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The phage T4 DNA ligase mediates bacterial chromosome DSBs repair as single component non-homologous end joining

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ABSTRACT

DNA double-strand breaks (DSBs) are one of the most lethal forms of DNA damage that is not efficiently repaired in prokaryotes. Certain microorganisms can handle chromosomal DSBs using the error-prone non-homologous end joining (NHEJ) system and ultimately cause genome mutagenesis. Here, we demonstrated that Enterobacteria phage T4 DNA ligase alone is capable of mediating *in vivo* chromosome DSBs repair in *Escherichia coli*. The ligation efficiency of DSBs with T4 DNA ligase is one order of magnitude higher than the NHEJ system from *Mycobacterium tuberculosis*. This process introduces chromosome DNA excision with different sizes, which can be manipulated by regulating the activity of host-exonuclease RecBCD. The DNA deletion length reduced either by inactivating *recB* or expressing the RecBCD inhibitor Gam protein from λ phage. Furthermore, we also found single nucleotide substitutions at the DNA junction, suggesting that T4 DNA ligase, as a single component non-homologous end joining system, has great potential in genome mutagenesis, genome reduction and genome editing.

1. Introduction

In cells, DNA double-strand breaks (DSBs) are one of the most lethal DNA damage that requires immediate and efficient repair [1]. Generally, it is repaired by either homologous-recombination (HR) or non-homologous end-joining (NHEJ) process [2,3]. HR repair necessitates a homologous intact donor DNA duplex as the template for the faithful re-synthesis of the broken strands, which is the basis for many genetic operation, including deletion, insertion and site mutagenesis [4]. However, HR is unable to mediate DSBs repair in stationary phase bacteria when there is no DNA replication.

NHEJ involves direct bridging of two DNA ends and subsequent end joining catalyzed by the specific DNA ligase [5]. In contrast with its wide prevalence in eukaryotes, prokaryotic NHEJ system has only been identified and characterized in a few bacteria, such as *Mycobacterium tuberculosis, Mycobacterium smegmatis, Bacillus subtilis, Pseudomonas aeruginosa, etc* [5–8], providing an alternative DSB repair pathway when HR cannot fulfill the repair of DSBs [9]. NHEJ is considered to confer the environmental tolerance of such pathogenic or heat-resistant microorganisms [7,9]. However, there is no similar DSB repair mechanism in the common laboratory strains such as *E. coli*.

In *M. tuberculosis*, DNA binding protein Mt-Ku and ATP-dependent DNA ligase Mt-LigD bi-component were implicated in NHEJ system [10,11]. Mt-Ku binds to the ends of DNA breaks as homodimer and protects the DNA ends from nuclease degradation. Mt-Ku is also able to recruit DNA ligase Mt-LigD and stimulate its end-joining activity [10]. Mt-LigD is a multifunctional protein that consists of three domains, the N-terminal polymerase domain, the central ligase domain, and the C-terminal nuclease domain [12]. The polymerase and nuclease domains enable Mt-LigD to process the unpaired DNA ends and promote stable synapsis, while the ligase domain is in charge of the eventual repairing of DSBs [9]. This process frequently causes DNA deletions of various lengths at the break site, suggesting that NHEJ could serve as a source of deletion mutations [13]. Therefore, the NHEJ system has the potential to be developed as a powerful genetic engineering tool with application in gene deletion and genome reduction [14,15]. However,

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introducing NHEJ system from *M. tuberculosis* into *E. coli* gave relatively low ligation efficiency of chromosomal DSBs according to our previous study [14].

T4 DNA ligase is capable of catalyzing reactions such as DNA ends relaxation [16]; duplex DNA gap sealing [17]; ligation of DNA with base pair mismatched [18]; nick-closing [19] and oligomerization of bacteriophage [20,21]. These catalytic properties suggest that T4 DNA ligase is able to mediate ligation of various DNA terminis. It has been demonstrated that T4 DNA ligase repaired chromosome damage induced by restriction endonucleases or radiation in mammalian cells [22,23]. A clear reduction of chromosomal aberrations was observed when T4 DNA ligase was introduced into cells with chromosome damage by electroporation [22]. However, direct expression of T4 DNA ligase *in vivo* for the repair of DSBs has not been investigated.

Here, we demonstrated that T4 DNA ligase from Enterobacteria phage T4 alone can efficiently mediate *in vivo* DSBs repair, just as *M. tuberculosis* bi-component NHEJ system but with higher efficiency. DSBs repair mediated by T4 DNA ligase introduces wide ranges of chromosome deletions. The deletion length of chromosomal DNA can be modulated via knockout of host-nuclease RecBCD or expressing RecBCD inhibitor Gam protein from λ phage. We proposed that the T4 DNA ligase can be used to exploit new genetic engineering tools and will promote genome streamlining.

2. Material and methods

2.1. Bacterial strains and culture conditions

All *E. coli* strains used in this work are listed in Supplementary Table S1. Bacteria were routinely cultured in Luria-Bertani (LB) broth with aeration at 220 rpm at 37 °C or 30 °C as indicated. Antibiotics were added to the following concentration when needed: Chloramphenicol (25 μg/ml), Spectinomycin (50 μg/ml) and Kanamycin (50 mg/ml). *E. coli* DH5α strain was used for molecular cloning and plasmids propagation. *E. coli* strains MG1655 (Δ*recB*) was obtained by the one-step gene inactivation method described previously [24]. *E. coli* MG1655 and MG1655 (Δ*recB*) were employed as the host for CRISPR-Cas9 targeting system and the DSBs repair system. When appropriate, anhydrotetracycline (aTc) was added to a final concentration of 1 μM to induce gene expression from pLtet promoter. LB agar plate containing 0.2 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) and 40 mg/L 5-bromo, 4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal) was used for blue/white screening.

2.2. Plasmid construction

All plasmid and primers used in this study are listed in Supplementary Table S2 and Table S3. The pUCLR4 plasmid was assembled from the LR4 spacer amplified from previously reported plasmid p15A-LR4 [14] using primers gRNA Spc-F/gRNA Spc-R, the pLtet promoter amplified from pwtCas9 plasmid using pLtet-F/pLtet-R and pucOri amplified from pUC19 plasmid using Ori-F/Ori-R by Gibson assembly [25]. Phanta HS Super-Fidelity DNA Polymerase was purchased from Vazyme Biotech (Nanjing, China). To construct the Ku-LigD expression plasmid pUCLR4-Ku-LigD, the *ku* gene and the *ligD* gene were amplified from *Mycobacterium tuberculosis* H37Rv (NC_000962.3) genome using primers ku-F/ku-R and ligD-F/ligD-R respectively. The two genes were cloned under the control of constitutive PJ23104 promoter (http://parts.igem.org/Part:BBa_K1468000). The resulting PCR products were cloned into pUCLR4 backbone using Trelief™ SoSoo Cloning Kit (TsingKe Biotech, Beijing, China).

Plasmid pUCLR4-T4 was assembled from T4 DNA ligase gene amplified from Enterobacteria phage T4 using primers T4-F/T4-R and pUCLR4 backbone amplified using primers T4 (ori terminator)-F/ori (terminator)-R by Gibson assembly [25]. Plasmid pUCLR4-T4-Gam was assembled from the Gam encoding gene was amplified from pTKRed plasmid using primers and Gam-F/Gam-R pUCLR4 backbone amplified by gam (terminator pLtet)-F/gRNA-Spc (terminator)-R by Gibson assembly [25]. Both T4 DNA ligase and Gam protein genes were cloned under the control of constitutive PJ23104 promoter.

The pSC101Cas9 plasmid was assembled from the pCas9 (TS) backbone amplified using primers pSC101 ori (Cas9)-F/pSC101 ori (pLtet)-R and *cas9* amplified from pwtCas9 plasmid (Addgene plasmid #42876) using primers pLtet Cas9-F/pLtet Cas9-R by FastPfu DNA Polymerase (TransGen Biotech, Beijing, China).

2.3. In vivo circularization of linear plasmid assay

The pACYCDuet-1 plasmid was digested by restriction endonucleases *Eco*NI, *Hind*III, *Eco*RI and *Hpa*I respectively *in vitro*. The linearized plasmids were purified from agarose gels prior to electroporation. Then, equal amount of linearized plasmids were electroporated respectively into *E. coli* MG1655 containing the pUCLR4, pUCLR4 T4 or the pUCLR4 Ku-LigD plasmid. The resulting transformants were plated onto LB agar plates supplemented with chloramphenicol. Colony forming unit (CFU) was counted to quantify circularization of linear plasmid. The end-joining efficiency was calculated as the ratio of colony-forming units per nanogram of transformed linear DNA versus colony-forming units per nanogram of circular DNA.

2.4. E. coli genomic DNA double-strand breaks repair assay

Overnight culture was sub-cultured into 50 ml fresh LB medium supplemented with chloramphenicol and spectinomycin. To introduce double-strand break at the chromosomal *lacZ* gene, aTc was added to a final concentration of 1 μM to induce expression of Cas9 and sgRNA LR4. Cultures were cultured at 30 °C with aeration at 220 rpm for 2 h. Cells were collected and standardized to OD_{600} = 1.0. Then, series diluted samples were plated onto LB agar plates supplemented with Spectinomycin, X-Gal and IPTG for white/blue screening and CFU.

2.5. CRISPR-mediated DBS repair analysis

To analyze the DNA fragment resected after DSBs repair at junction site, polymerase chain reaction (PCR) analysis and Sanger sequencing were performed. For each colony analyzed, primer pairs lacZ-JF1/lacZ-JR1 and lacZ-JF2/lacZ-JR2 amplifying 3.5 kb and 6.9 kb respectively flanking the LR4 targeted site were used as primers. PCR reaction was carried out using LA Taq™ version 2.0 Plus dye DNA polymerase (TaKaRa Bio Inc, Dalian, China). Sanger sequencing was performed by Ruibiotech (Qingdao, China).

3. Results

3.1. T4 DNA ligase mediates in vivo DNA ligation

T4 DNA ligase was widely used *in vitro* for DNA ligation, but its function *in vivo* was not systematically investigated. To determine whether T4 DNA ligase also efficiently mediates DNA ligation *in vivo*, a linear plasmid circularization assay was performed. It is well known that linear DNA molecule would be degraded by the intracellular exonuclease complex. Only re-circularized plasmids can be stably replicated and endow host cell with the corresponding antibiotic resistance. Thus, the survival rate indicated by colonies formed on antibiotic agar plate is able to reflect the *in vivo* ligation efficiency of linear DNA.

The linearized plasmid pACYCDuet-1 with both sticky ends (digested by restriction endonucleases *Eco*NI, *Hind*III and *Eco*RI) and blunt ends (digested by restriction endonuclease *Hpa*I) were electroporated into *E. coli* MG1655. As shown in Fig. 1, very few colonies appeared on the control plate, which indicated the inefficient endogenous re-

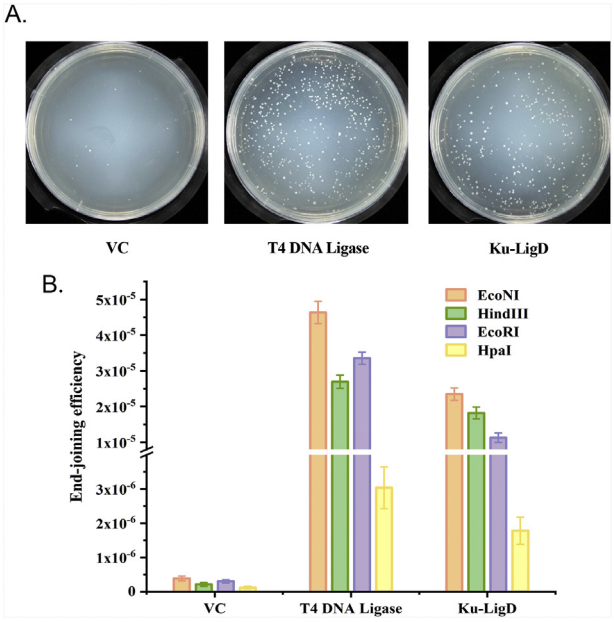

Fig. 1. T4 DNA ligase mediates DSBs repair *in vivo*. (A) Circularization of *Hin*dIII-digested linear plasmid in *E. coli* MG1655/pUCLR4 (VC), MG1655/pUCLR4-T4 (T4) and MG1655/pUCLR4-Ku-ligD (Ku-LigD) strains. Colonies formed on the chloramphenicol agar plates. (B) The end-joining efficiency of linear pACYCDuet-1 plasmids with either sticky ends or blunt-ends using T4 DNA ligase or Ku-LigD(Mt). Equal amount of linear pACYCDuet-1 plasmids with either sticky ends (digested with *Eco*RI, *Hin*dIII and *Eco*RN) or blunt-ends (digested with *Hpa*I) were electroporated into *E. coli* MG1655/pUCLR4 (VC), MG1655/pUCLR4-T4 (T4) and MG1655/pUCLR4-Ku-ligD (Ku-LigD) strains. The resulting transformants were plated on agar plates containing chloramphenicol. The end-joining efficiency was calculated as the ratio of colony-forming units per nanogram of transformed linear DNA versus colony-forming units per nanogram of circular DNA.

circularization of linear plasmid in wild-type *E. coli* cell. When T4 DNA ligase was expressed, the survival of colonies with *Hin*dIII-digested linear plasmid increased to $10^2 \sim 10^3$, hundreds times more than the control (Fig. 1A). Both of sticky and blunt ended dsDNA can be joined by T4 DNA ligase, but the ligation efficiency of sticky ended dsDNA was one order of magnitude higher than that of blunt ends (Fig. 1B). It is worth mentioning that T4 DNA ligase showed even higher linear plasmid ligation efficiency than Ku-LigD from *M. tuberculosis* (Fig. 1B), which has been shown to mediate non-homologous end-joining (NHEJ) process *in vivo* [13].

3.2. T4 DNA ligase alone repairs the chromosomal DNA DSBs generated by CRISPR-cas9

The efficient re-circularization of linear plasmid *in vivo* suggests that T4 DNA ligase maybe homologous to Ku-LigD bi-component NHEJ system and able to repair chromosomal DSBs. We then investigated whether T4 DNA ligase alone is capable of repairing chromosome DSBs like NHEJ. The CRISPR-Cas9 system was employed to introduce chromosome DSBs at *lacZ* gene. Either T4 DNA ligase or Ku-LigD bi-component proteins was co-expressed with CRISPR-Cas9 system in *E. coli* MG1655. After 2 h induction of Cas9 and sgRNA, cells were spread onto X-gal agar plate and counted to evaluate the repair efficiency of DSBs introduced by CRISPR-Cas9 system. Consistent with previous report [14], expression of CRISPR-Cas9 system targeting chromosomal *lacZ* gene killed host cells efficiently as very few CFU were observed for wild-type *E. coli* MG1655 (Fig. 2). Expression of Ku-LigD increased survival with CFU reached to $\sim 3 \times 10^2$. More importantly, expression of T4 DNA Ligase increased host cell survival even more efficiently and was ~5-fold of the strain harboring Ku-LigD (Fig. 2). This result indicated that T4 DNA ligase alone is sufficient for fulfilling the function of Ku and LigD bi-component NHEJ system and can repair the chromosomal DSBs *in vivo* with high efficiency.

3.3. DSBs repair mediated by T4 DNA ligase introduces wide ranges of chromosome deletions

With the evidence that T4 DNA ligase alone function as a reminiscent NHEJ of Ku-LigD in *E. coli*, we sought to analyze if the ligation mediated by T4 DNA ligase also introduced chromosome DNA mutation. As shown on the X-gal plates, about 60% of colonies repaired by T4 DNA ligase turned into white, indicating that *lacZ* gene has been mutated (Fig. 2). Therefore, fifty randomly selected white colonies were analyzed by PCR and DNA sequencing. Primers distributed at various distances from the target site were designed to determine the deletion length of chromosome DNA. As shown in Fig. 3, various lengths of chromosome deletions occurred during the repair of DSBs with T4 DNA ligase, of which 0–5 Kb, 5–10 Kb, 10–15 Kb, 15–20 Kb, 20–25 Kb, 25–30 Kb, 30–35 Kb and larger than 35 Kb deletions takes up 18%, 20%, 14%, 16%, 4%, 20%, 4% and 4%, respectively. Further, DNA fragments were sequenced to analyze the feature of ligation junction (Table 1). T4 DNA ligase-mediated end-joining is greatly facilitated by micro-homology at the end but not necessarily requires its presence. Also, it showed no preferential deletion length or direction towards the DSBs. Similar pattern was also found in *Arabidopsis thaliana* [26] and in *E. coli* [13] with NHEJ system repairing chromosomal DSBs.

3.4. Host-nuclease RecBCD regulates the DNA deletion length

T4 DNA ligase repaired DSBs tend to introduce large DNA deletion at the ligation junction, more than 82% of the white colonies had DNA deletions exceeding 5 Kb (Fig. 3), suggesting that the broken dsDNA ends were subjected to degradation before repaired by T4 DNA ligase. If the DNA deletions are introduced by the host DNA end processing system, the chromosome deletion size may be modulated by interfering the activity of RecBCD exonuclease. RecBCD is known the main intracellular exonuclease that involved in the degradation of linear duplex DNA [27]. The deficiency of *recB* has been demonstrated to promote the accurate repair of DSBs by the NHEJ system from *M. tuberculosis* [28].

To investigate whether RecBCD influences the DNA deletion size during DSBs repair by T4 DNA ligase, we expressed the T4 DNA ligase into a *recB* mutant strain. CRISPR-Cas9 system was employed to generate chromosome DSBs at *lacZ*. The DNA deletions of white colonies growth on the X-gal plate were analyzed by PCR and DNA sequencing. As shown in Fig. 4A, repair of DSBs in *recB*-deficient strain was inefficient compared with the wild-tpye *E. coli* strain, only $1–2 \times 10^2$ CFU growth on the plate. However, the deletion length of chromosome DNA in *recB*-deficient strain appeared to be shortened. The deletion length smaller than 3.5 Kb increased from 12% to 49%, while the deletion length larger than 6.9 Kb dropped from 88% to 46%. These indicated that RecBCD nuclease is capable of regulating the DNA deletion length. However, completely deactivation of *recB* decreased the DNA ligation efficiency, probably because of the inefficient of DNA processing (Fig. 4).

The Gam protein from λ phage inhibits the exonuclease RecBCD by competing at the DNA binding site by molecular mimicry of the DNA substrate [29]. We assumed that the inhibition of RecBCD in the presence of Gam probably modulated the DNA deletion and relieved the inefficient ligation in *recB*-deficient strain. To test this hypothesis, Gam from λ phage was expressed together with T4 DNA ligase in *E. coli* MG1655. As shown in Fig. 4B, co-expression of T4 DNA ligase with Gam did reduced the DNA deletion length. Gam expression resulted in increased population with deletion size smaller than 3.5 Kb in size (from 12% to 72%) and dramatically decreased population with deletions larger than 6.9 Kb in size (from 88% to only 24%). At the same time, the

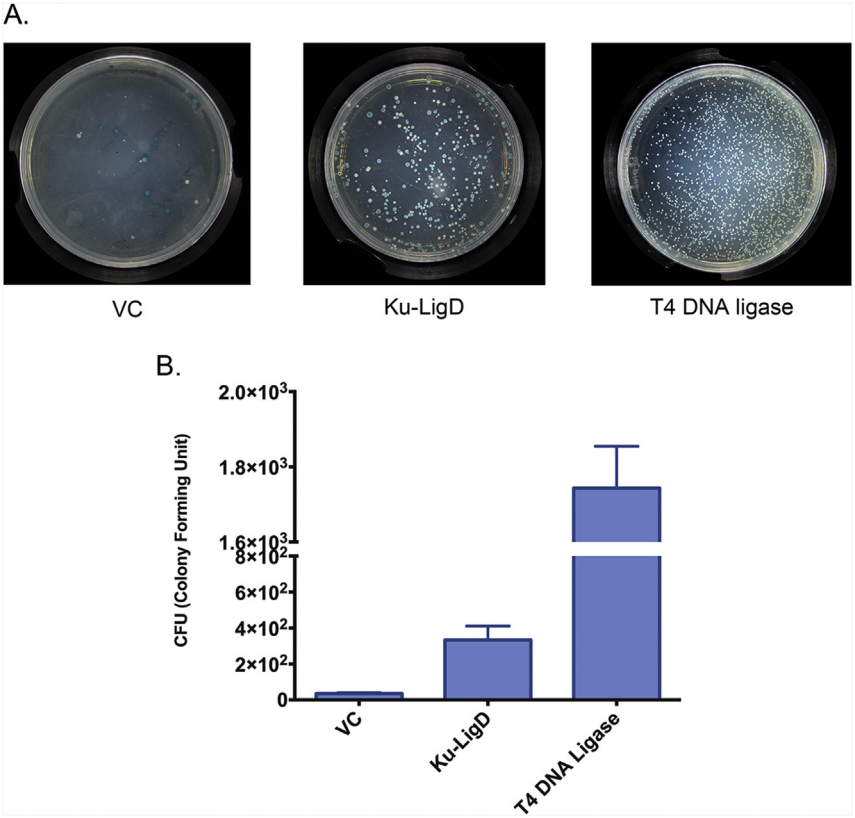

Fig. 2. T4 DNA ligase mediates DSBs repair introduced by CRISPR-Cas9. (A) Repair of DSBs introduced by CRISPR-Cas9 at *lacZ* gene locus in *E. coli* MG1655/pUCLR4 (VC), MG1655/pUCLR4-Ku-ligD (Ku-LigD) and MG1655/pUCLR4-T4 (T4) strains. Colonies formed on the X-gal agar plates. (B). The number of CFUs formed on the X-gal plates. Data shown are representative of three replicates and standard deviations were presented as error bars.

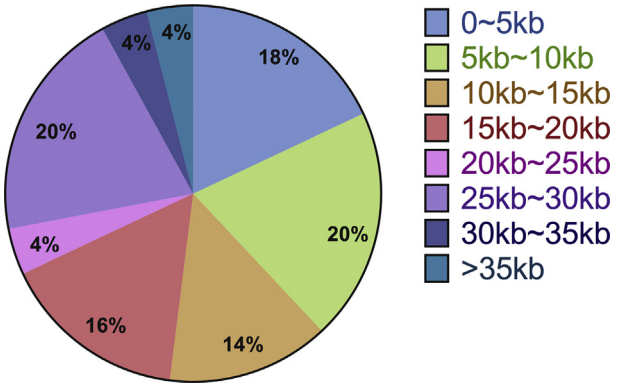

Fig. 3. Distribution of deletion lengths introduced at the junction sites with T4 DNA ligase mediated DSBs repair (sample size: 50 colonies).

DSBs ligation efficiency increased 220% compared with the *recB*-deficient strain (Fig. 4A). These results provided evidence that regulating the activity of exonuclease RecBCD was able to balance the DNA ending process and DNA fragment degradation.

Co-expression of Gam with T4 DNA ligase showed overall smaller deletion fragments in comparison to expression of T4 DNA ligase alone. To explore whether T4 DNA ligase in associated with λGam can cause smaller mutations, such as point mutations. Blue colonies were randomly selected and analyzed by PCR and DNA sequencing. The results revealed that 5 of the 24 sequenced strains contained single nucleotide mutations at the cleavage site of Cas9 without destroying the function of *lacZ* (Table S4). One of the remaining strains has a 21bp DNA deletion in the *lacZ* gene. These suggested that T4 DNA ligase in associated with Gam not only shortened the length of DNA deletions, but also was able to generate mutants with single nucleotide mutations.

4. Discussion

T4 DNA ligase has been extensively used in molecular biology applications *in vitro* given its ability of joining both sticky and blunt DNA ends [30,31]. We have established, in this work, that expression of T4 DNA ligase mediated *in vivo* DSBs repair for both linear plasmid and CRISPR-Cas9 introduced DSBs with high efficiency. The crystal structure of T4 DNA ligase shown that T4 DNA ligase had a unique structure consisting of a compact N-terminal α-helical DNA-binding domain (DBD), a nucleotidyl-transferase (NTase) domain, and a oligonucleotide-binding-fold (OB-fold) domain [32]. Both of DBD and OB-fold domains could contribute to DSBs ends binding during end joining. It has been reported that T4 DNA ligase bound to DNA ends and reduced DNA diffusivity by forming DNA-ligase complex [33]. Efficient binding of T4 DNA ligase to the DNA ends by DBD and OB-fold domains directly puts the NTase domain in close proximity for the subsequently ligation. These allow T4 DNA ligase to function as a single-component NHEJ system for DNA binding and ligation. On the contrary, DSBs repair mediated by Ku-LigD system takes two steps of initially binding of Ku to DNA ends and subsequently recruiting of LigD. It may explains how T4 DNA ligase alone is enough to mediate *in vivo* chromosome DNA DSBs repair (Fig. 2).

It was interesting to observe that T4 DNA ligase mediated genome DNA repair was more prone to deletion of larger size DNA fragments than NHEJ systems, which indicated that it may be used as a tool for genome reduction. This is very likely due to the lack of Ku for protecting the DNA ends. Also, the DNA end-joining by T4 DNA ligase was greatly facilitated by micro-homology at the DNA ends and no preferential deletion length or direction towards the DSBs. The DNA ends are likely to be degraded by intracellular nucleases, such as RecBCD [6], and the exposed sticky ends maybe more conducive to synapsis and end-joining. We showed that it was possible to regulate the DNA deletion size by manipulating DNA nucleases activity. RecBCD deficient strain reduced the chromosomal end-joining efficiency due to inefficient DNA ends resection in *E. coli* [34]. It is consistent with our

Table 1
Sequences of the DSBs junction repaired by T4 DNA ligase.

Sample	Sequence (5'-3') *	Deletion length (left,bp)†	Deletion length (right,bp)†
wt	……TAACAACCCGTCGGATTCTCCGTGGGAACAAACG…	-	-
S1	……TAACAACCCGT–GGATTCTCCGTGGGAACAAACG…	1	0
S2	……CGGTGCCGC…----------…CGCCAGGGT…	466	281
S3	……GACGTCACG…----------…ACGCCAATC…	268	1353
S4	……ATCCCAGCG…----------…CGCCATTCA…	2389	172
S5	……GTGCGGTCG…----------…CGCCATCAA…	1467	2368
S6	……CCGGATGCG…----------…CGTAACGCC…	2270	2979
S7	……AGTCACGCA…----------…ACTGGATGC…	388	5069
S8	……TGCCGTGCA…----------…GAGCGCACA…	2113	2550
S9	……AACTTTATA…----------…CCAGCCAGA…	4671	948

*Sequences colored in red represent the spacer sequences. Sequences highlighted in yellow indicate the deleted sequences. Sequences underlined are micro-homology sequences. The blue arrow indicates the cutting site of Cas9.

†The deletion length is the length of nucleotides deleted. Left and right are relative to the cutting site of Cas9.

finding that deactivation of *recB* decreased the efficiency of DSBs repair but shorten the length of DNA deletion. In fact, there is a range of strategies used by bacteriophages to manipulate DNA replication and repair in host cells [29,35,36]. Gam protein from transposable phage Mu has been reported possessed the function of reducing RecBCD activity and promoting NHEJ in concert with *E. coli* ligase [37]. The λ phase evolved an alternate mechanism to inhibit RecBCD by directly binding to the nuclease using λGam [38]. Co-expression of λGam and

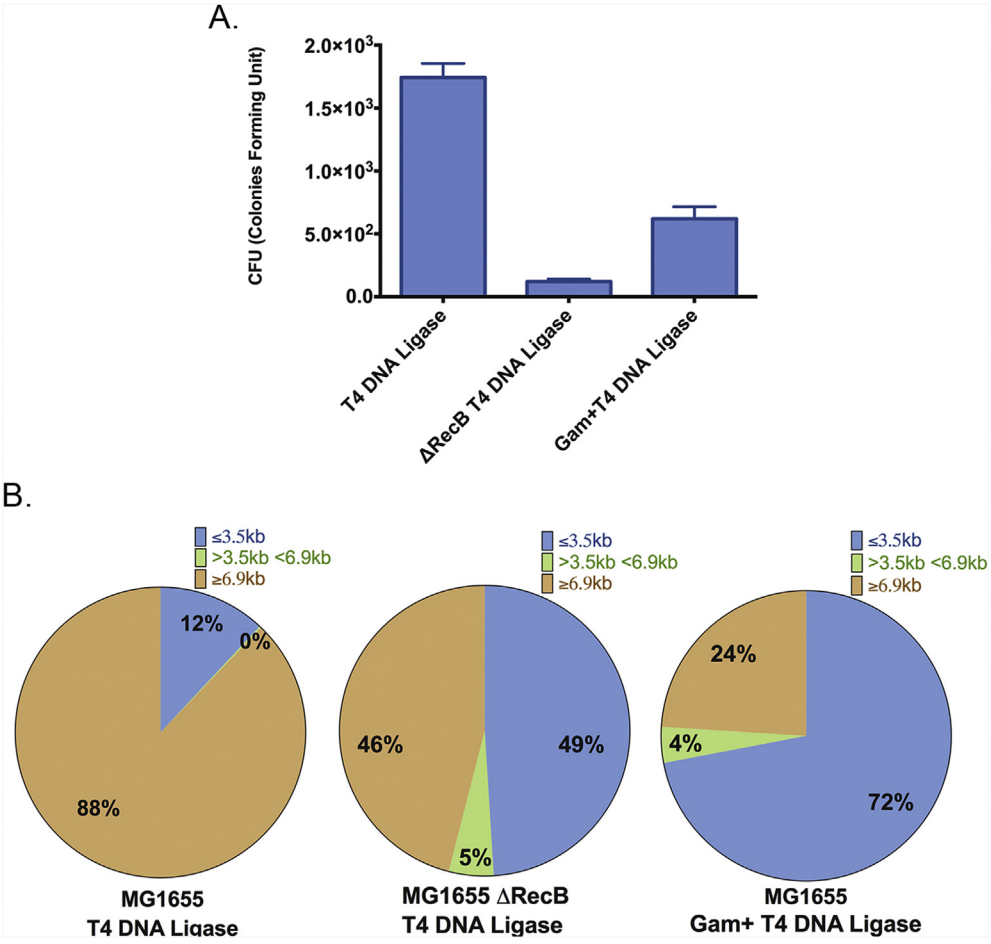

Fig. 4. The activity of host-exonuclease RecBCD interposes T4 DNA ligase mediated DSBs repair. A. Repair efficiency of DSBs introduced by CRISPR-Cas9 at *lacZ* gene locus in *E. coli* overexpressing T4 DNA ligase, *E. coli* Δ*recB* overexpressing T4 DNA ligase and *E. coli* overexpressing both T4 DNA ligase and Gam protein. Statistical of the number of CFUs on the agar plates supplemented with X-Gal and IPTG. Data shown are representative of three replicates and standard deviations were presented as error bars. B. Distribution of DNA deletion lengths introduced during DSBs repair in wild type *E. coli* overexpressing T4 DNA ligase, *E. coli* Δ*recB* overexpressing T4 DNA ligase and wild type *E. coli* overexpressing both T4 DNA ligase and Gam protein (sample size: 50 colonies).

T4 DNA ligase, in this study, showed overall smaller deletion fragments in comparison to expression of T4 DNA ligase alone (Fig. 4B). Meanwhile, single nucleotide substitutions was also observed among blue colonies. Since T4 DNA ligase itself does not have polymerase activity, other intracellular DNA polymerases are likely to be involved in this rare single base substitution event. The possibility of controlling the introduced DNA deletions size makes the T4 DNA ligase mediated-DNA ligation a suitable tool not only for genome reduction but also for genome editing. It is possible to explore a set of proteins that modulate the host cell nuclease activity to achieve manageable range of deletion size in the future.

In summary, we demonstrated that T4 DNA ligase could mediated DSBs ligation efficiently *in vivo* as a single component non-homologous end joining system and introduced variable length chromosome excisions. DNA deletion size can be managed by modulating the activity of host exonuclease RecBCD, providing us a potential genetic tool for gene deletion and genome reduction. Furthermore, expression of T4 DNA ligase was also able to improve the efficiency of microbial mutation breeding using DSBs-inducing mutagens (unpublished data). Since the entire system is only composed of a single T4 DNA ligase, this technology is expected to be applied in different microorganisms. These indicate that intracellular expression of T4 DNA ligase will have a broader application scope in the field of biotechnology.

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Conflicts of interest

The authors declare no financial or commercial conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2019.04.001.

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