Cloning and characterization of the gene encoding the primary \( \sigma \)-factor of *Campylobacter jejuni*

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Abstract

The \( rpoD \) gene encoding the primary \( \sigma \)-factor of *Campylobacter jejuni* was amplified from genomic DNA with degenerate oligonucleotide primers. The complete gene encodes a polypeptide of 622 amino acids and has a deduced \( M_r \) of 72.6 kDa. This polypeptide is 40% identical to the RpoD \( (\sigma^{70}) \) protein of *Escherichia coli* and has 66% identity with the *Helicobacter pylori* RpoD protein. A *C. jejuni* \( \sigma^{70} \) promoter, not recognized by the *E. coli* \( \sigma^{70} \) factor, could be activated in this bacterium in the presence of the cloned *C. jejuni* RpoD protein. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

Keywords: *Campylobacter jejuni*; \( rpoD \); \( \sigma^{70} \)

1. Introduction

*Campylobacter jejuni* is the major causative agent of human diarrheal disease [1]. The DNA of this Gram-negative bacterium has a very low G+C content. *C. jejuni* is one of only a few bacterial species with a known requirement for microaerophilic growth conditions [1]. Although the number of characterized *C. jejuni* genes is increasing rapidly, still little is known about the regulation of gene expression in this organism. Knowledge of the mechanisms of gene regulation is, however, necessary for a better understanding of how *C. jejuni* causes disease and survives and multiplies inside or outside its host.

Transcription in prokaryotes is mediated by the RNA polymerase holoenzyme, which contains two major functional components: the core RNA polymerase and a sigma factor. The core RNA polymerase consists of two \( \alpha \) subunits and one each of the \( \beta \) and \( \beta' \) subunits. It has a low DNA binding affinity for any DNA sequence and possesses a DNA polymerization activity. The sigma factor, once bound to the core enzyme, dramatically increases the affinity of the enzyme for promoter sequences.

Sequence analysis of bacterial \( \sigma \) factors related to \( \sigma^{70} \) of *Escherichia coli* has revealed four conserved regions, regions 1–4 [2]. Segments of region 2 and 4 interact directly with the promoter [2,3]. The --35
and -10 regions of the promoter sequences are recognized by subregions 4.2 and 2.4 of these σ factors [4], respectively. The difference of the C. jejuni σ^70 promoter sequence with the canonical promoter sequence recognized by enteric bacteria [5] and the lack of recognition of typical E. coli σ^70 promoters [6] in C. jejuni and vice versa prompted us to isolate and characterize the σ^70 gene of C. jejuni.

2. Materials and methods

2.1. Strains and plasmids

E. coli strain MOSblue (Pharmacia, Uppsala Sweden) was used for cloning PCR products in the T cloning vector pMOS (Pharmacia). For all other transformations of plasmids to E. coli strain DH5α was used [7]. Chromosomal DNA fragments of C. jejuni strain 129108 [8] were cloned in the vector pBluescript KS M13 † (Stratagene, La Jolla, CA). Plasmids 2A12 and 12G7 have been described [5]. E. coli strains were routinely cultured in Luria broth (LB) or on LB agar. The antibiotics ampicillin (100 μg ml⁻¹), kanamycin (30 μg ml⁻¹) or tetracycline (15 μg ml⁻¹) were added when required.

2.2. Polymerase chain reaction (PCR)

To amplify a fragment of the σ^70 factor from C. jejuni genomic DNA two degenerate primers DP1 and DP2 derived from conserved σ^70 amino sequences were used [9]. Primer DP1 (5'-ACT TAT GC(A/T) AC(A/T) TGG TGG AT-3') was derived from the amino acids sequence TYATWWI and DP2 (5'-ATT TG(A/G) CG(A/T) ATG TCA CGT TCA CG-3') was derived from the heptapeptide sequence RERIRQI (Fig. 1). A PCR with 2 U Taq DNA polymerase (Promega, Madison, WI) was performed for 35 cycles of 1 min at 94°C, 1 min at 48°C and 1 min at 72°C, followed by a final extension of 5 min at 72°C.

2.3. Colony blot

To clone the complete rpoD gene a colony blot was performed on a genomic HindIII library of C. jejuni 129108 in pBluescript KS M13+ (Stratagene, La Jolla, CA) made as described previously [10] and on a cosmid library of C. jejuni 81116 [11]. The PCR fragment amplified with the degenerate primers was isolated from an agarose gel [7] using the GeneCleanII kit (Bio-101, La Jolla, CA) radiolabelled with [α³²P]dATP by random priming [12] and used as probe. The probe was used to screen the colonies from both genomic libraries that were blotted on a membrane (Hybond-N; Amersham, Amersham, UK). Hybridization was performed at 60°C and was followed by five washes in 2×SSPE [7]. Hybridization signals were detected by autoradiography.

2.4. DNA sequencing and analysis

E. coli plasmid DNA was isolated using the Qia-gen plasmid kit (Qiagen Inc., Chatsworth, CA). Nucleotide sequencing was performed using the dideoxy chain termination method [13], with the Autoread sequencing kit using T7 DNA polymerase (Pharmacia), Cy5-labelled nucleotide primers (Pharmacia) and an Automated Laser Fluorescent DNA Sequencer (Pharmacia). The sequence analysis program PC/Gene 6.70 [14] was used to analyze nucleotide and amino acid sequences. Amino acid sequences were aligned with the program Multalin version 4.0 [15].

2.5. β-Galactosidase assay

The amount of β-galactosidase produced under the influence of a C. jejuni promoter in E. coli was measured in duplicate in the presence and absence of the C. jejuni rpoD gene by the conversion of O-nitrophenyl-β-D-galactopyranoside (ONPG) in nitrophenol as described by Miller [16].
3. Results and discussion

3.1. Cloning of the C. jejuni rpoD gene

PCR with the degenerate primers DP1 and DP2 with C. jejuni genomic DNA as template yielded a product of 488 bp. This PCR product was cloned and sequenced and appeared to contain part of the rpoD gene of C. jejuni. The PCR fragment was also used as a probe in a Southern blotting experiment, on genomic DNA digests of C. jejuni 129108 (data not shown). The probe hybridized to a single TaqI DNA fragment of 2.2 kb indicating that C. jejuni possesses only one rpoD homologue in its chromosome. To obtain the remainder of the C. jejuni rpoD gene, primers S701 and S702, derived from the sequence of the PCR product, were used in an inverse PCR on circularized TaqI chromosomal DNA fragments. Two independently obtained inverse PCR products (1.8 kb) were cloned in pMOS. Sequencing of the inverse PCR products revealed that a small part of the 3' end of the rpoD gene was still not cloned. Therefore a colony blot on chromosomal HindIII fragments cloned in pBluescript was performed. Clone pMB4 containing a 2.8-kb HindIII fragment hybridized with the probe. The sequence of this fragment was in accordance with the sequence derived from the PCR products. The complete rpoD sequence is shown in Fig. 1.

3.2. Analysis of the C. jejuni rpoD sequence

The rpoD gene of C. jejuni consists of a 1866-bp open reading frame (ORF) which encodes a protein of 72.6 kDa. A search of the databases revealed that this predicted protein has a high degree of similarity to other bacterial c70 genes. The C. jejuni RpoD protein was 66% and 40% identical to those of Helicobacter pylori and E. coli, respectively. A Shine-Dalgarno sequence and the PCR primers S701 and S702 used in this study are indicated in bold letters. The regions 2.4, 2.5 and 4.2 are underlined. The nucleotide sequence of the C. jejuni 129108 rpoD gene has been assigned accession number AJ002379.

Table 1: β-Galactosidase activity given in Miller units with standard deviations (S.D.) of E. coli strain DH5α with or without the C. jejuni promoter constructs 2A12, 12G7 and/or the rpoD gene on cosmid 2C9

<table>
<thead>
<tr>
<th>Strain</th>
<th>β-Galactosidase activity (Miller units ± S.D.)</th>
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<tbody>
<tr>
<td>DH5α</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>DH5α+2C9</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>DH5α+2A12</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>DH5α+2A12+2C9</td>
<td>25 ± 7</td>
</tr>
<tr>
<td>DH5α+12G7</td>
<td>35 ± 16</td>
</tr>
<tr>
<td>DH5α+12G7+2C9</td>
<td>70 ± 9</td>
</tr>
</tbody>
</table>
Dalgarno sequence is located 6 bp in front of the rpoD gene. No promoter consensus sequence could be identified (Fig. 1).

3.3. Expression of C. jejuni promoters in E. coli by the C. jejuni RpoD

A number of Campylobacter promoters are not or only poorly recognized in E. coli [5,1]. A reason for this might be a difference between $\sigma^{32}$ promoter sequences of C. jejuni and E. coli. In E. coli the promoter sequence is characterized by two nucleotide sequences, TTGACA and TATAAT, respectively, centered around positions −35 and −10, from the transcription start site +1 [17]. In Campylobacter the $\sigma^{32}$ promoter consensus sequence consists of three regions: a −35, −16 and a −10 region with the sequences TTTAAGTnTT, TTTTTTTG and TAAATT, respectively [5]. To determine whether the C. jejuni rpoD gene would recognize promoters similar to the C. jejuni consensus sequence, we expressed the rpoD gene in E. coli and investigated whether this led to activation of Campylobacter promoters. To obtain a functional rpoD gene a cosmid library of C. jejuni 81116 was screened. Cosmid 2C9 hybridized with the 488-bp rpoD probe. Southern blot analysis of cosmids 2C9 revealed that the complete rpoD gene was located on the insert of about 27 kb of this low copy number cosmids (data not shown). This C. jejuni rpoD gene was used to activate C. jejuni promoters located upstream of a lacZ gene on plasmids 2A12 and 12G7 [5] in E. coli. Plasmid 2A12 contains a promoter which is not recognized in E. coli, while plasmid 12G7 contains the poorly activated C. jejuni glu-tRNA promoter (Table 1). The presence of cosmids 2C9 increased the activity of both C. jejuni pro-

<table>
<thead>
<tr>
<th>2.4 region</th>
<th>2.5 region</th>
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<tbody>
<tr>
<td><strong>E. coli RpoD</strong></td>
<td><strong>H. influenzae RpoD</strong></td>
</tr>
<tr>
<td>IRQAI TRSI ADQ ARTIR</td>
<td>................. L....</td>
</tr>
<tr>
<td><strong>H. influenzae RpoD</strong></td>
<td><strong>B. subtilis SigA</strong></td>
</tr>
<tr>
<td>........................</td>
<td>........V........ I.VQ...L...F</td>
</tr>
<tr>
<td><strong>B. subtilis SigA</strong></td>
<td><strong>C. glutamicum SigA</strong></td>
</tr>
<tr>
<td>...........AM..........</td>
<td>.......V.V...G.Q.EL...L</td>
</tr>
<tr>
<td><strong>C. glutamicum SigA</strong></td>
<td><strong>S. coelicolor HrdA</strong></td>
</tr>
<tr>
<td>...........AM..........</td>
<td>.......VV.L...RVV.VQ.R...R</td>
</tr>
<tr>
<td><strong>S. coelicolor HrdA</strong></td>
<td><strong>H. pylori RpoD</strong></td>
</tr>
<tr>
<td>...........AM..........</td>
<td>.......K.S.A........ I...D...RI.KVM.KHI.T</td>
</tr>
<tr>
<td><strong>H. pylori RpoD</strong></td>
<td><strong>C. jejuni RpoD</strong></td>
</tr>
<tr>
<td>...........AM..........</td>
<td>.......I.OQ.I.K.I.EH.KD</td>
</tr>
</tbody>
</table>

![Fig. 2. Alignment of the subregions 2.4, 2.5 and 4.2 of the primary $\sigma$ factors of E. coli (J01687), Haemophilus influenzae (L45174), B. subtilis (M10089), Corynebacterium glutamicum (Z49822), Streptomyces coelicolor (X52983), H. pylori (U83703) and C. jejuni. Codes in parentheses are GenBank accession numbers. The double underlined E. coli amino acids bind to the $\sigma^{32}$ promoter sequence.](https://academic.oup.com/femsle/article-abstract/162/1/97/630228)
3.4. Comparison of subregions 4.2, 2.5 and 2.4 of RpoD proteins

The −35 and −10 promoter sequences are recognized by the subregions 4.2 and 2.4 of RpoD, respectively [18]. Recently a substitution study in the RpoD protein of E. coli led to the identification of a functional region (region 2.5) immediately adjacent to region 2.4. This region is involved in making contact with the nucleotides TG in the −16 promoter consensus sequence [19]. This −16 region is more common and extended in Bacillus subtilis, TnTG [20]. Divergences in these RpoD regions change sequence specificity for the promoter [3,19]. In the E. coli RpoD 4.2 region the arginines at positions 588 and 584 bind to the G and C nucleotides in the hexamer sequence TTGACA of the promoter region. The glutamic acid residue at position 458 of region 2.5 binds to the G at position −14 and the glutamine residue at position 437 (region 2.4) binds to the 5′ T of the hexamer TATAAT. Comparison of the C. jejuni RpoD subregions revealed that the promoter sequence-binding amino acids of the E. coli RpoD protein are conserved in the C. jejuni RpoD protein (Fig. 2). Subregion 2.4 is similar to subregions 2.4 of E. coli and B. subtilis, which is in agreement with the similarity between the −10 consensus sequences found for these bacteria [17,21]. However, subregions 2.5 and 4.2 in the C. jejuni RpoD protein are less conserved. This is a reflection of the fact that these subregions bind to different −16 and −35 promoter regions of C. jejuni [5]. We observed that both the number of T residues in the −16 region and the number of isoleucine residues in the 2.5 region of C. jejuni RpoD are increased. This suggests that there is a connection between these two phenomena, since statistically an increase in the number of T residues does not give rise to an increase in isoleucine residues. All known σ70 factors contain the amino acid sequence VTRERIRQIE in subregion 4.2 except those of C. jejuni and H. pylori [9]. In these bacteria the isoleucine between the two arginines that bind to the G and C residues of the hexamer sequence TTGACA in the E. coli σ70 promoter sequence is changed into valine. This, as well as the other differences observed in subregion 4.2, may be needed for the recognition of the totally different canonical −35 promoter sequence found in C. jejuni. The almost identical sequences of C. jejuni and H. pylori in the 4.2 region suggest that H. pylori may have a −35 promoter sequence similar to that of C. jejuni.

References