Interaction of the putative tyrosine recombinases RipX (UU145), XerC (UU222), and CodV (UU529) of Ureaplasma parvum serovar 3 with specific DNA

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Abstract
Phase variation of two loci (‘mba locus’ and ‘UU172 phase-variable element’) in Ureaplasma parvum serovar 3 has been suggested as result of site-specific DNA inversion occurring at short inverted repeats. Three potential tyrosine recombinases (RipX, XerC, and CodV encoded by the genes UU145, UU222, and UU529) have been annotated in the genome of U. parvum serovar 3, which could be mediators in the proposed recombination event. We document that only orthologs of the gene xerC are present in all strains that show phase variation in the two loci. We demonstrate in vitro binding of recombinant maltose-binding protein fusions of XerC to the inverted repeats of the phase-variable loci, of RipX to a direct repeat that flanks a 20-kbp region, which has been proposed as putative pathogenicity island, and of CodV to a putative dif site. Co-transformation of the model organism Mycoplasma pneumoniae M129 with both the ‘mba locus’ and the recombinase gene xerC behind an active promoter region resulted in DNA inversion in the ‘mba locus’. Results suggest that XerC of U. parvum serovar 3 is a mediator in the proposed DNA inversion event of the two phase-variable loci.

Introduction
Ureaplasma (U.) parvum and U. urealyticum are commensals and potential pathogens of the human genital tract. The organism has been associated with nongonococcal, nonchlamydial urethritis in men, chorioamnionitis in pregnant women as well as bronchopulmonary dysplasia in newborn infants (Waites et al., 2005). Fourteen serovars have been identified, of which serovars 1, 3, 6, and 14 belong to the U. parvum species and the remaining to the U. urealyticum species (Robertson et al., 2002). The genomes of all 14 described serovars have been sequenced (Glass et al., 2000; Paralanov et al., 2012).

Both species express a distinct immunodominant, size-and phase-variable surface protein, the multiple-banded antigen, whose gene is one member of a paralogous gene family dispersed throughout the chromosome (Teng et al., 1994; Zheng et al., 1994, 1995; Glass et al., 2000; Monecke et al., 2003). In U. parvum serovar 3, two loci (‘mba locus’ and ‘UU172 phase-variable element’) have been identified that undergo high-frequency phase variation that is achieved by site-specific DNA inversions at short inverted repeats (Fig. 1a and b). Phase variation between UU375 (GenBank: AAF30784.1) (mba for multiple banded antigen) and UU376 (GenBank: AAF30785.1) (upvmp for Ureaplasma phase-variable membrane protein) is believed to be the result of site-specific DNA recombination at the inverted repeats 5′-ATTTG AATTATCAAACAGAAAAAG-3′ and occurs when the ORFs are oriented in opposite directions (Zimmerman et al., 2009). The second, more conserved phase-variable locus among the Ureaplasma species ‘UU172 phase-variable element’, like the ‘mba locus’ of U. parvum serovar 3, comprises two coding sequences (UU172 and UU171), which are oriented in opposite direction. Two inverted repeats (5′-ATAATTAAAAATTATCAACACAGTTTTTGAAACAGTTCT-3′), one located in the 5′ sequence of UU172 and another in the intergenic spacer region between UU172 and UU171,
share partial identity (letters in bold and Fig. 1c) to the inverted repeats of the ‘mba locus’. It is believed that phase-variable expression of the UU172 element is governed by site-specific DNA inversion analogous to that occurring in the ‘mba locus’ (Zimmerman et al., 2011).

Three potential tyrosine recombinases (RipX, XerC, and CodV) have been annotated in the genome of U. parvum serovar 3 (Glass et al., 2000). To date, these three proteins have neither been functionally characterized nor have their binding sites been determined. Of the three genes, ripX (UU145) is located near the ‘mba locus’ in the ATCC strains of serovars 4, 5, 6, 7, 8, 9, 10, 11, and 12, suggesting an involvement of RipX in the site-specific recombination event in the ‘mba locus’. The gene is, however, also located at the boundary of a 20-kbp genomic region that has previously been proposed as a potential pathogenicity island (Momynaliev et al., 2007).

Absence of this 20-kbp region and ripX has been documented for serovars 1, 2, 13, 14 (Paralanov et al., 2012), and clinical isolates of serovars 1 and 6 (Momynaliev et al., 2007), which questions the protein’s involvement in the site-specific recombination event of the phase-variable loci. Two 22-bp direct repeats (5′-TAATCGTGATTATTGAACCTTG-3′) that are located at the boundaries of the 20-kbp region in serovar 10 suggest that the region has been acquired by horizontal gene transfer. Mobility of the region can be inferred from its different location in serovar 3, where the region disrupts a gene encoding a putative membrane protein of the ‘UU172 phase-variable element’ (Zimmerman et al., 2011).

Recombinases belonging to the tyrosine family are integrases that recombine DNA duplexes by executing
two consecutive strand breakage and rejoining steps and a
topoisormerization of their substrate (Esposito & Scocca,
1997). The first member of this family that was described
is the λ-Int protein, which promotes integration and exci-
sion of the phage genome from that of the host (Nash,
1981). Other family members related to the λ-Int, such as
the Flp from the yeast 2μ plasmid, the XerC/D of Escheri-
chia (E.) coli, the Cre recombinase of phage P1, the HvsR
of Mycoplasma (M.) pulmonis, and the Xer1 of M. agalac-
tiae, function in the amplification/maintenance of plasmid
copy number (Hoess et al., 1984), the elimination of
chromosome dimers from replicated chromosomes
(Hayes & Sherratt, 1997), the cyclization of virion DNA
and the life cycle of temperate phages (Sternberg et al.,
1986), the alteration of the type I restriction modification
system and of cell-surface components (Sitarman et al.,
2002), and in phase variation of membrane proteins
(Czurda et al., 2010), respectively. In E. coli, the proteins
XerC and XerD (CodV and RipX in Bacillus subtilis) act
in concert at a sequence designated dif ‘deletion-induced
filamentation’ to resolve dimeric chromosomes after chro-
mosome replication (Blakely et al., 1991, 1993; Sciochetti
et al., 1999, 2001). The dif site is usually a 28-nucleotide
motif associated with the chromosome’s replication ter-
minus and serves as template for chromosome dimer res-
olution. The sequence often contains palindromic motifs
separated by a central hexanucleotide. In numerous bacte-
ria, each side of the dif sequence is specifically targeted by
one of the two Xer recombinases. An exception to this
was documented for Bacillus subtilis

\[ \text{dif} \]

was found in the mba locus’ and the ‘UU172 phase-variable element’,
(ii) the two potential dif sites, and (iii) the direct repeat
flanking the 20-kb region. We demonstrate protein–DNA
interaction for the three recombinases and discuss their
possible functional roles.

**Materials and methods**

**Southern blot**

Genomic DNA from *U. parvum* serovar 3 cultures
(strains ATCC 27815, DRI, M14, V397, V890, V892)
was isolated as described (Zimmerman et al., 2011) from
500 mL overnight cultures. DNA pellets were air-dried
and re-suspended in 100 μL 1 × TE buffer for digestion
with HindIII. The digested DNA (20 μL per lane) was
separated in a 1% agarose gel and transferred onto nylon
membranes (Sambrook et al., 1989). Three DIG-
deoxyUTP-labeled PCR products were synthesized with
recombinant Taq DNA polymerase for use as hybridiza-
tion probes: UU145 (#145) with primers 5′-GGGATCC
CATGGAGCGACAAAGATATG-3′ and 5′-CGAAGCTTTAT
TATCATTTTCAAATTC-3′, UU222 (#222) with primers
5′-GGGATCCATGAAAGATATTTATAGATA-3′ and 5′-CGAAGCTTTATCTGATCATATTGGG-3′, and UU529
(#529) with primers 5′-GGGATCCTGAAATATTTAT
AAAT-3′ and 5′-CGAAGCTTTAATACCTTTAT-3′.
Hybridization and detection were carried out as described
(Zimmerman et al., 2011). Hybridization was carried out
in 5× SSC/1% SDS at 53°C. In two separate blots,
UU222 was detected prior to detection of either UU145
or UU529.

Genomic DNA from *Mycoplasma pneumoniae* M129
was isolated as described above from adhesive cells grow-
ing in 75-cm² cell culture flasks and was re-suspended
in 300–500 μL 1 × TE. Genomic DNA was digested with
HindIII and BglII. Three DIG-deoxyUTP-labeled PCR
products were synthesized for use as hybridization probes:
400 bp of the 5′ region of the gentamicin resistance gene
from plasmid pMT85 (Zimmerman & Herrmann, 2005)
with primers 5′-GATGATGTATTTCCTTTGTAG-3′ and
5′-ATGCCTTTATGCTTGTGAT-3′, the repeat region of
the mba gene with primers 5′-ATGGGATCCTACATCAC
AACAGGT-3′ and 5′-TTATTTCCAGATGATT-3′, and
322 bp of the 5′ region of UU376 with primers 5′-AT
CTGGACTCCAGCTCC-3′ and 5′-TTCAATCCTACACCT
TGAAAT-3′.

**Purification of recombinant proteins**

**MBP::RipX, MBP::XerC, and MBP::CodV**

Three recombinant proteins were expressed as fusions
with the maltose-binding protein (MBP) of the expres-
sion vector pMAL-c2X (New England Biolabs) and puri-

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Eurofins MWG Operon, with optimized codon usage for E. coli (accession # HF558294 and HF558295). Genes were fused between the restriction sites BamHI and HindIII of pMAL-c2X and constructs were cloned in E. coli DH10B (Invitrogen). Fusion proteins MBP::XerC and MBP::RipX were expressed from 400 mL broth cultures for 2 h with 0.5 mM IPTG. Fusion protein MBP::CodV was purified from 7 L broth culture. The soluble fractions of cell lysates were loaded onto 5 mL amylose, and fusion proteins were purified as described by the manufacturer (NEB; pMAL™ Protein Fusion and Purification System (Expression and Purification of Proteins and Cloned Genes) Instruction Manual, #E8000S Version 5.3 11/07, Affinity Chromatography, Method 1).

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) analysis was carried out with the LightShift® Chemoluminescent EMSA Kit (PIERCE) according to the product manual. Reactions were carried out in a final volume of 20 μL at 20°C for 20 min, prior to loading onto a polyacrylamide gel in 0.5 × TBE buffer. Labeled DNA always had a concentration of 10 fmol per reaction. The protein concentration was 500 ng (ca. 490 nM for MBP and 340 nM for fusion proteins) per reaction, and the MgCl2 concentration was 7.5 mM, unless otherwise specified.

A 145-bp PCR product with the 24-bp IRmba located between positions 87 and 111 was synthesized from the mba locus with biotinylated primers 5′-ATCGATAAACATTATTAGATAT-3′ and 5′-TTGTTGGCTTGGAGCTGAAG-3′.

Short double-stranded DNA was generated by annealing 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. The following biotinylated probes (Fig. S2) were constructed: IRmba (5′-TTCAAAAGTCTACCTTCTGTTGATAATTCAAAATGATAATTAAATTATCAACAGAATTCTT-3′), IRuu222 (5′-TTAAATGATATTAATTATATTATATTAT-3′), IRfu227 (5′-TGAAGGAAATAGGATATGAGGTAATAC-3′), and DR20kb (5′-AA CAAGGTGACAATACGATTATTTA-3′). Two non-biotinylated competitor DNA probes were generated: IRmba (5′-TTACCTTCTGTTGATAATTCAAAATGATAATTAAATTATCAACAGAATTCTT-3′) and IRuu222 (5′-TTAAATGATATTAATTATATTATATTAT-3′).

Construction of vectors for transformation of M. pneumoniae

Construction of pMT::mba\textsuperscript{trunc}

An ‘mba locus’ was constructed by ligating two PCR products together, exchanging the TGA codon in the 5′ region of the mba gene to TGG and adding a HindIII restriction site 3′ to the stop codon of the mba gene. This mba locus was digested with the restriction endonucleases HindIII and HpaI, truncating the UU376 gene at the 3′ end by 21 nucleotides (six amino acids) with HpaI to eliminate the third IRmba found in the intergenic region 3′ of UU376. This truncated locus (mba\textsuperscript{trunc}) was ligated between the BstZ17I and HindIII sites of a modified Tn4001 vector plasmid pMT85 (Zimmerman & Herrmann, 2005) that contains a resistance cassette against gentamicin, yielding pMT::mba\textsuperscript{trunc} (Fig. S3). Mycoplasma pneumoniae M129 was transformed with pMT::mba\textsuperscript{trunc} by electroporation (Hederyda et al., 1993), and a clone (MPmba\textsuperscript{trunc}) with single genomic integration at position 495,321 at the 3′ end of the hypothetical gene MPN411 was chosen for further experiments.

Construction of pCT::UU222

Gene UU222 was PCR amplified from genomic DNA of U. parvum serovar 3 and ligated with a 275-bp upstream region of UU529 (529P) that served as active promoter, using an NdeI site as linker between promoter and gene. Activity of the putative promoter region in M. pneumoniae was first tested by linking the 275 bp to the gene mrfp1 (Campbell et al., 2002) in pMT85 (Zimmerman & Herrmann, 2005) and following Mrfp1 (monomeric red fluorescent protein) expression by Western blot from subclones (Fig. S4). The 529P::UU222 construct was ligated between the BamHI and EcoRI sites of the modified Tn4001 vector plasmid pCT461 (Herrmann, unpublished) that contains a resistance cassette against chloramphenicol, yielding pCT::UU222. Clone MPmba\textsuperscript{trunc} was transformed with pCT::UU222 as described above.

Results

Screening of recombinase genes in U. parvum serovar 3 strains

Three recombinases belonging to the tyrosine family have been annotated in U. parvum serovar 3 (ATCC 700970) (Glass et al., 2000). In this strain, the genes received the locus tags UU145, UU222, and UU529 for ripX, xerC, and codV, respectively. Of the three potential tyrosine recombinases, only orthologs of xerC have been annotated in all 14 Ureaplasma serovars (Paralanov et al., 2012); ripX seems to be absent in several strains, while codV has been annotated only in U. parvum strains (Table S1). Momynaliev et al. (2007) likewise documented the absence of ripX and codV in several U. parvum strains. We carried out Southern blot analyses with genomic DNA from five clinical U. parvum serovar 3 strains and the sequenced type strain ATCC 27815\textsuperscript{T} and screened for
the presence of the three recombinase-encoding genes with gene-specific probes. Results indicated that only orthologs of xerC (UU222) are present in all six strains (Fig. 2); UU529 was detected in three strains, while UU145 was found present only in the type strain.

**In vitro binding of fusion proteins to DNA substrates**

All three putative recombinase genes were cloned and expressed in *E. coli*. After removal of internal TGA codons, genes were cloned into the expression vector pMAL-c2X as fusions with the MBP encoding gene. This system was chosen, as His-tagged fusions of XerC proved to be highly insoluble (data not shown). Expression of

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**Fig. 2.** Southern blot detection of genes: ripX, xerC, and codV. Detection of UU145, UU222, and UU529 in different *Ureaplasma parvum* serovar 3 strains (lanes 1–6: ATCC27815ᵀ, DR-1, M14, V397, V890, V892) that showed phase variation in the ‘mba locus’ and the ‘UU172 phase-variable element’. Chromosomal DNA was digested with HincII, separated in a 1% agarose gel, and transferred onto nylon membranes. Expected fragment sizes: 3193 bp for #222, 6890 bp for #145, and 6201 bp for #529. Bands were detected with Dig-11-dUTP-labeled PCR probes (#145, #222, #529) comprising the entire sequences of the genes UU145, UU222, and UUS29.

**Fig. 3.** Binding of RipX, XerC, and CodV to substrate DNA. (a) Protein–DNA interaction of purified proteins and the soluble protein fraction of *Escherichia coli* DH10B with different biotinylated substrate DNAs. For each reaction, 250 ng of protein was used (except MBA::CodV, where 400 ng was used). Specific interactions of recombinant fusion proteins with substrate DNA are labeled with an asterisk. (b) Specific binding of XerC to IR<sub>mba</sub>. EMSA analysis using a purified MBP::XerC fusion (●) and a biotinylated (*) PCR product of 145 bp containing one inverted repeat (◄) (IR<sub>mba</sub>). Lane 1, PCR; lane 2, MBP::XerC; lane 3, PCR + MBP::XerC; lanes 4–7, PCR + MBP::XerC + increasing concentrations of a short 23-bp IR<sub>mba</sub> competitor DNA (1, 3, 10, and 30 pmol); lane 8, MBP; lane 9, PCR + MBP. (c) Binding of XerC to IR<sub>UU172</sub>. EMSA analysis using purified MBP::XerC or MBP::RipX and the biotinylated inverted repeat IR<sub>UU172</sub>. Lane 1, IR<sub>UU172</sub>; lane 2, IR<sub>UU172</sub> + MBP::RipX; lane 3, IR<sub>UU172</sub> + MBP::XerC; lanes 4–7, IR<sub>UU172</sub> + MBP::XerC + increasing concentrations of the IR<sub>UU172</sub> competitor DNA (1, 3, 10, and 30 pmol).
MBP::CodV was meager and required greater amounts of cells for a higher protein yield. We attribute this to the lethal properties of CodV to *E. coli* as the cell titer dropped upon induction and protein expression was low (Figs S5 and S6). Moreover, DAPI staining of DNA from induced cells indicated DNA degradation (Fig. S7). The soluble protein fraction of *E. coli* DH10B and the purified MBP alone were used as controls. Expressed proteins were purified by affinity chromatography, observed by SDS-PAGE (Fig. S8), and used in EMSA experiments. Express purifying protein–DNA complex with the purified proteins and the annealed templates indicated a binding specificity of XerC for the inverted repeat IR*mba*, of RipX for the direct repeat DR 20-kb, and of CodV for the potential difUP site (Fig. 3a). Interaction of XerC with DR 20-kb was also observed (left panel) and the signal enhanced with increased protein concentration (Fig. S9). MBP alone did not bind to the DNA substrates. A further protein–DNA complex was observed with probes MBP::CodV and difUP; however, this band ran above the expected height and is attributable to binding of background *E. coli* proteins in the protein preparation. This false-positive band can be observed in reactions using the soluble protein fraction of *E. coli* with the same substrate DNA (Fig. 3a, right panel, and Fig. S10).

To enhance the signal and to test whether binding of XerC to IR*mba* was specific, we synthesized a 145-bp-long PCR product from the ‘mba locus’ that contained one inverted repeat IR*mba* and applied it in competition analysis using a short 23-bp IR*mba* as competitor DNA. Binding of XerC proved to be specific for the IR*mba* sequence (Fig. 3b). Similar results were obtained with XerC and IRUU172, using a short 23-bp competitor DNA (Fig. 3c).

Fig. 4. Divalent cation-dependent protein–DNA interaction. Magnesium- and manganese-dependent binding of MBP::XerC and MBP::RipX to substrate DNA *in vitro*. (a) Left panel: EMSA analysis using a purified MBP fusion of XerC and a biotinylated PCR product of 145 bp containing one inverted repeat IR*mba* Lane 1, MBP::XerC; lane 2, PCR; lane 3, PCR + MBP::XerC; lanes 4–9, PCR + MBP::XerC, and increasing MgCl₂ concentration (0.5, 0.75, 1, 2.5, 5, and 7.5 mM) in the binding reaction. Right panel: EMSA analysis using a purified MBP fusion of XerC and a biotin-labeled PCR product. Lane 1, PCR; lane 2, PCR + MBP::XerC; lanes 3–8, PCR + MBP::XerC and increasing MnSO₄ concentration (0.5, 0.75, 1, 2.5, 5, and 7.5 mM) in the binding reaction; lane 9, PCR + MBP::XerC and 7.5 mM MgCl₂ in the binding reaction. (b) Left panel: EMSA analysis using a purified MBP fusion of RipX and the biotinylated substrate DR 20-kb. Lane 1, MBP::RipX; lane 2, DR 20-kb; lane 3, DR 20-kb + MBP::RipX; lanes 4–9, DR 20-kb + MBP::RipX and increasing MgCl₂ concentration (0.5, 0.75, 1, 2.5, 5, and 7.5 mM) in the binding reaction. Right panel: Lane 1, DR 20-kb; lane 2, DR 20-kb + MBP::RipX; lanes 3–8, DR 20-kb + MBP::RipX and increasing MnSO₄ concentration (0.5, 0.75, 1, 2.5, 5, and 7.5 mM) in the binding reaction; lane 9, DR 20-kb + MBP::RipX and 7.5 mM MgCl₂ in the binding reaction.
EDTA inhibited protein–DNA interaction (Fig. S11). We therefore tested whether binding of XerC and RipX to their DNA substrates was cation dependent. Interactions of MBP::XerC with IRmba and MBP::RipX with DR20-kb could be enhanced with either MgCl2 or MnSO4, indicating divalent cation-dependent binding (Fig. 4).

XerC-mediated inversion of the mba locus

The EMSA results suggested XerC as potential mediator in the DNA inversion event associated with MBA phase variation. To test whether DNA inversion is mediated by XerC, the model organism M. pneumoniae was co-transformed with two plasmids, one carrying a truncated ‘mba locus’ with two IRmba sequences and the other harboring the recombinase gene xerC fused behind an active promoter. An M. pneumoniae clone (MPmba trunc) with the mba locus integrated at the genomic position 495,321 was first generated by transforming M. pneumoniae M129 with plasmid pMT::mba trunc. MBA and UU376 protein expression in MPmba trunc was screened by Western blot and colony blot throughout eight passages, showing no alternating expression (data not shown); that is, only MBA and no UU376 protein was expressed at all times in subclones. Clone MPmba trunc from the fourth passage was transformed with pCT::UU222 and subcloned. Although the xerC gene had not been integrated into the genome, subclones of transformed MPmba trunc now showed either MBA (variant A) or UU376 (variant B) expression (Fig. 5). Southern blot analysis with genomic DNA showed that DNA inversion had taken place in variant B (Fig. 6). We repeated the transformation experiment, however, obtained the same result; that is, subclones showed phase-locked expression for either MBA or UU376, but not the desired integration of xerC into the genome.

Discussion

We have identified binding sites of the three potential tyrosine recombinases of U. parvum serovar 3. XerC was found to interact with the short inverted repeats located within the two phase-variable gene clusters that have been described as the ‘mba locus’ and the ‘UU172 phase-variable element’, suggesting its involvement in promoting the postulated site-specific recombination event that leads to antigenic variation of major surface proteins. DNA inversion was observed within the ‘mba locus’ after cotransformation of M. pneumoniae with both the ‘mba locus’ and the xerC gene located behind an active promoter. Unfortunately, we were unable to follow alternating expression of MBA and UU376 in M. pneumoniae, as the recombinase gene had not integrated into the organism’s genome. We believe that the active XerC protein in the transformed clone MPmba trunc processed DNA inversion of the ‘mba locus’ before the vector was degraded, and subclones were phase-locked for either MBA or UU376 expression. Chloramphenicol resistance is frequently acquired by M. pneumoniae after electroporation, which explains the antibiotic resistance of the false-positive subclones.

The fact that only xerC is present in some Ureaplasma strains that showed high-frequency phase variation in the two loci supports the idea that only one tyrosine recombinase is involved in the site-specific recombination event of these loci. Recombination mechanisms in mycoplasmas, where only a single recombinase mediates site-specific recombination, have been described for the hsd and vps systems of M. pulmonis (Sitaraman et al., 2002), the mpl system of M. penetrans (Horino et al., 2009), and the vpsn system of M. agalactiae (Czurda et al., 2010). Because all analyzed Ureaplasma strains showed high-frequency phase variation in both the ‘mba locus’ and the
Fig. 6. DNA inversion in the 'mba locus'. (a) Southern blot analysis with genomic DNA of Mycoplasma pneumoniae M129 (MP), MP that was transformed with plasmid pMT::mba\textsuperscript{trunc} (MP\textsubscript{mba\textsuperscript{trunc}}), and two clonal variants (A and B) of MP\textsubscript{mba\textsuperscript{trunc}} that had been isolated after transformation with plasmid pCT::UU222 (indicated by XerC). Genomic DNA of variants A and B was isolated after the second and sixth growth passage (P2 and P6). DNA was digested with HindIII and BglII and hybridized with the DIG-11-dUTP-labeled probes #Genta\textsuperscript{r}, #UU375, and #UU376. Probe #Genta\textsuperscript{r} was used for determining single integration of the insert and detected an 8402-bp fragment in HindIII-digested DNA. Probes #UU375 and #UU376 were used for detecting mba locus configuration and DNA inversion within the mba locus before and after co-transformation of MP\textsubscript{mba\textsuperscript{trunc}} with pCT::UU222. Probe #UU375 detected a 1007-bp fragment in the unaltered mba locus of BglII/HindIII-digested DNA and a 1288-bp fragment in the locus that had undergone DNA inversion. Similarly, probe #UU376 detected a 1622-bp fragment in the unaltered mba locus of BglII/HindIII-digested DNA and a 1342-bp fragment in the locus that had undergone DNA inversion. (b) Schematic illustration of the DNA inversion event in the 'mba locus' that had been integrated in the genome of M. pneumoniae. Integration of the mba locus (mba\textsuperscript{trunc}) had occurred at chromosome position 495,321 via the inverted repeats (IR) of the insertion element located in plasmid pMT::mba\textsuperscript{trunc} with concurrent elimination of the transposase gene (see Fig. S12). The mba locus of variant A corresponds to that of MP\textsubscript{mba\textsuperscript{trunc}}, while that of variant B has undergone DNA inversion. Captions and labeling: Genta\textsuperscript{r}, gentamicin resistance gene; black triangle, short inverted repeat; and black cross, DNA inversion.
'UU172 phase-variable element' in our previous studies, the absence of UU145 and UU529 suggests that their encoded proteins are neither required for site-specific recombination in these phase-variable loci nor essential for in vivo or in vitro growth.

The core-binding domain of Ureaplasma RipX has previously been aligned with other integrases (Swalla et al., 2003). This, and the proposal of a putative dif site within the Ureaplasma genome (Yen et al., 2002), prompted us to investigate possible binding of the potential tyrosine recombinases to this site. Interestingly, none of the tested fusion proteins bound to the proposed dif site. However, CodV was found to interact with another potential dif site that is located 181° away from the origin of replication. The finding suggests an involvement of Ureaplasma CodV in a chromosome dimer resolution event. The fact that codV is not present in all Ureaplasma strains, however, indicates that the proposed event might be processed by other enzymes, such as the translocase FtsK and the topoisomerase IV complex, whose genes are present in all sequenced Ureaplasma genomes (Table S1). It could likewise be that the gene codV encodes an unessential protein of the chromosome dimer resolution mechanism, left over after genome reduction in some strains, or has been acquired by horizontal gene transfer, or is responsible for yet another unknown mechanism. Horizontal gene transfer has recently been described to occur among ureaplasmas, but also with other Mycoplasma species (Pereyre et al., 2009; Xiao et al., 2011; Paralanov et al., 2012), and has been suggested for the occurrence of ripX which, like codV, is present only in a subset of isolates. Interestingly, orthologs of codV have so far only been annotated for the U. parvum species and seem to be missing in U. urealyticum.

Our results suggest that XerC of U. parvum serovar 3 is a mediator in the proposed DNA inversion event of the two phase-variable loci. We postulate that RipX is a potential mediator in the integration of a mobile element. Further analyses focusing on the recombination mechanisms are needed to elucidate the direct functional roles of these potential enzymes in the proposed recombination events.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Alignment of three putative tyrosine recombinases of Ureaplasma parvum serovar 3 (ATCC 700970 and ATCC 27815T).
Fig. S2. Annealed oligonucleotides for EMSA analyses.
Fig. S3. Plasmid pMt::mba(trunc).
Fig. S4. Promoter fusion and Mrf1 expression from Ureaplasma promoters in Mycoplasma pneumoniae M129.
Fig. S5. Growth curve of MBP::CodV expressing Escherichia coli.
Fig. S6. Protein expression and purification of MBP fusion proteins.
Fig. S7. Cell morphology of MBP::CodV expressing Escherichia coli.
Fig. S8. Protein preparations for EMSA analyses.
Fig. S9. Protein concentration-dependent binding of MBP::RipX and MBP::XerC to DR 20-kb.
Fig. S10. Protein-DNA interaction.
Fig. S11. Inhibition of XerC binding to IRmba by EDTA.
Fig. S12. Integration of mba(trunc) into the Mycoplasma pneumoniae chromosome.
Fig. S13. Protein concentration-dependent binding of MBP::RipX and MBP::XerC to IRmba.
Fig. S14. Protein concentration-dependent binding of MBP::XerC to IRmba on a 145-bp PCR product.
Fig. S15. Protein concentration-dependent binding of MBP::CodV and the soluble fraction of Escherichia coli DH10B to difCB.
Table S1. Occurrence of the three putative tyrosine recombinase-encoding genes ripX, xerC and codV in Ureaplasma serovars, the translocase encoding gene ftsK and genes encoding topoisomerase subunits parE and parC.
Table S2. Growth of MBP::CodV expressing Escherichia coli.
Table S3. Features of proteins used for EMSA analyses.