Analysis of a *Helicobacter pylori* operon incorporating flagellar export genes

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Abstract

Motility of *Helicobacter* species has been shown to be essential for successful colonization of the host. Previous studies indicated that the regulation of flagellar biosynthesis in the human gastric pathogen *Helicobacter pylori* differs from the suggested model for Gram-negative Enterobacteriaeceae.

In this study, the organization of two *H. pylori* genes involved in export of flagellar structural proteins was investigated. A 7 kb fragment of the *H. pylori* 17874 genome was cloned. Sequence determination and analysis revealed a putative operon comprising an ORF of unknown function (ORF03), and genes for the isoleucyl-tRNA synthetase (*ileS*), an *Agrobacterium tumefaciens* VirB11 homolog (*virB11*), an ATPase involved in flagellum-specific protein export (*fliI*), a presumptive flagellar export channel component (*fliQ*), and a homolog of an enzyme necessary for cell wall biosynthesis (*murB*). The genetic organization of this region was found to be conserved in a panel of clinical *H. pylori* isolates, and in *H. pylori* 915 and SS1. The locus was also identified in the genome sequences of the *H. pylori* strains J99 and 26695.

Cotranscription of ORF03, *ileS, virB11, fliI, fliQ* and *murB* was demonstrated by RT-PCR. Primer extension experiments identified the major transcription start site, which coincided with the A residue of the initiation codon of ORF03. A promoter element was inferred that resembled the *E. coli* σ^{70} consensus sequence. In addition, a minor transcription start site was detected upstream from *ileS*.

Non-polar mutation of *virB11*, *fliI* and *fliQ* was generated by an allele replacement strategy. Engineered *H. pylori fliI* and *fliQ* mutant strains were completely aflagellate and nonmotile, whereas a *virB11* mutant still produced flagella and displayed slightly greater motility. The *fliI* and *fliQ* mutant strains produced severely reduced levels of flagellin and the hook protein FlgE, although reduction was less stable in the *fliI* mutant. Production of OMP4, a member of the outer membrane protein family identified in *H. pylori* 26695, was diminished in both the *virB11* and the *fliI* mutant. This suggested related functions of the putative virulence factor transport protein (VirB11) and the flagellar export component (FliI).

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Related Publications

Some of the material presented in this thesis has been published.

Porwollik, S., Noonan, B. & O'Toole, P.W. (1999). Molecular characterization of a flagellar export locus of *Helicobacter pylori*. *Infect Immun* 67:2060-2070.

Porwollik, S. & O'Toole, P.W. (1998). Molecular characterization of a flagellar export locus of *Helicobacter pylori*. *Gut* 43 (Suppl 2): A02/31

Abbreviations

A+T content of deoxyadenylate and deoxythymidylate in DNA
aa amino acid
ab antibody
ABI Applied Biosystems
AMV avian myeloblastosis virus
Ap ampicillin
APS ammonium persulphate
ATP adenosine triphosphate
BBH basal body-hook complex
BCIP 5-bromo-4-chloro3-indolyl phosphate
BLAST basic local alignment search tool
BSA bovine serum albumine
cAMP cyclic adenosine monophosphate
CAP catabolite gene activator protein
CBA columbia base agar
cDNA complementary DNA
Cm chloramphenicol
colE1 colicin E1
CRP cyclic adenosine monophosphate receptor protein
CSPD disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1.]decan}-4-yl)phenyl phosphate
CTP cytidine triphosphate
dATP 2' deoxyadenosine triphosphate
DEPC diethylpyrocarbonate
dGTP 2' deoxyguanosine triphosphate
DIG digoxigenin
DNA deoxyribonucleic acid
DNase deoxyribonuclease
dNTP deoxynucleoside triphosphate
dTTP 2' deoxythymidine triphosphate
dUTP 2' deoxyuridine triphosphate
EDTA ethylenediaminetetraacetic acid
EPB electroporation buffer
f1 bacteriophage f1
F_{ab} variable sequence fragment of immunoglobulin

FSB final sample buffer
FP forward primer
G+C content of deoxyguanylate and deoxycytidylate in DNA
GSP general secretory pathway
GTP guanosine triphosphate
IL interleukin
IPTG isopropyl-ß-D-galactoside
Kan kanamycin
LB Luria-Bertani broth
LBA Luria-Bertani agar
LPS lipopolysaccharide
MALT mucosa-associated lymphoid tissue
mcl monoclonal
MCS multicloning site
MOPS 3-(N-morpholino) propanesulphonic acid
n/a not applicable
NBT 4-nitro blue tetrazolium chloride
NCBI National Center for Biotechnology Information
Neo neomycin
OD optical density
ORF open reading frame
ori origin of replication
PBS phosphate-buffered saline
pcl polyclonal
PCR polymerase chain reaction
PEG polyethylene glycol
pI isoelectric point
PIR protein information resource
^{r} resistant
RNA ribonucleic acid
RNase ribonuclease
RP reverse primer
rpm revolutions per minute
RT room temperature
RT-PCR polymerase chain reaction involving an initial reverse transcriptase step
SDS sodium dodecyl sulphate
SM-TBS skim milk powder in Tris-buffered saline

SV40 simian virus 40
TB terrific broth
TBS Tris-buffered saline
TEMED NNN'N' tetramethylethylenediamine
TIGR The Institute for Genomic Research
T_m melting temperature
TNF tumor necrosis factor
Tris tris(hydroxymethyl)methylamine
TSB tryptic soy broth
U unit
UTP uridine triphosphate
Vol volume
w/v weight per volume
X-gal 5-bromo-4-chloro-3-indolyl-ß-D-galactoside

In addition, the conventional one-letter codes for amino acids, deoxyribonucleosides and ribocucleosides were applied:

amino acids: G, A, V, L, I, P, F, Y, W, S, T, C, M, N, Q, D, E, K, R, H for glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, tyrosine, tryptophan, serine, threonine, cysteine, methionine, asparagine, glutamine, aspartate, glutamate, lysine, arginine and histidine, respectively
deoxyribonucleosides: A, C, G, T for deoxyadenylate, deoxycytidylate, deoxyguanylate and deoxythymidylate, respectively
ribonucleosides: A, C, G, U for adenylate, cytidylate, guanylate and uridylate, respectively

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