The VirR/VirS regulatory cascade affects transcription of plasmid-encoded putative virulence genes in Clostridium perfringens strain 13

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Abstract

We analyzed the transcriptional regulation of the putative virulence genes encoded on the plasmid pCP13 from Clostridium perfringens strain 13. The transcription of the beta2-toxin (cpb2) and possible collagen adhesin (cna) genes were regulated in both a positive and negative manner, respectively, by the two-component VirR/VirS system. The secondary regulator of the VirR/VirS system, VR-RNA, also affects the expression of both of these genes in the same fashion as the VirR/VirS system. This indicates that the global regulatory cascade of the VirR/VirS system controls the expression of virulence genes located on the plasmid, as well as those found chromosomally in C. perfringens strain 13.

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1. Introduction

The Gram-positive anaerobic pathogen, Clostridium perfringens, produces numerous extracellular toxins and enzymes that are believed to be involved in the ability of this organism to cause gas gangrene (clostridial myonecrosis) in humans. A variety of studies have defined the mechanistic basis for the contribution of identified toxins and enzymes to this particular pathology [1,2]. However, there seem to be other, as yet uncharacterized, virulence factors that will also participate in the pathogenicity of C. perfringens.

The structural genes for toxins produced by C. perfringens have been located on the chromosome or plasmid. The genes encoding alpha-toxin (ple), theta-toxin (pfoA), kappa-toxin (colA), hyaluronidase (nagH), and two sialidases (nanH and nanI) are reported to be chromosomally located, while those encoding beta-toxin (cpb), epsilon-toxin (etx), iota-toxin (iap and ibp), and lambda-toxin (lam) are found on plasmids of various sizes [1]. In contrast, the enterotoxin gene (cpe) has been localized to the chromosome in C. perfringens strains involved in food poisoning of humans, while also found on a large plasmid in isolates from patients with non-food-borne human gastrointestinal diseases and veterinary cases [1].

Recently, the complete nucleotide sequences of the 3.04-Mb chromosome and 54.3-kb plasmid (pCP13) of C. perfringens strain 13 were determined [3]. This plasmid was found to carry the genes for beta2-toxin (cpb2) and possible collagen adhesin (cna), which may contribute to the pathogenicity of C. perfringens. On a post-sequencing level, we report here that the expression of cpb2 and cna is affected in C. perfringens strain 13 by both the two-component VirR/VirS system and its secondary RNA regulator, VR-RNA.

2. Materials and methods

2.1. Strains, media, and culture conditions

C. perfringens strains 13 [4], TS133 [5], TS140 [6], and their derivative strains were cultured in GAM medium
containing appropriate antibiotics at 37°C under anaerobic conditions, as described previously [5].

2.2. Northern hybridization

Total RNA from *C. perfringens* was extracted as described previously [5]. Northern blot analysis was carried out as described elsewhere [6] and signals were detected by CDPstar chemiluminescence (Amersham Biosciences). Gene probes were made by PCR amplification of *cna*, *cpb2*, and PCP16 with appropriate primer sets and were labeled with an AlkPhos-direct kit (Amersham Biosciences).

2.3. Primer extension analysis

The primer extension experiment was carried out as described previously [7] using a 5'-oligolabeling for fluorescein kit (Amersham Bioscience) and a primer extension system (Promega). Synthetic oligonucleotide primers (*cna*-PE2, 5'-AGTAATCCACTTAATCTTCTCCTTCCT-3', and *cpb2*-PE, 5'-TGCAGTTGCTTTCATTGGACTTAT-GCTCCAACA-3') were used to determine the transcription start sites of the *cna* and *cpb2* genes, respectively. Signals were detected using a FluorImager image analyzer (Amersham Biosciences).

3. Results and discussion

3.1. Analysis of the nucleotide sequence of pCP13

Previously, putative open reading frames (ORFs) were deduced from the nucleotide sequence of pCP13. A total of 63 ORFs (PCP01–PCP63, Fig. 1) were identified as residing on the plasmid [3]. BLAST-based searching revealed that the majority of these ORFs appear to encode proteins of unknown function (Fig. 1). Among the ORFs, the gene (PCP17) encoding beta2-toxin is known to be a novel virulence factor of *C. perfringens* [8]. The putative product of PCP17 showed 91% identity with the *cpb2* gene product identified in another *C. perfringens* strain [8]. It has been reported that the beta2-toxin-producing *C. perfringens* is associated with digestive tract diseases of piglets, horses and calves [8–11]. Since the *cpb2* gene is thought to be localized within large plasmids [8], pCP13 should be one of the plasmids carrying the beta2-toxin gene in *C. perfringens*. Another possible virulence-associated gene (*cna*) whose putative product (encoded by PCP57) showed similarity (23% identity across an 813-aa overlap) to a collagen adhesin from *Staphylococcus aureus* [12] was also found in pCP13 (Fig. 1). The *cna* gene might encode a cell wall-anchored protein possibly involved in the attachment of *C. perfringens* to connective tissues in the host.

![Fig. 1. Representation of the ORF map of plasmid pCP13. The direction of genes on the two strands is indicated by arrow-shaped symbols. Hatched symbols represent genes whose functions could be deduced from homology searches, while white symbols are genes with unknown function (encoding hypothetical proteins). Filled symbols indicate genes identified as putative virulence-associated genes. This figure was drawn from the nucleotide sequence of the plasmid pCP13 (accession number AP003515) [3].](https://academic.oup.com/femsl/issue/10962/7-5-03)
3.2. Transcriptional regulation of the cna and cpb2 genes by the VirR/VirS system

Many toxin genes, such as alpha-toxin (plc), kappa-toxin (colA), and theta-toxin (pfoA), are under the control of the two-component VirR/VirS system at the level of transcription in C. perfringens strain 13 [2,13]. Therefore, we investigated whether the VirR/VirS system is involved in the transcriptional regulation of the cna and cpb2 genes. Northern blot analysis was carried out on total RNA preparations from the wild-type strain 13 with pJIR418 vector [14], the virR mutant strain TS133 with pJIR418 [5], and TS133 with pBT405 (virR/virS⁺). Growth curves of strain 13 and TS133 were described in [15], and they showed no significant difference. In Northern analyses, we used PCP16 located upstream of the cpb2 gene (Fig. 1) as an internal control whose transcript showed no significant change in all the strains at any growth stages tested in this study (Fig. 2A,B).

The 5.0-kb transcript representing the cna gene was significantly increased in TS133(pJIR418) in comparison to wild-type strain 13(pJIR418) and the complemented strain TS133(pBT405) (Fig. 2A). Expression of cna mRNA was maximal after 2 h of culture (Fig. 2A). These results indicated that the VirR/VirS system negatively regulates the transcription of cna during the early exponential growth phase of C. perfringens strain 13. On the other hand, the 1.4-kb transcript derived from the beta2-toxin gene was apparently decreased in TS133(pJIR418), and its expression was clearly restored by transformation of pBT405 carrying intact virR/virS genes (Fig. 2A). The cpb2 gene was transcribed maximally at the mid exponential growth phase (3 h) (Fig. 2A). These findings clearly indicated that transcription of the cpb2 gene on the plasmid pCP13 is positively regulated by the VirR/VirS system.

3.3. Effect of VR-RNA on the expression of cna and cpb2

In previous studies, it was found that the VirR/VirS system involves a secondary RNA regulator (VR-RNA) to control several genes including alpha-toxin (plc), kappa-toxin (colA), 2’,3’-cyclic nucleotide phosphodiesterase (cpd), protein tyrosine phosphatase (ptp), and the ycgJ-metB-cysK-luxS operon [6,15,16]. To elucidate whether the cna and cpb2 genes are under the control of VR-RNA, we checked the level of transcription of these genes in the VR-RNA mutant strain TS140 [6]. Growth curves of strain 13 and TS140 showed no significant difference (data not shown). Compared with the transcript of PCP16, the mRNA level of cna increased in TS140(pJIR418) and decreased to a wild-type level in TS140(pSB1031) (Fig. 2B).
2B). On the contrary, the transcription of the cphb2 gene apparently decreased in TS140(pJIR418), and its expression was restored in TS140(pSB1031) carrying intact VR-RNA on pJIR418 [15] (Fig. 2B). The expression pattern of both genes in TS140 was quite identical to that found in TS133 (Fig. 2A,B). These results indicate that the expression of the cna and cphb2 genes is regulated by VR-RNA and that the VirR/VirS-VR-RNA regulatory cascade controls the transcription of both genes on pCP13.

3.4. Identification of the VR-RNA-regulated promoters

To characterize the VR-RNA-regulated promoters of the cna and cphb2 genes, primer extension analyses were carried out. The mRNA start site of the cna gene was located 183 bases upstream of the putative start codon GTG (Fig. 3A). The extension product of cna mRNA of wild-type strain 13 was apparently less than that of the VR-RNA mutant strain TS140 (Fig. 3A), which is in good agreement with the finding that the cna gene is negatively regulated by VR-RNA (see above). Similarly, the transcription start site for the cphb2 gene was located 28 bases upstream of the start codon ATG (Fig. 3B). Contrary to cna, the amount of the extended product of cphb2 was greater in the wild-type strain than that seen in TS140 (Fig. 3B), confirming the positive regulation of the cphb2 gene by VR-RNA.

3.5. Promoter analysis of the VR-RNA-regulated genes

According to the results of the primer extension experiments, we deduced possible promoter sequences (−35 and −10) in the upstream regions of the cna and cphb2 genes (Fig. 4). The deduced −35 and −10 sequences (AAGAAA and TGAAAT for cna, and TTGTTAA and TATAAT for cphb2) were similar to the typical consensus sequences seen in other promoter-determined genes such as plc, colA, pfoA, and VR-RNA of C. perfringens [6,17]. Furthermore, when the regions upstream of the deduced promoters were compared with the plc and colA genes, whose expression is affected by VR-RNA [6], no evidence of DNA sequence similarity was found (Fig. 4). Finally, it is now apparent that the VirR/VirS cascade regulates the expression of plasmid-encoded putative virulence genes in both a positive and negative manner in C. perfringens strain 13. This indicates that the global regulatory function of the VirR/VirS system also affects the expression of the genes on pCP13, as well as those on the chromosome. It is well known that the VirR/VirS system regulates the expression of many toxin genes on the chromosome and plays an important role in the pathogenicity of C. perfringens [5,6,15,17,18]. Therefore, it is highly possible that the cphb2 and cna genes also play important roles in the pathogenesis of gas gangrene in humans, as well as the intestinal diseases of animals. In other bacteria, several global regulators have been reported to control both chromosomal and plasmid-encoded genes. In E. coli K-12, a two-component system cusRS is required for copper-induced expression of plasmid-borne gene as well as at least one chromosomal gene [19]. A two-component sensor protein ChvG that is important for the virulence of Agrobacterium tumefaciens regulates acid-inducible genes on its two chromosome and Ti plasmid [20]. It is interesting to investigate how the chromosomal VirR/VirS system has evolved to regulate plasmid-encoded genes. Further comprehensive studies, e.g. using DNA microarray-based analysis, will be required to elucidate the precise mode of regulation of the chromosome- and plasmid-encoded genes in C. perfringens strain 13.

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References


