Gene 457 (2010) 42–49

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

# Chitin disaccharide $(GlcNAc)_2$ induces natural competence in *Vibrio cholerae* through transcriptional and translational activation of a positive regulatory gene *tfoX*$^{VC}$

Shouji Yamamoto *, Masatomo Morita, Hidemasa Izumiya, Haruo Watanabe

*Department of Bacteriology I, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan*

ARTICLE INFO

*Article history:*
Received 10 December 2009
Received in revised form 4 March 2010
Accepted 4 March 2010
Available online 17 March 2010

Received by D.L. Court

*Keywords:*
*Vibrio cholerae*
Natural competence
Chitin
*tfoX*
Transcriptional control
Translational control

ABSTRACT

A pathogenic marine bacterium *Vibrio cholerae* shows natural competence for genetic transformation in the presence of chitin, a polymer of *N*-acetylglucosamine (GlcNAc). In this study, we extensively analyzed the regulatory mechanisms of *tfoX*$^{VC}$, encoding an activator protein for the chitin-induced competence. Using a chromosomal *tfoX*$^{VC}$-*lacZ* reporter system, we showed that a disaccharide of chitin, $(GlcNAc)_2$, at least was needed to activate both the transcription and translation of *tfoX*$^{VC}$. This activation was moderate at the transcriptional level but was strong at the translational level. We also identified two sequence elements, one for transcription and another for translation. The transcriptional control element (TCE) included a 34-bp potential transcriptional operator overlapped by the *tfoX*$^{VC}$ promoter, while the translational control element (TLE) consisted of a 42-bp sequence located within the 5′-untranslated region. Deletion of either TCE or TLE still resulted in $(GlcNAc)_2$-dependent competence for exogenous DNA. However, the deletion in both elements induced competence for transformation at high efficiency regardless of the presence or absence of $(GlcNAc)_2$. These results suggested the dual activation of *tfoX*$^{VC}$ expression to be essential to induce competence. The highly transformable strain created here should aid the study of natural competence in *V. cholerae*.


## 1. Introduction

Natural competence is the ability of bacteria to actively take up exogenous DNA and integrate it into the recipient chromosome, ultimately leading to "transformation", which is universally recognized as a strategy of horizontal gene transfer as well as transduction and conjugation. In well-characterized naturally competent bacteria (e. g., *Haemophilus influenzae* for Gram-negative bacteria, and *Streptococcus pneumoniae* and *Bacillus subtilis* for Gram-positive bacteria), the development of a competent state is tightly controlled (Claverys et al., 2006).

Natural competence is also observed in the pathogenic marine bacterium *Vibrio cholerae*, whose competence program is induced in response to at least three environmental signals: 1) increasing cell density, 2) nutrient limitation, and 3) the presence of chitin, a polymer of β-1,4-linked *N*-acetylglucosamine (GlcNAc) (Meibom et al., 2005). The former two signals activate *hapR*, encoding a central regulator of the well-characterized quorum-sensing system of *V. cholerae* (Lenz et al., 2004; Nielsen et al., 2006; Liang et al., 2007), whereas the latter activates *tfoX*$^{VC}$, encoding an ortholog of the *H. influenzae* competence regulator, TfoX (Meibom et al., 2004, 2005; Cameron and Redfield, 2006). HapR up-regulates the expression of *VC1917*, encoding an essential competence protein homologous to the *B. subtilis* ComEA, but down-regulates the expression of *dns*, encoding an extracellular nuclease that degrades exogenous DNA (Blokesch and Schoolnik, 2008). TfoX$^{VC}$ activates the expression of competence genes more widely than HapR, including not only *VC1917* but also structural genes for type IV pilus-like DNA uptake machinery (*pilA*, *pilB*, and *pilQ etc*) (Meibom et al., 2005). In addition, overproduction of TfoX$^{VC}$ induces competence even in the absence of chitin (Meibom et al., 2005). Thus, TfoX$^{VC}$ plays a central role in the chitin-induced competence of *V. cholerae*. However, the mechanisms underlying the chitin-dependent activation of *tfoX*$^{VC}$ remain unknown.

In this report, we describe several novel aspects of the regulatory mechanisms of *tfoX*$^{VC}$ expression. First, chitin disaccharide $(GlcNAc)_2$ acted as a minimum inducer for chitin-dependent competence. Second, this induction involved both transcriptional and translational activation of *tfoX*$^{VC}$. Third, we identified, within the region upstream of *tfoX*$^{VC}$, sequence elements responsible for the transcriptional and translational control. Finally, disruption of these elements conferred a $(GlcNAc)_2$-independent competence phenotype on *V. cholerae*.

## 2. Materials and methods

### 2.1. Bacterial strains, plasmids, media, and DNA manipulation

All the strains and plasmids used in this study are listed in Table 1. The *Escherichia coli* strain JM109 (Yanisch-Perron et al., 1985) was used for DNA cloning. The bacteria were grown in Luria–Bertarni (LB) broth and

*Abbreviations:* bp, base pair(s); CFUs, colony forming units; Cm, chloramphenicol; DB, downstream box; DSE, downstream element; FRT, FLP recognition target; IR, inverted repeat; LB, Luria–Bertarni; GlcNAc, *N*-acetylglucosamine; PCR, polymerase chain reaction; TCE, transcriptional control element; TLE, translational control element.

* Corresponding author. Tel.: +81 3 5285 1111; fax: +81 3 5285 1163.
*E-mail address:* yshouji@nih.go.jp (S. Yamamoto).

0378-1119/$ – see front matter 
doi:10.1016/j.gene.2010.03.003

**Table 1**
Strains and plasmids used in this study.

| Strain or plasmid | Characteristics | Source or reference |
|---|---|---|
| Strains | | |
| N16961 | Biovar El Tor | Heidelberg et al. (2000) |
| SY1001 | N16961 Δ*ctxB*::FRT::*cat*::FRT | Yamamoto et al. (2009) |
| SY1005 | N16961 Δ*lacZ*::FRT *ctxA*::*lacZ*::FRT::*cat*::FRT | Yamamoto et al. (2009) |
| SY1012 | N16961 FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }+92}$ | This study |
| SY1012S | N16961 FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }+92}$ | This study |
| V060002 | Biovar El Tor | Clinical isolate, Japan |
| SY0603 | V060002 Δ*lacZ*::FRT::*cat*::FRT | This study |
| SY0603S | V060002 Δ*lacZ*::FRT | This study |
| SY0605 | SY0603S *ctxA*::*lacZ*::FRT::*cat*::FRT | This study |
| SY0608 | V060002 Δ*tfoX*::FRT::*cat*::FRT | This study |
| SY0608S | V060002 Δ*tfoX*::FRT | This study |
| SY0609 | V060002 Δ*pilA*::FRT::*cat*::FRT | This study |
| SY0609S | V060002 Δ*pilA*::FRT | This study |
| SY0610 | V060002 *tfoX*$_{\Delta+7\text{ to }+92}$:: FRT::*cat*::FRT | This study |
| SY0610S | V060002 *tfoX*$_{\Delta+7\text{ to }+92}$:: FRT | This study |
| SY0611 | V060002 FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }-1}$ | This study |
| SY0611S | V060002 FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }-1}$ | This study |
| SY0612 | V060002 FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }+92}$ | This study |
| SY0612S | V060002 FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }+92}$ | This study |
| SY0612S09 | V060002 FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }+92}$ Δ*pilA*::FRT::*cat*::FRT | This study |
| SY0612S09S | V060002 FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }+92}$ Δ*pilA*::FRT | This study |
| SY0613 | SY0603S *tfoX*$_{TC}$::*lacZ*::FRT::*cat*::FRT | This study |
| SY0613S | SY0603S *tfoX*$_{TC}$::*lacZ*::FRT | This study |
| SY0613SP | SY0603S FRT::*cat*::FRT::*tfoX*$_{TC\Delta-38\text{ to }-8}$::*lacZ*::FRT | This study |
| SY0615 | SY0603S FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{TC\Delta-38\text{ to }-1}$::*lacZ*::FRT | This study |
| SY0624 | SY0603S FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{TC\Delta-38\text{ to }+42/\Delta+107\text{ to }+712}$::*lacZ*::FRT | This study |
| SY0616 | SY0603S *tfoX*$_{TL}$::*lacZ*::FRT::*cat*::FRT | This study |
| SY0616S | SY0603S *tfoX*$_{TL}$::*lacZ*::FRT | This study |
| SY0625 | SY0603S *tfoX*$_{TL\Delta+107\text{ to }+712}$::*lacZ*::FRT::*cat*::FRT | This study |
| SY0625S | SY0603S *tfoX*$_{TL\Delta+107\text{ to }+712}$::*lacZ*::FRT | This study |
| SY0617 | SY0603S FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{TL\Delta-38\text{ to }-1}$::*lacZ*::FRT | This study |
| SY0618 | SY0603S FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{TL\Delta-38\text{ to }+42}$::*lacZ*::FRT | This study |
| SY0619 | SY0603S FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{TL\Delta-38\text{ to }+84}$::*lacZ*::FRT | This study |
| SY0620 | SY0603S FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{TL\Delta-38\text{ to }+92}$::*lacZ*::FRT | This study |
| SY0621 | SY0603S FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{TL\Delta-38\text{ to }+42/\Delta+134\text{ to }+712}$::*lacZ*::FRT | This study |
| SY0622 | SY0603S FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{TL\Delta-38\text{ to }+42/\Delta+107\text{ to }+712}$::*lacZ*::FRT | This study |
| SY0623 | SY0603S *pilA*::*lacZ*::FRT::*cat*::FRT | This study |
| SY062308K | SY0623 Δ*tfoX*::FRT::*kan*::FRT | This study |
| V070035 | Biovar El Tor | Clinical isolate, Japan |
| SY0708 | V070035 Δ*tfoX*::FRT::*cat*::FRT | This study |
| SY0708S | V070035 Δ*tfoX*::FRT | This study |
| SY0709 | V070035 Δ*pilA*::FRT::*cat*::FRT | This study |
| SY0709S | V070035 Δ*pilA*::FRT | This study |
| SY0712 | V070035 FRT::*cat*::FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }+92}$ | This study |
| SY0712S | V070035 FRT::$P_{tac}$::*tfoX*$_{\Delta-38\text{ to }+92}$ | This study |
| Plasmids | | |
| pKD3 | Template plasmid for Red system, *bla* FRT::*cat*::FRT *ori* R6K | Datsenko and Wanner (2000) |
| pKD4 | Template plasmid for Red system, *bla* FRT::*kan*::FRT *ori* R6K | Datsenko and Wanner (2000) |
| pKD46 | Red expression plasmid, *bla* $P_{BAD}$ *gam bet exo ori* pSC101 | Datsenko and Wanner (2000) |
| pCP20 | Flp expression plasmid, *bla cat cI*857 λ$P_R$ *flp ori* pSC101 | Datsenko and Wanner (2000) |
| pGEM-T | TA cloning vector, *bla* | Promega |
| pRL124 | *lacZ* transcriptional fusion vector, *bla lacZYA* | Malo and Loughlin (1988) |
| pTrc99A | Expression vector, *bla* $P_{tac}$ | Amann et al. (1988) |

agar, and SOC. The antibiotic concentrations were 100 μg/ml ampicillin, 1 μg/ml chloramphenicol (Cm), and 30 μg/ml kanamycin. L-arabinose was used at 5 mg/ml. Polymerase chain reaction (PCR) amplification of DNA was carried out with a GeneAmp®PCR System 9700 (Applied Biosystems) using the Herculase® II Fusion Enzyme (Stratagene). Customized primers, the nucleotide sequences of which are listed in Table S1, were purchased from Greiner Bio-One. Chromosomal DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen).

## 2.2. Sequencing of the *tfoX*$^{VC}$ and *hapR* genes

Sequencing of *tfoX*$^{VC}$ and *hapR* in the *V. cholerae* strains used was done using the primer pairs TFOXf2/TFOXr7 and HAPRf2/HAPRr2, respectively.

## 2.3. Chromosomal engineering

Genetically modified strains were constructed using the λ Red-FLP recombination system optimized for *V. cholerae* (Yamamoto et al., 2009). Briefly, the procedure includes three processes: 1) preparation of a PCR fragment containing two FRT (Flp recognition target)-linked antibiotic resistance gene cassette with flanking regions homologous to the target gene (*X*::FRT::*cat*::FRT::*X*′ or *X*::FRT::*kan*::FRT::*X*′ fragment: *X*, the upstream homologous sequence; *X*′, the downstream homologous sequence; the size of *X* or *X*′ = ~1000 bp), 2) introduction of the PCR fragment into the recipient strain expressing λ Red recombinase to integrate it into the chromosome, and 3) elimination of the antibiotic resistance gene by FLP recombinase if necessary.