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Regulation of toxin gene expression in *Clostridium perfringens*

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Abstract

The Gram-positive, anaerobic, spore-forming, rod-shaped *Clostridium perfringens* is widely distributed in nature, especially in soil and the gastrointestinal tract of humans and animals. *C. perfringens* causes clostridial myonecrosis (or gas gangrene), enteritis and enterotoxemia in humans and livestock by producing numerous extracellular toxins and enzymes. The toxin gene expression is regulated by a two-component regulatory system and regulatory RNA VirR/VirS-VR-RNA cascade. The VirR/VirS system was originally found in a type A strain, but a recent report showed that it is also important for the toxin gene regulation in other types of strains. Two types of cell–cell signaling, i.e., *agr*-system and AI-2 signaling, are also important for the regulation of toxin genes. Several regulatory systems independent from the VirR/VirS system, including *virX*, the orphan histidine kinase ReeS and orphan response regulator RevR, are also involved in the regulation of toxin genes. In addition, the expression of toxin genes is upregulated after contact with Caco-2 cells. *C. perfringens* has a complex regulatory network for toxin gene expression and thus the coordination of toxin gene expression is important for the process of infection.

Keywords: Substance A; Two-component system; Cell–cell signaling; Regulatory RNA; Cell–pathogen signaling

1. Introduction

Clostridium perfringens is a Gram-positive, spore-forming anaerobic bacterium widely distributed in the environment. *C. perfringens* is classified into five types (A to E) according to the productivity of the main toxins (alpha-, beta-, epsilon-, and iota-toxins) [13,43]. The type A strains are recognized as a major pathogen in humans [13,43]. *C. perfringens* is the causative agent of several human and animal diseases, including clostridial myonecrosis, or gas gangrene, enteritis and/or enterotoxemia in humans and livestock [13]. *C. perfringens* produces various extracellular enzymes and toxins. These toxins and enzymes have specific activities and roles in the disease process, and it is thought that a synergistic action of the toxins and enzymes on the host tissue is needed for the development of unique infectious lesions [44].Figs. 1 and 2.

Since the late 1980s, many genes of known toxins and enzymes have been cloned and sequenced, and studies on these genes, the functions of their gene products, and their interactions with hosts have been intensively undertaken [44]. In conjunction with improvements in gene-analysis systems, including the construction of shuttle vectors [48] and identification of transformable strains, *C. perfringens* has been recognized as a model organism for the genetic analysis of clostridia.

Complete genome analysis of *C. perfringens* has suggested that *C. perfringens* lacks many of the genes required for amino acid biosynthesis [46,31]. To obtain the required nutrients under infectious conditions, *C. perfringens* must secrete toxins and enzymes that degrade macromolecules in the host, which is critical for bacterial survival in the host. A global regulatory system of toxin gene expression is needed to govern the process of infection and to derive nutrients from the host tissue quickly and effectively.

Therefore, the study of toxin gene regulation in *C. perfringens* is one of the most important approaches for understanding the pathogenicity of this organism.

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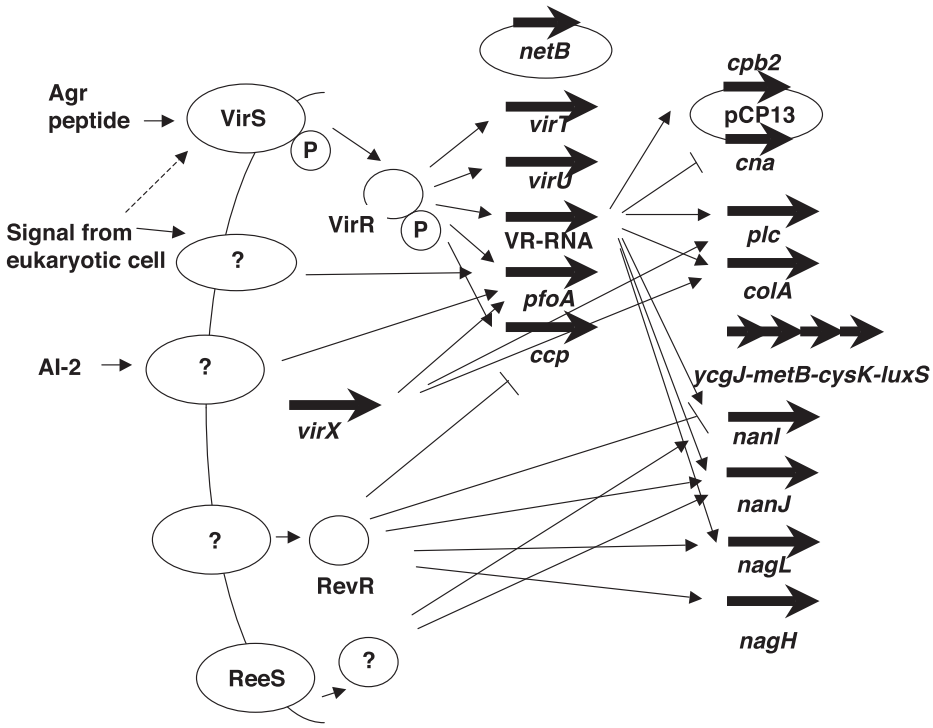

Fig. 1. Schematic representation of the global regulatory network of toxin gene expression in *Clostridium perfringens*. The diagram was constructed using the results of studies mentioned in this chapter.

Recent progress in the investigation of toxin gene regulation in *C. perfringens* is summarized below. It is hoped that this review will establish a fresh starting point for future research and application.

2. Identification of two-component VirR/VirS system

Since 1970s, it has been suggested that a specific regulatory mechanism for toxin production exists in *C. perfringens*. In an early study, θ-toxin (or perfringolysin O, PFO) -negative strains were isolated by using a chemical mutagenesis system [14]. These strains were divided into two groups, group 'a' and 'b'. When the group 'a' and 'b' strains were cross-streaked on a blood agar plate, the group 'b' strain recovered θ-toxin production just after the crossing point. The activities of κ-toxin and hemagglutinin were also recovered in the group 'b' mutant [19]. Further analysis showed that the group 'a' supernatant could activate the θ-toxin production in group 'b' mutants [18]. These data suggested that there is an extracellular signal molecule (called substance A) produced by group 'a' strains. Group 'a' strains could produce substance A but had a mutation in the θ-toxin encoding gene [19]. Group 'b' strains were unable to produce substance A but had an intact θ-toxin gene. If group 'b' strains sensed the substance A, the substance A activated gene expression and recovered θ-toxin production. This complementation of θ-toxin production occurred even when two strains were co-cultured but separated by a cellophane membrane, indicating that the substance A is a small molecule which can pass through the cellophane membrane [18,19]. A small signaling molecule has been reported to be involved in the regulation of the production of κ- and θ-toxins, and the data suggest that there is a global regulatory system for toxin production. However, the genetic nature of the regulatory mechanism for toxin production has been unclear [14,19].

The identity of the global regulator was investigated by random chemical mutagenesis, NTG-mutagenesis and construction of toxin-negative strains [45]. When a chromosomal library of the wild-type strain 13 was transformed into a toxin-negative strain, SI112, 5 out of 10,000 transformants restored θ-toxin production on a blood agar plate [45]. All transformants contained a recombinant plasmid carrying the same 2.5-kb DNA fragment from strain 13. Further deletion and nucleotide sequencing analyses showed that a 1.25-kb region of the original 2.5-kb fragment was responsible for the trans-regulatory activity and that there is an intact ORF (ORF2) in this region. A homology search for the deduced amino acid sequence revealed that ORF2 was homologous to a response regulator of a two-component regulatory system and thus designated as *virR* [45].

In another study, Tn916 mutagenesis was used to isolate θ-toxin-negative strains [26]. An inverse PCR method was used to clone the mutated region. The isolated toxin-negative strain could restore the toxin production by transformation of the 4.3-kb *Pst*I fragment. The analysis of this 4.3-kb fragment showed that the fragment contained a response regulator and histidine kinase, *virR* and *virS*, for a two-component regulatory system [26].

Two-component systems are signal transduction systems used by bacteria to regulate gene expression in response to