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CinA is regulated via ComX to modulate genetic transformation and cell viability in *Streptococcus mutans*

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Abstract

The *Streptococcus mutans* ComX-regulon encompasses >200 mostly uncharacterized genes, including *cinA*. Here we report that *cinA* is regulated by ComX in the presence of the competence stimulating peptide (CSP), wherein loss of CinA (strain SmuCinA) results in reduced transformability with or without added CSP by 74- and 15-fold, respectively ($p<0.003$). In CSP-supplemented cultures, a 2-fold increase in cell viability was noted for SmuCinA relative to UA159 ($p<0.002$), suggesting CinA's involvement in the CSP-modulated cell killing response. Relative to UA159, loss of CinA also rendered the mutant hypersensitive to killing by methyl methanesulfonate (MMS), which impairs homologous recombination. Despite our use of a non-polar mutagenesis strategy to knockout *cinA*, which is the first gene of the multicistronic operon harboring *cinA*, we noted a drastic reduction in *recA* expression. By using a CinA-complemented mutant, we were able to partially, but not completely restore all phenotypes to UA159 levels. Complementation results suggested that although *cinA* participates in modulating competence, viability and MMS tolerance, genes downstream of the *cinA* transcript may also regulate these phenotypes, a finding that warrants further examination. This is the first report that describes a role for *S. mutans'* CinA in contending with DNA damage, genetic transformation and cell survival.

Keywords

Streptococcus mutans; *cinA*; *comX*; CSP; genetic competence; cell death

Introduction

Genetic competence is a transient physiological state that facilitates horizontal gene transfer that enables recipient bacteria to acquire novel genes by the uptake of exogenous DNA from the environment (Claverys & Martin, 1998). The dental biofilm harbors copious amounts of DNA that is released via cell lysis, which acts as a major component of the biofilm matrix and contributes both to its structural integrity and provides a source of genetic material for naturally transformable plaque bacteria such as *Streptococcus mutans* (Perry, *et al.*, 2009).

S. mutans is an opportunistic pathogen considered as one of the principle etiological agents of dental caries. Natural genetic transformation of this bacterium was shown to be modulated by a quorum sensing (QS) signaling system comprised of a ComDE two component signaling system, which responds to a peptide signaling molecule designated the competence stimulating peptide (CSP) (Li, *et al.*, 2001). In addition to eliciting the competence phenotype, the CSP signaling pathway also contributes to proper biofilm formation, bacteriocin production and stress tolerance in *S. mutans* (Senadheera &

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Cvitkovitch, 2008). Intriguingly, the CSP-induced genetic transformation pathway also modulates cellular lysis in a fraction of the population in *S. mutans* cultures (Qi, et al., 2005, Perry, et al., 2009). Development of genetic competence is directly correlated with activation of an alternate sigma factor, ComX, which depends on ComE activity and that of another regulatory protein, ComR that responds to an internalized signaling peptide called XIP (Mashburn-Warren, et al., 2010). Recently, it was demonstrated that *ComX* was expressed only in a fraction of the CSP-induced population, which resulted in the bifurcation of the population into fractions undergoing competence or cell death (Mashburn-Warren, *et al.*, 2010, Lemme, *et al.*, 2011). Although transcriptome analysis has shown the regulation of nearly 240 genes by ComX (Perry, et al., 2009), most of these putative "late competence genes" modulating competence and cell lysis remain uncharacterized to date. Here, we studied a ComX-regulated gene designated the competence induced protein A (*cinA*) in *S. mutans*. Recently, Okinanga et al. showed that the HdrRM system regulated expression of *cinA* via ComX in *S. mutans* (Okinaga, *et al.*, 2010). While *cinA*'s putative functions have not been closely examined in *S. mutants,* in *S. pneumoniae*, its ortholog belongs to the ComX-activated "late competence" regulon (Masure, *et al.*, 1998, Mortier-Barriere, *et al.*, 1998). In pneumococci, *cinA* is part of the *rec* locus, which includes *recA* that facilitates homologous recombination between single- and double-stranded DNA during genetic transformation (Kowalczykowski, 1994, Camerini-Otero & Hsieh, 1995). While CinA in *S. pneumoniae* was shown to facilitate transport of RecA to the membrane during genetic transformation (Masure, *et al.*, 1998), studies in *Bacillus subtilis* suggested that CinA is not specific to competence, but instead is a nucleoid-associated protein that serves a general role in cells entering stationary phase (Kaimer & Graumann, 2010).

Here we report that *cinA* transcription is modulated by ComX in response to CSP, and that *cinA* is required for optimal genetic transformation in *S. mutans*. Deficiency of *cinA* drastically reduces the ability of synthetic CSP (sCSP) to induce cell lysis within the population, and to survive DNA damaging conditions induced by methyl methanesulfonate. This is the first report that describes functional roles for *cinA* in *S. mutans*.

Materials and Methods

Bacterial Strains and culture conditions

S. mutans wild type UA159 strain (J. Ferretti, University of Oklahoma), its isogenic CinA deficient mutant (SmuCinA, this study) and a CinA complimented mutant (strain SmuCinA +pCinAHis, this study) were utilized (Table 1). All strains were grown overnight at 37°C in a 5% (vol/vol) CO_2 atmosphere as standing cultures in Todd-Hewitt-yeast extract (THYE) broth (Becton Dickinson, Sparks, MD). Strains were propagated on THYE plates supplemented with agar 1.5% (wt/vol) agar (Bioshop, Burlington) in the presence or absence of 10 μg/ml erythromycin.

Construction of mutant strains

S. mutans wild type UA159 was used to construct a *cinA* knockout mutant (strain SmuCinA) using PCR-ligation mutagenesis with primers in Table 1, as described previously (Lau, *et al.*, 2002). Briefly, 5′ and 3′ flanking regions of *cinA* (NCBI gene ID: SMU.2086) were ligated to an erm^r cassette, which were then amplified and transformed into UA159. From these, an Erm^r transformant was selected and successful deletion of *cinA* was validated using PCR and nucleotide sequence analysis. The SmuCinA complimented strain (SmuCinA+pCinAHis) was constructed by amplifying *cinA* from the UA159 genome with its corresponding 129 bp promoter sequence upstream of the ATG start site. A penta His-tag sequence was also added to the 3′ end of the reverse primer (Table 1). PCR amplicons were then cloned into $pDL277^{Spec}$ (LeBlanc, *et al.*, 1992) and the plasmid construct (pCinAHis)

was transformed into DH5α *Escherichia coli* cells (Invitrogen). Following plasmid extraction, successful cloning was confirmed using DNA sequencing and SmuCinA was transformed with pCinAHis using standard in-house transformation protocols.

Northern Blot hybridization

Total RNAs were isolated from UA159 and SmuCinA using the Trizol method as described previously (Senadheera, *et al.*, 2007) and used for Northern hybridization according to the protocol outlined in the DIG High Prime DNA labeling and Detection Starter Kit II (Roche) with the following modifications. To prepare RNA probes, 330bp and 558bp fragments of the *cinA* and *recA* genes were PCR amplified, respectively, using primers listed in Table 1 and labeled according to the DIG High Prime DNA Labeling Starter Kit (Roche Applied Science). Total RNA was separated using a 3.5% polyacrylamide gel, which was electro-transferred to a Sensiblot Plus Nylon membrane (Fermentas). Hybridization, washing and detection were all performed using appropriate protocols and solutions in the Detection Starter Kit II (Roche Applied Science). Images were captured every 5 min using BioRad ChemiDoc Gel Docking System and Quantity One software (BioRad, Hercules, CA, USA). A second hybridization was performed by stripping the same blot with NaOH and reprobing with a *recA* RNA probe (Table 1).

Transcriptional Analysis

Quantitative real-time PCR (qRTPCR) was performed using cells grown to mid-exponential phase (OD_{600} ~ 0.4) in THYE medium, in the presence or absence of 1μg/ml of CSP. Cells were harvested by centrifugation and snap frozen in liquid nitrogen, and used for cDNA synthesis as described previously (Senadheera, *et al.*, 2007). Fold expression of a target gene was calculated relative to the no-CSP control or wild-type levels set at a user-defined value of 1.0. Expression was calculated using three to five biological replicates, each subjected to triplicate amplifications.

Genetic Transformation

Cultures treated without CSP (natural transformation) or supplemented with 1μg/ml of CSP were grown to early exponential phase (OD_{600} 0.1) and transformation frequency (TF) assays were conducted using streptococcal vector pDL289 as described previously (Senadheera, *et al.*, 2007).

Growth kinetics

Overnight cells were diluted 1:30 in pre-warmed sterile THYE with or without 1μg/ml of synthetic CSP. Growth was monitored as described previously (Senadheera, *et al.*, 2007) using a Bioscreen microbiology workstation (Bioscreen C Labsystems, Finland).

Cell viability

For cell viability assays, *S. mutans* strains were grown to stationary phase (OD_{600} 0.8 to 1.0) in the presence or absence of 1μg/ml CSP. Following incubation, cells were sonicated, serially diluted, and grown on THYE plates at 37°C in 5% CO_2 for 48 h. Percentage survival was calculated as CFU of cells treated with CSP divided by cells not treated with CSP, times 100. Statistical significance was calculated using the Student's t-test using results from 3 independent experiments.

Determination of sensitivity to DNA damaging agents

To assess sensitivity to DNA damaging agents, mitomycin C (MMC, 0.05 μg/ml) and methyl methanesulfonate (MMS, 0.1%) were added to cells in mid-log phase (OD_{600} 0.4). MMC-treated cells were incubated for 20 and 60 min while MMS-treated cells were

incubated for 90 min. Untreated cells were used as controls. Following incubation, cells were sonicated, serially diluted, spotted on THYE agar plates in triplicate and incubated at 37°C in 5% CO_2 for 48 h. Percentage survival was calculated by counting CFUs of treated cells divided by untreated cells, times 100.

Results and Discussion

CinA locus

The *cinA* locus (NCBI ID SMU.2086) is framed by several genes primarily involved in DNA recombination and repair, processes important for genetic competence (Figure 1a). In the vicinity of *cinA*, two terminator sequences were identified downstream of SMU.2083c and SMU.2090c (WebGesTer DB: http://pallab.serc.iisc.ernet.in/gester/dbsearch.php), suggesting that *cinA* may be a component of a 7-gene operon as indicated in Figure 1A. It was previously shown that in *S. pneumoniae,* the *cinA* transcript was only present during genetic competence induced by CSP and was co-transcribed with *recA* (Martin, et al., 1995). Since repeated attempts at determining the nature of transcripts originating from the putative *cinA* promoter using a series of reverse-transcription PCR provided inconclusive results, we employed northern blot analysis to determine whether *cinA* and *recA* were co-transcribed during CSP-induced competence development. Using a *cinA* probe, we identified 2 transcripts in CSP-supplemented UA159 cells, wherein one corresponded to ~ 1.2 kb *cinA* transcript alone and the other corresponded to ~ 2.4 kb *cinA*-*recA* transcript (Figure 1B). No transcripts were identified in SmuCinA mutant cells grown in the presence of CSP (negative control) and also when UA159 was grown in the absence of CSP. In UA159 cells without CSP supplementation, it was likely that we could not detect bands due to the low abundance of the transcripts without added CSP. To validate that *cinA* and *recA* were indeed co-transcribed, we further probed CSP-supplemented RNAs with a *recA* probe, which resulted in a single transcript corresponding to a size representing the *cinA*-*recA* transcript (Figure 1B). Hence, these results suggested that *cinA* and *recA* were co-transcribed under conditions favoring DNA uptake, and that *cinA* was likely to produce transcripts in excess of *recA* when CSP was added.

cinA and *recA* are upregulated by CSP and are transcriptionally regulated by ComX

In *S. pneumoniae,* the *cinA* and *recA* orthologs belong to the ComX-activated “late competence” regulon (Masure*, et al.*, 1998, Mortier-Barriere*, et al.*, 1998). Our search of the *cinA* promoter in *S. mutans* revealed a putative ComX binding site (Figure 1), suggesting that *cinA* and *recA* were perhaps part of the CSP-inducible ComX regulon (Peterson*, et al.*, 2004, Rathsam*, et al.*, 2005). To test this, we examined *cinA* and *recA* expression using cDNAs derived from *S. mutans* UA159 grown in the absence or presence of CSP. In CSP-supplemented UA159 cells, the expression of *cinA* and *recA* were increased by 5.5- and 2.4-fold, respectively, relative to the no-CSP control (Figure 2). Without added CSP, fold-expression of *recA* was reduced by 63% (i.e. ~ 0.37) relative to that in UA159, suggesting a polar effect on *recA* transcription by *cinA* mutagenesis (Figure 2). Supplementing the SmuCinA strain with CSP increased *recA* expression to 0.64, which still reduced *recA* transcription by 36% compared with wild type levels. Taken together, these results can be used to summarize that CinA is independently and highly driven by its own promoter, likely in the presence of CSP, and that the recA is co-transcribed with *cinA*, but not transcribed independently.

To understand the regulatory role of ComX on *cinA* and *recA* expression, we also performed qRTPCR using cDNAs isolated from a *comX*-deficient mutant (SmuComX) and its wild type parent grown in the presence of CSP. Compared with UA159 supplemented with CSP, we could not detect *cinA* and *recA* transcripts in the *comX* mutant (Figure 2). These results

are in accordance with previous finding by Okinaga et al. (2010), which suggested that the alternate sigma factor ComX was necessary for transcription of *cinA* and *recA* in the presence of CSP. As shown in Figure 1A, a conserved com-box sequence was identified in the *cinA* promoter, suggesting that ComX directly binds to the *cinA* promoter for transcriptional regulation, although more research is warranted to validate this finding.

CinA modulates genetic transformation

In pneumococci, ComX occupies a central role in the latter stages of the transformation process, by facilitating uptake of extracellular DNA and modulating genetic recombination (Luo & Morrison, 2003, Peterson, et al., 2004). Since we showed that ComX regulated *cinA* expression increases dramatically in response to CSP, we investigated the role of CinA in genetic transformation by assessing the transformation frequency (TF) of *S. mutans* UA159 wild type and SmuCinA in the presence and absence of synthetic CSP. Relative to wild type, the natural transformability and TF with added CSP of SmuCinA was significantly decreased by 15-fold and 74-fold, respectively ($p<0.001$) (Figure 3).

Since we showed that *cinA* was co-transcribed with *recA* under competence-inducing conditions, and because deletion of *cinA* caused polar effects on *recA* expression, we constructed a CinA complemented strain SmuCinA+pCinAHis that was used in TF assays to validate CinA's role in genetic transformation. In the CinA complemented strain, although transformability was drastically increased relative to the CinA-deficient mutant, TF was not restored to wild type levels as observed under no-CSP and plus-CSP conditions (Figure 3). More specifically, an approximate 5-fold decrease in TF was observed in the SmuCinA +pCinAHis strain relative to UA159 in the presence or absence of CSP ($p<0.001$) (Figure 3).

Despite polar effects on *recA* as judged by expression analysis using SmuCinA and UA159 strains, partial restoration of the competence phenotype by the CinA complemented strain demonstrates a clear role for *cinA* in genetic transformation in *S. mutans.* However, we cannot ignore the possible contribution of *recA* to the transformation results we observed. RecA is a major component of the bacterial homologous recombination apparatus and is essential for the transformation of both plasmid and chromosomal DNA in *S. pneumoniae* (Mortier-Barriere*, et al.*, 1998). Our inability to fully complement the CinA deficiency was likely caused by diminished *recA* expression in the SmuCinA mutant.

Recently Mashburn-Warren*, et al.* showed that *S. mutans* ComR serves as the proximal regulator of ComX, that ComR is activated by exogenous XIP (Mashburn-Warren*, et al.*, 2011). Hence, it is likely that the ComRS system also regulates *cinA* transcription by activating ComX. Examination of other two component signaling systems in *S. mutans* suggests that in addition to the CSP-activated ComDE, other systems including RelRS, CiaRH, and VicRK also modulate ComX activity [(Ahn*, et al.*, 2006), unpublished data], thus affecting expression of *cinA*. While here we focused on understanding ComX-mediated effects on *cinA* transcription and function, the regulatory roles of these other systems on ComX and CinA also warrant additional experimentation.

CinA modulates CSP-mediated cell death

Since *cinA* was up-regulated in the presence of CSP, and CSP was shown to modulate cell death via activity of ComX (Perry*, et al.*, 2009, Lemme*, et al.*, 2011), we hypothesized that CinA participated in CSP-induced cell lysis. To test this, we first monitored the growth of *S. mutans* UA159, SmuCinA and SmuCinA+pCinAHis in the absence and presence of 1μg/ml of sCSP. While growth in the absence of CSP was not drastically affected by the loss of *cinA* (Figure 4A), supplementing CSP resulted in an increased growth yield of SmuCinA relative to UA159 (Figure 4B). In fact, the negative effect of CSP on growth was partially

abolished when CinA was complemented (Figure 4B), suggesting that killing effects of CSP was modulated by *comX* via the *cinA*. To validate *cinA's* role in cell lysis, we performed cell viability assays in the presence of synthetic CSP. As expected, a significant increase in CFUs was observed in SmuCinA (54%) relative to UA159 (24%) ($p<0.002$, Figure 4C). Complementation of *cinA* did not bring the percentage survivors to wild type levels, although percentage viability of the SmuCinA+pCinAHis strain was substantially reduced to 35% relative to wild type ($p<0.01$). These results clearly demonstrate a role for CinA in CSP-induced cell lysis in *S. mutans*.

A role for CinA in cell lysis of pneumococci was previously suggested by Novak *et al* who showed that a zinc metalloprotease (ZmpB) mutant had a lysis defect when treated with penicillin (Novak*, et al.*, 2000). It was suggested that this defect was caused by co-localization of the autolysin LytA with CinA within the cytoplasm, wherein LytA was normally located in the cell membrane (Novak*, et al.*, 2000), a finding that could not be confirmed by a different group (Berge*, et al.*, 2001). Despite these conflicting results in *S. pneumoniae*, the possibility of CinA interacting with a putative autolysin protein in *S. mutans* to initiate cell lysis should be considered.

Loss of CinA affects sensitivity to DNA damaging agents

In *S. pneumoniae,* competent cells or those exposed to DNA damaging agents produced a 5.7 kb polycistronic transcript that included *cinA* and *recA* (Martin*, et al.*, 1995). From this transcript, the product encoded by *recA* serves a critical step during transformation and DNA repair where it identifies homologous regions of incoming DNA and incorporates them into the host chromosome (Kowalczykowski, 1994). Martin *et al* also demonstrated that CinA and RecA interacted to modulate genetic competence and facilitate survival under DNA damaging conditions (Martin*, et al.*, 1995). Hence, we next studied CinA's role in contending with DNA damage by assessing cell survival under chemical agents that either damaged DNA directly or disrupted the replication process. We used mitomycin C (MMC) which inhibits growth by causing DNA cross-linkage (Tomasz, 1995) and methyl methanesulfonate (MMS) that stalls the replication fork in areas where homologous recombination occurs (Lundin*, et al.*, 2005). Following MMC treatment, survival of SmuCinA was not significantly altered relative to wild type (data not shown), which was similar to the results obtained for the CinA mutant in *B. subtilis* (Kaimer & Graumann, 2010). In contrast, a 22-fold reduction in survival was observed in SmuCinA, when exposed to 0.1% MMS for 90 minutes as compared to UA159 ($p<0.0002$, Figure 5). The growth was partially restored by complementation with *cinA* resulting in percentage survival of a 2.3-fold reduction as compared with the wild-type strain ($p < 0.007$, Figure 5). The loss of CinA, therefore, enhances the mutant's sensitivity to killing by MMS, which is likely caused by diminished expression of *recA* in our SmuCinA mutant or due to a possible interaction with RecA at the DNA replication fork. However, our ability to partially restore viable CFUs by using the CinA complemented strain clearly suggests an important role for CinA in contending with MMS-induced stress in *S. mutans.*

Conclusions

Here we have demonstrated that *cinA* is transcriptionally regulated by ComX, which in turn, modulates genetic competence and cell death in *S. mutans*. Although, we only investigated CSP's effects on *cinA* upregulation, it is likely that *cinA* also transcriptionally responds to XIP, which was shown to activate ComX (Mashburn-Warren*, et al.*, 2010, Lemme*, et al.*, 2011). In addition to ComDE, we know that other signaling systems also modulate ComX activity (e.g. ComRS, LiaRS, VicRK) (Mashburn-Warren*, et al.*, 2010; unpublished data). Hence, it stands to reason that ComX-dependent transcription of *cinA* relies on multiple signaling inputs for optimal activity. Further, our results support the findings of Lemme *et*

al, who showed that ComX can modulate cell death versus competence depending on its activity (Mashburn-Warren*, et al.*, 2010, Lemme*, et al.*, 2011). Here, we have further shown that these ComX-regulated phenotypes are, at least in part, regulated via CinA. In this report, we also showed that *S. mutans'* ability to withstand DNA damage induced by MMS was also dependent on CinA. Taken together, we have demonstrated novel roles for the CinA in *S. mutans* in modulating genetic transformation, cell viability and tolerance to MMS.

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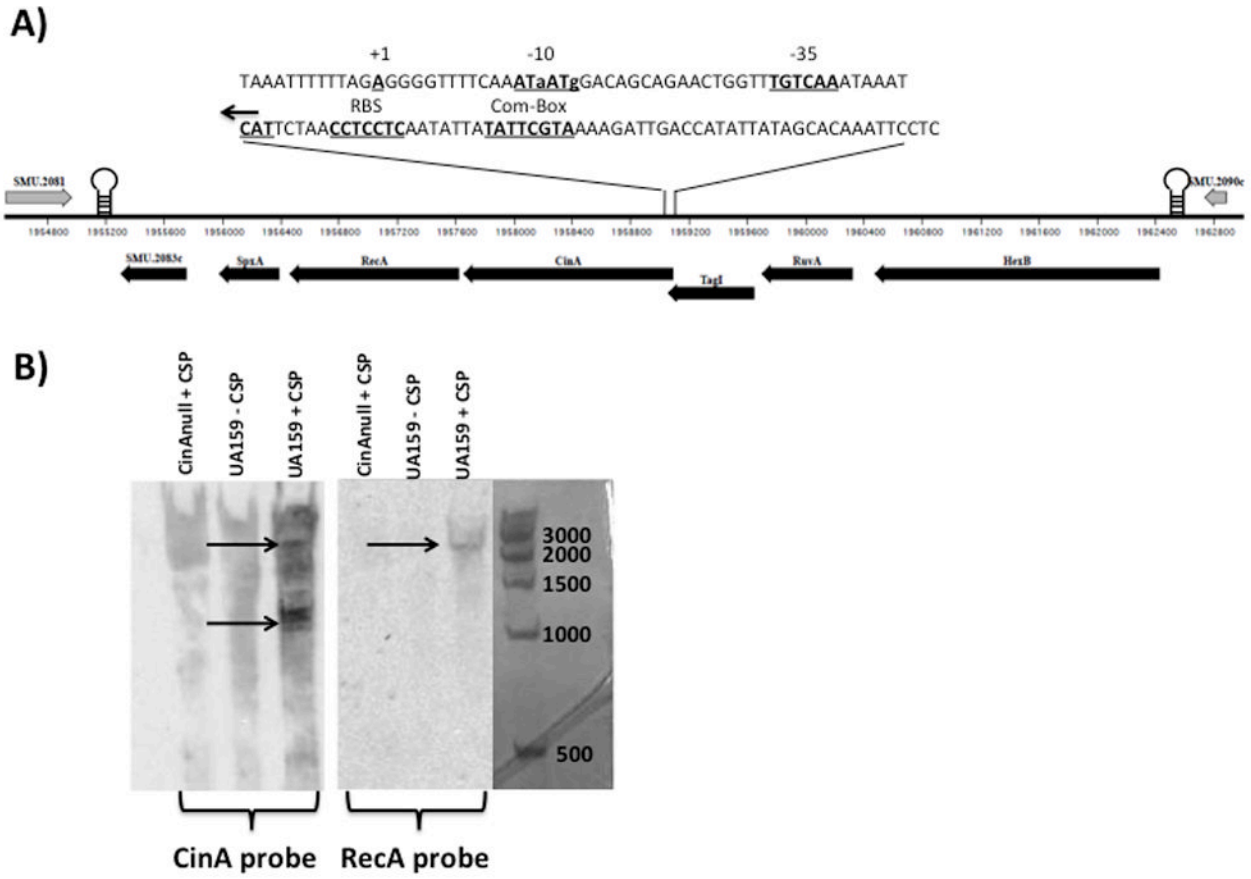

Figure 1.

(A) The genetic locus of c*inA* (SMU.2086c) and its neighboring genes are highlighted by the dark arrows and include genes that are primarily involved in DNA recombination and repair. Two predicted termination sequences represented by hairpin loop structures were found in the vicinity of SMU.2080 and SMU.2090 (grey arrows). Classical promoter elements including a ribosomal binding site (RBS), transcriptional start site (+1), −10 TATAAT box and −35 TTGACA box, were detected in the complementary strand harboring the putative *cinA* promoter (bold and underlined). A putative Cin-Box or Com-Box (Rathsam, *et al.*, 2005) was identified 27 bp upstream of the ATG start site. (B) Northern analysis of the CinA null mutant and UA159 strains probed with *cinA* or *recA*. All strains were grown to OD_{600} 0.4 in the presence or absence of 1ug/ml of synthetic CSP. The initial blot was probed for *cinA*, chemically stripped, and then re-probed for the *recA* transcript. The arrows correspond to a predicted transcripts representing *cinA* (1,256 bp) and *cinA-recA* (2,407 bp).

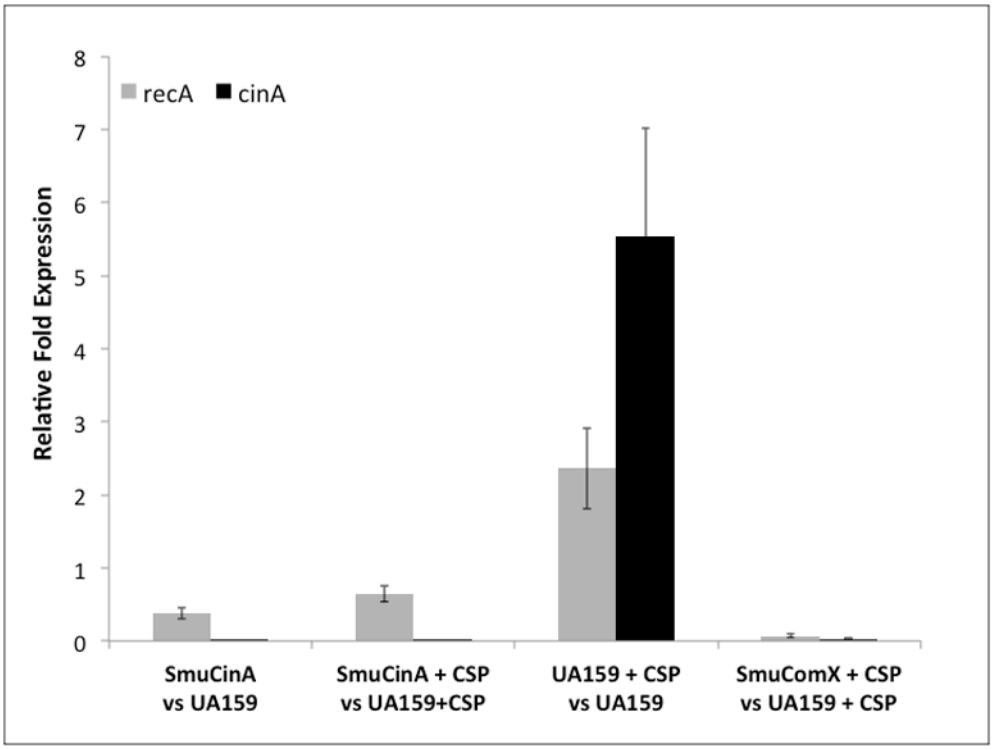

Figure 2.
Real-time gene expression of *cinA* and *recA* in *S. mutans* strains UA159 wild-type, SmuCinA and SmuComX. Each strain was grown in the presence or absence of 1μg/ml of sCSP Fold-expression was calculated using three biological replicates and normalized to the house keeping gene 16sRNA. Graphs represent mean ± SE.

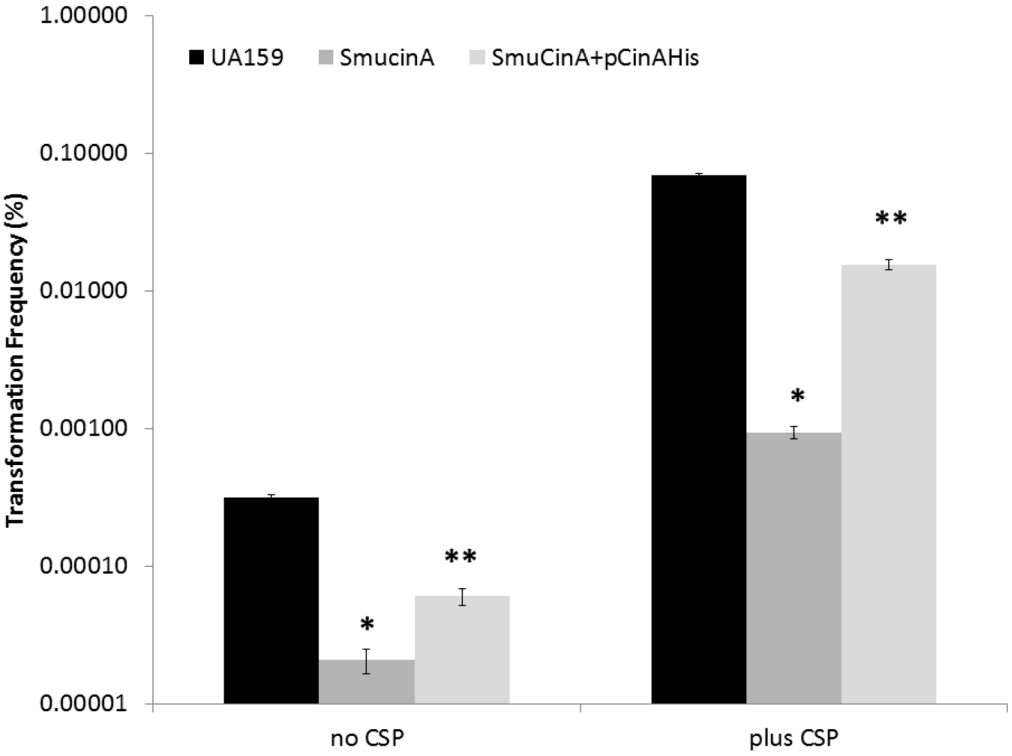

Figure 3.

Transformation frequencies (TF) of *S. mutans* strains UA159, SmuCinA and SmuCinA +pCinAHis cells. Experiments were performed using cells in early-log phase (OD_{600} 0.1) supplemented with 1μg/ml of CSP or without CSP (natural TF). All experiments were performed with a minimum of three biological replicates and Student's t-test was used to calculate statistical significance (* $p<0.001$; ** $p<0.003$). Graphs represent mean ± SE.

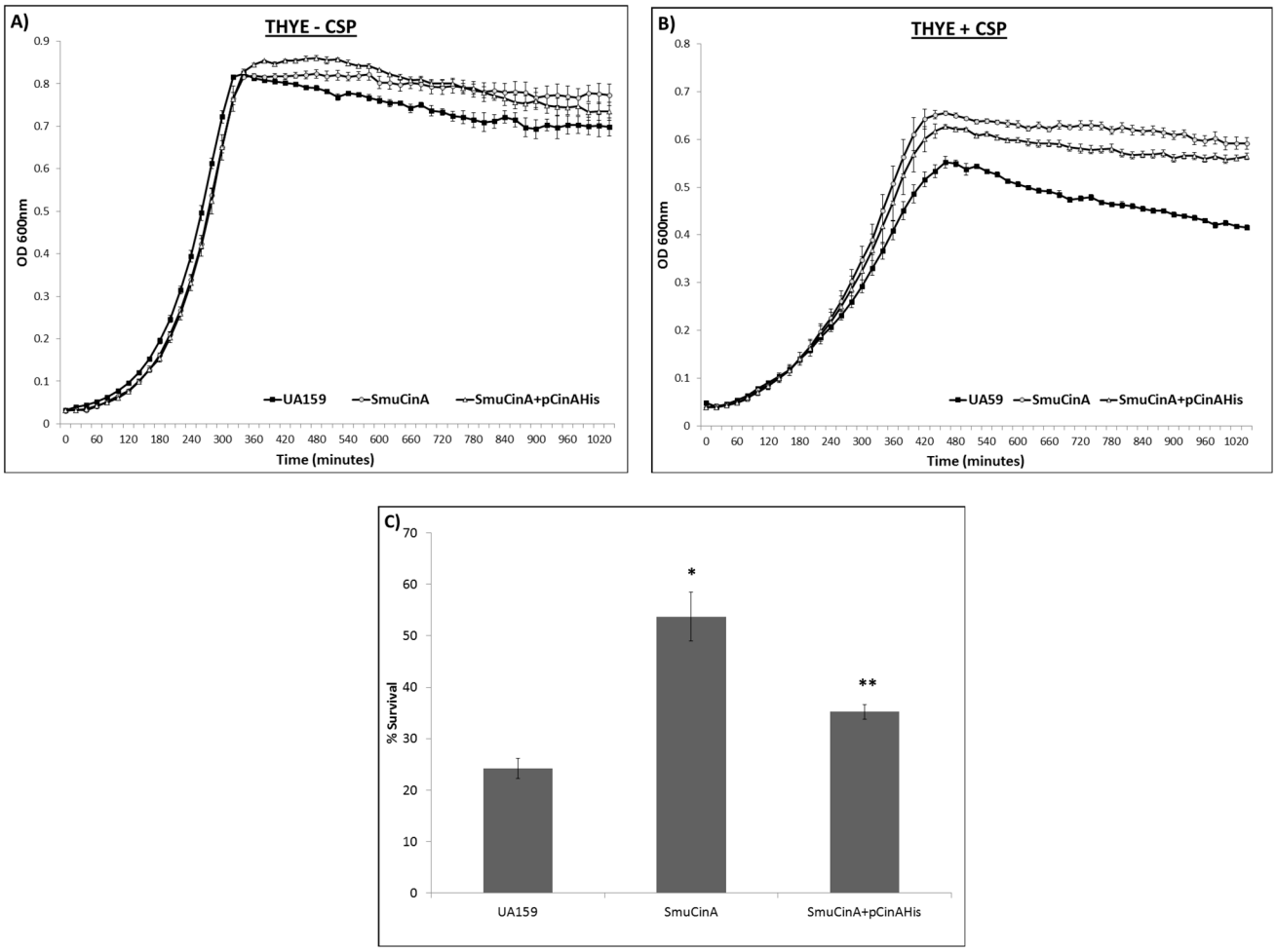

Figure 4.

Growth kinetics of *S. mutans* strains UA159, SmuCinA and SmuCinA+pCinAHis in THYE (A) and THYE supplemented with 1 μg/ml of CSP (B). Graphs represent mean ± SE (n=5). (C) Cell viability assays were performed by growing each strain to post-exponential phase in the presence and absence of 1μg/ml CSP. Statistical significance was calculated using Student's T-test wherein * $p<0.002$ and ** $p<0.01$. Graphs represent mean ± SE (n=4).

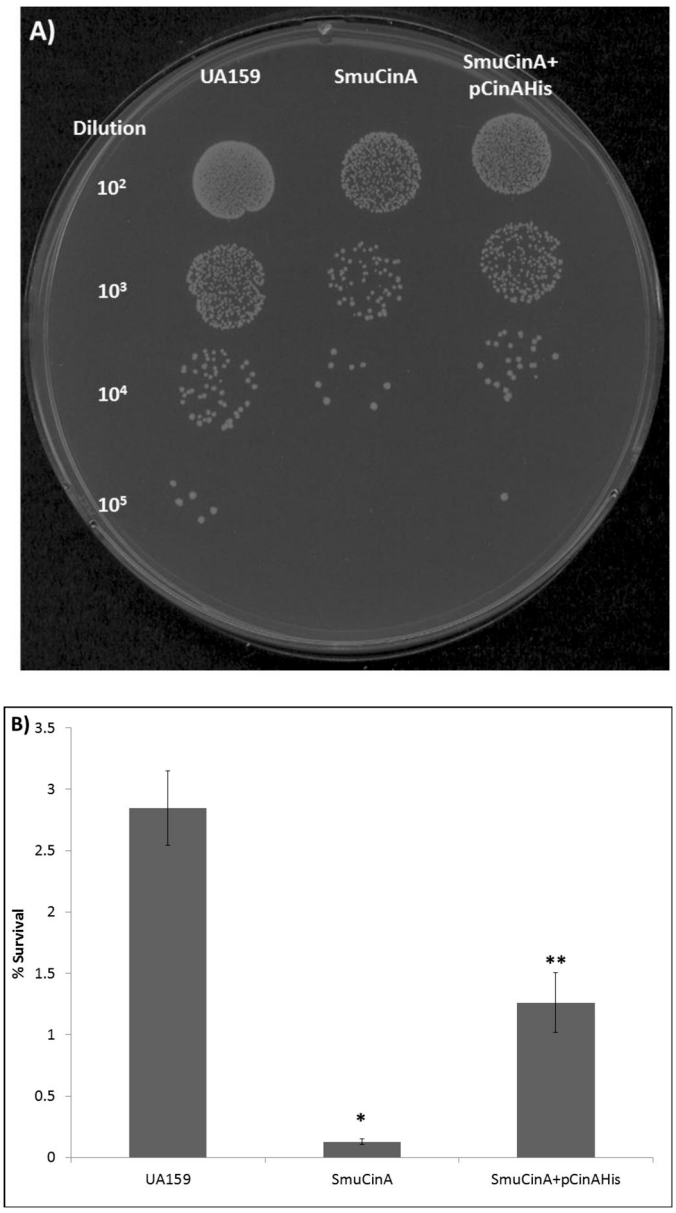

Figure 5.

A) Effects of 0.1% methyl methanesulfonate (MMS) on viability of *S. mutans* strains UA159, SmuCinA and SmuCinA+pCinAHis.B) Graphical representation of results from (A) subjected to statistical analysis using Student's T-test; * $p<0.0002$ and ** $p<0.007$. Graphs represent mean ± SE (n=4).

Table 1

Strains and primers used in this study

Primer	**Sequence (5′ - 3′)**	**Description**
cinA-P1	GAAGGAACGACTGATACG	*cinA*mutagenesis
cinA-P2	ggcgcgccCAGATTTCATTCTAACCTCC	*cinA*mutagenesis
cinA-P3	ggccggccGAACAATGGCAATCAAAGTG	*cinA*mutagenesis
cinA-P4	ACGGACATCAAGACGCACAG	*cinA*mutagenesis
erm-F	ggcgcgccCCGGGCCCAAAATTTGTTTGAT	Erythromycin cassette
erm-R	ggccggccAGTCGGCAGCGACTCATAGAAT	Erythromycin cassette
ProHisCinA-F	CAgagctcCTATGTGTGATGGTGATGATGAATTTTTATGAGATAATAAAGT	pCinAHisA complimentation
ProCinA-R	AGgaattcAAGAGGCTTAACTAGCTCAA	pCinAHisA complimentation
cinAprobe-F	CCTATGGGCTTTTGTTGATAAACG	cinA probe, Northern blot
cinAprobe-R	GCAGTTGGTGATAATGAAGAACGC	cinA probe, Northern blot
recAprobe-F	TTGCCACGGACATCAAGACG	recA probe, Northern blot
recAprobe-R	ACCAGATTCAGGAGAACAGGGTC	recA probe, Northern blot
cinArt-F	GCGTAACACACGAGAATAAAGC	*cinA* qRT-PCR
cinArt-R	TTGGAGGCAGATGGAGTGAC	*cinA* qRT-PCR
recArt-F	TATCTCCGTCAATCTCCGCACG	recA qRT-PCR
recArt-R	CTATGCTGCTGCTCTTGGTG	recA qRT-PCR
16sRNArt-F	CTTACCAGGTCTTGACATCCCG	16S qRT-PCR
16sRNArt-R	ACCCAACATCTCACGACACGAG	16S qRT-PCR
Strain	**Relevant Charateristics**	**Reference/source**
UA159	*S. mutans* wild-type strain	J. Ferretti Univ. of Oklahoma
SmuCinA	Δsmu.2086; Em^r	This study
SmuCinA+pCinAHIs	Δsmu.2086; Em^r + pCinAHis; $Spec^r$	This study
Plasmids	**Relevant Charateristics**	**Reference/source**
pDL277	$Spec^r$	(LeBlanc, *et al.*, 1992)
pDL289	Kan^r	(Buckley, *et al.*, 1995)

Note: Restriction Enzyme sites are in lower case AscI - ggcgcgcc; FseI – ggccggcc; EcoRI – gaattc; SacI – gagctc. Penta-His Tag is underlined.