD analysis revealed that the β-sheet content increases from 15% to 26% and the α-helix content is reduced from 37% to 21% with the increase of temperature from 20° to 60°C. However, in the presence of 4 M urea at pH 7.8, the spectra showed a mixture of α-helix, β-sheet, and perhaps other secondary structures which are nearly independent of temperature. Both thermal and urea denaturation are irreversible.

6. Conclusion

T7 RNA polymerase has a wide array of biological applications and thus has been a protein of interest for last 49 years since its first isolation. As we have seen, a large number of T7RNAP structures have been solved with different substrates and in various stages of transcription process along with various experimental studies. Complementing to these experimental studies, we have also seen a vast number of computational studies to analyse the conformational dynamics and understanding transcription mechanism of this polymerase. With the recent developments in both experimental and computational methods, T7RNAP can be subjected to various analyses to completely understand the molecular mechanism of transcription of this polymerase and to explore its full potential. These analyses may also lead to new avenues to expand its usage and help the highly efficient T7RNAP to find a place in the uncharted territories of biological experiments.

References

[1] M. Chamberlin, J. McGrath, L. Waskell, New RNA polymerase from Escherichia coli infected with bacteriophage T7, Nature 228 (1970) 227-31.

[2] U. Qimron, S. Tabor, C.C. Richardson, New Details about Bacteriophage T7-Host Interactions, Microbe 5 (2010) 117-122.

[3] L.R. Garcia, I.J. Molineux, Rate of translocation of bacteriophage T7 DNA across the membranes of Escherichia coli, Journal of bacteriology 177 (1995) 4066-76.

[4] R. Sousa, S. Mukherjee, T7 RNA polymerase, Progress in nucleic acid research and molecular biology 73 (2003) 1-41.

[5] V.L. Tunitskaya, S.N. Kochetkov, Structural-functional analysis of bacteriophage T7 RNA polymerase, Biochemistry. Biokhimiia 67 (2002) 1124-35.

[6] J.F. Klement, M.B. Moorefield, E. Jorgensen, J.E. Brown, S. Risman, W.T. McAllister, Discrimination between bacteriophage T3 and T7 promoters by the T3 and T7 RNA polymerases depends primarily upon a three base-pair region located 10 to 12 base-pairs upstream from the start site, Journal of molecular biology 215 (1990) 21-9.

[7] G.M. Cheetham, T.A. Steitz, Insights into transcription: structure and function of single-subunit

DNA-dependent RNA polymerases, Current opinion in structural biology 10 (2000) 117-23.

[8] P.A. Pavco, D.A. Steege, Characterization of elongating T7 and SP6 RNA polymerases and their response to a roadblock generated by a site-specific DNA binding protein, Nucleic acids research 19 (1991) 4639-46.

[9] D.L. Lyakhov, B. He, X. Zhang, F.W. Studier, J.J. Dunn, W.T. McAllister, Pausing and termination by bacteriophage T7 RNA polymerase, Journal of molecular biology 280 (1998) 201-213.

[10] L.E. Macdonald, R.K. Durbin, J.J. Dunn, W.T. McAllister, Characterization of two types of termination signal for bacteriophage T7 RNA polymerase, Journal of molecular biology 238 (1994) 145-158.

[11] N. Tatsumi, M. Inui, Corynebacterium glutamicum: biology and biotechnology, Springer Science & Business Media2012.

[12] M. Kortmann, V. Kuhl, S. Klaffl, M. Bott, A chromosomally encoded T7 RNA polymerase-dependent gene expression system for Corynebacterium glutamicum: construction and comparative evaluation at the single-cell level, Microb Biotechnol 8 (2015) 253-265.

[13] T.R. Fuerst, E.G. Niles, F.W. Studier, B. Moss, Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase, Proceedings of the National Academy of Sciences of the United States of America 83 (1986) 8122-6.

[14] A. Grakoui, C. Wychowski, C. Lin, S.M. Feinstone, C.M. Rice, Expression and identification of hepatitis C virus polyprotein cleavage products, Journal of virology 67 (1993) 1385-95.

[15] V.E. Volchkov, S. Becker, V.A. Volchkova, V.A. Ternovoj, A.N. Kotov, S.V. Netesov, H.D. Klenk, GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases, Virology 214 (1995) 421-430.

[16] A. Bridgen, R.M. Elliott, Rescue of a segmented negative-strand RNA virus entirely from cloned complementary DNAs, Proceedings of the National Academy of Sciences of the United States of America 93 (1996) 15400-4.

[17] J.J. Dunn, B. Krippl, K.E. Bernstein, H. Westphal, F.W. Studier, Targeting bacteriophage T7 RNA polymerase to the mammalian cell nucleus, Gene 68 (1988) 259-66.

[18] Y. Aoki, H. Aizaki, T. Shimoike, H. Tani, K. Ishii, I. Saito, Y. Matsuura, T. Miyamura, A human liver cell line exhibits efficient translation of HCV RNAs produced by a recombinant adenovirus expressing T7 RNA polymerase, Virology 250 (1998) 140-50.

[19] D.L. Shis, M.R. Bennett, Synthetic biology: the many facets of T7 RNA polymerase, Molecular systems biology 10 (2014) 745.

[20] D.L. Shis, M.R. Bennett, Library of synthetic transcriptional AND gates built with split T7 RNA polymerase mutants, Proceedings of the National Academy of Sciences of the United States of America 110 (2013) 5028-33.

[21] T.H. Segall-Shapiro, A.J. Meyer, A.D. Ellington, E.D. Sontag, C.A. Voigt, A 'resource allocator' for transcription based on a highly fragmented T7 RNA polymerase, Molecular systems biology 10 (2014) 742.

[22] E. Wons, B. Furmanek-Blaszk, M. Sektas, RNA editing by T7 RNA polymerase bypasses InDel mutations causing unexpected phenotypic changes, Nucleic acids research 43 (2015) 3950-3963.

[23] S. Saurabh, A.S. Vidyarthi, D. Prasad, RNA interference: concept to reality in crop improvement, Planta 239 (2014) 543-64.

[24] G.J. Hannon, RNA interference, Nature 418 (2002) 244-51.

[25] S. Enayati, M. Azizi, E. Aminollahi, M. Ranjvar Shahrivar, V. Khalaj, T7-RNA polymerase dependent RNAi system in Aspergillus fumigatus: a proof of concept study, FEMS microbiology letters 363 (2016) fnw029.

[26] B.A. Moffatt, J.J. Dunn, F.W. Studier, Nucleotide sequence of the gene for bacteriophage T7 RNA polymerase, J Mol Biol 173 (1984) 265-9.

[27] S.N. Kochetkov, E.E. Rusakova, V.L. Tunitskaya, Recent studies of T7 RNA polymerase mechanism, Febs Lett 440 (1998) 264-267.

[28] D. Jeruzalmi, T.A. Steitz, Structure of T7 RNA polymerase complexed to the transcriptional inhibitor T7 lysozyme, The EMBO Journal 17 (1998) 4101-4113.

[29] G.M.T. Cheetham, D. Jeruzalmi, T.A. Steitz, Structural basis for initiation of transcription from an RNA polymerase-promoter complex, Nature 399 (1999) 80-83.

[30] R. Sousa, Y.J. Chung, J.P. Rose, B.-C. Wang, Crystal structure of bacteriophage T7 RNA polymerase at 3.3 Å resolution, Nature 364 (1993) 593-599.

[31] S. Wu, Molecular dynamics simulation study of the "stay or leave" problem for two magnesium ions in gene transcription, Proteins 85 (2017) 1002-1007.

[32] J. Lykke-Andersen, J. Christiansen, The C-terminal carboxy group of T7 RNA polymerase ensures efficient magnesium ion-dependent catalysis, Nucleic Acids Res 26 (1998) 5630-5.

[33] L.T. Da, C. E, B. Duan, C. Zhang, X. Zhou, J. Yu, A Jump-from-Cavity Pyrophosphate Ion Release Assisted by a Key Lysine Residue in T7 RNA Polymerase Transcription Elongation, PLoS computational biology 11 (2015) e1004624.

[34] D. Temiakov, V. Patlan, M. Anikin, W.T. McAllister, S. Yokoyama, D.G. Vassylyev, Structural basis for substrate selection by T7 RNA polymerase, Cell 116 (2004) 381-391.

[35] D. Nayak, Q. Guo, R. Sousa, Functional architecture of T7 RNA polymerase transcription complexes, J Mol Biol 371 (2007) 490-500.

[36] Y. Watanabe, K. Igarashi, K. Mitsui, S. Hirose, Differential stimulation by polyamines of phage DNA-directed in vitro synthesis of proteins, Biochimica et biophysica acta 740 (1983) 362-8.

[37] M. Frugier, C. Florentz, M.W. Hosseini, J.M. Lehn, R. Giege, Synthetic Polyamines Stimulate in-Vitro Transcription by T7 RNA-Polymerase, Nucleic acids research 22 (1994) 2784-2790.

[38] J.F. Milligan, D.R. Groebe, G.W. Witherell, O.C. Uhlenbeck, Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates, Nucleic acids research 15 (1987) 8783-98.

[39] M. Iwata, M. Izawa, N. Sasaki, Y. Nagumo, H. Sasabe, Y. Hayashizaki, T7 RNA polymerase activation and improvement of the transcriptional sequencing by polyamines, Bioorganic &

medicinal chemistry 8 (2000) 2185-94.

[40] A.A. Tokmakov, Y. Fukami, Activation of T7 RNA polymerase in Xenopus oocytes and cell-free extracts, Genes to cells : devoted to molecular & cellular mechanisms 15 (2010) 1136-44.

[41] Z. Chen, Y. Zhang, Dimethyl sulfoxide targets phage RNA polymerases to promote transcription, Biochemical and biophysical research communications 333 (2005) 664-70.

[42] S. Borkotoky, C.K. Meena, A. Murali, Interaction Analysis of T7 RNA Polymerase with Heparin and Its Low Molecular Weight Derivatives - An In Silico Approach, Bioinformatics and biology insights 10 (2016) 155-66.

[43] M. Chamberlin, J. Ring, Characterization of T7-specific ribonucleic acid polymerase. II. Inhibitors of the enzyme and their application to the study of the enzymatic reaction, The Journal of biological chemistry 248 (1973) 2245-50.

[44] V.V. Novikov, O.A. Varzatskii, V.V. Negrutska, Y.N. Bubnov, L.G. Palchykovska, I.Y. Dubey, Y.Z. Voloshin, Size matters, so does shape: Inhibition of transcription of T7 RNA polymerase by iron(II) clathrochelates, J Inorg Biochem 124 (2013) 42-45.

[45] X. Zhang, F.W. Studier, Multiple roles of T7 RNA polymerase and T7 lysozyme during bacteriophage T7 infection, Journal of molecular biology 340 (2004) 707-730.

[46] X.D. Cheng, X. Zhang, J.W. Pflugrath, F.W. Studier, The Structure of Bacteriophage-T7 Lysozyme, a Zinc Amidase and an Inhibitor of T7 RNA-Polymerase, P Natl Acad Sci USA 91 (1994) 4034-4038.

[47] N.M. Stano, S.S. Patel, T7 lysozyme represses T7 RNA polymerase transcription by destabilizing the open complex during initiation, Journal of Biological Chemistry 279 (2004) 16136-16143.

[48] M. Sharma, D. Kumar, K.M. Poluri, Elucidating the pH-Dependent Structural Transition of T7 Bacteriophage Endolysin, Biochemistry 55 (2016) 4614-25.

[49] S. Borkotoky, A. Murali, A computational assessment of pH-dependent differential interaction of T7 lysozyme with T7 RNA polymerase, BMC structural biology 17 (2017) 7.

[50] O. Bensaude, Inhibiting eukaryotic transcription: Which compound to choose? How to evaluate its activity?, Transcription 2 (2011) 103-108.

[51] Y. Griko, N. Sreerama, P. Osumi-Davis, R.W. Woody, A.Y.M. Wood, Thermal and urea-induced unfolding in T7 RNA polymerase: Calorimetry, circular dichroism and fluorescence study, Protein Sci 10 (2001) 845-853.

[52] S. Borkotoky, C.K. Meena, G.M. Bhalerao, A. Murali, An in-silico glimpse into the pH dependent structural changes of T7 RNA polymerase: a protein with simplicity, Sci Rep-Uk 7 (2017).

[53] M. Maslak, C.T. Martin, Effects of Solution Conditions on the Steady-State Kinetics of Initiation of Transcription by T7 Rna-Polymerase, Biochemistry 33 (1994) 6918-6924.

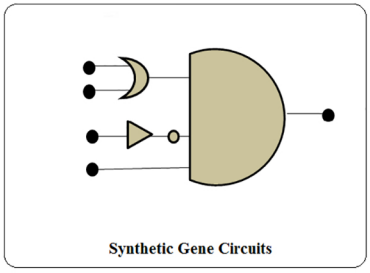
[54] M. Greif, R. Mueller, C. Rudolph, A. Ruf, M. Schmidt, H. Sobek, J.P. Thalhofer, Novel T7 RNA polymerase variants with enhanced thermostability, Google Patents, 2011.

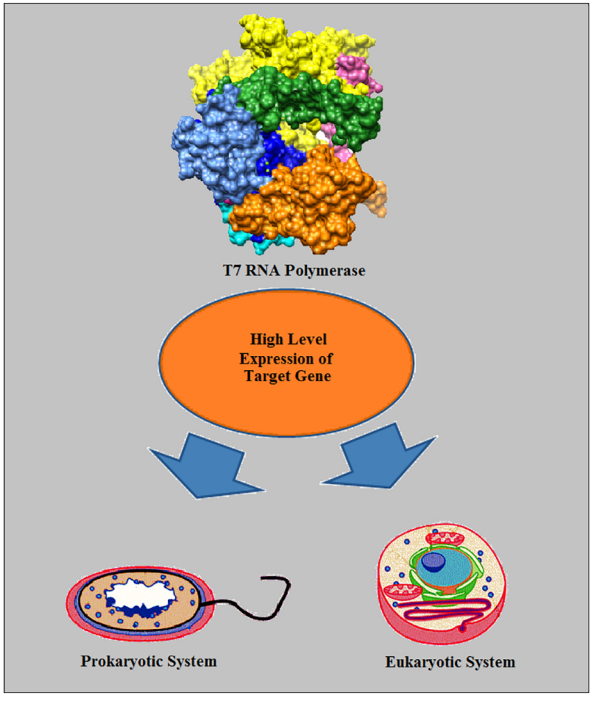
Legends for Figures:

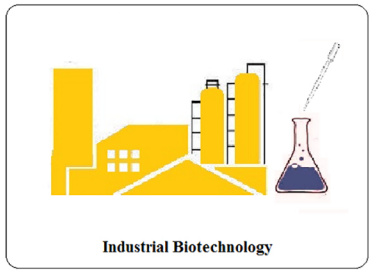
Figure 1 Multifaceted role of T7RNAP in biological realm.

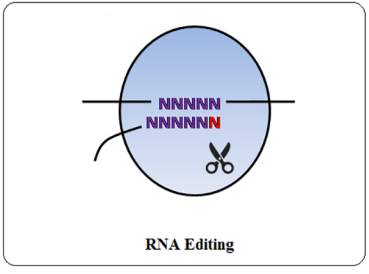
Figure 2 T7RNAP three dimensional structure. The color representation is as follows, with the N-terminal domain (1–325), yellow, the thumb (326–411), green, the palm (412–449, 528–553, 785–879), dark blue, the palm insertion module (450–527), light blue, the fingers (554–739, 769–784), orange, specificity loop (740–769), pink, extended foot module (838–879), cyan and C-terminal (880-883), violet. Two metal ions at the active site are shown as green spheres.

Figure 3 A general flowchart of T7 RNA polymerase: depicting domains and sub-domains and their respective role in the transcription process.


Synthetic Gene Circuits


T7 RNA Polymerase
High Level Expression of Target Gene
Prokaryotic System
Eukaryotic System


Industrial Biotechnology


NNNNN
NNNNNN
RNA Editing

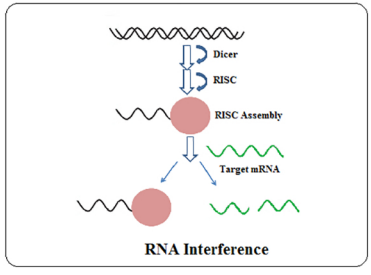

Dicer
RISC
RISC Assembly
Target mRNA
RNA Interference

Figure 1

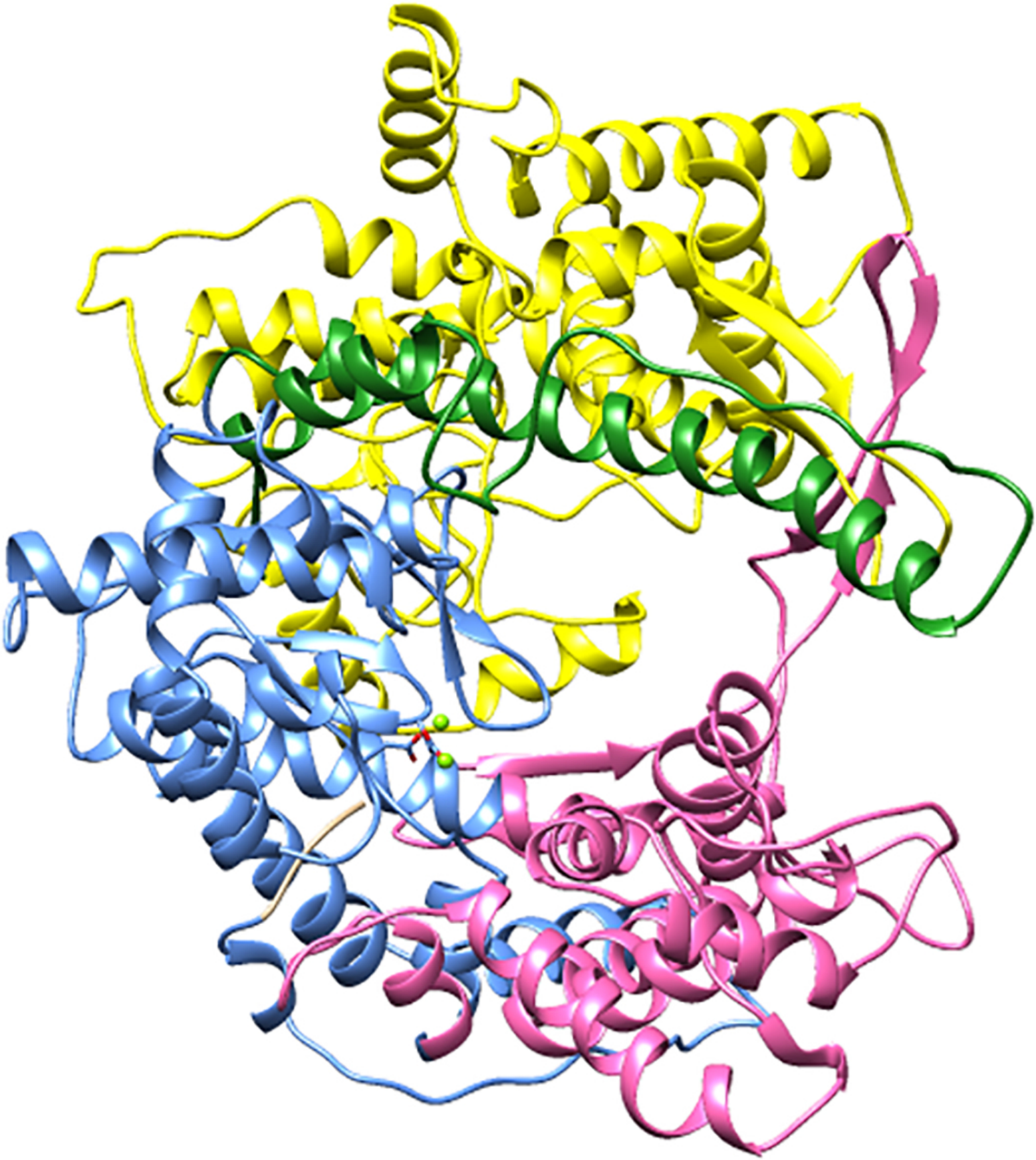


Figure 2

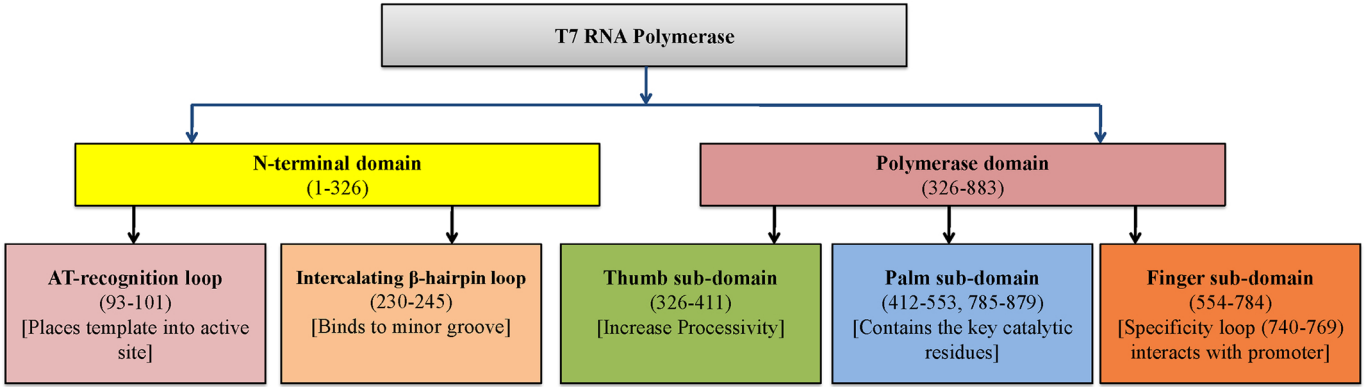

T7 RNA Polymerase
N-terminal domain
(1-326)
Polymerase domain
(326-883)
AT-recognition loop
(93-101)
[Places template into active site]
Intercalating β-hairpin loop
(230-245)
[Binds to minor groove]
Thumb sub-domain
(326-411)
[Increase Processivity]
Palm sub-domain
(412-553, 785-879)
[Contains the key catalytic residues]
Finger sub-domain
(554-784)
[Specificity loop (740-769) interacts with promoter]

Figure 3