2-26-2018

Pol I DNA polymerases stimulate DNA end-joining by Escherichia coli DNA ligase

Yanling Yang
Louisiana State University

Vince J. LiCata
Louisiana State University

Follow this and additional works at: https://digitalcommons.lsu.edu/biosci_pubs

Recommended Citation

Yang, Y., & LiCata, V. (2018). Pol I DNA polymerases stimulate DNA end-joining by Escherichia coli DNA ligase. *Biochemical and Biophysical Research Communications*, 497 (1), 13-18. <https://doi.org/10.1016/j.bbrc.2018.01.165>

This Article is brought to you for free and open access by the Department of Biological Sciences at LSU Digital Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Digital Commons. For more information, please contact ir@lsu.edu.

Pol I DNA polymerases stimulate DNA end-joining by *Escherichia coli* DNA ligase

Yanling Yang¹, Vince J. LiCata^{*}

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA, 70803, USA

ARTICLE INFO

Article history:

Received 28 December 2017

Accepted 26 January 2018

Available online 31 January 2018

Keywords:

DNA ligase

DNA polymerase

DNA repair

DNA replication

Double strand break repair

Non-homologous end-joining

ABSTRACT

Klenow and KlenTaq are the large fragment domains of the Pol I DNA polymerases from *Escherichia coli* and *Thermus aquaticus*, respectively. Herein, we show that both polymerases can significantly stimulate complementary intermolecular end-joining ligations by *E. coli* DNA ligase when the polymerases are present at concentrations lower than that of the DNA substrates. In contrast, high polymerase concentrations relative to the DNA substrates inhibit the intermolecular ligation activity of DNA ligase. Neither polymerase was able to stimulate the DNA ligase from T4 bacteriophage. Additionally, nick-closure by *E. coli* DNA ligase (but not T4 ligase) is slightly stimulated by both polymerases, but only at about 10% of the magnitude seen for end-joining enhancement. The data represent one of the first observations of direct polymerase-ligase interactions in prokaryotes, and suggest that the polymerases stabilize the associated DNA ends during intermolecular ligation, and that such a complex can be taken advantage of by some, but not all, DNA ligases.

Published by Elsevier Inc.

1. Introduction

Interactions between DNA polymerases and DNA ligases have been documented in a number of different eukaryotic systems, both in the context of base-excision repair and single-strand break repair [1–3]. In this study, we report a prokaryotic polymerase-ligase cooperation that functions in the context of DNA end-joining. On their own, DNA ligases can catalyze the end-joining of two DNA fragments with complementary ends and the repair of a single-stranded nick within double stranded DNA. In conjunction with different cooperating proteins, including ones that hold two DNA fragments together or that perform nuclease or polymerase dependent processing, DNA ligases can also end-join non-complementary DNA fragments in processes such as non-homologous end-joining (NHEJ) [4,5].

Both Klenow and KlenTaq DNA polymerases (the large fragment domains of the Pol I polymerases from *E. coli* and *T. aquaticus*, respectively) show tight binding affinity to DNA fragments with

different types of end-structures including 5' overhangs, 3' overhangs, and blunt ends [6]. Furthermore, previous studies of the end-joining abilities of Pol I DNA polymerases have shown that both *E. coli* and *T. aquaticus* DNA polymerases are able to use discontinuous templates to produce fill-in products between two DNA fragments [7–10]. This DNA synthesis-dependent end-joining activity has been demonstrated with fragments having fully complementary overlapping ends, with fragments having limited complementary regions that are interior to non-complementary end sequences, and with blunt ended fragments [7–10]. *In vivo* evidence for this synthesis-dependent end-joining activity in *E. coli* has also been described [10].

The present study demonstrates that *E. coli* and *Taq* Pol I DNA polymerases will also facilitate DNA end-joining activity in the absence of DNA synthesis by enhancing the complementary end-joining activity of *E. coli* DNA ligase when the polymerases are present at sub-stoichiometric concentrations relative to DNA fragments. The effects of both Pol I polymerases on ligation are specific to *E. coli* DNA ligase, with neither polymerase able to enhance T4 DNA ligase. We hypothesize that this effect is due to an ability of the Pol I DNA polymerases to stabilize association of the two DNA fragments combined with a productive interaction with *E. coli* DNA ligase.

Abbreviations: KLN, Klenow DNA polymerase; KTQ, KlenTaq DNA polymerase; BSA, bovine serum albumin; NHEJ, non-homologous end-joining.

^{*} Corresponding author.

E-mail address: licata@lsu.edu (V.J. LiCata).

¹ Present address for Y.-Y.: Proteomics Core, NHLBI/National Institutes of Health, 10 Center Drive, Room 8C103A, Bethesda, MD 20892.

2. Experimental procedures

2.1. Materials

Oligonucleotides were obtained from Integrated DNA Technologies (Coralville, IA). The DNA substrate sequences are shown in Table 1. Oligonucleotide concentrations were determined by measuring the absorbance at 260 nm and using extinction coefficients provided by the manufacturer. All DNAs are 5' phosphorylated. The DNA molecules used in this study have stable terminal hairpin structures flanking the duplex region to circumvent the difficulty in analyzing the various products produced by multiple ligations. Hairpin structures are closed using a stable tetraloop [11]. Hairpin DNAs were annealed from single-strand DNA by heating at 95 °C for 5 min and slowly cooling down to ambient temperature.

Klenow (KLN) and KlenTaq (KTQ) polymerases were purified in our laboratory as previously described [12]. The Klenow clone used in this study contains the D424A mutation (Klenow exo-) and was provided by Catherine Joyce from Yale University. This mutant has only residual 3'-5' exonuclease activity, but retains DNA binding affinity for the proofreading site [13]. Protein concentrations were measured at 280 nm and calculated by using ϵ_{280} values of $5.88 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for Klenow and $7.04 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for KlenTaq. *E. coli* DNA ligase and T4 DNA ligase were purchased from New England Biolabs (Ipswich, MA).

2.2. Methods

Ligation reactions were performed in each DNA ligase's corresponding commercially supplied reaction buffer containing identical amounts of DNA substrates, a fixed amount of one of the DNA ligases, and increasing amounts of one of the DNA polymerases or bovine serum albumin (BSA). The reaction volume was 10 μL . Intermolecular ligations with two pieces of DNA having complementary 5-base 3' overhangs (hp27 and hp57 in Table 1) or intramolecular ligations with nicked DNA (Table 1) were performed at 25 °C for the times indicated in each of the figures. For end-joining reactions: 10 μL reactions contained 250 nM of hp57, 500 nM of hp27, 0.3 units of *E. coli* or T4 DNA ligase, and variable amounts of added polymerase or BSA as indicated in each figure. Higher concentration of the smaller hairpin were added simply to help with visualization on the gels. For nick closure reactions: 10 μL reactions contained 150 nM of nicked DNA, 0.2 units of *E. coli* DNA ligase, or 0.1 units of T4 DNA ligase, and increasing amounts of polymerase. Ligation reactions were stopped with 6 μL of a stop buffer containing 0.2% SDS, 10 mM EDTA, 90% formamide, and 0.1% bromophenol blue, by heating at 95 °C for 10 min, and then immediately putting on ice for 5 min. A 4.5 μL of sample of each stopped reaction

was loaded onto a 20% denaturing polyacrylamide gel containing 7.5 M urea and electrophoresed in TAE (80 mM Tris acetate, 2 mM EDTA, pH 8.0). Following electrophoresis, gels were stained with SYBR Green (Invitrogen) and imaged using a Bio-Rad gel imager. Each ligation reaction was performed 2–3 times.

The amount of ligated product on the denaturing polyacrylamide gels was quantitated using the program ImageQuant 5.1 (GE Healthcare). Rectangles of the same size were drawn around all product bands, and equally sized rectangles were also drawn in the corresponding positions below each product band as background controls. The difference in intensities between each product band and that of the corresponding background control yields the relative amount of ligation product formed, which is reported herein as an approximate enhancement of ligation in all figures. The variability in band quantification upon repeated trials was ± 4 –8%.

3. Results

3.1. Both Klenow and KlenTaq polymerases stimulate *E. coli* DNA ligase activity at sub-stoichiometric concentrations

Hairpin DNA fragments (hp27 and hp57 in Table 1) containing complementary 5-base 3' overhangs were used as the substrates to assess the DNA ligase catalyzed intermolecular end-joining to form a fully ligated 84 base construct *in vitro*. Fig. 1 shows the enhancing effects of increasing amounts of Klenow and KlenTaq polymerases in reactions containing a constant amount of *E. coli* DNA ligase over differing reaction incubation times. Reactions were performed in the presence of excess starting DNA substrates relative to DNA ligase units (i.e. more DNA than the DNA ligase should react with in the time frame of the experiments). In addition, all polymerase concentrations used in Fig. 1 are well below stoichiometric concentrations relative to the DNA fragments (i.e. the starting concentrations of the DNA fragments are 250 and 500 nM while the highest polymerase concentration in these reactions is 50 nM). It can be seen that shorter incubation times (6 min) remain well below the point of substrate exhaustion, while the longer incubation times (20 min) are reaching substrate exhaustion and maximal product formation.

To rule out a generic protein effect or a molecular crowding effect, the ligation reactions were also characterized in the presence of increasing amounts of bovine serum albumin (BSA), which, as shown in Fig. 2, displays no enhancement of the DNA ligation activity, and in fact shows a slight (~20–25%) inhibition of ligation activity. To examine the specificity of the functional interaction between Pol I DNA polymerase and the NAD^+ -dependent *E. coli* DNA ligase, we examined whether the Pol I polymerases could simulate the ATP-dependent T4 DNA ligase. In contrast to their effects on *E. coli* DNA ligase, Fig. 2 shows that neither polymerase enhances

Table 1
Sequences of DNAs Used in this Study*.

hp27	$\left[\begin{array}{l} \text{AACGACGTCCGGGTTT-3'} \\ \text{AGGCTGCAGGC-5'} \end{array} \right]$
hp57	$\left[\begin{array}{l} \text{AACGGCTATGCTCACC GCCACTACGCAAACC-3'} \\ \text{GAGCCGATACGAGTGGCGGTGATGCG-5'} \end{array} \right]$
nickedDNA	$\left[\begin{array}{l} \text{AACGGGTGACACGGCATCCGCG} \quad \text{CGCGTAGTGACTGTGAGTCCGAG} \\ \text{GAGCCCACTGTGCCGTAGGCGC} \quad \text{GCGCATCACTGACACTCAGGC AA} \end{array} \right]$

*Regions of the sequences shown in bold are base-paired double-strands.

Fig. 1. DNA polymerase I stimulation of *E. coli* DNA ligase end-joining activity by Klenow polymerase (KLN, panels A and B) and by Klenotaq polymerase (KTQ, panels C and D). A) *E. coli* DNA ligase end-joining reaction products in the absence and presence of Klenow polymerase shown on denaturing polyacrylamide gels. Light grey bars indicate incubation with ligase for the specified times (lane 1 of the top gel in Panel A shows an incubation in the absence of both ligase and polymerase). Increasing amounts of Klenow polymerase were added to reactions in successive lanes (concentrations indicated in the associated graph). B) The graph shows the [Klenow] versus the relative enhancement of ligation estimated from quantitation of band intensity for the gels in panel A. C) *E. coli* DNA ligase end-joining reaction products in the absence and presence of Klenotaq polymerase shown on denaturing polyacrylamide gels. Increasing amounts of Klenotaq polymerase were added to reactions in successive lanes (concentrations indicated in the associated graph). D) The graph shows the [Klenotaq] versus the relative enhancement of ligation estimated from quantitation of band intensity.

the activity of T4 DNA ligase, instead Klenow has little or no effect on T4 ligase while Klenotaq inhibits T4 ligase by up to 40–50%.

3.2. High concentration of Klenow and Klenotaq inhibit ligation by *E. coli* DNA ligase

To further explore the potential mechanism of stimulation, we examined the effects of significantly higher concentrations of polymerases – approaching stoichiometric values relative to the DNA fragments (in all end-joining experiments the DNA fragment concentrations are 250 nM for hp57 and 500 nM for hp27). Fig. 3 shows that in the presence of 500 nM of either polymerase, ligation of the two DNA fragments is inhibited, while the earlier demonstrated enhancement at lower concentrations is shown again in the 50 nM lanes. Similar inhibition was seen in the presence of 300 nM of either polymerase (data not shown). Fig. 3 also shows that high concentrations of BSA do not effect ligase activity—i.e. BSA neither enhances ligation at lower concentrations (Fig. 2) nor inhibits ligation at higher concentrations (Fig. 3).

Interestingly, Fig. 3 also shows that higher concentrations of Klenow and Klenotaq polymerases also inhibited DNA end-joining by T4 DNA ligase, despite the absence of an enhancement effect on T4 ligase at lower polymerase concentrations. The 84bp ligated

band is similar in intensity in the 0 and 50 nM [polymerase] lanes for T4 ligase in Fig. 3, while the 500 nM [polymerase] lanes for both polymerases show inhibition of T4 ligase. Similar inhibition of T4 ligase was also seen at 300 nM concentrations of both polymerases (data not shown). Quantitation of band intensities at the high polymerase concentrations in Fig. 3 indicated that the end-joining reactions are inhibited relative to the absence of polymerases by about 10–20% for both *E. coli* and T4 ligases (i.e. compare 0 polymerase lanes and 500 nM polymerase lanes).

3.3. Polymerases slightly enhance ligation of a nicked DNA by *E. coli* ligase

It is possible that Klenow and Klenotaq polymerases could enhance the *E. coli* DNA ligase reaction via a direct polymerase-ligase interaction, or by forming a complex with both DNAs and stabilizing their non-covalent association, or both. The data suggest that both modes of action are involved, but that stabilization of the bi-molecular DNA association is a dominating effect. The fact that both polymerases stimulate *E. coli* DNA ligase but not T4 DNA ligase suggests a direct polymerase-protein interaction. To further examine these two possibilities, a double-hairpin DNA construct with a single interior nick was examined. In these reactions there is

Fig. 2. Bovine serum albumin (BSA) does not stimulate *E. coli* DNA ligase end-joining activity (Panel A), and polymerases do not stimulate T4 DNA ligase (Panels B&C). A) End-joining ligation reaction products shown on a denaturing polyacrylamide gel where increasing amounts of BSA are added to reactions in successive lanes at concentrations indicated in the associated graph. B&C) T4 DNA ligase end-joining ligation reaction products in the presence of increasing amounts of Klenow polymerase (B) and Klenaq polymerase (C). D) The graph shows the [protein] (BSA or Klenow or Klenaq) versus the relative change in end-joining ligation estimated from quantitation of band intensities.

no need for two DNAs to associate before the ligation reaction can occur. Fig. 4 shows that nick closure by *E. coli* DNA ligase is slightly enhanced at up to 20 nM concentrations of either polymerase, while T4 DNA ligase is slightly inhibited at all concentrations of either polymerase. Fig. 4 further supports the hypothesis of a direct favorable interaction between *E. coli* ligase and the polymerases, but both of these effects are within ± 20 –25% of the ligation activity in the absence of polymerases and are thus substantially more subtle than the 250–600% effects of the polymerases on end-joining activity by *E. coli* DNA ligase seen in Fig. 1. This >10 fold difference in the magnitude of the effects thus suggests that polymerase involvement in holding complementary fragments together in the reactions in Fig. 1 is a major part of the enhancement of DNA

Fig. 3. High concentrations of Klenow (KLN) and Klenaq (KTQ) inhibit the end-joining activity of both *E. coli* and T4 DNA ligases. Different ligase+polymerase (or BSA) combinations are shown at 0, 50, and 500 nM concentrations of polymerases (or BSA). Top 3 sets of gels show *E. coli* DNA ligase +0, 50, or 500 nM of Klenow (KLN), Klenaq (KTQ), or BSA. Bottom two sets of gels show T4 ligase +0, 50, or 500 nM of Klenow (KLN) or Klenaq (KTQ) polymerases. All reactions shown were incubated at 25 °C for 6 min. Substrate and ligation product sizes are indicated in nucleotides.

end-joining. Much higher polymerase concentrations (up to 500 nM of each polymerase) showed no further enhancement or inhibition of nick closure (data not shown).

4. Discussion

4.1. A prokaryotic version of a known eukaryotic protein-protein interaction

The data presented in this study demonstrate that both *E. coli* and *T. aquaticus* Pol I DNA polymerases can stimulate inter-

Fig. 4. Klenow (KLN) and Klenotaq (KTQ) effects on *E. coli* DNA ligase or T4 DNA ligase closure of a single nick. a) T4 DNA ligase and b) *E. coli* DNA ligase nick-closure reactions are shown in the presence of increasing amounts of Klenow (KLN) or Klenotaq (KTQ) polymerases. Reactions were incubated at 25 °C for 6 min. c) Relative nick-closure effects as a function of [polymerase], estimated from band intensities in Panels A&B.

molecular DNA ligation by *E. coli* DNA ligase. Interactions between polymerases and DNA ligases have been directly documented previously in several eukaryotic systems within the context of different DNA repair processes, but not in prokaryotic systems. For example DNA polymerase β and DNA ligase I have long been shown to cooperate during base excision repair [1], where instead of mediating DNA end-joining, the DNA ligase catalyzes nick closure at completion of the repair process. A direct interaction between mitochondrial DNA polymerase γ and DNA ligase III has also been documented, again with mechanistic links to base excision repair [3]. Interaction between DNA ligase III α and poly (ADP-ribose) polymerase-1 (PARP-1) has been shown to be involved in single-strand break repair [2]. Interestingly, in prokaryotic systems, Ignatove and Kramarov have reported that adding ligase enhances the PCR amplification of long DNA sequences, and suggested that a polymerase-ligase interaction might be involved, but did not further characterize the hypothesized interaction [14].

Enhancement of *E. coli* DNA ligase by small molecules has been documented by a number of researchers. The original studies of *E. coli* DNA ligase by Lehman and associates demonstrated the enhancement of ligase activity by cations, especially ammonium ion [15]. Several different researchers have shown that molecular crowding agents and other small molecules enhance ligase activity

[16–19]. The present report appears to be the first demonstration of a protein-based prokaryotic DNA ligase enhancement effect that is not crowding based. Beyond its potential fundamental roles in DNA replication and repair, enhancement of DNA ligation in general also has some biotechnological utility as evidenced by the patenting of small molecule enhancers of DNA ligation [18] and by the recent ligation efficiency enhancement studies of Suzuki et al. [20]. The approximately 2.5–6 fold (250–600%) enhancements of DNA ligase activity seen in the presence of Pol I polymerases in this study (Fig. 1) are similar to the range of cation enhancement effects originally described Modrich and Lehman [15] and similar to the range of enhancements described in the Kucera and Evans patent, which covers small molecules that enhance DNA ligation “by at least 25%” and up to about 9 fold [18].

4.2. Hypothetical model for Polymerase-DNA ligase cooperation

The data described herein suggest a simple model to explain the stimulation of ligation by the Pol I polymerases where at low concentrations of protein, one polymerase molecule could simultaneously bind both pieces of DNA, stabilizing the complementary association between the fragments. DNA ligase would then need to either interact directly with the polymerase to gain access to the ligation sites, or the ligation sites may be exposed in such a way in the putative polymerase-double DNA complex that *E. coli* DNA ligase can access them. At high polymerase concentrations, polymerases would be available to bind to individual DNA ends to form 1:1 (DNA-polymerase) complexes rather than 1:2 (DNA-polymerase-DNA) complexes. This would impede rather than enhance association of the DNA fragments, and result in the high concentration inhibition of ligation demonstrated in this study. The series of studies by Morgan and associates on DNA synthesis-dependent DNA end-joining by Pol I polymerases hypothesized a similar 1:2 DNA-polymerase-DNA complex to facilitate the end-joining results they observed [8,9]. Neither Morgan and associates, nor our laboratory, has (yet) been able to empirically demonstrate the existence of such a 1:2 complex, suggesting that, if it exists, it may be a transient or very low affinity complex.

4.3. Species specificity of the polymerase-DNA ligase interaction

The data in this study show that while both Pol I polymerases stimulate the activity of *E. coli* DNA ligase, neither stimulates the activity of T4 DNA ligase. This species specificity indicates that the mechanism of polymerase stimulation involves more than just holding the two DNA fragments together for longer than they might associate on their own – if this were the only mechanism for stimulation, both DNA ligases should be stimulated. This species specificity implies some sort of direct interaction and/or structurally specific characteristics of *E. coli* DNA ligase that allow it to cooperate/interact with the polymerases. It is notable that all of the documented eukaryotic polymerase-ligase interactions either demonstrate or postulate a direct protein-protein interaction between the two enzymes [1–3]. *E. coli* DNA ligase is a NAD⁺-dependent DNA ligase while T4 DNA ligase is an ATP dependent DNA ligase with only about 2% identity to *E. coli* DNA ligase [21,22]. Interestingly, the original cation enhancement effect seen for *E. coli* DNA ligase is also absent for T4 ligase [15]. The significant evolutionary/sequence distance between *E. coli* and T4 DNA ligases certainly make it plausible that the structure/function motif(s) on *E. coli* DNA ligase that allows it to cooperate/interact with the polymerases never evolved in T4 DNA ligase. Interestingly

for T4 ligase, in contrast to the absence of stimulation by polymerases at low stoichiometric ratios, higher concentrations of polymerases inhibited DNA end-joining by both *E. coli* and T4 DNA ligases (Fig. 3), further suggesting that when too many polymerase molecules are present and binding to individual DNA ends, any species of ligase may be occluded from accessing the DNA.

4.4. Possible relationship to non-homologous end-joining (NHEJ)?

Pursuant to DNA end-joining, DNA ligases are known to directly associate with several different types of partner proteins in different systems, a notable interaction being the Ku-LigD association involved in prokaryotic NHEJ, where Ku protein bridges the two DNA fragments and LigD (DNA ligase D) is a multifunctional enzyme having both polymerase and DNA ligase activities (as well as a 3' phosphoesterase domain) (for reviews see [4,5]). In eukaryotic NHEJ, human Ku protein has also been shown to stimulate the end-joining activity of other human DNA ligases, and notably similar to the results shown herein for Klenow and Klenoq polymerases, Ku also does not significantly enhance ligation at isolated single nicks [23]. One of the hypothesized modes of action in the present study is also similar to the model for the stimulation of Ku on ligation of DNA ligase IV in mammalian cells, where Ku stabilizes the association of the two DNA fragments, then moves inward on DNA when DNA ligase IV binds to allow ligation [23]. Also similar in this mammalian Ku-Ligase IV interaction is the fact that higher (super-stoichiometric) concentrations of Ku inhibit Ligase IV activity [24].

It has been noted that *E. coli* does not contain a Ku homologue [25]. For *T. aquaticus*, we performed iterative PSI-BLAST database searches [26] using the sequences of YkoV, MgKU70, Yku70p-Yku80p and LigD as queries, but no homologies to Ku or LigD were detected in *Thermus thermophilus* HB27 or *Thermus thermophilus* HB8, each having high similarity to *Thermus aquaticus*. Thus, it appears that there is not a homologous Ku-LigD or Ku-Ligase IV NHEJ system in *Thermus* bacteria either.

The data in the present study focus exclusively on DNA fragments with complementary ends, but such complementary double-strand breaks will also be repaired by the prokaryotic Ku-LigD or eukaryotic Ku-Ligase IV NHEJ systems. The data from this study combined with the previous studies by Morgan and associates on synthesis-dependent end-joining suggest that it may be fruitful to further examine whether in *E. coli* and *T. aquaticus*, the Pol I DNA polymerases may at least partially fulfill the NHEJ function that the Ku protein system performs in other systems, by facilitating DNA ligase end-joining repair of both homologous double-strand breaks (this study) and DNA-synthesis-dependent repair of non-homologous double-strand breaks [7–10].

Acknowledgements

This work was funded by the NSF Division of Molecular and Cellular Biosciences.

Transparency document

Transparency document related to this article can be found online at <https://doi.org/10.1016/j.bbrc.2018.01.165>.

References

- [1] R. Prasad, R.K. Singhal, D.K. Srivastava, J.T. Molina, A.E. Tomkinson, S.H. Wilson, Specific interaction of DNA polymerase β and DNA ligase I in a multiprotein base excision repair complex from bovine testis, *J. Biol. Chem.* 271 (1996) 16000–16007.
- [2] J.B. Leppard, Z. Dong, Z.B. Mackey, A.E. Tompkinson, Physical and functional interaction between DNA ligase IIIa and poly (ADP-ribose) polymerase 1 in DNA single-strand break repair, *Mol. Cell Biol.* 23 (2003) 5919–5927.
- [3] A. De, C. Campbell, A novel interaction between DNA ligase III and polymerase γ plays an essential role in mitochondrial DNA stability, *Biochem. J.* 402 (2007) 175–186.
- [4] S. Shuman, M.S. Glickman, Bacterial DNA repair by non-homologous end-joining, *Nat. Rev. Microbiol.* 5 (2007) 852–861.
- [5] R.S. Pitcher, N.C. Brissett, A.J. Doherty, Non-homologous end-joining in bacteria: a microbial perspective, *Annu. Rev. Microbiol.* 61 (2007) 259–282.
- [6] A.J. Wowor, K. Datta, H.S. Brown, G.S. Thompson, S. Ray, A. Grove, V.J. LiCata, Thermodynamics of the DNA structural selectivity of the Pol I DNA polymerases from *Escherichia coli* and *Thermus aquaticus*, *Biophys. J.* 98 (2010) 3015–3024.
- [7] J.M. Clark, DNA synthesis on discontinuous templates by DNA polymerase I of *Escherichia coli*, *Gene* 104 (1991) 75–80.
- [8] J.S. King, C.F. Fairley, W.F. Morgan, Bridging the gap. Joining of nonhomologous ends by DNA polymerases, *J. Biol. Chem.* 269 (1994) 13061–13064.
- [9] J.S. King, C.F. Fairley, W.F. Morgan, DNA end joining by the Klenow fragment of DNA polymerase I, *J. Biol. Chem.* 271 (1996) 20450–20457.
- [10] J.S. King, C.F. Fairley, W.F. Morgan, The joining of blunt DNA ends to 3'-protruding single strands in *Escherichia coli*, *Nucleic Acids Res.* 26 (1998) 1749–1754.
- [11] V.P. Antao, I. Tinoco Jr., Thermodynamic parameters for loop formation in RNA and DNA hairpin tetraloops, *Nucleic Acids Res.* 20 (1992) 819–824.
- [12] K. Datta, V.J. LiCata, Salt dependence of DNA binding by *Thermus aquaticus* and *Escherichia coli* DNA polymerases, *J. Biol. Chem.* 278 (2003) 5694–5701.
- [13] V. Derbyshire, P.S. Freemont, M.R. Sanderson, L. Beese, J.M. Friedman, C.M. Joyce, T.A. Steitz, Genetic and crystallographic studies of the 3',5'-exonucleolytic site of DNA polymerase I, *Science* 240 (1988) 199–201.
- [14] K.B. Ignatov, V.M. Kramarov, DNA ligases from thermophilic bacteria enhance PCR amplification of long DNA sequences, *Biochemistry (Moscow)* 74 (2009) 557–561.
- [15] P. Modrich, I.R. Lehman, Dextroxyribonucleic acid ligase: a steady state kinetic analysis of the reaction catalyzed by the enzyme from *Escherichia coli*, *J. Biol. Chem.* 248 (1973) 7502–7511.
- [16] K. Hayashi, M. Nakazawa, Y. Ishizaki, A. Obayashi, Influence of monovalent cations on the activity of T4 DNA ligase in the presence of polyethylene glycol, *NAR* 13 (1985) 3261–3271.
- [17] K. Hayashi, M. Nakazawa, Y. Ishizaki, N. Hiraoka, A. Obayashi, Stimulation of intermolecular ligation with *E. coli* DNA ligase by high concentrations of monovalent cations in polyethylene glycol solutions, *NAR* 13 (1985) 7979–7992.
- [18] R. Kucera, T. Evans, Ligation Enhancement, US Patent Number 20120283144 A1, 2012.
- [19] Z.X. Xiao, H.M. Cao, X.H. Luan, J.L. Zhao, D.Z. Wei, J.H. Xiao, Effects of additives on efficiency and specificity of ligase detection reactions, *Mol. Biotechnol.* 35 (2007) 129–133.
- [20] M. Suzuki, H. Hayashi, T. Mizuki, et al., Efficient DNA ligation by selective heating of DNA ligase with a radio frequency alternating magnetic field, *Biochemistry and Biophysics Reports* 8 (2016) 360–364.
- [21] I.R. Lehman, DNA ligase: structure, mechanism, and function, *Science* 186 (1974) 790–797.
- [22] M. Takahashi, E. Yamaguchi, T. Uchida, Thermophilic DNA ligase. Purification and properties of the enzyme from *Thermus thermophilus* HB8, *J. Biol. Chem.* 259 (1984) 10041–10047.
- [23] D.A. Ramsden, M. Gellert, Ku protein stimulates DNA end joining by mammalian DNA ligases: a direct role for Ku in repair of DNA double-strand breaks, *EMBO J.* 17 (1998) 609–614.
- [24] B. Kysela, A.J. Doherty, M. Chovanec, et al., Ku stimulation of DNA ligase IV-dependent ligation requires inward movement along the DNA molecule, *J. Biol. Chem.* 278 (2003) 22466–22474.
- [25] R. Bowater, A.J. Doherty, Making ends meet: repairing breaks in bacterial DNA by non-homologous end-joining, *PLoS Genet.* 2 (2006) e8.
- [26] S.F. Altschul, T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, *Nucleic Acids Res.* 25 (1997) 3389–3402.