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Derepression of Excision of Integrative and Potentially Conjugative Elements from *Streptococcus thermophilus* by DNA Damage Response: Implication of a cI-Related Repressor[▽]

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A DNA-damaging agent, mitomycin C, derepresses the site-specific excision of two integrative and potentially conjugative elements from *Streptococcus thermophilus*, ICE*St1* and ICE*St3*. The regulation pathway involves a repressor related to phage lambda cI repressor. It could also involve a putative regulator related to another type of phage repressors, the "cI-like" repressors.

Whereas in silico analyses revealed that numerous genomic islands could be integrative and conjugative elements (ICEs) or elements derived from ICEs (2, 4, 9, 13, 15, 19, 25, 26), only a few ICEs have been described. ICEs excise by site-specific recombination, transfer through conjugation, and integrate into a replicon of the recipient cell (8). Whereas the regulation of the excision of numerous prophages is well known, the regulation of site-specific excision of only a few ICEs, including Tn*916*, ICEs from *Bacteroides*, pSAM2, and *clc*, has been described (6, 11, 22, 23). These few regulation systems are very different from each other. Recently, DNA-damaging agents were found to derepress the excision and transfer of two other ICE types, ICE*Bs1* from *Bacillus subtilis* (1) and IncJ elements, including SXT from *Vibrio cholerae* (3) and SXT-related elements from enterobacteria (18). Such regulation systems are similar to the derepression of the site-specific excision of numerous prophages by DNA damage.

Two putative ICEs, ICE*St1* and ICE*St3*, are integrated in the 3′ end of the *fda* locus of the lactic acid bacteria *Streptococcus thermophilus* CNRZ368 and CNRZ385, respectively (Fig. 1A). These ICEs harbor almost identical recombination and conjugation modules (20). The tyrosine integrase and the excisionase encoded by the ICE*St1* recombination module catalyze its excision by recombination between the *attL* and *attR* flanking sites, leading to an excised circular ICE harboring an *attI* site and to a chromosomal *attB* site (10). Furthermore, ICE*St1* carries an internal recombination site related to *attL*, *attL′* (Fig. 1A) (20). Recombination between *attL′* and *attR* leads to excision of the circular form of a shorter putative ICE, ICE*St2*, carrying an *attI′*site. A genomic island corresponding to the left part of ICE*St1* and flanked by *attL* and *attB′* sites remains integrated in *fda*.

The closely related regulation modules of ICE*St1*/ICE*St2* and ICE*St3* contain three shared open reading frames (ORFs) (*arp1*, *orfQ*, and *arp2*) (Fig. 1B). The ICE*St3* recombination module also includes three specific ORFs or pseudogenes (*orf385A*, *orf385B*, and Δ*orf385C*), and the ICE*St1*/ICE*St2* recombination module contains two specific ORFs (*orfP* and *orfR*). The putative regulatory proteins encoded by *arp1*, *arp2*, and *orf385A* have a helix-turn-helix (HTH) DNA binding domain. The functions of the putative proteins encoded by the other ORFs are unknown.

The 5′ part of *arp1* encodes an HTH domain, and its 3′ part encodes a region characteristic of the COG2932 protein family, including the cI repressor of phage λ (9, 20). This region has two functions, cI autoproteolysis and cI oligomerization. In the presence of damaged DNA, the RecA protein, activated by single-stranded DNA (RecA*), induces autoproteolysis of cI and related proteins. Cleaved proteins are not able to oligomerize and therefore are not able to repress their target genes (12). In silico analysis suggested that DNA damage could derepress excision of ICEs from *S. thermophilus*.

To test this hypothesis, strains harboring ICE*St1*/ICE*St2* (CNRZ368) or ICE*St3* (CNRZ385) were grown at 42°C in HJL medium (24). Exponentially growing cells (optical density at 600 nm for MIC/4 and MIC/2, 0.04; optical density at 600 nm for 2× MIC and 4× MIC, 0.4) were treated with mitomycin C (MC) concentrations close to the MIC for 2.5 h to induce DNA damage. Then the recombination sites resulting from excision, *attI* and *attB* (ICE*St1* and ICE*St3*) or *attI′* (ICE*St2*), were amplified by PCR using 1 μg genomic DNA for CNRZ368 and 1 ng genomic DNA for CNRZ385. PCR experiments were performed in 25-μl mixtures using 0.5 U of *Taq* DNA polymerase (Biolabs) according to the manufacturer's specifications and primers (melting temperatures, 49.6 to 53.1°C) described in the legend to Fig. 1. After an initial denaturation step consisting of 4 min at 95°C, PCR was performed for 30 cycles of denaturation for 30 s at 95°C, annealing for 30 s at 47.5°C, and extension for 30 s at 72°C, followed by final extension for 7 min at 72°C.

Treatment of CNRZ368 with MC induced an increase in the PCR signal intensity corresponding to the *attB* and *attI* sites, while this treatment did not induce an increase in the PCR signal intensity corresponding to the *fda* gene used as a control (Fig. 2). The most intense signal for both *attB* and *attI* sites was

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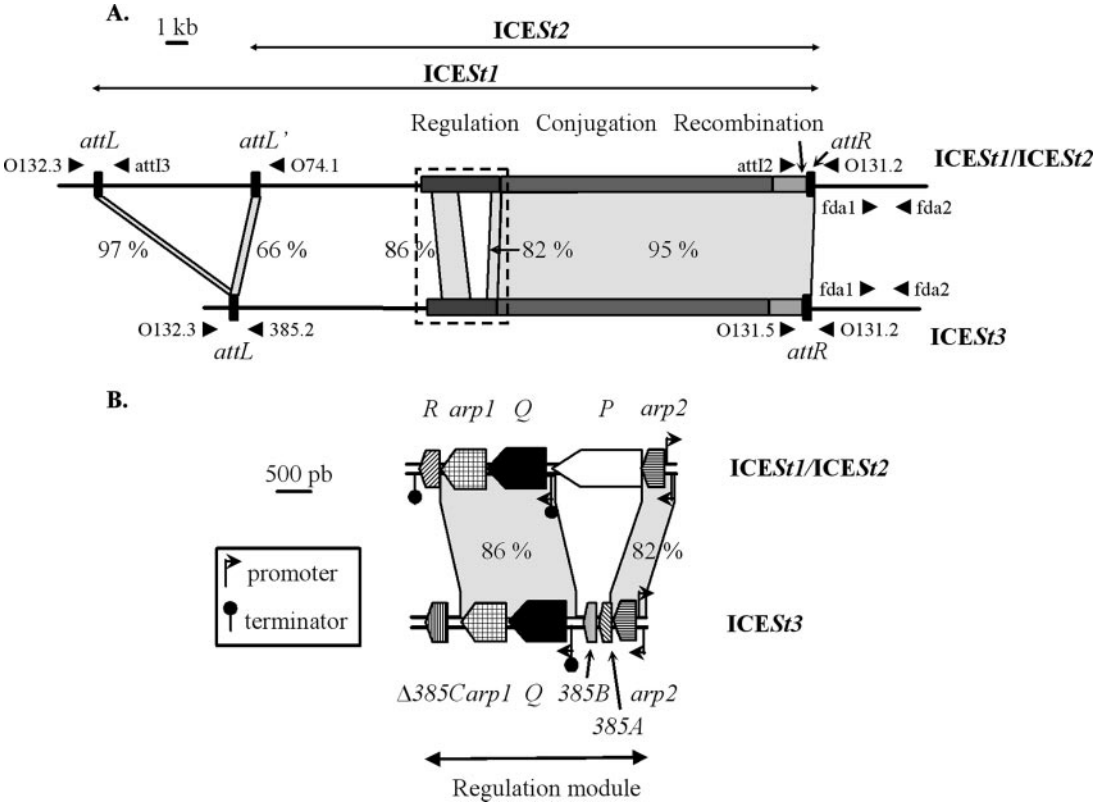

FIG. 1. Comparison of ICE*St1*/ICE*St2* and ICE*St3* maps. (A) Location of the recombination, conjugation, and regulation modules and the recombination sites on the ICE*St1*/ICE*St2* and ICE*St3* maps. The recombination sites are indicated by solid rectangles and are magnified. The schematic localizations and orientations of oligonucleotides used for PCRs are indicated by arrowheads. The following primer pairs were used for amplification of fragments containing the recombination sites resulting from ICE excision: primers O131.2 and O132.3 (*attB* from ICE*St1* and ICE*St3*), primers attI2 and attI3 (*attI* from ICE*St1*), primers attI2 and O74.1 (*attI'* from ICE*St2*), and primers O131.5 and 385.2 (*attI* from ICE*St3*). The fda1-fda2 primer pair was used for amplification of an *fda* internal region. The dotted rectangle indicates the area shown in panel B. (B) Map of regulation modules of ICE*St1*/ICE*St2* and ICE*St3*. ORF designations beginning with "*orf*" are abbreviated with the corresponding letter or name. The locations and orientations of ORFs and truncated ORFs (Δ385C) belonging to the regulation modules are indicated by arrow boxes. The angled arrows and the lollipops indicate the putative promoters and rho-independent transcription terminators deduced from in silico analyses. The gray areas join closely related modules and *att* sites belonging to the ICEs (>65% nucleotide identity); the levels of identity are indicated. The close relationships between short sequences and insertion elements are not shown.

obtained when 0.1 μg/ml MC (MIC/2) was used. The effects of MC were also determined by PCR amplification of fragments harboring *attI* and *attB*, using 1 μg to 1 ng genomic CNRZ368 DNA. The minimum amounts of template DNA that resulted in positive PCR amplification of the *attB* and *attI* sites were 100 ng using CNRZ368 DNA in the absence of MC and 10 ng after treatment with MC (MIC/2), suggesting that DNA damage caused by MC induced at least a 10-fold increase in ICE*St1* excision.

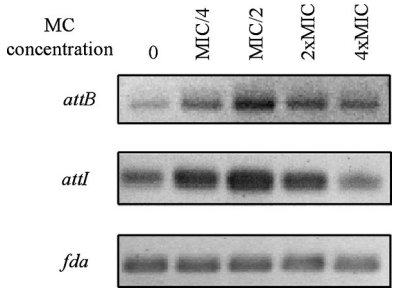

FIG. 2. Effects of MC treatment of exponentially growing cells on the excision frequency of ICE*St1*. Fragments containing the *attB* (361 bp) or *attI* (400 bp) recombination site were amplified using 1 μg template DNA from MC-treated cells and primers described in the legend to Fig. 1. An internal fragment of the *fda* gene (339 bp) was amplified as a control using 1 ng template DNA.

In the absence of MC, the following ratios of the excised circular form to the genome were found in the stationary phase of growth: 10^{-6} for ICE*St1*, $<10^{-6}$ for ICE*St2*, and 9×10^{-3} for ICE*St3* (20). However, treatment of the strains harboring ICE*St2* or ICE*St3* with MC (MIC/2) was also found to induce excision (data not shown). This suggests that DNA damage induced excision of the three ICEs.

In order to examine the *arp1* function, two fragments containing the 5′ and 3′ ends of *arp1* of ICE*St1*/ICE*St2* were cloned in the thermosensitive vector pG$^+$host9 (5, 17). The recombinant plasmid, carrying a very short deleted ORF, Δ*arp1*, was introduced into *S. thermophilus* CNRZ368. A strain harboring the Δ*arp1* ORF instead of *arp1* (CNRZ368 Δ*arp1*) was obtained by two successive homologous recombination events.

The effects of the *arp1* deletion were determined by PCR

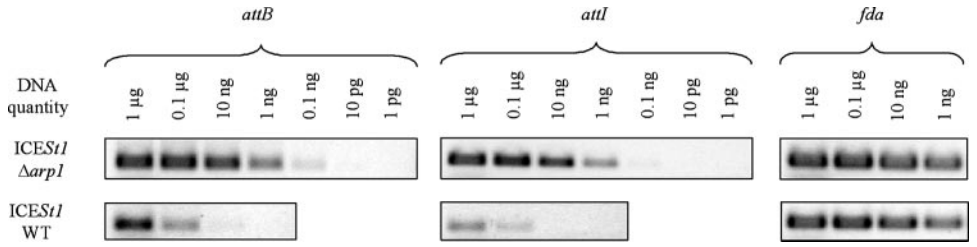

FIG. 3. Effects of *arp1* deletion on the excision frequency of ICE*St1*. Fragments containing the *attB* (361 bp) and *attI* (400 bp) recombination sites from the strains harboring wild-type ICE*St1* (ICE*St1* WT) or ICE*St1* Δ*arp1* were amplified by PCR using primers described in the legend to Fig. 1 and amounts of template DNA ranging from 1 μg to 1 pg for CNRZ368 Δ*arp1* and from 1 μg to 1 ng for CNRZ368. An internal fragment of the *fda* gene (339 bp) was amplified as a control.

amplification of fragments harboring *attI* and *attB*, using amounts of genomic DNA ranging from 1 μg to 1 ng for wild-type ICE*St1* and from 1 μg to 1 pg for ICE*St1* Δ*arp1* (Fig. 3). The minimum amounts of DNA that produced a positive result for the *attB* site were 10 ng of CNRZ368 template DNA and 10 pg of CNRZ368 Δ*arp1* template DNA. Using the same procedure, the minimum amounts of DNA that produced a positive PCR result for the *attI* site were 0.1 μg of CNRZ368 DNA and 0.1 ng of CNRZ368Δ*arp1* DNA. The *arp1* deletion did not induce an increase in the PCR signal intensity when the *fda* gene was used as a control. Thus, deletion of the *arp1* gene resulted in at least a 1,000-fold increase in the concentration of ICE*St1 attB* and *attI* sites. Furthermore, whereas ICE*St1* and ICE*St2* were excised at different frequencies, the concentrations of a fragment carrying the *attI′* site from wild-type ICE*St2* or ICE*St2* Δ*arp1* (Fig. 1A) increased by a factor similar to that observed for ICE*St1* (data not shown). Therefore, the *arp1* gene repressed the excision of ICE*St1* and ICE*St2*.

To validate the assumption that Arp1 autoproteolysis is involved in the induction of ICE excision by MC, exponentially growing cells of CNRZ368 Δ*arp1* were treated with MC at a concentration of 0.1 μg/ml (MIC/2). In three replicate experiments, the minimal amount of DNA that allowed detection of *attB* amplification (1 ng) was the same in the presence and in the absence of MC. Furthermore, in these three replicate experiments, the minimal amounts of DNA that allowed detection of *attI* amplification were 0.1 ng or 1 ng in the absence of MC and 0.1 ng after treatment with MC. Therefore, the inducibility of ICE*St1* Δ*arp1* excision by MC was reduced or suppressed compared to the results for the same MC treatment in the wild-type strain. This suggests that MC treatment alleviates the repression of ICE*St1* excision mediated by Arp1. The minimal amount of DNA that allowed detection of *attI′* amplification (1 ng) was the same in the presence and in the absence of MC. This suggests that MC treatment also alleviates the repression of ICE*St2* excision mediated by Arp1. In the same way, MC treatment derepressed the excision and transfer of another ICE, SXT from *V. cholera*e, probably by promoting the autocleavage of a cI homologue encoded by the element (3).

The functions of the other genes harbored by the ICE*St1*/ICE*St2* and ICE*St3* regulation modules (Fig. 1B) remain unknown. Nevertheless, the regulation modules of numerous *Firmicutes* prophages, such as TP901-1 from *Lactococcus lactis* (16), and of another ICE, ICE*Bs1* from *B. subtilis* (1), encode an OrfQ homologue and a "cI-like" repressor (i.e., a repressor related to Arp2), while none of these elements encode a genuine homologue of the cI repressor. OrfQ and the OrfQ homologues have a DUF955 domain that has a conserved H-E-X-X-H motif, suggesting that these proteins could be Zn^{2+} metalloproteinases (21). However, the activity of these proteins has not been demonstrated. Whereas the "cI-like" proteins encoded by prophages repress their lytic growth, these proteins are shorter than genuine cI homologues. Indeed, the cI-like repressors contain an HTH DNA binding domain but lack a region harboring autoproteolytic and oligomerization functions related to the genuine cI. However, the TP901-1 "cI-like" repressor binds to operators as dimers and higher multimers (14). Furthermore, the excision of prophages with *cI*-like genes and *orfQ* homologues, such as ϕSfi21 from *S. thermophilus* (7) or TP901-1 (16), is inducible by MC, and the prophage TP901-1 *cI*-like gene is involved in the lytic phase induction pathway. Moreover, the induction of the prophage TP901-1 (16) and of ICE*Bs1* by MC (1) is RecA dependent. Thus, *arp2* and *orfQ* might also be involved in the derepression pathway of the excision of the *S. thermophilus* ICEs.

To our knowledge, ICE*St1*/ICE*St2* and ICE*St3* are the only integrative elements (i.e., phages, ICEs, or related elements) that encode a genuine cI homologue and might encode a cI-like homologue. Since MC induces the conjugative transfer of SXT, an ICE from *V. cholerae* coding for a genuine cI (3), and of ICE*Bs1*, an ICE from *B. subtilis* coding for a cI-like repressor (1), DNA damage could regulate not only the excision but also the conjugative transfer of the ICEs from *S. thermophilus*.

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