



Bacteriophage T7 DNA polymerase – sequenase

Bin Zhu*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

Edited by:

Andrew F. Gardner, New England Biolabs, USA

Reviewed by:

Kirk Matthew Schnorr, Novozymes A/S, Denmark

Samir Hamdan, King Abdullah University of Science and Technology, Saudi Arabia

*Correspondence:

Bin Zhu, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, C2-221, 240 Longwood Avenue, Boston, MA 02115, USA
e-mail: bin_zhu@hms.harvard.edu

An ideal DNA polymerase for chain-terminating DNA sequencing should possess the following features: (1) incorporate dideoxy- and other modified nucleotides at an efficiency similar to that of the cognate deoxynucleotides; (2) high processivity; (3) high fidelity in the absence of proofreading/exonuclease activity; and (4) production of clear and uniform signals for detection. The DNA polymerase encoded by bacteriophage T7 is naturally endowed with or can be engineered to have all these characteristics. The chemically or genetically modified enzyme (Sequenase) expedited significantly the development of DNA sequencing technology. This article reviews the history of studies on T7 DNA polymerase with emphasis on the serial key steps leading to its use in DNA sequencing. Lessons from the study and development of T7 DNA polymerase have and will continue to enlighten the characterization of novel DNA polymerases from newly discovered microbes and their modification for use in biotechnology.

Keywords: bacteriophage T7, DNA polymerase, sequenase, DNA sequencing, marine phages

INITIAL CHARACTERIZATION

DNA polymerases catalyze the synthesis of DNA, a pivot process in both living organisms and in biotechnology (Hamilton et al., 2001; Reha-Krantz, 2008). Family A DNA polymerases including *Escherichia coli* DNA polymerase I, Taq DNA polymerase, and T7 DNA polymerase have served as prototypes for biochemical and structural studies on DNA polymerases and have been widely used as molecular reagents (Patel et al., 2001; Loh and Loeb, 2005).

A DNA polymerase activity from bacteriophage T7 was first observed in an *E. coli* mutant deficient in DNA polymerase I infected with bacteriophage T7 (Grippe and Richardson, 1971). The initial characterization of T7 DNA polymerase was intriguing. Although the gene responsible for the polymerase activity was mapped to gene 5 (Hinkle and Richardson, 1974; Hori et al., 1979b), gene 5 protein (gp5) itself had what appeared to be no DNA polymerase activity but only ssDNA exonuclease activity (Hori et al., 1979a). Apparently a host component was required to reconstitute the full DNA polymerase (Modrich and Richardson, 1975a). This host factor turned out to be a small redox protein – *E. coli* thioredoxin (Modrich and Richardson, 1975b; Mark and Richardson, 1976). The redox capacity of thioredoxin, however, is not required for stimulation of the DNA polymerase activity (Huber et al., 1986). Instead thioredoxin plays a structural role in stabilizing the binding of gene 5 protein to a primer-template (Huber et al., 1987) and increase the processivity of the polymerase more than 100-fold (Tabor et al., 1987a), representing a unique function of this universal protein. Thioredoxin binds to a 71-residue loop of T7 gene 5 protein (Doublé et al., 1998), which is not present in other Pol I-type polymerases, resulting in a stable 1:1 complex ($K_D = 5$ nM; Tabor et al., 1987a).

Another intriguing finding during the initial characterization of T7 DNA polymerase is on its exonuclease activity. T7 DNA polymerase lacks the 5′–3′ exonuclease activity found in *E. coli* DNA polymerase I but does possess a strong 3′–5′ single and

double stranded DNA exonuclease activity (Hori et al., 1979b). The double-stranded DNA exonuclease activity requires the presence of thioredoxin. Interestingly, various protein purification procedures, depending on the presence or absence of EDTA in the buffer, can generate T7 DNA polymerases that differ significantly in their exonuclease activity, resulting in two forms of DNA polymerase (Fischer and Hinkle, 1980; Engler et al., 1983). By comparison of the two forms of polymerase and careful tracking of the purification procedures, it was revealed that the exonuclease activity of T7 DNA polymerase could be specifically inactivated in an oxidation reaction by oxygen, a reducing agent and ferrous ion (Tabor and Richardson, 1987b). The easily modifiable exonuclease and extraordinary processivity of T7 DNA polymerase kindled the emergence of a powerful tool in the DNA sequencing era.

SEQUENASE ERA

Invented by Sanger et al. (1977), the method of chain-terminating sequencing initiated a revolution toward the genome-sequencing era. However, the enzymes initially used for chain-terminating sequencing, the Klenow fragment of *E. coli* DNA polymerase I and avian myeloblastosis virus (AMV) reverse transcriptase, had low processivity (~15 nt for Klenow fragment and 200 for AMV reverse transcriptase, the latter has a relatively higher processivity but its rate of DNA synthesis is only several nucleotides per second). Processivity describes the number of nucleotides continuously incorporated by a DNA polymerase using the same primer-template without dissociation. Thus if the DNA polymerase used for chain-terminating sequencing is non-processive, artifactual bands will arise at positions corresponding to the nucleotide at which the polymerase dissociated. Frequent dissociation will create strong background that obscures the true DNA sequence. Although the issue can be partially improved by long time incubation with high concentration of substrates that may “chase” those artifactual bands up to higher molecular weight,

this procedure is not an ideal solution since reinitiation of primer elongation at dissociation sites (usually regions of compact secondary structure or hairpins) is inefficient and may result in the incorporation of incorrect nucleotides. Although T7 DNA polymerase itself has a processivity of only a few nucleotides, the association with *E. coli* thioredoxin dramatically increases its processivity. Consequently, with T7 DNA polymerase termination of a sequencing reaction will occur only at positions where a chain-terminating agent (such as a dideoxynucleotide) is incorporated, yielding a long DNA sequence (Tabor and Richardson, 1987c).

A more severe problem with DNA polymerases used prior to T7 DNA polymerase is the discrimination against dideoxynucleotides, the chain-terminating nucleotides used in Sanger sequencing. Most of known DNA polymerases strongly discriminate against ddNTP. For example, T4 DNA polymerase, *E. coli* DNA polymerase I, Taq DNA polymerase, and Vent DNA polymerase incorporate a dideoxynucleoside monophosphate (ddNMP) at least a 1000 times slower than the corresponding deoxynucleoside monophosphate (dNMP). To use these polymerases in DNA sequencing a high ratio of ddNTP to dNTP must be used for efficient chain-termination. Even though the overall incorporation of ddNMP can be improved in such an uneconomic way, wide variation in the intensity of adjacent fragments still occur because the extent of discrimination varies with different DNA sequences and structures. T7 DNA polymerase, however, is at the other end of the spectrum, discriminating against ddNTP only several-fold. Thus a much lower concentration of ddNTP can be used with T7 DNA polymerase and the uniformity of DNA bands on the gel is much higher (Tabor and Richardson, 1987c). The discrimination was further lowered by replacing magnesium with manganese in the sequencing reaction (Tabor and Richardson, 1989a). With Mn^{2+} in an isocitrate buffer, T7 DNA polymerase incorporates dNMP and ddNMP at same rate, resulting in uniform terminations of sequencing reactions.

With the naturally endowed high processivity and the lack of discrimination against ddNTP, the only hindrance for T7 DNA polymerase as a DNA sequencing enzyme is its robust 3'-5' exonuclease activity. Exonuclease activity increases the fidelity of DNA synthesis by excising newly synthesized bases incorrectly base-paired to the template. For applications like PCR it is often a desired feature. While for DNA sequencing such activity is detrimental since when the dNTP concentration falls, the rate of exonuclease activity increases close to that of polymerase activity, resulting in no net DNA synthesis or degradation of DNA. The associated exonuclease activity will also cause DNA polymerase to idle at regions with secondary structures in the template, producing variability in the intensity of signals. The iron-catalyzed oxidation mentioned above can produce modified T7 DNA polymerase with greatly reduced exonuclease activity, and this chemically modified enzyme was the basis for Sequenase and the first easy-to-use DNA sequencing kits commercialized by United States Biochemical Co. However, the residual exonuclease activity can still result in some loss of labeled DNA bands upon prolonged incubation (Tabor and Richardson, 1987b). Tabor and Richardson carried out an extensive chemical and mutagenesis screen for selective elimination of the

exonuclease activity of T7 DNA polymerase. The rapid screen of a large number of mutants was based on the observation that exonuclease minus mutants of T7 DNA polymerase can synthesize through a specific hairpin region in the DNA template (Tabor and Richardson, 1989b). As a result many mutants deficient in exonuclease activity were revealed and among them a mutant lacking 28 amino acids in the N-terminal exonuclease domain had no detectable exonuclease activity, while its polymerase activity is significantly higher than that of the wild-type protein. This mutant was the basis of version 2 of Sequenase. Sequenase pioneered development of the most stable enzymes and facilitated the automation for high-throughput sequencing.

Degradation of a DNA fragment can occur via a nucleophilic attack on the 3'-terminal internucleotide linkage by H_2O or pyrophosphate (PPi). The 3'-5' exonuclease catalyzes the former reaction, generating dNMP or ddNMP. The latter reaction is called pyrophosphorolysis. As the reversal of polymerization, pyrophosphorolysis generates dNTP or ddNTP, sometimes resulting in "holes": the disappearance of ddNMP labeled DNA fragments on the gel. By adding pyrophosphatase to the reaction to cleave PPi the pyrophosphorolysis can be eliminated (Tabor and Richardson, 1990). The combination of modified T7 DNA polymerase, manganese ion, and pyrophosphatase can generate accurate and uniform bands on a DNA sequencing gel to the extent that, the DNA sequence can be directly determined by the relative intensity of each band if different amount of the four ddNTPs are added at certain ratio (Tabor and Richardson, 1990).

Thermostability is a highly desired feature for DNA polymerase. A thermostable enzyme like Taq DNA polymerase is superior for cycle sequencing, in which multiple rounds of DNA synthesis are carried out from the same template, with the newly synthesized DNA strand released after each cycle by heat denaturation. The heat stable DNA polymerase survives the denaturation step and is available for the next cycle of polymerization. Cycle sequencing allows much less DNA template and polymerase to be used in a sequencing reaction. In cycle sequencing low processivity is an advantage because a polymerase with low processivity cycles rapidly, decreasing the chance of strong specific stops. However, the strong discrimination against ddNTP (at least 100-fold, often 10,000-fold) by most thermostable DNA polymerase was a significant obstacle for their use in cycle sequencing. Although the use of manganese ion can decrease the discrimination (Tabor and Richardson, 1989a), manganese has several disadvantages compared with magnesium such as narrow working concentration, precipitation, and less activity of DNA polymerase than that supported by magnesium ion.

Studies on T7 DNA polymerase led to one of the most elegant demonstrations of enzyme engineering and turned Taq DNA polymerase into "Thermo Sequenase." To pursue the molecular mechanism underlying the discrepancy in discrimination against ddNTP among family A DNA polymerases, Tabor and Richardson swapped the five most conserved regions in the crevice responsible for binding DNA and NTPs between T7 DNA polymerase and *E. coli* DNA polymerase I (Tabor and Richardson, 1995), based on the 3D structure of *E. coli* DNA polymerase I. By an SDS-DNA activity assay, the "Helix O" from *E. coli* DNA polymerase

I was observed to confer strong discrimination against ddNTP to T7 DNA polymerase. Further mutagenesis in this region revealed that the tyrosine-526 in T7 DNA polymerase or the homologous position phenylalanine-762 in *E. coli* DNA polymerase I was the single determinant for discrimination against ddNTP. When the corresponding residue, F667 in Taq DNA polymerase was replaced with tyrosine, the modified Taq DNA polymerase F667Y actually preferred ddNTP 2-fold over dNTP, comparing to the 6000-fold discrimination against ddNTP by the wide-type enzyme (Tabor and Richardson, 1995). Taq DNA polymerase F667Y, with its naturally endowed superior thermostability and engineered elimination of discrimination against ddNTP, was the basis for “Thermo Sequenase,” an enzyme that greatly expedited the Human Genome Project. The structure of T7 DNA polymerase in complex with a primed-template and a nucleoside triphosphate solved later (Doubl  e et al., 1998) revealed that the 3'-hydroxyl of the incoming nucleotide and the hydroxyl of the pro Tyr 526 are both within hydrogen-bonding distance of the pro S_p-oxygen of the β-phosphate and suggested that one or both of these interactions may be required for nucleotide incorporation. However, even with the structure one could not have predicted the dramatic effect of tyrosine-526 on nucleotide analog discrimination.

AN IDEAL MODEL TO STUDY INTERACTIONS WITHIN A REPLISOME

T7 DNA polymerase consisting of T7 gene 5 protein and *E. coli* thioredoxin, together with T7 gp4 bifunctional primase-helicase, and gene 2.5 ssDNA-binding proteins constitute the simplest known replisome that mediates coordinated leading- and lagging-strand DNA synthesis (Richardson, 1983; Debyser et al., 1994; Lee et al., 1998; Hamdan and Richardson, 2009). The concise organization of the T7 replisome makes it ideal for studies of the multiple interactions of DNA polymerase during the movement of the replisome such as loading of the polymerase (Zhang et al., 2011), polymerase exchange (Johnson et al., 2007), processive synthesis (Hamdan et al., 2007), and translesion synthesis (Zhu et al., 2011). Critical interactions for coordinated DNA synthesis including polymerase-thioredoxin (Johnson and Richardson, 2003; Ghosh et al., 2008; Akabayov et al., 2010; Tran et al., 2012), polymerase-helicase (Zhang et al., 2011; Kulczyk et al., 2012), polymerase-primase (Chowdhury et al., 2000; Zhu et al., 2010), and polymerase-gene 2.5 single-stranded DNA binding protein (He et al., 2003; Hamdan et al., 2005; Ghosh et al., 2009, 2010) interaction were extensively studied. The solid biochemical background of T7 DNA polymerase also attracted investigations using single-molecular methods (Lee et al., 2006; Hamdan et al., 2009; Pandey et al., 2009; Etson et al., 2010; Loparo et al., 2011; Geertsema et al., 2014).

NOVEL T7-LIKE DNA POLYMERASES

DNA polymerases from microbes advanced DNA sequencing technology that in turn unveiled a much larger, diverse and unexplored microbial world. Metagenomics data indicates that the marine phages are the most abundant and diverse organisms on the earth (Suttle, 2005), of which 60–80% potential gene products do not match any in the database. A large portion

of these gene products must be involved in the nucleic acid metabolism, thus one can expect numerous novel nucleic acid enzymes that can enrich the present toolbox of enzymes derived from a small group of characterized microbes. Indeed, our own initial effort on the characterization of marine phage polymerases have revealed unique features of a single-subunit RNA polymerase from marine cyanophage Syn5 that can complement the predominantly used T7 RNA polymerase for *in vitro* RNA synthesis (Zhu et al., 2013a,b). Characterization of marine phage DNA polymerases appears more promising since one can easily target numerous interesting DNA polymerases from the reported marine phage genomes, even just for T7-like or family A DNA polymerases such as those from cyanophage Syn5 (Pope et al., 2007) and P-SSP7 (Sullivan et al., 2005), phages infecting SAR116-clade bacterium (Kang et al., 2013) and marine ssDNA phages (Schmidt et al., 2014). Considering the high probability that the 60–80% unmatched genes may harbor novel polymerase genes, the marine phage is an unlimited treasure to contribute new polymerase tools that can fulfill niches in biotech industry. Characterization and engineering of T7 DNA polymerase has shown the value of identifying novel properties of nucleic acid enzymes.

REFERENCES

- Akabayov, B., Akabayov, S. R., Lee, S. J., Tabor, S., Kulczyk, A. W., and Richardson, C. C. (2010). Conformational dynamics of bacteriophage T7 DNA polymerase and its processivity factor, *Escherichia coli* thioredoxin. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15033–15038. doi: 10.1073/pnas.1010141107
- Chowdhury, K., Tabor, S., and Richardson, C. C. (2000). A unique loop in the DNA-binding crevice of bacteriophage T7 DNA polymerase influences primer utilization. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12469–12474. doi: 10.1073/pnas.230448397
- Debyser, Z., Tabor, S., and Richardson, C. C. (1994). Coordination of leading and lagging strand DNA synthesis at the replication fork of bacteriophage T7. *Cell* 77, 157–166. doi: 10.1016/0092-8674(94)90243-7
- Doubl  e, S., Tabor, S., Long, A. M., Richardson, C. C., and Ellenberger, T. (1998). Crystal structure of a bacteriophage T7 DNA replication complex at 2.2 Å resolution. *Nature* 391, 251–258. doi: 10.1038/34593
- Engler, M. J., Lechner, R. L., and Richardson, C. C. (1983). Two forms of the DNA polymerase of bacteriophage T7. *J. Biol. Chem.* 258, 11165–11173.
- Etson, C. M., Hamdan, S. M., Richardson, C. C., and van Oijen, A. M. (2010). Thioredoxin suppresses microscopic hopping of T7 DNA polymerase on duplex DNA. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1900–1905. doi: 10.1073/pnas.0912664107
- Fischer, H., and Hinkle, D. C. (1980). Bacteriophage T7 DNA replication in vitro. Stimulation of DNA synthesis by T7 RNA polymerase. *J. Biol. Chem.* 255, 7956–7964.
- Geertsema, H. J., Kulczyk, A. W., Richardson, C. C., and van Oijen, A. M. (2014). Single-molecule studies of polymerase dynamics and stoichiometry at the bacteriophage T7 replication machinery. *Proc. Natl. Acad. Sci. U.S.A.* doi: 10.1073/pnas.1402010111 [Epub ahead of print].
- Ghosh, S., Hamdan, S. M., Cook, T. E., and Richardson, C. C. (2008). Interactions of *Escherichia coli* thioredoxin, the processivity factor, with bacteriophage T7 DNA polymerase and helicase. *J. Biol. Chem.* 283, 32077–32084. doi: 10.1074/jbc.M805062200
- Ghosh, S., Hamdan, S. M., and Richardson, C. C. (2010). Two modes of interaction of the single-stranded DNA-binding protein of bacteriophage T7 with the DNA polymerase-thioredoxin complex. *J. Biol. Chem.* 285, 18103–18112. doi: 10.1074/jbc.M110.107656
- Ghosh, S., Marintcheva, B., Takahashi, M., and Richardson, C. C. (2009). C-terminal phenylalanine of bacteriophage T7 single-stranded DNA-binding protein is essential for strand displacement synthesis by T7 DNA polymerase at a nick in DNA. *J. Biol. Chem.* 284, 30339–30349. doi: 10.1074/jbc.M109.024059

- Grippo, P., and Richardson, C. C. (1971). Deoxyribonucleic acid polymerase of bacteriophage T7. *J. Biol. Chem.* 246, 6867–6873.
- Hamdan, S. M., Johnson, D. E., Tanner, N. A., Lee, J. B., Qimron, U., Tabor, S., et al. (2007). Dynamic DNA helicase-DNA polymerase interactions assure processive replication fork movement. *Mol. Cell* 27, 539–549. doi: 10.1016/j.molcel.2007.06.020
- Hamdan, S. M., Loparo, J. J., Takahashi, M., Richardson, C. C., and van Oijen, A. M. (2009). Dynamics of DNA replication loops reveal temporal control of lagging-strand synthesis. *Nature* 457, 336–339. doi: 10.1038/nature07512
- Hamdan, S. M., Marintcheva, B., Cook, T., Lee, S. J., Tabor, S., and Richardson, C. C. (2005). A unique loop in T7 DNA polymerase mediates the binding of helicase-primase, DNA binding protein, and processivity factor. *Proc. Natl. Acad. Sci. U.S.A.* 102, 5096–5101. doi: 10.1073/pnas.0501637102
- Hamdan, S. M., and Richardson, C. C. (2009). Motors, switches, and contacts in the replisome. *Annu. Rev. Biochem.* 78, 205–243. doi: 10.1146/annurev.biochem.78.072407.103248
- Hamilton, S. C., Farchaus, J. W., and Davis, M. C. (2001). DNA polymerases as engines for biotechnology. *Biotechniques* 31, 370–376.
- He, Z. G., Rezende, L. F., Willcox, S., Griffith, J. D., and Richardson, C. C. (2003). The carboxyl-terminal domain of bacteriophage T7 single-stranded DNA-binding protein modulates DNA binding and interaction with T7 DNA polymerase. *J. Biol. Chem.* 278, 29538–29545. doi: 10.1074/jbc.M304318200
- Hinkle, D. C., and Richardson, C. C. (1974). Bacteriophage T7 deoxyribonucleic acid replication in vitro. Requirements for deoxyribonucleic acid synthesis and characterization of the product. *J. Biol. Chem.* 249, 2974–2980.
- Hori, K., Mark, D. F., and Richardson, C. C. (1979a). Deoxyribonucleic acid polymerase of bacteriophage T7. Characterization of the exonuclease activities of the gene 5 protein and the reconstituted polymerase. *J. Biol. Chem.* 254, 11598–11604.
- Hori, K., Mark, D. F., and Richardson, C. C. (1979b). Deoxyribonucleic acid polymerase of bacteriophage T7. Purification and properties of the phage-encoded subunit, the gene 5 protein. *J. Biol. Chem.* 254, 11591–11597.
- Huber, H. E., Russel, M., Model, P., and Richardson, C. C. (1986). Interaction of mutant thioredoxins of *Escherichia coli* with the gene 5 protein of phage T7. The redox capacity of thioredoxin is not required for stimulation of DNA polymerase activity. *J. Biol. Chem.* 261, 15006–15012.
- Huber, H. E., Tabor, S., and Richardson, C. C. (1987). *Escherichia coli* thioredoxin stabilizes complexes of bacteriophage T7 DNA polymerase and primed templates. *J. Biol. Chem.* 262, 16224–16232.
- Johnson, D. E., and Richardson, C. C. (2003). A covalent linkage between the gene 5 DNA polymerase of bacteriophage T7 and *Escherichia coli* thioredoxin, the processivity factor: fate of thioredoxin during DNA synthesis. *J. Biol. Chem.* 278, 23762–23772. doi: 10.1074/jbc.M301366200
- Johnson, D. E., Takahashi, M., Hamdan, S. M., Lee, S. J., and Richardson, C. C. (2007). Exchange of DNA polymerases at the replication fork of bacteriophage T7. *Proc. Natl. Acad. Sci. U.S.A.* 104, 5312–5317. doi: 10.1073/pnas.0701062104
- Kang, I., Oh, H. M., Kang, D., and Cho, J. C. (2013). Genome of a SAR116 bacteriophage shows the prevalence of this phage type in the oceans. *Proc. Natl. Acad. Sci. U.S.A.* 110, 12343–12348. doi: 10.1073/pnas.1219930110
- Kulczyk, A. W., Akabayov, B., Lee, S. J., Bostina, M., Berkowitz, S. A., and Richardson, C. C. (2012). An interaction between DNA polymerase and helicase is essential for the high processivity of the bacteriophage T7 replisome. *J. Biol. Chem.* 287, 39050–39060. doi: 10.1074/jbc.M112.410647
- Lee, J., Chastain, P. D. 2nd, Kusakabe, T., Griffith, J. D., and Richardson, C. C. (1998). Coordinated leading and lagging strand DNA synthesis on a minicircular template. *Mol. Cell* 1, 1001–1010. doi: 10.1016/S1097-2765(00)80100-8
- Lee, J. B., Hite, R. K., Hamdan, S. M., Xie, X. S., Richardson, C. C., and van Oijen, A. M. (2006). DNA primase acts as a molecular brake in DNA replication. *Nature* 439, 621–624. doi: 10.1038/nature04317
- Loh, E., and Loeb, L. A. (2005). Mutability of DNA polymerase I: implications for the creation of mutant DNA polymerases. *DNA Repair (Amst.)* 4, 1390–1398. doi: 10.1016/j.dnarep.2005.09.006
- Loparo, J. J., Kulczyk, A. W., Richardson, C. C., and van Oijen, A. M. (2011). Simultaneous single-molecule measurements of phage T7 replisome composition and function reveal the mechanism of polymerase exchange. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3584–3589. doi: 10.1073/pnas.1018824108
- Mark, D. F., and Richardson, C. C. (1976). *Escherichia coli* thioredoxin: a subunit of bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. U.S.A.* 73, 780–784. doi: 10.1073/pnas.73.3.780
- Modrich, P., and Richardson, C. C. (1975a). Bacteriophage T7 Deoxyribonucleic acid replication in vitro. A protein of *Escherichia coli* required for bacteriophage T7 DNA polymerase activity. *J. Biol. Chem.* 250, 5508–5514.
- Modrich, P., and Richardson, C. C. (1975b). Bacteriophage T7 deoxyribonucleic acid replication in vitro. Bacteriophage T7 DNA polymerase: an enzyme composed of phage- and host-specific subunits. *J. Biol. Chem.* 250, 5515–5522.
- Pandey, M., Syed, S., Donmez, I., Patel, G., Ha, T., and Patel, S. S. (2009). Coordinating DNA replication by means of priming loop and differential synthesis rate. *Nature* 462, 940–943. doi: 10.1038/nature08611
- Patel, P. H., Suzuki, M., Adman, E., Shinkai, A., and Loeb, L. A. (2001). Prokaryotic DNA polymerase I: evolution, structure, and “base flipping” mechanism for nucleotide selection. *J. Mol. Biol.* 308, 823–837. doi: 10.1006/jmbi.2001.4619
- Pope, W. H., Weigle, P. R., Chang, J., Pedulla, M. L., Ford, M. E., Houtz, J. M., et al. (2007). Genome sequence, structural proteins, and capsid organization of the cyanophage Syn5: a “horned” bacteriophage of marine *Synechococcus*. *J. Mol. Biol.* 368, 966–981. doi: 10.1016/j.jmb.2007.02.046
- Reha-Krantz, L. J. (2008). Recent patents of gene sequences relative to DNA polymerases. *Recent Pat. DNA Gene Seq.* 2, 145–163. doi: 10.2174/187221508786241729
- Richardson, C. C. (1983). Bacteriophage T7: minimal requirements for the replication of a duplex DNA molecule. *Cell* 33, 315–317. doi: 10.1016/0092-8674(83)90411-7
- Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* 74, 5463–5467.
- Schmidt, H. F., Sakowski, E. G., Williamson, S. J., Polson, S. W., and Wommack, K. E. (2014). Shotgun metagenomics indicates novel family A DNA polymerases predominate within marine viroplankton. *ISME J.* 8, 103–114. doi: 10.1038/ismej.2013.124
- Sullivan, M. B., Coleman, M. L., Weigle, P., Rohwer, F., and Chisholm, S. W. (2005). Three *Prochlorococcus* cyanophage genomes: signature features and ecological interpretations. *PLoS Biol.* 3:e144. doi: 10.1371/journal.pbio.0030144
- Suttle, C. A. (2005). Viruses in the sea. *Nature* 437, 356–361. doi: 10.1038/nature04160
- Tabor, S., and Richardson, C. C. (1989a). Selective inactivation of the exonuclease activity of bacteriophage T7 DNA polymerase by in vitro mutagenesis. *J. Biol. Chem.* 264, 6447–6458.
- Tabor, S., and Richardson, C. C. (1989b). Effect of manganese ions on the incorporation of dideoxynucleotides by bacteriophage T7 DNA polymerase and *Escherichia coli* DNA polymerase I. *Proc. Natl. Acad. Sci. U.S.A.* 86, 4076–4080. doi: 10.1073/pnas.86.11.4076
- Tabor, S., and Richardson, C. C. (1990). DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. Effect of pyrophosphorolysis and metal ions. *J. Biol. Chem.* 265, 8322–8328.
- Tabor, S., and Richardson, C. C. (1995). A single residue in DNA polymerases of the *Escherichia coli* DNA polymerase I family is critical for distinguishing between deoxy- and dideoxyribonucleotides. *Proc. Natl. Acad. Sci. U.S.A.* 92, 6339–6343. doi: 10.1073/pnas.92.14.6339
- Tabor, S., Huber, H. E., and Richardson, C. C. (1987a). *Escherichia coli* thioredoxin confers processivity on the DNA polymerase activity of the gene 5 protein of bacteriophage T7. *J. Biol. Chem.* 262, 16212–16223.
- Tabor, S., and Richardson, C. C. (1987b). Selective oxidation of the exonuclease domain of bacteriophage T7 DNA polymerase. *J. Biol. Chem.* 262, 15330–15333.
- Tabor, S., and Richardson, C. C. (1987c). DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. *Proc. Natl. Acad. Sci. U.S.A.* 84, 4767–4771. doi: 10.1073/pnas.84.14.4767
- Tran, N. Q., Lee, S. J., Akabayov, B., Johnson, D. E., and Richardson, C. C. (2012). Thioredoxin, the processivity factor, sequesters an exposed cysteine in the thumb domain of bacteriophage T7 DNA polymerase. *J. Biol. Chem.* 287, 39732–39741. doi: 10.1074/jbc.M112.409235

- Zhang, H., Lee, S. J., Zhu, B., Tran, N. Q., Tabor, S., and Richardson, C. C. (2011). Helicase-DNA polymerase interaction is critical to initiate leading-strand DNA synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 9372–9377. doi: 10.1073/pnas.1106678108
- Zhu, B., Lee, S. J., and Richardson, C. C. (2010). Direct role for the RNA polymerase domain of T7 primase in primer delivery. *Proc. Natl. Acad. Sci. U.S.A.* 107, 9099–9104. doi: 10.1073/pnas.1004220107
- Zhu, B., Lee, S. J., and Richardson, C. C. (2011). Bypass of a nick by the replisome of bacteriophage T7. *J. Biol. Chem.* 286, 28488–28497. doi: 10.1074/jbc.M111.252023
- Zhu, B., Tabor, S., Raytcheva, D. A., Hernandez, A., King, J. A., and Richardson, C. C. (2013a). The RNA polymerase of marine cyanophage Syn5. *J. Biol. Chem.* 288, 3545–3552. doi: 10.1074/jbc.M112.442350
- Zhu, B., Tabor, S., and Richardson, C. C. (2013b). Syn5 RNA polymerase synthesizes precise run-off RNA products. *Nucleic Acids Res.* 42:e33. doi: 10.1093/nar/gkt1193
- Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
- Received: 10 March 2014; paper pending published: 31 March 2014; accepted: 01 April 2014; published online: 16 April 2014.
- Citation: Zhu B (2014) Bacteriophage T7 DNA polymerase – sequenase. *Front. Microbiol.* 5:181. doi: 10.3389/fmicb.2014.00181
- This article was submitted to *Evolutionary and Genomic Microbiology*, a section of the journal *Frontiers in Microbiology*.
- Copyright © 2014 Zhu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.