論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	A biochemical study on the site-specific recombinase XerA from the archaeon Thermoplasma acidophilum
著者(和文)	JoMinji
Author(English)	Minji Jo
出典(和文)	学位:博士(理学), 学位授与機関:東京工業大学, 報告番号:甲第10607号, 授与年月日:2017年7月31日, 学位の種別:課程博士, 審査員:岩﨑 博史,一瀬 宏,相澤 康則,中戸川 仁,梶川 正樹
Citation(English)	Degree:Doctor (Science), Conferring organization: Tokyo Institute of Technology, Report number:甲第10607号, Conferred date:2017/7/31, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（論文博士）

論　文　要　旨（英文）　（800語程度）

（Summary）

報告番号	乙第　　号	氏　名	Jo　Minji

（要　旨）

In organisms with circular chromosomes, an odd number of homologous recombination (HR) events between circular chromosomes can induce a chromosome dimer generated from two circular chromosome monomers. This chromosome dimer cannot be segregated to daughter cells, resulting in a failure of inheritance of genomic information. To resolve these dimers and ensure faithful inheritance of the genome, Xer-mediated site-specific recombination has evolved. The Xer family of site-specific recombinases have been extensively studied in the bacterium *Escherichia coli*, where two tyrosine-recombinases, XerC and XerD, form a complex that resolves chromosome dimers. The XerC and XerD recombinases bind to a specific chromosomal site known as *dif* and mediate site-specific recombination at this site. An ATP-dependent DNA translocase, FtsK, further promotes the resolution of chromosome dimers by activating XerC and XerD to mediate recombination. Like bacteria, archaea also have circular chromosomes, thus a system for resolution of chromosome dimers also exists. In contrast, only a single orf (referred to as XerA) with sequence similarity to common Xer recombinases has been found in the genome of the archaeon *Thermoplasma acidophilum*. No apparent FtsK homologue can be identified in this organism's genome. Here, we provide evidence that XerA is a site-specific recombinase that can resolve chromosome dimers in *T. acidophilum*.

By employing ChIP-Seq analysis with an anti-XerA antibody, we identified two potential XerA binding sites in the *T. acidophilum* genome, which we named Peak 1 and Peak 2. ChIP-qPCR experiments suggested that, of these two tentative *dif* sites, Peak 2 has a higher affinity for XerA than Peak 1. To confirm whether the identified XerA binding sites correspond to the *dif* sites of XerA in *T. aciophilum*, plasmid dimers containing either Peak 1 or Peak 2 sites were generated. In vitro site-specific recombination assays with XerA revealed that the plasmid dimer carrying the Peak 2 site generated monomeric sized plasmids as recombination products. Sequence alignment of recombination sites in other archaeal species allowed us to minimalize the two peaks to two sequences, *dif1* (27 bp) and *dif2* (28 bp), which contain consensus *dif* sequence in their left and right arms. Notably, the *dif2* site contains a 6 bp spacer between the two arms, whereas the *dif1* site has a 5 bp spacer. In addition, one nucleotide (C) of the right arm in *dif1* is different from one (T) in *dif2* site. Similarly to the plasmid dimers, I also generated plasmids containing two direct repeat of either *dif1* or *dif2* site and confirmed XerA-mediated site-specific recombination. As expected, XerA generated recombination products through both intra- and

inter-recombination between plasmids with two *dif2* sites.

To further elucidate the molecular mechanism of XerA-mediated site-specific recombination, I adopted a half-site strand-transfer assay. Two left- and right-half sites containing left or right arm (putative XerA binding sites) and a 6 nt spacer region were used as substrates. This assay revealed that the XerA monomer binds to each half site to form a covalent complex and mediates recombination between the cleaved half-sites. Furthermore, electrophoretic mobility shift assays using full and half-sites of *dif1* and *dif2* sequences revealed that XerA binds differentially to *dif1* and *dif2* sites. That is, XerA-mediated site-specific recombination requires two XerA binding to each left and right arm on the *dif* site and a XerA monomer firstly binds to the left arm and another XerA monomer binds to the right arm in *dif* site. In order to validate the critical nucleotides in the *dif2* site for XerA recognition and binding, I generated a series of *dif2* mutations. Point mutations were introduced into each consensus arm of *dif2* sequence including flanking region and gel shift assays (with full and half sites) and half-site strand-transfer assays with those mutated *dif2* sequences were performed. The results indicated that not only the core consensus sequence (left and right arm) of *dif2*, but also the flanking regions (including the spacer region) play important roles in the XerA-recognition and site-specific recombination reactions mediated by XerA in *T. acidophilum*. I also sought to identify *dif1*-like sequences on other archaea genomes, and we may have found similar sequences in other archaea suggesting that they may play an important role *in vivo*.