Characterizing the Regulation of tfoX *in* Vibrio fischeri

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Abstract

Natural transformation describes how some bacteria can incorporate exogenous DNA into their genome and acquire new traits. This phenomenon occurs in *Vibrios* and is induced by the biopolymer chitin. In *Vibrios*, TfoX is a primary regulator of natural transformation. The mechanisms underlying chitin-induced natural transformation and activation of TfoX in *Vibrio fischeri* remain unclear. This project examines the response of *tfoX* expression to various genetic factors and environmental signals, such as *N*-acetyl-glucosamine, which is derived from chitin. The findings from this study will help us construct a model for the regulatory pathway of *tfoX* in *Vibrio fischeri*.

Introduction

Unicellular organisms greatly outnumber multicellular organisms. This could be credited to the ability of certain microbes to adapt and transform to their environment at a remarkable rate. Some microbes are able to incorporate DNA from their surroundings into their genome. This phenomenon is called natural transformation and contributes to the emergence of new traits and characteristics within the bacterium that is competent or able take in DNA material (Sun et al 2013).

Recent studies have shown that species of the genus *Vibrio* exercise natural transformation. The *Vibrionaceae* family consists of numerous Gram-negative bacteria that mostly reside in aquatic environments. Some Vibrios specifically interact with their respective multicellular host. Competence and natural transformation could be vital in these host-microbe interactions. *Vibrio cholerae*, the causative agent of the cholera disease, is predicted to competently attain genetic elements pertaining to interactions with its host from neighboring cells (Sun et al 2013). The pathogenic bacterium has garnered attention from biomedical researchers due to its impact on human health.

In addition to *Vibrio cholerae*, other Vibrios including *Vibrio fischeri* have demonstrated natural transformation (Pollack-Berti et al 2010). Despite being ancestrally related to *V. cholerae*, *V. fischeri* is a non-pathogenic bacteria that leads a symbiotic relationship with the Hawaiian bobtail squid, *Euprymna scolopes* (Verma et al 2013). With regards to natural transformation, the rate of transformation is much lower in *V. fischeri* than that of *V. cholerae*. The reason for the difference in the transforming ability between the two Vibrios remains unclear. Regardless of this difference, much of the components needed for natural transformation are conserved in Vibrios.

Previous work has shown that the biopolymer chitin appears to be a factor in the Vibrios' natural transformation process. Chitin, a biopolymer composed on β1,4-linked *N*-acetyl-glucosamine monomers, is a constituent of exoskeletons of crustaceans and the cell wall of fungi. Vibrios have been shown to thrive in environments in which chitin is abundant such as surfaces of copepods (Dalia et al 2013). Vibrios are able to use chitin as carbon and nitrogen source. Several marine Vibrios are even able to utilize carbon as a sole carbon source (Meibom et al 2003). The monomer of chitin, *N*-acetylglucosamine (GlcNAc) is a preferred carbon source that Vibrios are able to process. Hence, they have evolved to acquire GlcNAc utilization genes that are needed for the uptake and metabolism of GlcNAc. NagE, encoded by *nagE,* transports and phosphorylates GlcNAc (GlcNAc-6P). The *nagBAC* genes encode for proteins that break down the GlcNAc (Miyashiro et al 2011).

In the absence of GlcNAc, Vibrios, as well as other γ-proteobacteria, use the transcriptional repressor NagC to inhibit expression of the *nag* locus (Miyashiro et al 2011). NagC binds to two distinct sites of the *nagE* and *nagBAC* operons to inhibit transcription. This repression is alleviated by the allosteric binding of GlcNAc-6P to NagC. Activation of the *nag* genes leads to the processing of the GlcNAc as a nutrient source.

In addition to serving as a nutrient source, chitin also induces competency by transcriptionally and post-transcriptionally activating the transcription factor TfoX (Yamamoto et al 2010). All sequenced *Vibrionaceae* members possess a homologue of *tfoX* (Pollack-Berti et al 2010). TfoX activates genes that encode for the competence machinery.

Preliminary findings of this study revealed that NagC also binds and represses *tfoX* in *V. fischeri*. Therefore, we hypothesized that the presence of GlcNAc would lead to the activation of the GlcNAc utilization genes and *tfoX*. This hypothesis was tested using a GlcNAc dose response assay in which the expression of each gene was measured at increasing concentration of GlcNAc. We found that genes of the *nag* locus were activated by high concentrations of GlcNAc. However, *tfoX* expression did not increase at these high concentrations inferring a GlcNAc-independent regulation. Constructing a model that explains this *tfoX* regulation in *V. fischeri* will help us understand natural transformation better in Vibrios.

Results

GFP used to follow gene expression

The expression of the different genes was followed via reporter plasmids containing transcriptional fusions of specific promoters with *gfp*, which encodes green fluorescent protein (GFP). A gene encoding for a red fluorescent protein, mCherry, is transcribed from a constitutively expressed promoter also contained in the reporter plasmid. This serves as a control as levels of mCherry expression should be similar for all samples.

GlcNAc dose response assay

The genes *nagA*, *nagB*, *nagE*, and *tfoX* were subjected to increasing concentrations of GlcNAc to determine their corresponding responses (fig 1). The concentrations ranging from 0.01mM to 100mM were sufficient to compare expression of the genes under study. Each plot represents the effect of GlcNAc concentrations 0.01, 0.1, 1, 10, 100 mM has on the represented gene. Overall the results show that the expression of *nagA* and *nagB* was noticeably affected by increasing the concentration of GlcNAc. At higher concentrations of GlcNAc, the expression of these genes increased by about a ten-fold. For *nagE*, expression increased at 1mM and doubled to about two-fold at 10mM. In contrast, the expression of *tfoX* does not change as the concentration of GlcNAc is increased. This leads us to believe that another regulator specific to *tfoX* might be the cause of this anomaly.

GlcNAc dose-response curves for the four genes in WT

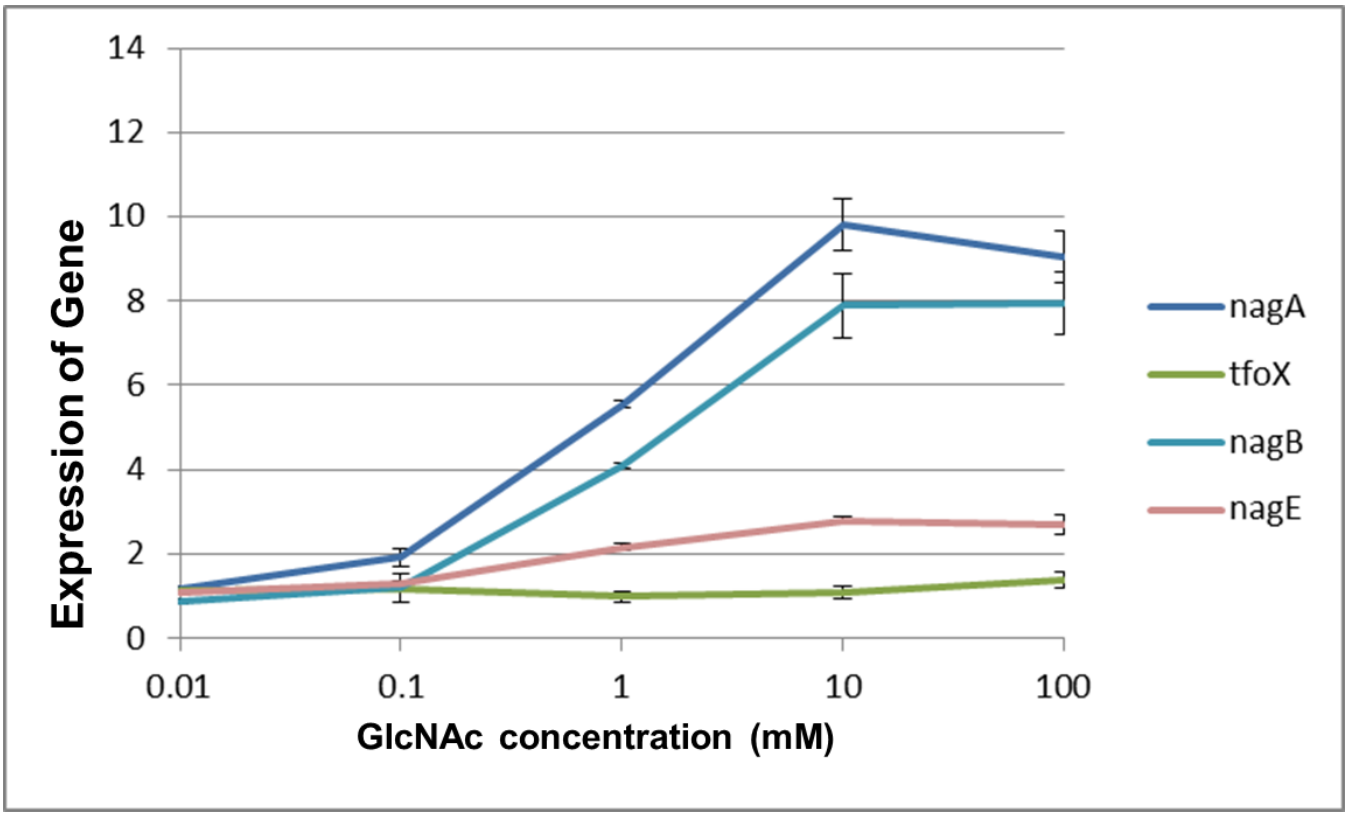

Figure 1: Expression for WT strains for four different promoters over increasing GlcNAc conc.

After seeing that *tfoX* expression was not activated by the increasing GlcNAc concentration, we wanted to test if the repression would be alleviated if NagC was removed and GlcNAc concentration was increased. Figure 2 shows that the response for *tfoX* expression in the *nagC* mutant (Δ*nagC*/*tfoX*), does not change when GlcNAc concentration is increased to 100mM. This indicates that the possible unknown regulator of *tfoX* is independent of GlcNAc levels.

GlcNAc dose response for *tfoX* WT and ΔnagC

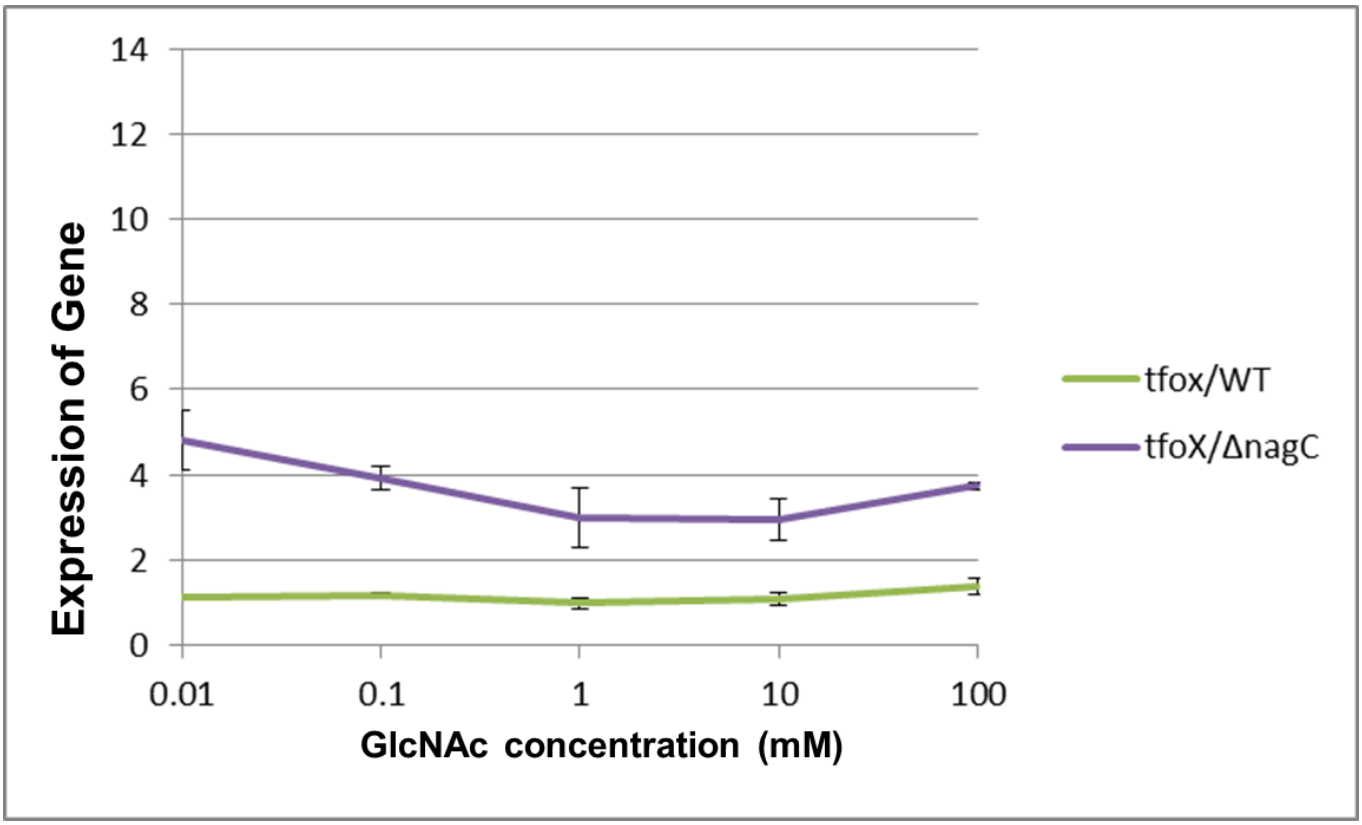

Figure 2: Expression of *tfoX* in WT and the *nagC* mutant for increasing GlcNAc concentration

Discussion

The present study was performed in order to characterize the regulatory network of *tfoX*. The fusion of the color reporter, GFP, with the promoters of *tfoX* and the GlcNAc utilization genes allowed for the monitoring of the genes' response to different concentrations of GlcNAc. Surprisingly, *tfoX* is not activated by the presence of GlcNAc like demonstrated for *nagA*, *nagE*, and *nagB*. This indicates that there could be another regulator for *tfoX* that is repressing it. We predict that this regulator is independent of GlcNAc. One possibility is that the binding pocket of GlcNAc-6P on NagC is blocked due to the dimerization of NagC. This could lead to the lack of activation of *tfoX* by GlcNAc. However, recent experiments have demonstrated that the media that *V. fischeri* cells are grown in might be the reason activation is not apparent for *tfoX* when GlcNAc is present. The latest experiment showed that induction of *tfoX* by GlcNAc is achieved

when the cultures are grown in minimal media. This indicates that the repression of *tfoX* demonstrated in the dose response curves above is due to a factor present in the LBS media. This media is a complex media with components that are not defined. I plan on doing dose response experiments with the minimal media to test if *tfoX* is activated by higher concentrations of GlcNAc, supporting the GlcNAc-NagC regulation model.

Methodology

Growth media

V. fischeri cultures are grown on LBS media at 28°C with 2.5 µg/mL chloramphenicol (CAM). CAM is the antibiotic needed so the plasmid is retained. When inoculating strain into liquid media, one micro liter is used per one mL of media.

Dose-response fluorescence assay

Cultures are grown overnight in the liquid media and antibiotic for the fluorescence experiment the next day. Before diluting overnight cultures, falcon tubes with the different concentrations of GlcNAc are prepared. From a 0.5M GlcNAc stock, GlcNAc concentrations of 0.01, 0.1, 1, 10, 100mM are prepared in a conical tube containing 10 ml of LBS+CAM. 2 ml of each concentration is dispensed into three falcon tubes for biological replicates.

Dilution of overnight cultures is 1:100. 20 µl of the overnight culture is introduced to 2ml media of each falcon tube. Afterwards, the diluted cultures are grown to optical density (O.D.) of 1. When cultures reach O.D. ~1, they are placed in an ice bucket to stop their growth. 1 ml from each falcon tube is transferred to a 1.5 ml centrifuge tube. The centrifuge tubes are spun at 15000 rpm, 4 degree Celsius for 5 minutes. The supernatant is then discarded and the pellets resuspended in 350 µl of Tris minimal media.

A plate reader is used to measure the fluorescence of cells resuspended in the minimal media. 100 µl from each centrifuge tube is transferred three times to three wells of a 96-well plate. These represent technical replicates.

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