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XerC-mediated DNA inversion at the inverted repeats of the UU172-phase-variable element of *Ureaplasma parvum* serovar 3

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ABSTRACT

Phase variation of the UU172 phase-variable element of *Ureaplasma parvum* is governed by a DNA inversion event that takes place at short inverted repeats. The putative tyrosine recombinase XerC of *Ureaplasma* has been suggested as a mediator in the proposed site-specific recombination event. Here, we provide evidence that XerC mediates DNA inversion at the inverted repeats located on a synthetic locus that was introduced into the model organism *Escherichia coli*. Synthetic loci were created by exchanging the genes UU171 and UU172 with the two reporter genes *gfp* (green fluorescent protein) and *mrfp1* (monomeric red fluorescent protein 1) either containing or missing the inverted repeats of the UU172 phase-variable element. *E. coli* was transformed with these loci and also co-transformed with the expression vector pBAD24 that contained the *xer*C gene behind the arabinose inducible pBAD promoter. Upon XerC expression, DNA inversion was observed only in the locus that contained the inverted repeat regions. We also demonstrate that XerC can process the recombination event with both an N-terminal maltose binding protein tag and a C-terminal 6×His tag in *E. coli*. A XerC mutant, where the proposed catalytic tyrosine residue 228 was exchanged with an alanine, did not process the recombination event.

Introduction

Ureaplasma urealyticum and *Ureaplasma parvum* are potential pathogens and commensals of the human urogenital tract. Non-gonococcal/non-chlamydial urethritis in men, chorioamnionitis, spontaneous abortion, preterm labor in pregnant women and bronchopulmonary dysplasia in neonates are only a few of the complications that have been attributed to these species (Waites et al. 2005), yet little is known about the virulence factors of this microorganism. The highly immunogenic multiple banded antigen (MBA), a strongly expressed size- and phase-variable membrane protein (Monecke et al. 2003; Zheng et al. 1994), has the potential to induce inflammatory responses both *in vitro* (Shimizu et al. 2008; Triantafilou et al. 2013) and *in vivo* (Uchida et al. 2013), all good criteria for classifying MBA as a potent virulence factor.

Aside from the proteins expressed from the phase-variable *mba* locus, other loci likewise contribute to antigenic variation and membrane plasticity of this bacterium, such as the membrane proteins expressed from the UU172 phase-variable element, an *Ureaplasma*-specific locus present in both human *Ureaplasma* species. Both, the *mba* locus and the UU172 phase-variable element share in common, that alternating expression is governed by a DNA inversion event that takes place at short inverted repeats (Zimmerman et al. 2009, 2011), a mechanism that is usually processed by site-specific recombinases. In a previous study, the putative recombinase XerC (NP_078055.1) was shown to bind to the inverted repeats of both phase variable loci *in vitro* (Zimmerman et al. 2013). Also, a XerC-mediated DNA inversion at the inverted repeats of the *mba* locus had been suggested, based on studies conducted with the model organism *M. pneumoniae*. This organism was co-transformed with the *mba* locus and the *xerC* behind an active promoter. Although DNA inversion in subclonal lineages was observed, results were incomplete because *xerC* did not remain present in the cells, wherefore the reversible DNA inversion and alternating expression was not observed.

The goal of this study was to provide evidence that XerC is a tyrosine recombinase that can mediate DNA inversion at the inverted repeats of the UU172 phase-variable element. To reach these goals, the suggested catalytic tyrosine at position 228 of the XerC protein was mutated to alanine and the mutated and the native protein was expressed in clonal lineages of *Escherichia coli* that also contained a synthetic UU172 phase-variable element with or without the inverted repeats. Upon expression of XerC, the recombination

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event was monitored by restriction analysis of the plasmids containing the synthetic phase-variable element.

Materials and methods

Cultivation and transformation of microorganisms

E. coli Top10 F′ (F´{lacIq Tn10 (TetR)} mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara-leu)7697 galU galK rpsL endA1 nupG) (LifeTechnologies) was grown in broth or on agar containing 10 g L^{-1} trypton, 10 g L^{-1} yeast extract and 5 g L^{-1} NaCl. Competent cells were transformed by heat shock at 43 °C for 2 min.

Purification of recombinant proteins

The recombinant MBP (maltose binding protein) fusion protein MBP::XerC-6×His was purified as described (Zimmerman et al. 2013) by affinity chromatography over amylose resin according to the manufacturer's protocol (NEB; http://www.neb.com/nebecomm/manualfiles/manuale8000.pdf, Affinity Chromatography, Method I). The elution was then further purified over Ni^{2+}-charged ProBond™ Resin (LifeTechnologies) and eluted with buffer (50 mM Tris, 300 mM NaCl, pH 7.5).

Electrophoretic mobility shift assay (EMSA)

EMSA analysis was carried out with the LightShift® Chemoluminescent EMSA Kit (PIERCE) according to the product manual in a final volume of 20 μl. A 39 bp double stranded DNA substrate IR_{UU172} (5′-TTAAATAATGATAATTTAAATTATCAAACAGTAACTTTT-3′) was generated by annealing two 5′-biotinylated oligonucleotides in 10 mM Tris/HCl pH 7.5, 100 mM NaCl and 1 mM EDTA during a temperature gradient from 85 °C to 25 °C. Per EMSA reaction, 10 fmol biotinylated IR_{UU172} was used. The reaction time was 20 min. Samples were loaded onto 8% polyacrylamide gels in 0.5 × TBE. Gels were pre-run for 45 – 60 min at 10 mA and 80 V before samples were loaded and samples were run at the same electrophoretic conditions.

Western blot

Total cell protein was separated by 10% SDS/PAGE and transferred to nitrocellulose by semi-dry electroblotting. The His-tagged UU172N::Mrfp1 and all XerC proteins were detected with a polyclonal α-Mrfp1-His antibody. The antibody against the monomeric red fluorescent protein 1 (Campbell et al. 2002) was kindly provided by R. Herrmann.

Construction of gfp

A synthetic *gfp* pseudogene (accession #: LN515608) with optimized codon usage for *M. pneumoniae*, that also encoded a C-terminal 7×-His tag, was generated as described (Fuhrmann et al. 1999). Protocol for synthesis: Fifteen forward primers (each app. 50 nt in length), comprising the *gfp* sequence, were phosphorylated in an equal molar ratio (500 pmol total) in a final volume of 100 μl with 10 μl ATP (2 mM), 10 U T4-PNK (New England BioLabs), PNK buffer and H_2O for 4 h. The forward ssDNA was synthesized in a final volume of 100 μl, using 10 μl of phosphorylated forward primer mixture, reverse primer mixture (50 pmol total), *Pfu* DNA ligase buffer and 12 U *Pfu* DNA ligase (Stratagene). The reaction was carried out in an Eppendorf thermocycler. The mixture was preheated for 3 min at 95 °C and 3 min at 80 °C before adding the heat stable ligase. The following cycle was repeated three times: 1 min 95 °C, a temperature drop to 70 °C within 1 min followed by a linear drop to 65 °C within 1 h, and 2 h at 65 °C. The ligation mixture was ethanol precipitated and resuspended in 50 μl 1 × TE buffer. The gene was amplified by PCR, using 1 μl of ligation mixture per 50 μl PCR reaction mixture. The addition of an *Eco*RI restriction site at the 5′-end and an *Xho*I site at the 3′-end allowed cloning into the expression vector PGEX-5x-3 (Amersham), in frame with the glutathione S-transferase gene. Transformed *E. coli* clones that showed positive fluorescence were used for further cloning experiments. An *Xho*I endonuclease restriction site at the 5′-end and a *Bam*HI site at the 3′-end was used for ligation into the vector pMT (see below).

Construction of plasmid vectors

pMT-gfp::172IR::mrfp1 and pMT-gfp::172::mrfp1

Two PCR products (172IR and 172) were amplified from the UU172 phase-variable element. Product 172IR was synthesized with primers (5′-GG**CTCGAG**TACTGGTGTAGAGTTTTGATT-3′) and (5′-GG**CCCGGG**TTTTGGTTCTAATTTTTGATC-3′) and contained the inverted repeats. Product 172 was synthesized with primers (5′-GG**CTCGAG**ATCATTATTTAAAACTAATGC-3′) and (5′-GG**CCCGGG**ATAATTTATATTAACTTTAATTTA-3′) and was missing the inverted repeats. Both PCR products were digested with restriction endonucleases *Xho*I and *Sma*I (letters in bold) and were ligated together with the *Xho*I/*Bam*HI-digested *gfp* pseudogene into the vector pMT-*mfrp1* (Zimmerman and Herrmann 2005), which contained an *mrfp1* pseudogene with a 5′ *Sma*I endonuclease restriction site prior to the second codon (Fig. 1). The final plasmids were designated pMT-*gfp*::172IR::*mrfp1* and pMT-*gfp*::172::*mrfp1*.

pBAD-xerC and pBAD-xerCmut

The *xerC* gene encoding a C-terminal 6×His tag (accession #: HG977142) was ligated between the endonuclease restriction sites *Nco*I and *Hind*III of the plasmid pBAD24 (Guzman et al. 1995). The final construct was designated pBAD-*xer*C.

The amino acid exchange Y232A was introduced into XerC-6×His through primer mutagensis, exchanging the original triplet TAT with GCA. Two PCR products were synthesized with primer pairs (5′-ATGCCATAGCATTTTTATCC-3′ and 5′-GG**AGTACT**-AAACGTTGTTTGAATGTTTGCATG-3′) and (5′-GG**TGCGCA**TTAAAT-TTAAATGAACAAACAAATCG-3′ and 5′-GCGTTTCACTTCTGAGTTCG-3′), using pBAD-*xer*C as template. The 5′ product was digested with *Nco*I/*Sca*I and the 3′ product was digested with *Fsp*I/*Hind*III. The ligated gene product (accession #: HG977143) was cloned in pBAD24 and the final construct was designated pBAD-*xer*Cmut.

pBAD-malE::xerC

The gene *malE*, encoding the maltose binding protein (MBP) was linked in frame to the *xerC*-6×His gene and the fusion construct was cloned into pBAD24. The final construct was designated pBAD-*malE*::*xer*C.

Arabinose induction of XerC proteins

E. coli Top10 F′ that were co-transformed with plasmids (Table 1) for inversion experiments were grown overnight at 28 °C in broth

Table 1
Clones for arabinose induction experiments. *E. coli* Top10 F′ were co-transformed with pMT and a pBAD plasmid vectors generating clones C1–C5.

Designation of clone	Plasmid 1	Plasmid 2
C1	pMT-*gfp*::172IR::*mrfp1*	pBAD24
C2	pMT-*gfp*::172IR::*mrfp1*	pBAD-*xerC*
C3	pMT-*gfp*::172::*mrfp1*	pBAD-*xerC*
C4	pMT-*gfp*::172IR::*mrfp1*	pBAD-*xer*Cmut
C5	pMT-*gfp*::172IR::*mrfp1*	pBAD-*malE*::*xerC*