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TRANSCRIPTIONAL ANALYSIS OF CELL DIVISION GENES IN *NEISSERIA GONORRHOEAE*

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ABSTRACT

This work represents the first study of the regulation of cell division genes in the Gram negative coccus *Neisseria gonorrhoeae*, and this regulation was studied under aerobic and anaerobic growth conditions using two different gonococcal strains: CH811 and FA1090. Three different clusters of cell division genes were investigated: the *mur-fts* cluster, the *ftsEX* cluster, and the *min* cluster corresponding to homologues at the 2 min, the 78 min, and the 26 min region of the *Escherichia coli* chromosome, respectively. Driven by initial DNA sequencing of the pivotal cell division gene *ftsZ* in our laboratory, further genomic analysis using the raw data from the *N. gonorrhoeae* FA1090 Genome Project was used to identify the genetic organization of the *mur-fts* cluster as well as the *min* cluster. The *ftsZ* gene was located close to the 3' end of a large cluster of 13 genes, most of which are believed to be involved in cell growth and division. The gonococcal homologues identified and their order are as follows: 5'-*murE-hyp1-murF-mraY-murD-ftsW-murG-murC-ddl-ftsQ-ftsA-ftsZ-hyp2*-3', which was similar to the gene order from *E. coli* (5'-*ftsL-ftsI-murE-mraY-murD-ftsW-murG-murC-ddl-ftsQ-ftsA-ftsZ-envA*-3'). The intergenic spaces within this cluster were generally larger with no overlapping genes as compared to the similar cluster from *E. coli*. Within three of these intergenic spaces, transcriptional terminators presumptively identified by paired Neisserial uptake sequences, were shown to be functioning as terminators using reverse-transcriptase polymerase chain reaction (RT-PCR) techniques. Using RT-PCR, four major transcripts were observed in this cluster; this differs from the similar cluster from *E. coli* in which all of the genes are thought to be co-transcribed. The genes from the *min* cluster, *minC*, *minD*, and *minE*, which are important

for the localization of septation were also identified and were determined to be co-transcribed along with two other unrelated genes using RT-PCR. The *ftsEX* genes were also shown to be co-transcribed by RT-PCR.

Northern hybridizations showed that the cell division genes *ftsE*, *ftsX*, and *ftsZ* are expressed differently under aerobic and anaerobic growth conditions, and that this difference is likely due to an increase in RNA turnover anaerobically. Promoters upstream of *ftsE*, *ftsQ*, *ftsA*, and *ftsZ* were identified by primer extension using RNA from both aerobically grown and anaerobically grown *N. gonorrhoeae*. Two promoters were found to regulate expresssion of *ftsE*, and the more distal of these resulted in a one nucleotide difference in the transcriptional start site when aerobic and anaerobic conditions were compared. One weak promoter was identified upstream of *ftsQ*, and two promoters were immediately upstream of *ftsA*; these three promoters initiated transcription at the same sites and in similar quantities when aerobic and anaerobic growth conditions were compared. Three promoters were found upstream of *ftsZ*; the most proximal of these was strongly used anaerobically, but was only slightly used aerobically. The second promoter was used preferentially aerobically in some cases, but more evenly used when the experiment was repeated. The third promoter was used equally under both growth conditions.

Finally, the promoter regions of *ftsE*, *ftsQ*, and *ftsZ* were cloned upstream of a promoterless *lacZ* gene in the vector pLES94. The 466 bp amplicon of the upstream region of *ftsE*, the 606 bp amplicon upstream of *ftsZ*, and the 790 bp amplicon upstream of *ftsQ* were cloned into the unique *Bam*HI site of pLES94. These clones will be used in future studies for quantifying the expression of the promoters under different environmental

conditions.

Therefore, these data show that the cell division genes from *N. gonorrhoeae* are highly regulated, and this regulation differs with respect to RNA turnover and promoter strength and usage under aerobic and anaerobic growth conditions.

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"One can't believe impossible things", (said Alice).
'I daresay you haven't had much practice,' said the Queen. 'When I was your age, I always did it for half-an-hour a day. Why, sometimes I've believed as many as six impossible things before breakfast.'

Lewis Carroll

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ATP	adenosine 5'-triphosphate
bp	base pairs
BLAST	basic local alignment search tool
cDNA	complementary DNA
contig	contiguous sequence
dATP	deoxyadenosine 5'-triphosphate
dCTP	deoxycytidine 5'-triphosphate
DEPC	diethyl pyrocarbonate
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
DTT	dithiothreitol
dNTP	deoxynucleotide triphosphate
EDTA	ethylenediamineteraacetate
GCMB	GC medium base
GTP	guanosine triphosphate
GTPase	guanosine triphosphate dephosphorylase
IHF	integration host factor
kb	kilobase pair
LB	Luria Bertani
LOS	lipooligosaccharide
min	map minutes
min.	minutes
mM	millimolar
mra	murein A
mRNA	messenger ribonucleic acid
nt	nucleotide
ORF	open reading frame
PCR	polymerase chain reaction
PID	pelvic inflammatory disease
PPNG	penicillinase-producing *Neisseria gonorrhoeae*
RNA	ribonucleic acid
RNase	ribonuclease
RT-PCR	reverse-transcriptase polymerase chain reaction
SDS	sodium dodecyl sulphate
sec.	seconds
SSC	sodium chloride, sodium citrate buffer
STD	sexually transmitted disease
U	unit
V	volt
X-gal	5-bromo-4-chloro-3-indoyl-β-D-galactoside

1. INTRODUCTION

Cell division is an integral component of bacterial growth, survival, and propagation, and is therefore a fundamental process that warrants detailed study. An understanding of cell division is an important aspect of understanding the bacterial cell cycle. Very little is known of cell division in *Neisseria gonorrhoeae* at present, therefore our laboratory has begun identifying and characterizing genes necessary for division to occur in order to elucidate this essential process in this Gram negative coccus. This work focuses on the identification and the regulation of cell division genes in *N. gonorrhoeae* building on previous work from both our laboratory, as well as the raw data supplied by the Gonococcal Genome Project. The expression of cell division genes was studied under both aerobic and anaerobic growth conditions using Northern hybridizations, reverse-transcriptase polymerase chain reaction (RT-PCR), and primer extension analysis.

1.1 *NEISSERIA GONORRHOEAE*: THE ORGANISM

N. gonorrhoeae (gonococcus) is an important human pathogen to which no vaccine has yet been developed. It is a Gram negative diplococcus and a member of the family *Neisseriaceae*. This family also includes the closely related pathogen *Neisseria meningitidis*, as well as numerous commensal *Neisseria* species (Vedros, 1984). The gonococcus is an obligate human pathogen that is the causative agent of the sexually transmitted disease (STD) gonorrhea. The World Health Organization (1995) estimated that the global incidence of new cases of gonorrhea was 62 million in 1995. These numbers are of particular concern when the fact that *N. gonorrhoeae* can act as a co-factor in the transmission of the Human

Immunodeficiency Virus, increasing the risk of transmission of this virus three to five fold is considered (Wasserheit, 1992; Quinn, 1996). *Chlamydia trachomatis* and *Trichomonas vaginalis* are also commonly found co-existing with *N. gonorrhoeae* and therefore reducing the transmission of these STDs would also have an impact on several other STDs. Gonorrhea causes a wide range of disease from asymptomatic to more serious disseminated infection (Cohen and Sparling, 1992). In uncomplicated infections, the gonococcus is localized to the primary site of infection and is easily treated (Howard, 1994). The primary site of infection is the endocervix in women and the urethra in men (Howard, 1994). Complications occur predominantly in women and can result in pelvic inflammatory disease (PID) which occurs when the organism spreads to the fallopian tubes and causes endometritis, salpingitis, and peritonitis (Howard, 1994). PID may also result in more serious sequelae such as ectopic pregnancy and infertility (Howard, 1994). In rare cases, the gonococcal infection can spread from the genitourinary tract to the bloodstream causing bacteraemia which may lead to infections of the skin, synovium, and joints, and occasionally even meningitis or endocarditis (Howard, 1994; Cohen and Sparling, 1992). Gonorrhea can also be spread to the eyes of newborns during childbirth causing ophthalmia neonatorum, but this can be prevented by using silver nitrate or erythromycin drops. (Howard, 1994).

Because infection by the gonococcus consists of adhesion to and invasion of epithelial cells of mucosal surfaces, most studies have focused on the surface structures of *N. gonorrhoeae* such as the pilins, the opacity proteins, and the lipoolygosaccharide (LOS). These surface structures are antigenically variable which has made the development of a vaccine elusive. This variability also helps the gonococcus to evade the host's immune

system (Sparling *et al.*, 1990; Cohen and Sparling, 1992).

Piliated gonococci are naturally competent for transformation and horizontal transfer of DNA between different *Neisseria* species has been shown to occur (Biswas *et al.*, 1989). This competence allows the exchange of genetic information between cells and therefore genomic rearrangements can occur causing the genome of *N. gonorrhoeae* to be "plastic" (Goodman and Scocca, 1988; Seifert and So, 1991) The uptake of DNA can only occur when a 10 bp Neisserial uptake sequence (5'-GCCGTCTGAA-3') is present on the DNA fragment; one uptake sequence is sufficient for transformation to occur. This uptake sequence is prevalent throughout the genome of *N. gonorrhoeae*, occurring approximately once per kilobase on the chromosome (Goodman and Scocca, 1991). Uptake sequences are also commonly found as inverted repeats that form stem-loop structures downstream of open reading frames (ORFs) and these act as transcriptional terminators (Goodman and Scocca, 1988; Barber *et al.*, 1994).

Although gonorrhea is currently treatable using antibiotics, the emergence of resistance is a constant problem. By the late 1980s, resistance to penicillin and tetracycline was so high that these drugs were no longer recommended for treatment (Dillon and Yeung, 1989). Over the past decade the use of cephalosporins (cefixime and ceftriaxone), or the fluoroquinolones (ciprofloxacin and ofloxacin) has been effective. However, incidences of low-level resistance to fluoroquinolones have been reported world-wide, and high level resistance and treatment failures have also occurred (Yeung and Dillon, 1991; Handsfield and Whittington, 1996; Tapsall *et al.*, 1997).

Several physical and genetic maps have been constructed using hybridization studies

for *N. gonorrhoeae* strains MS11 and FA1090 (Bihimaier *et al.*, 1991; Dempsey *et al.*, 1991; Dempsey and Cannon, 1994), however these will soon be dated compared to the genomic information anticipated from genome project of *N. gonorrhoeae* strain FA1090, which is almost complete. The raw sequence data from this project have been released such that it can be searched using a Basic Local Alignment Search Tool (BLAST) engine (Dr. B. A. Roe, Dr. S. Clifton and Dr. W. Dyer, University of Oklahoma, OK, 1997).

1.2 ANAEROBIOSIS IN THE GONOCOCCUS

Historically, *N. gonorrhoeae* was thought to be an obligate aerobe despite the fact that it was isolated along with obligate anaerobes from the genitourinary tracts of patients (Vedros, 1984; Burnakis and Hildebrandt, 1986). It was not until relatively recently that Knapp and Clark (1984) determined that *N. gonorrhoeae* could use nitrite as a terminal electron acceptor in order to grow anaerobically. Growth rates under aerobic and anaerobic conditions were found to be almost the same when *N. gonorrhoeae* was grown in a rich broth (GC Broth), with generation times of approximately 70 min. anaerobically as compared to 60 min. aerobically (Knapp and Clark, 1984). Therefore, anaerobic growth is an efficient method of gonococcal propagation (Knapp and Clark, 1984). Clark *et al.* (1987) identified three outer membrane proteins that were induced under anaerobic growth conditions (Pan 1, Pan 2, and Pan 3). Pan 1 was detected only under anaerobic conditions, while some Pan 2 was detected aerobically, though expression was increased anaerobically (Clark *et al.*, 1987). Pan 3 was a minor protein that was difficult to study because it had a similar molecular weight to protein III. Other proteins that were repressed under aerobic conditions

were also identified (Pox 1-5). The proteins Pan 2 and Pan 3 were also induced when cells were grown under conditions of nutrient limitation; thus Pan 1 may be the only truly anaerobically induced protein (Clark *et al.*, 1987).

The *aniA* gene encoding Pan 1 has been identified and sequenced. Pan 1 had 30-35% homology with copper containing nitrite reductases from other species (Cardinale *et al.*, 1996). Aerobically grown gonococci also exhibited nitrite reductase activity however, which indicates that two reductases may be functional in the gonococcus, though the second enzyme has not yet been identified (Cardinale *et al.*, 1996).

Sera from patients with uncomplicated, disseminated, or PID infections all had antibodies to Pan 1, while patients with no history of a gonococcal infection did not have these antibodies (Clark *et al.*, 1988). Therefore, anaerobiosis may be an important part of infection, and maybe even pathogenesis. The differentially expressed outer membrane proteins could play a role in adhesion or pathogenesis at different sites. Studying *N. gonorrhoeae* under anaerobic conditions may also more closely reflect the growth of this organism *in vivo* instead of the aerobic growth conditions that are commonly used in the laboratory (Clark *et al.*, 1987). Another study demonstrating the biological importance of anaerobic growth in the pathogenesis of *N. gonorrhoeae* showed that anaerobically grown gonococci could convert to serum resistance at two to three times the rate of aerobically grown cells by increasing the rate of sialylation of the LOS, thus more effectively evading the immune system (Frangipane and Rest, 1993). Serum resistance is a mechanism by which the gonococcus evades the bactericidal activity of human serum by the sialylation of its LOS, which blocks binding by immunoglobulin M. It is a characteristic that is lost upon passage

in vitro (Frangipane and Rest, 1993).

1.3 PROMOTERS AND THE REGULATION OF GENE EXPRESSION IN BACTERIA

A critical component of the bacteria's ability to survive and adapt to different environmental conditions is by the regulation of gene expression. This regulation can be mediated at several different levels. Gene regulation at the transcriptional level is the most efficient method for the cell because energy need not be wasted on synthesizing unnecessary mRNA. However, when quick responses to changes of environment are required, this may not be the most adaptive method of regulation because transcription from the genes important for adaptation would have to be initiated, which would result in a delayed response time (Harwood, 1992). Regulation may also be mediated at the level of mRNA stability. The average half-life of bacterial mRNA is two minutes, however this can increase when conditions require increased protein synthesis (Harwood, 1992). RNA degradation occurs by endoribonucleases such as RNase III and RNAse E, as well as exoribonucleases which cleave RNA at the 3' end. No 5' exonucleases have yet been identified in *E. coli* (Harwood, 1992).

The promoter is responsible for the regulation of genes at the level of transcription. The promoter is the DNA sequence required for binding by RNA polymerase (Lewin, 1990). Bacterial RNA polymerase consists of the core enzyme as well as a sigma factor which confers the specificity to the promoter. Both of these components are required for gene transcription, but the sigma factor is responsible for binding to specific promoters, and it is released after the initiation of transcription (Lewin, 1990). Most bacterial promoters are

recognized by a σ^{70}-like factor which recognizes the consensus -10 (TATAAT) and -35 (TTGACA) sequences (Busby and Ebright, 1994). Alternate sigma factors have been identified which are specific for genes involved in the heat shock response, stationary phase, flagellar activity, and sporulation. These sigma factors are all closely related to the σ^{70} family (Kusto *et al.*, 1989; Harwood, 1992). A second type of sigma factor, σ^{54} which is encoded by *rpoN*, is responsible for the transcription of genes involved in a wide range of functions such as toluene and xylene catabolism, hydrogenase biosynthesis, pilus production, and nitrogen fixation. σ^{54} is not related in either structure or function to σ^{70}, and its mechanism of action is different requiring completely different consensus sequences found at the -12 and -24 positions (Merrick, 1993). The primary structure of the σ^{54} proteins contains a highly conserved RpoN box consisting of 10 amino acid residues. This is a signature sequence of this type of sigma factor, and has not been identified in any other proteins to date (Merrick, 1993). σ^{54} promoters differ from σ^{70} promoters because an activator protein is absolutely required for open complex formation. In addition it has been proposed that σ^{54} binds to a different site altogether than σ^{70} on the core RNA polymerase (Merrick, 1993).

Numerous bacterial genes have expression that is inversely related to the growth rate of the culture, with greatest expression occurring upon entry into stationary phase (Aldea *et al.*, 1990). The sigma factor σ^{S}, (encoded by *rpoS*) is a member of the σ^{70} family of sigma factors and is responsible for the induction of genes of this type whose gene products have a wide variety of functions (Hengge-Aronis, 1996). The σ^{S}-dependent promoter is closely related to the σ^{70} type promoter, which can sometimes be recognized by the σ^{S} factor *in vitro* (Espinosa-Urgel *et al.*, 1996). A comparison of the amino acid sequences showed that σ^{S}

is the most closely related sigma factor to σ^{70} in *E. coli*, which along with many other sigma factors of this group contain the characteristic RpoD box (Hengge-Aronis, 1996). The consensus sequence for the σ^S-dependent promoter has been recently defined as having a -10 sequence of CTATACT with no consensus -35 sequence, though an upstream curvature of the DNA is likely to be important (Espinosa-Urgel *et al.*, 1996). The gearbox promoters are a group of closely related promoters upstream of genes such as *bolA* (a morphogene whose overexpression in *E. coli* results in a round morphology), *ftsQ* (an essential cell division gene), and *mcbA* (a plasmid-encoded gene responsible for the synthesis of microcin B17) which are similarly induced in stationary phase and have very high sequence similarity in the region encompassing the -10 consensus sequence. Interestingly, only *bolA* and *ftsQ* are σ^S-dependent, while *mcbA* which does not contain a curvature of the DNA in the upstream region, is transcribed using the σ^{70} holoenzyme (Espinosa-Urgel *et al.*, 1996). It has been shown for the gearbox promoters that the -10 sequence is required for promoter strength and inverse relation to growth rate, but the -10 sequence alone was not sufficient for complete promoter activity (Aldea *et al.*, 1990). The upstream secondary structure of the DNA may fulfill this requirement for the gearbox and all σ^S-dependent promoters (Espinosa-Urgel *et al.*, 1996).

Many promoters also require activator proteins for expression to occur, and these usually bind at the promoter region and increase the rate of open complex formation, possibly by bending the DNA (Harwood, 1992). An example of an activator is the catabolite activator protein which is activated when glucose levels are low, which in turn causes it to promote expression from the lactose, galactose, and arabinose operons (Harwood, 1992).

Repressors are also important in the negative control of gene regulation (Harwood, 1992). Repressors bind to operators which are normally found within the promoter region, and this binding prevents transcription from occurring (Harwood, 1992).

1.4 OVERVIEW OF GENE REGULATION IN *N. GONORRHOEAE*

Gene regulation by bacteria is necessary so that they can adapt and survive in different environments. This may be particularly true for pathogenic bacteria which often encounter hostile environments in their hosts. There are only a few genes in *N. gonorrhoeae* that have been studied extensively in terms of gene regulation in response to environmental conditions, and four of these will be described as models of gene regulation in the gonococcus.

The pilin genes are one such example. Pilin, encoded by *pilE*, is a major virulence factor in *N. gonorrhoeae* and recombination within this gene is responsible for phase and antigenic variation of pilin subunits (Arvidson and So, 1995). *pilE* is highly regulated at the transcriptional level, with three promoters identified which appear to be regulated by DNA binding proteins PilA/PilB and integration host factor (IHF; Taha *et al.*, 1992; Arvidson and So, 1995; Fyfe *et al.*, 1995; Hill *et al.*, 1997). PilA and PilB are homologous to a family of two-component regulatory systems that are used to regulate gene transcription in response to changes in environmental conditions. PilB is homologous to the sensor component of this system which is often found in the cytoplasmic membrane, while PilA is homologous to the response-regulator which is usually found in the cytoplasm. Communication between the two proteins occurs via the transfer of a phosphoryl group from ATP to the sensor which is

followed by the transfer of this group to the response regulator (Arvidson and So, 1995). PilA, which binds to the promoter region of the *pilE* gene, was shown to be an activator of transcription of *pilE* in the absence of PilB, however when both gene products were present, transcription of *pilE* was inhibited (Arvidson and So, 1995). Mutations in *pilA* were found to be lethal in the gonococcus, therefore it is proposed that the PilA/PilB regulatory proteins are responsible for the expression of additional genes other than *pilE* in response to changes of environment (Arvidson and So, 1995). Recently it was shown that PilA is homologous to FtsY from *E. coli,* and that the PilA binding site in the promoter region of *pilE* is not required for expression of *pilE*, and thus it has been proposed that PilA is in fact involved in targeting of secretory proteins and does not act in a regulatory role (Fyfe and Davies, 1996). Of the three promoters identified upstream of the *pilE* gene, two of these were σ^{70} dependent (P1 and P2), while the third was homologous to a RpoN (σ^{54}) type promoter (P3). Site-directed mutagenesis and *cat* fusions showed that the activities of these three promoters differed when expressed in *E. coli*, *Pseudomonas aeruginosa*, or in *N. gonorrhoeae* (Fyfe *et al.*, 1995). In an *E. coli* background, RNA polymerase-σ^{54} molecule bound to P3 and completely repressed expression from this promoter, and also partially repressed expression from the overlapping P1. Expression did occur from P2 in the *E. coli* background. The repression at P3 was likely due to the absence of an appropriate activator protein (Fyfe *et al.*, 1995). In an *rpoN* mutant, *pilE* was overexpressed to the point that it was lethal to the cells, indicating that this promoter is used to inhibit transcription rather than promote it in *E. coli* (Fyfe *et al.*, 1995). Transcription from this promoter was however shown to occur in *P. aeruginosa*, but the expression from P1 and P2 was not investigated in *P. aeruginosa* (Fyfe

et al., 1995). In *N. gonorrhoeae*, no transcription was detected from the σ^{54} promoter nor from one of the σ^{70} promoters (P2), however since the promoters upstream of *pilE* are likely dependent on environmental conditions, the *in vitro* conditions used were likely not sufficient for transcription to occur from these promoters (Fyfe *et al.*, 1995). It was interesting to note that P1 was the major transcriptional start site of *pilE* in *N. gonorrhoeae* considering that although this promoter had a perfect -10 consensus sequence, no -35 consensus was identified (Fyfe *et al.*, 1995). Taha and Giorgini (1995) reported conflicting data however. They found that the σ^{54} promoter was the major promoter upstream of *pilE* in *N. gonorrhoeae*. Another protein important in the transcription of *pilE* is IHF which binds in the region of the three promoters (Hill *et al.*, 1997). Transcription of *pilE* was ten fold less in the absence of IHF (Hill *et al.*, 1997). IHF commonly acts with σ^{54} promoters by either directly activating transcription, by bending the DNA to bring an upstream activator in contact with the RNA polymerase, or by causing the release of an inhibitor. Kinking of the *pilE* promoter was demonstrated by microscopic methods upon incubation with IHF (Hill *et al.*, 1997). From these studies, *pilE* is highly regulated and its transcription is dependent on changes in environmental conditions.

Another model of gonococcal gene expression which is regulated by environmental conditions is found in the *fbpA* gene. The gonococcus can exploit the iron resources of its host by using iron bound to transferrin, lactoferrin, and heme mediated by a receptor mechanism that binds to the iron-containing molecules (Berish *et al.*, 1993). In *E. coli*, the ferric uptake regulator (Fur) controls the expression of iron-regulated proteins, and a Fur homologue has been identified in *N. gonorrhoeae* (Berish *et al.*, 1993). In iron-restricted

conditions, *N. gonorrhoeae* makes several proteins that are not present when iron is plentiful, one example of which being FbpA (Berish *et al.*, 1993). *fbpA* lies upstream of *fbpB* and *fbpC* and these three genes are proposed to encode an iron transport system which has characteristics of an ABC (ATP-binding cassette) transporter (Adhikari *et al.*, 1996). Northern hybridizations demonstrated that *fbpA* mRNA is only present under conditions of iron restriction (Berish *et al.*, 1993), and the promoter of this gene was shown to bind the Fur protein (Berish *et al.*, 1993; Desai *et al.*, 1996). When *fbpA* promoter-*cat* fusions were expressed in *E. coli*, it was shown that Fur activated transcription in an iron deficient environment; Fur mutants however did not exhibit an increased transcription from the *fbpA* promoter (Desai *et al.*, 1996). The stability of the *fbpA* mRNA was also shown to be dependent on the presence of iron, with greater stability occurring under iron-restricted conditions (Forng *et al.*, 1997). A strong stem loop structure before the stop codon of *fbpA* may play a role in this differential stability (Forng *et al.*, 1997). Primer extension analysis identified one transcriptional start site for *fbpA*, and this was more prominent for cells grown in iron-deficient media as compared to cells grown when iron was plentiful. The consensus sequences of this promoter were consistent with a σ^{70} promoter, though both the -10 and -35 sequences had only 3/6 bp identical to the consensus sequences (Forng *et al.*, 1997). Therefore, the promoter of the *fbpA* gene in *N. gonorrhoeae* is regulated at the transcriptional level by the presence of iron, which is mediated by the Fur protein.

A third model whereby environmental conditions affect gene regulation in the gonococcus is demonstrated by the *mtr* (multiple transferrable resistance) efflux system which confers resistance to hydrophobic agents (HAs) which are often present in toxic fecal

lipids and bile salts (Hagman and Shafer, 1995; Lucas *et al.*, 1997). The *mtrCDE* operon encodes membrane proteins that comprise the pump of the efflux system, and their transcription is regulated by the *mtr* repressor (MtrR; Hagman and Shafer, 1995). MtrR is encoded by the upstream *mtrR* gene which is transcribed in the opposite direction from *mtrCDE* (Hagman and Shafer, 1995). Mutations in this gene resulted in increased expression of *mtrC* which in turn caused enhanced resistance to HAs (Hagman and Shafer, 1995). MtrR was shown to bind to the overlapping σ^{70} promoters of both the *mtrR* gene, as well as the *mtrCDE* operon (Lucas *et al.*, 1997). In addition, mutations within a cis-acting 13 bp inverted repeat identified within the promoter regions of both *mtrR* and *mtrC* inhibited expression of MtrR, but enhanced expression of MtrC in a MtrR independent fashion (Hagman and Shafer, 1995). Therefore both cis and trans acting transcriptional factors affect the expression of *mtrCDE*, which in turn affects HA resistance which is likely important for the gonococcus to adapt to different environments.

A final example of gene expression in the gonococcus is the anaerobically induced *aniA* gene described above. Northern hybridizations using the *aniA* gene as a probe showed that an mRNA transcript of *aniA* was present only in anaerobically grown gonococci, and not in aerobically grown cells, indicating that the expression of this gene is regulated at the level of transcription (Hoehn and Clark, 1992). Similarly, primer extension studies showed two transcriptional start sites upstream of *aniA* when RNA isolated from anaerobically grown cells was used as a template, but these were not used when aerobically grown cells were used (Hoehn and Clark, 1992). Sequence analysis indicated that the less significant of these promoters was a σ^{70} dependent promoter while the second more prominent promoter had

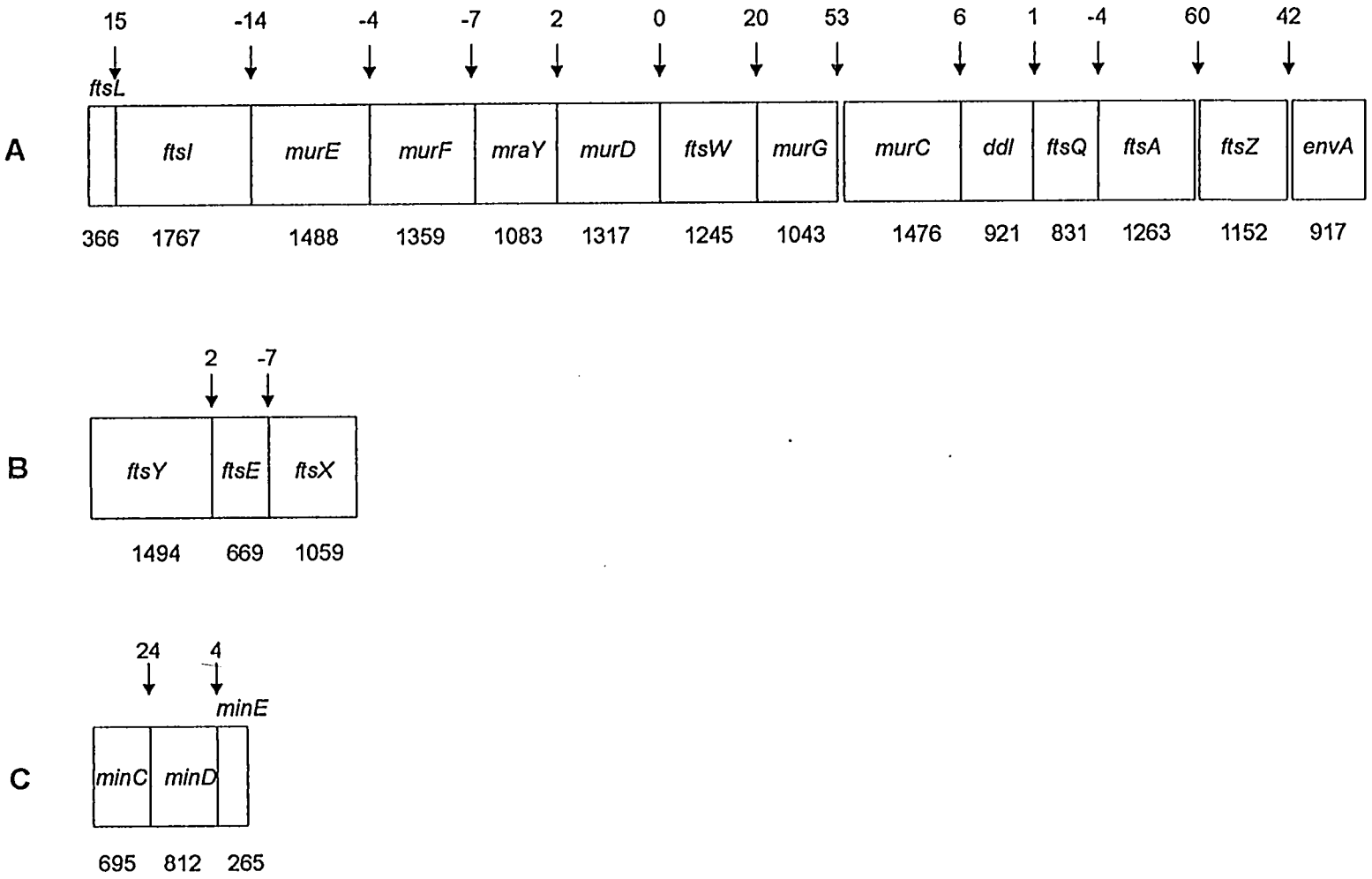
extensive similarity to the gearbox family of promoters (Hoehn and Clark, 1992).

1.5 CELL DIVISION AND THE CELL DIVISION CLUSTERS OF *E. COLI*

Bacterial cell division has been characterized most extensively in the Gram negative rod *E. coli*. Cell division consists of chromosome replication, partition of the chromosomes, and division of the cell by septation (Donachie, 1993). These mechanisms must be coordinated both temporally and spatially so that division occurs only once per cell cycle resulting in two new cells each containing one chromosome (Lutkenhaus and Mukherjee, 1996). Three clusters of cell division genes have been identified in *E. coli*: the *mra* (murein A) cluster consists of at least 16 genes involved in cell growth and biosynthesis, a number of which have been deemed essential. A second cluster is the *ftsYEX* cluster which contains genes which encode a putative ABC transporter, and a third cluster is the *min* cluster which controls the localization of the division site. (Lutkenhaus and Mukherjee, 1996).

The *mra* cluster is located at the 2 min region of the *E. coli* chromosome and contains the *ftsZ* gene (*fts*: filamentation temperature-sensitive), which is the most extensively studied of the cell division genes, and which plays a critical role in division by septation. This cluster is depicted in Figure 1A. Within this cluster are the following genes: *mraZ*, *mraW*, *ftsL*, *ftsI* (*pbp3*), *murE*, *murF*, *mraY*, *murD*, *ftsW*, *murG*, *murC*, *ddl*, *ftsQ*, *ftsA*, *ftsZ*, and *envA* (Lutkenhaus and Mukherjee, 1996). Most of these genes are involved in either peptidoglycan biosynthesis or in cell division though the function of the first two genes is as yet unknown (Lutkenhaus and Mukherjee, 1996). The organization of the above genes is interesting since the genes either overlap or are spaced very closely together, and are organized in a functional

Figure 1. Genetic organization of the cell division gene clusters from *E. coli*. **A.** The cell growth and cell division cluster at the 2 min region of the *E. coli* chromosome (Lutkenhaus and Mukherjee, 1996). **B.** The *ftsYEX* cluster at the 78 min region of the *E. coli* chromosome (Gill *et al.*, 1986). **C.** The *min* cluster at the 26 min region of the *E. coli* chromosome (Donachie, 1993). Gene sizes (bp) are indicated below the genes while the intergenic sizes are indicated above. Negative numbers indicate overlapping genes.


A
15
-14
-4
-7
2
0
20
53
6
1
-4
60
42
ftsL
ftsI
murE
murF
mraY
murD
ftsW
murG
murC
ddl
ftsQ
ftsA
ftsZ
envA
366
1767
1488
1359
1083
1317
1245
1043
1476
921
831
1263
1152
917
B
2
-7
ftsY
ftsE
ftsX
1494
669
1059
C
24
4
minE
minC
minD
695
812
265

operon with all of the genes being transcribed in the same direction. These genes are likely co-transcribed because only one transcriptional terminator was identified by sequence analysis, and this was located downstream of the *envA* gene (Lutkenhaus and Mukherjee, 1996). Multiple promoters are present throughout this cluster however, and these likely contribute to different levels of expression of the individual genes (Lutkenhaus and Mukherjee, 1996).

Similar clusters of cell growth and division genes have been identified by using information from genome projects (*Haemophilus influenzae*, *Bacillus subtilis*, *Helicobacter pylori*, *Mycoplasma genitalium*, and *Methanococcus jannaschii*) or by cloning and sequencing studies (*E. coli* initially, *Pseudomonas aeruginosa*, *Caulobacter crescentus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Haloferax volcanii*). Clusters were identified in *H. influenzae* which contained the same homologues in the same order as was found in *E. coli* though the sizes of the intergenic regions differed (Fleischmann *et al.*, 1995). Other Gram negative organisms such as *P. aeruginosa* and *C. crescentus* also contained cell division clusters (Sanschagrin *et al.*, 1997; Ohta *et al.*, 1997). Only *ftsA* and *ftsZ* were clustered together in *H. pylori* however (Tomb *et al.*, 1997). In the Gram positive *B. subtilis* a large cell division cluster was also identified containing *ftsA*, *ftsZ* and several *mur* genes (Biaudet *et al.*, 1996). The Gram positive cocci *S. aureus* and *E. faecalis* had *ftsA* and *ftsZ* clustered with other growth genes and preliminary work indicated similar clusters were also present in *S. pneumoniae* and *S. pyogenes* (Pucci *et al.*, 1997).

FtsZ is a highly conserved GTPase in eubacteria, and is even present in *Mycoplasma*

and the *Archaea*, neither of which have peptidoglycan cell walls (Lutkenhaus and Addinall, 1997). *ftsZ* is the most extensively studied of the cell division genes and its products have been shown to be essential for cell division to occur (Dai and Lutkenhaus, 1991). FtsZ, which has homology to eukaryotic tubulin, polymerizes to form a ring structure at the division site during cell septation (Erickson, 1995; Erickson *et al.*, 1996). This ring structure constricts during cell division such that the FtsZ ring remains at the leading edge of the septum (Lutkenhaus, 1993; Rothfield and Justice, 1997). Because FtsZ is present in organisms with no cell wall, it has been proposed that the role of FtsZ may be to promote the invagination of the cytoplasmic membrane, which may indirectly stimulate the formation of the septum (Lutkenhaus and Muhherjee, 1996). FtsZ is also a target for the inhibitors SulA and MinCD which block the formation of the FtsZ ring at division sites, thereby inhibiting cell division (Bi and Lutkenhaus, 1993). In *E. coli* the ratio of FtsZ to FtsA must be strictly maintained for viability of the cell (Dai and Lutkenhaus, 1992; Dewar *et al.*, 1992). FtsA, which has homology to ATPases, interacts with FtsZ, and perhaps FtsI, and has been localized to the FtsZ ring after FtsZ assembly has occurred, though its function remains to be determined (Addinall and Lutkenhaus, 1996). FtsQ is a membrane protein required for septum formation, though no function for this protein has yet been proposed (Guzman *et al.*, 1997).

A newly identified essential integral inner-membrane protein, ZipA, has also been shown to be required for septum formation and therefore cell division (Hale and de Boer, 1997). ZipA interacts directly with both FtsZ at the septal ring as well as with the cytoplasmic membrane. ZipA is proposed to control the assembly and function of the ring

structure, perhaps by stimulating the polymerization of the FtsZ molecules by recruiting FtsZ to the developing ring, though this model remains to be confirmed (Hale and de Boer, 1997)

Other genes involved in cell division include *ftsL* whose gene product is present in very low amounts in *E. coli*, and its function is as yet unknown. It is a membrane protein that contains a leucine zipper which indicates it may form a dimer. FtsI is a penicillin binding protein with transpeptidase activity and is essential for peptidoglycan biosynthesis at the septum during cell division. MurE, MurF, MurD, MurG, and MurC, MraY, and Ddl are all involved in murein biosynthesis (Van Heijenoort, 1996). *ftsW* is an essential gene which likely functions in the stabilization and constriction of the FtsZ ring during cell division (Boyle *et al.*, 1997). EnvA was thought to be responsible for cell separation after division, but it has recently been shown to be involved in lipopolysaccharide biosynthesis, and thus its role in separation may be indirect (Lutkenhaus and Mukherjee, 1996). *ftsN* is another essential cell division gene, though it is not located within the 2 min region. FtsN has been shown to localize to the FtsZ ring after FtsZ and FtsA, and this localization was dependent on FtsI and FtsQ (Addinall *et al.*, 1997).

A second cluster of cell division genes was found at the 78 minute region of the *E. coli* chromosome (Gill *et al.*, 1986; Figure 1B). *ftsE* encodes an ATP-binding protein, and both FtsE and FtsX are cytoplasmic membrane proteins with homology to the ABC family of transporters (Gill and Salmond, 1987). The roles of *ftsE* and *ftsX* in cell division remains controversial however. It was proposed by Gill and Salmond (1987) that FtsE may function with FtsY and FtsX to couple ATP hydrolysis to the cell division process, or that they may function to transport elements required for cell division across the cytoplasmic membrane.

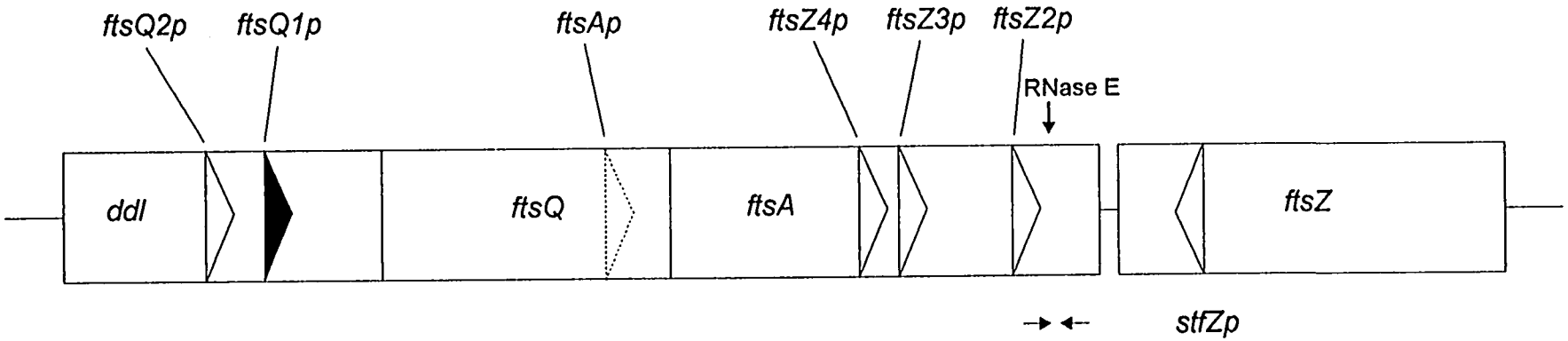
However there is no direct evidence that they are important for cell division. In *E. coli*, *ftsY*, *ftsE*, and *ftsX* are organized in an operon (Gill and Salmond, 1987). The promoters of *ftsYEX* have not been mapped, but insertional mutagenesis indicates that at least two promoters exist in this operon: the first upstream of *ftsY* which promotes transcription of all three genes, while the second is upstream of *ftsE* within *ftsY*, allowing for transcription of *ftsEX* alone (Gill and Salmond, 1987).

The *min* cluster which consists of *minC*, *minD*, and *minE* is responsible for the localization of septum formation during cell division. The organization of this cluster is shown in Figure 1C. When the *min* locus is deleted, septation occurs at the poles as well as the mid-cell, which results in the formation of mini-cells. *minC* and *minD* encode inhibitors of cell division which act at the poles of the cell at the sites of previous divisions of the cell. MinC and MinD may interact indirectly with FtsZ since overexpression of FtsZ overcomes inhibition by MinCD. The appropriate ratio of MinCD to MinE is required for normal cell division. An excess of MinCD results in inhibition of all division, while an excess of MinE overcomes the inhibitory effects of MinCD resulting in mini-cells (Lutkenhaus and Mukherjee, 1996). Analysis of the Min proteins using the yeast two-hybrid system indicated that MinC interacts with MinD while MinD interacts with MinE, however no direct interaction was identified between MinC or MinD, and FtsZ (Huang *et al.*, 1996). The model proposed by Huang *et al.* (1996) was that MinCD masks the previous division sites at the poles so that cell division at these sites is inhibited, while MinE binds to the midcell which allows FtsZ to bind there and form its ring structure.

1.6 TRANSCRIPTION AND REGULATION OF CELL DIVISION GENES IN *E. COLI*

The regulation of the cell division genes from the 2 min region of *E. coli* is highly complex, with multiple promoters and regulatory factors affecting gene expression (Figure 2). The ratio of the cell division proteins FtsQ, FtsA, and FtsZ has been shown to be critical for normal cell growth and division, and a two- to seven-fold overproduction of FtsZ results in the formation of minicells (Ward and Lutkenhaus, 1985). Therefore, regulation of these genes must be tightly controlled. No transcriptional terminators have been identified within the 2 min region except at the 3' end of the *envA* gene, and early studies showed that the *ftsQAZ* genes did not form a simple operon, but instead promoters were identified upstream of *ftsZ*, *ftsQ*, *ddl*, and *ftsA* (Robinson *et al.*, 1984; Robinson *et al*, 1986). Because no terminators are present within the cluster, the expression from each of these promoters must contribute to additive expression of the genes, where the downstream genes are more highly transcribed than those found upstream (Aldea *et al.*, 1990). Aldea *et al.* (1990) identified six promoters controlling the expression of *ftsQAZ* by S1 nuclease assays, though one of these promoters has recently been found to be the product of RNase E (an endonuclease in *E. coli*) cleavage rather than a product of transcription initiation (Cam *et al.*, 1996; Figure 2). Three of the promoters were localized upstream of *ftsZ* within the *ftsA* gene, while two others were upstream of *ftsQ* within *ddl* (Aldea *et al.*, 1990). One of the promoters upstream of *ftsQ* (*ftsQ1p*) was determined to be a gearbox promoter, which is defined as being a promoter from which the expression is inversely related to the growth rate of the culture, with greatest expression occurring upon entry into stationary phase (Aldea *et al.*, 1990). The FtsA and FtsZ proteins were quantified throughout the cell cycle, and their expression was also shown

Figure 2. Promoters responsible for the transcription of *ftsQAZ* in *E. coli*. White arrowheads indicate the sigma 70 promoters; the black arrowhead indicates the gearbox promoter, and the inverted arrowhead indicates the promoter responsible for transcription of antisense RNA (Cam *et al.*, 1996). The promoter upstream of *ftsA* is shown with dashed lines because its precise location has not been identified. The site of RNase E cleavage is indicated and the small arrows below the diagram represent the inverted repeat required for full expression of *ftsZ*.


ftsQ2p
ftsQ1p
ftsAp
ftsZ4p
ftsZ3p
ftsZ2p
RNase E
ddl
ftsQ
ftsA
ftsZ
stfZp

to be inversely dependent on growth rate which was not unexpected because the genes are co-transcribed (Aldea *et al.*, 1990). The -10 and -35 sequences upstream of *ftsQ1p* were similar to those found upstream of two other genes whose expression is also inversely dependent on growth rate: *bolA* and *mcb*. The alternate sigma factor RpoS (σ^S) is responsible for increased expression from a promoter in stationary phase, and it was shown that transcription from *ftsQ1p* was dependent on a functional *rpoS* gene (Sitnikov *et al.*, 1996). Therefore one of the promoters upstream of *ftsQ* is indeed a gearbox promoter dependent on the alternate sigma factor σ^S (Sitnikov *et al.*, 1996).

The other promoters identified upstream of *ftsQAZ* all had similarity to σ^{70} dependent promoters. No promoters were identified immediately upstream of *ftsA* by Aldea *et al.* (1990), however other studies have shown that at least one promoter is present upstream of *ftsA* within the *ftsQ* gene (Dewar and Donachie, 1990; Flardh *et al.*, 1997).

The promoter *ftsz1p* was initially identified by Aldea *at al.* (1990), but this was later determined to be a site of RNase E cleavage. RNase E therefore is important for its contribution to the differential expression of *ftsA* and *ftsZ*, and perhaps even *ftsQ*. A second cleavage site was also identified 1 nt downstream of the *ftsA* stop codon. RNase E was shown to affect the relative amounts of *ftsA:ftsZ* mRNA, with RNAse E decreasing the relative amount of *ftsA* mRNA by a factor of five. The cleavage affects the stability of both transcripts almost equally however, and therefore exonucleases do not explain the relative amounts of *ftsA:ftsZ* mRNA. The decreased stability of *ftsA* likely affects *ftsQ* similarly, though this was not shown.

Studies identifying which promoters within the *ftsQAZ* region are preferentially used

have been conflicting. Garrido *et al.* (1993) showed by transcription titration assays that the majority of *ftsZ* transcripts were generated from the two most proximal promoters (one of which was later shown to be a result of RNase E cleavage, Cam *et al.*, 1996), while only 21% of transcripts were generated by promoters further upstream from *ftsz2p*, and only 10% resulting from promoters upstream of *ftsQ*. However, using partial deletions of a 3 kb fragment containing *ftsQAZ* along with the identified promoters upstream of a promoterless *lacZ* gene, it was reported that *ftsZ2p* was actually a very weak promoter which contributed less than 5% of *ftsZ* transcription (Flardh *et al.*, 1997). Flardh *et al.* (1997) also showed that *ftsZ3p* and *ftsZ4p* contributed to about 37% of *ftsZ* transcription. They showed conclusively that at least one promoter (*ftsAp*) lies directly upstream of *ftsA* using *lacZ* fusions, and that it contributes significantly to *ftsA* and *ftsZ* expression, though its location has not yet been identified. *ftsAp* contributed 12%, and *ftsQ1p* and *ftsQ2p* contributed a total of 46%, though the individual contribution of each of these was not examined. Therefore more than half of the transcription of *ftsZ* originates from promoters upstream of *ftsQ* and *ftsA*. They also showed that the *ftsZ* proximal region was required for full expression, and when a 43 nt region containing the site of RNase E cleavage as well as a large part of an inverted repeat was deleted, transcription of *ftsZ* decreased considerably. Although *ftsZ4p*, *ftsZ3p*, and *ftsZ2p* are not gearbox promoters, Flardh *et al.* (1997) also showed that these are induced in stationary phase. Their experiments were done on log phase cells, but they predicted similar results in stationary phase since all of the promoters or promoter pairs were similarly induced in stationary phase. It is important to note that the studies by Flardh *et al.* (1997) examined the transcriptional start points, and not the actual mRNA levels. Garrido *et al.* (1993)

showed that about 80% of *ftsZ* transcripts had their 5' ends within 500 nt upstream of the start codon, and since it was shown that the *ftsZ* proximal promoter was actually weakly transcribed, this indicates that the majority of the transcripts have been cleaved by RNaseE at the sites identified by Cam *et al.* (1996).

Differential translation of FtsQAZ also plays a role in regulation of the expression of these genes (Mukherjee and Donachie, 1990). FtsA was shown to be translated at three times the rate of FtsQ from a polycistronic RNA which would contribute further to the regulation of the relative amounts of proteins produced from this cluster (Mukherjee and Donachie, 1990).

Studies have also been performed to attempt to determine the temporal regulation of *ftsZ* transcription. *ftsZ* was shown to be transcribed throughout the cell cycle, but this transcription is not uniform throughout the cell cycle (Robin *et al.*, 1990; Garrido *et al.*, 1993; Zhou and Helmstetter, 1994). Garrido *et al.* (1993) also showed that the transcription of *ftsZ* is not constant throughout the cell cycle; it reaches its maximum when DNA replication begins. They showed that this timing was not dependent on chromosome replication however. Therefore a factor is responsible for temporally regulating transcription of *ftsZ* at the time of chromosome replication, but the two events are not dependent on each other (Garrido *et al.*, 1993). Zhou and Helmstetter (1994) agreed with Garrido *et al.* (1993) that *ftsZ* is transcribed periodically, and concluded further that this temporal regulation is due to the inhibition of transcription around the time of gene replication instead of activation near the time of initiation of chromosome replication. Transcription from upstream of *ftsA* was shown to contribute significantly to periodic expression. They found the level of the *ftsZ*

transcript varied periodically over 2 cell cycles, with maximum expression midway in the cell cycle and minimum at time of cell division, which agreed with Garrido *et al.* (1993) in that maximal levels of transcripts were produced close to the time of chromosome replication.

Additional intracellular and extracellular factors have also been shown to regulate the promoters of the *ftsQAZ* genes. The SdiA (suppression of division inhibition) protein, which is homologous to the LuxR family of transcriptional activators whose function is mediated by autoinducers, was identified because it was found to suppress the MinC/MinD inhibitors (Wang *et al.*, 1991; Sitnikov *et al.*, 1996). SdiA acts to upregulate the transcription of *ftsQAZ* exclusively at the *ftsQ2p* promoter, and is not dependent on growth rate (Wang *et al.*, 1991). The effects of SdiA on activation of *ftsQ2p* were shown to be dependent on cell density, and the addition of conditioned media resulted in earlier effects by SdiA, conforming to the definition of an autoinduction system (Sitnikov *et al.*, 1996). Studies of *ftsQ1p*, the gearbox promoter, showed that transcription from this promoter is dependent on a functional *rpoS* gene which encodes the sigma factor responsible for increased transcription in stationary phase (Sitnikov *et al.*, 1996). They proposed a model whereby low levels of transcription from both *ftsQ1p* and *2p* occurred at low cell densities, and at a threshold level of growth, SdiA and σ^S induce P1 and P2 respectively, either directly or through another stimulatory molecule (Sitnikov *et al.*, 1996). SdiA is regulated by another extracellular factor that is released into the growth media by *E. coli* cells which acts to decrease production of SdiA, which in turn downregulates expression from *ftsQ2p* (Garcia-Lara *et al.*, 1996).

An antisense RNA, *stfZ*, which is approximately 140 nucleotides (nt) in length, is transcribed from the 5' end of the *ftsZ* gene of *E. coli* into the junction between *ftsA* and *ftsZ* (Figure 2). When this fragment was overexpressed, inhibition of cell division resulted. It is not known whether a protein is produced from this antisense RNA (Dewar and Donachie, 1993).

While there is no transcriptional terminator within the cell division cluster, there is no direct evidence that a single mRNA transcript containing all of the genes of this cluster is produced. One study has shown however that a transcript containing the first nine genes of the cluster is synthesized (Hara *et al.*, 1997). It is likely that transcription continues on through to the end of *envA*, though this has not yet been proven (Hara *et al.*, 1997).

The regulation of cell division genes with respect to the cell cycle has been extensively studied, however this regulation has not been studied under different environmental conditions, for example, under aerobic or anaerobic growth.

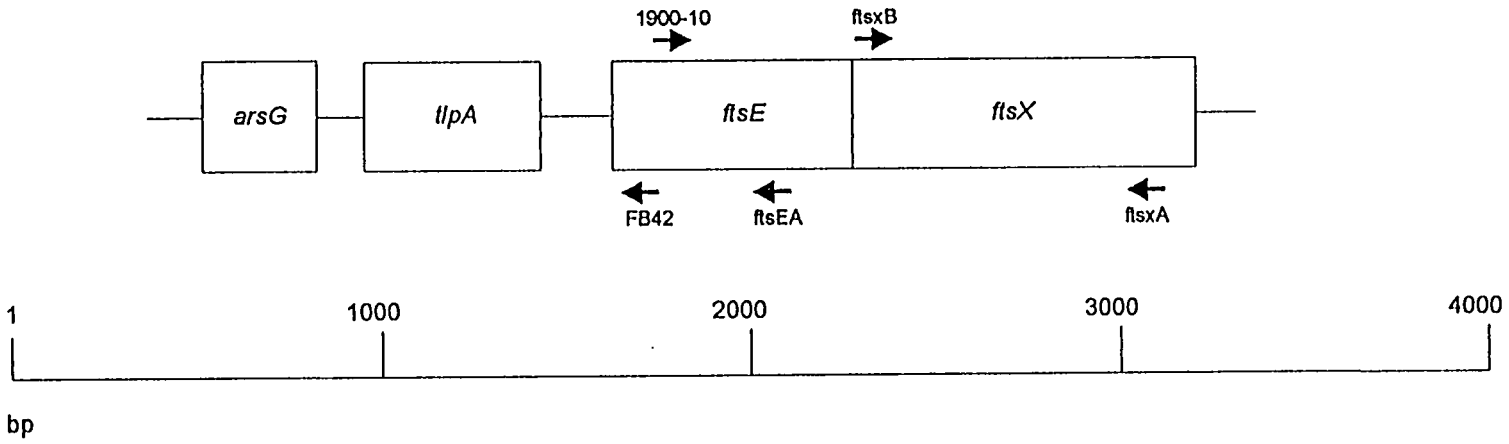
1.7 CELL DIVISION IN *N. GONORRHOEAE*

Little is known of the cell division process in *N. gonorrhoeae*. Early studies using microscopy showed that gonococcal cell growth is bidimensional and that division occurs by septation as seen in other Gram negative bacteria (Westling-Häggström *et al.*, 1977). Recently, the *ftsZ* gene was cloned and sequenced in our laboratory coupled with initial characterization of the FtsZ protein (Radia and Dillon, unpublished data). The *ftsZ* gene comprises 1179 bp encoding a protein of 392 aa with a predicted molecular weight of 41.5 kDa which contains a GTP binding site homologous to the eukaryotic tubulin signature

sequence (Radia and Dillon, unpublished data). A 15 bp inverted repeat downstream of the *ftsZ* stop codon did not contain the Neisserial uptake sequence, and was therefore not predicted to be a terminator of transcription when analyzed using the Genetics Computer Group software package. The gonococcal FtsZ protein shared 72% identity with FtsZ from *E. coli*, and 64% identity with that from *H. influenzae* and was highly conserved with FtsZ from other organisms in the amino portion of the sequence. The carboxy terminus was more variable in all of the FtsZ aa sequences examined (Radia and Dillon, unpublished data). Overexpression of the gonococcal FtsZ in *E. coli* resulted in filamentation of the cells, indicating that FtsZ from *N. gonorrhoeae* may interact with that from *E. coli* and interfere with the division process. *ftsZ* is likely to be an essential gene in *N. gonorrhoeae* as attempts to make a knock-out mutant in this gene have failed.

The *ftsE* and *ftsX* genes from *N. gonorrhoeae* CH811 have also been cloned and sequenced in our laboratory (Bernatchez and Dillon, unpublished data; Figure 3). These genes have been proposed to function in cell division, however this role has yet to be demonstrated (Sturgeon and Ingram, 1978; Taschner *et al.*, 1988). *ftsE* and *ftsX* overlap by 4 bp and encode proteins of 216 and 305 aa respectively. FtsE is homologous to proteins from a group of ABC (ATP Binding Cassette) transporters, and contains an ATP binding motif. FtsX has been proposed to be an integral membrane protein with 4 transmembrane domains. Together they may function as a transporter of components important for cell division, with FtsE comprising the ATP binding portion and FtsX acting as the membrane anchor (Bernatchez and Dillon, unpublished data). FtsX is not essential in *N. gonorrhoeae* as an insertional mutant showed no observed change in phenotype (Bernatchez and Dillon,

Figure 3. The genetic organization of the *ftsE* and *ftsX* genes and their flanking genes from *N. gonorrhoeae* CH811 (Bernatchez and Dillon, unpublished data). Primers used for Northern hybridizations, RT-PCR, and primer extension are indicated.


1900-10
ftsxB
arsG
tlpA
ftsE
ftsX
FB42
ftsEA
ftsxA
1
1000
2000
3000
4000
bp

unpublished data). *ftsY* was not found upstream of *ftsE* as it is in *E. coli*, however the *pilA* gene has been proposed to be a homologue of *ftsY* (Fyfe and Davies, 1996). PilA was previously reported to be involved in regulating the pilin-encoding *pilE* gene (Taha *et al.*, 1988), however Fyfe and Davies (1996) showed that the binding site of PilA upstream of *pilE* was not required for transcription of *ftsE*, and they proposed instead that PilA is involved in protein translocation as FtsY is in *E. coli*.

The *penA* gene from *N. gonorrhoeae* which encodes Penicillin Binding Protein 2 (PBP2) has been cloned and sequenced, and is homologous to the *ftsI* gene of *E. coli* which encodes PBP3 in that organism (Spratt, 1988). PBP3 in *E. coli* has been shown to function in peptidoglycan synthesis at the septum during cell division, however its role in cell division in *N. gonorrhoeae* has yet to be demonstrated. *ftsI* is located within the cell division cluster at the 2 min region of the *E. coli* chromosome (Figure 1A).

Another gene believed to function in cell division in *N. gonorrhoeae* is the *tpc* (tetrapac) gene which was cloned and sequenced by Fussenegger *et al.* (1996). Mutations in this gene result in the formation of tetrads in which two diplococci are joined together by a double murein layer with a continuous outer membrane. These mutants also show an altered colony morphology which is "cratered and crinkled". The *tpc* gene likely encodes a murein hydrolase which acts to cleave the murein for cell separation to occur (Fussenegger *et al.*, 1996).

1.8 HYPOTHESES AND OBJECTIVES

Cell division is an integral component of the cell cycle in bacteria. While cell

division has been extensively studied in the Gram negative rod *E. coli*, no studies of a Gram negative coccus have been reported. Based on the known sequences of the cell division genes from the 2 min region of the *E. coli* chromosome, I hypothesized that a similar cluster of cell division and growth genes was present on the chromosome of *N. gonorrhoeae*. Due to the general lack of operons in the gonococcus, it was also hypothesized that the regulation of the gonococcal cell division genes would be different from *E. coli*, in which the genes of this cluster are thought to be co-transcribed. I also proposed that environmental conditions such as aerobic and anaerobic growth affect the regulation of cell division genes.

In undertaking studies of cell division genes in *N. gonorrhoeae* in which I have proposed that their regulation is highly complex, the following objectives were developed:

1) To determine if *ftsZ* is located within a cluster of cell growth and division genes in *N. gonorrhoeae*, and if homologues of the *E. coli* genes *minC*, *minD*, and *minE* are also present on the gonococcal chromosome. The raw data from the gonococcal genome project will be used to determine the primary structure surrounding *ftsZ*, and data analysis for open reading frames, Neisserial uptake sequences, and transcriptional terminators will be undertaken to see if a cell division cluster is present in the gonococcus.

2) To identify promoters and transcriptional terminators surrounding cell division genes within the gonococcal *ftsZ* cluster in order to determine if cell division is a highly regulated process as it is in *E. coli*. Studies will be performed under both aerobic and anaerobic growth conditions to determine if environmental factors affect cell division. Reverse-transcriptase-polymerase chain reaction (RT-PCR), Northern blot analysis

and primer extension studies will be performed to identify promoter sites and transcripts.

3) To determine if the *ftsEX* genes are co-transcribed, and to identify promoters responsible for their transcription. RT-PCR will be used to determine co-transcription of *ftsE* and *ftsX*, and Northern hybridizations and primer extension will be used to study promoters and transcripts.

2. METHODS

2.1 BACTERIAL STRAINS AND GROWTH CONDITIONS

Neisseria gonorrhoeae strain CH811 [auxotype (A)/serotype (S)/plasmid content class (P) class]: non-requiring/IB-2/plasmid free was isolated by Moreno *et al.* (1987) and described by Picard and Dillon (1989), and *N. gonorrhoeae* strain FA1090 (A/S/P class P/IB-3/2.6) was described by Dempsey *et al.* (1991). These strains were grown on GC Medium Base (GCMB; Difco, Detroit, MI) supplemented with Kellogg's defined supplement (Kellogg, 1963) with the modifications of Dillon (1983) and were incubated overnight for 14 to 16 hours at 35°C with 5% CO_2 in a humid environment.

N. gonorrhoeae CH811 and FA1090 were grown anaerobically on GCMB with Kellogg's defined supplement onto which a sterile disk (Schleicher & Schuell, Keene, NH) inoculated with 50 µL of 2.4 M $NaNO_2$ had been centrally placed. Plates were incubated overnight in a humid environment in the presence of 5% CO_2, 7% H_2, and 88% N_2 at 35°C in an anaerobic chamber (Forma Scientific, Marietta, OH). Gonococcal strains were subcultured at least once before being used for RNA isolation, and all subculturing was performed within the anaerobic chamber to ensure no residual oxygen was present. A GCMB plate was also subcultured and incubated without the addition of nitrite as a control to ensure that the cultures were indeed growing anaerobically. No growth was present without the addition of nitrite. GCMB plates were pre-reduced for at least 24 h in the anaerobic chamber before use.

E. coli MC1061 (F$^-$*araD139 Δ(ara-leu)7696 galE15 galK16 Δ(lac)X74 rpsL* (Str^r) *hsdR2* ($r_k^-m_k^+$) *mcrA mcrB1*) and *E. coli* LigATor (*end*A1 *hsd*17 ($rk_{12}^-m_{k12}^+$) *sup*E44*thi*-1

*rec*A1 *gyr*A96 *rel*A1 *lac*[F'*pro*A$^+$B$^+$*lac*I^{q}ZΔM15::Tn10(Tc^R)]) (R&D Systems, Minneapolis, MN) were grown in Luria Bertani (LB) broth or on LB plates (Sambrook *et al.*, 1989) to which 100 μg/mL or 50 μg/mL of ampicillin, 15 μg/mL of tetracycline, and 50 μg/mL 5-bromo-4-chloro-3-indoyl-β-D-galactoside (X-gal) were added when required. *N. gonorrhoeae* and *E. coli* strains were stored frozen at -70°C in Brain-Heart Infusion (BHI; Difco) containing 20% glycerol (Dillon, 1983).

2.2 PLASMIDS AND PLASMID CONSTRUCTS

The following plasmids were used for sequencing reactions in the primer extension experiments. For *ftsE*, the plasmid pSB1900 (Table 1; Bernatchez and Dillon, unpublished data) was used which contained the complete coding and upstream region of the *ftsE* gene. The plasmids pCV11 and pCV21 were used for *ftsQ* and *ftsA* respectively (Table 1), and were constructed by cloning PCR amplicons into the pTAg cloning vector (R&D Systems) by Victor *et al.* (1997; unpublished data). pCV11 contained an 822 bp fragment produced by PCR using primers CVQ1 and CVQ2 (Table 2) which consisted of the complete coding sequence of *ftsQ* as well as 51 bp upstream of the start codon of *ftsQ* (Victor *et al.*, unpublished). pCV21 contained a 1338 bp amplicon produced by PCR using primers CVA3 and CVA4 (Table 2) which consisted of the complete coding sequence of *ftsA* as well as 63 bp upstream of the start codon of *ftsA* (Victor *et al.*, unpublished data). Cloning was performed according to the instructions of the LigATor cloning system (R&D systems): 50 ng of linear pTAg vector was ligated with 75 ng of the appropriate PCR amplicon in a reaction containing 3 U T4 DNA ligase (Pharmacia Biotech, Baie d'Urfé, PQ), 10 mM

dithiothreitol (DTT), 1 mM ATP, and 1X Ligase buffer (Pharmacia Biotech) in a 10 μL reaction volume. Ligations were incubated overnight at 16°C. Competent *E. coli* LigATor cells (R&D systems) were transformed according to the instructions of the manufacturer, and clones were screened by PCR using the T7 primer which is located on the vector, and either CVQ2 for pCV11 or CVA4 for pCV21 (Table 1 and Table 2). For *ftsZ*, pAR2 was used for the sequencing reaction (Radia and Dillon, unpublished data).

Table 1. Plasmids used in this work.

Plasmid	Characteristics	Reference
pSB1900	pCRII with 2.8 kb amplicon of *N. gonorrhoeae* CH811 DNA including the 5' end of *ftsE* and 2.3 kb upstream of *ftsE*	Bernatchez and Dillon, unpublished data
pTAg	Cloning vector for PCR amplicons (Amp^R, Kan^R)	R&D Systems
pCV11	pTAg with an 822 bp amplicon containing the coding sequence of *ftsQ* from *N. gonorrrhoeae* CH811	This study; unpublished data
pCV21	pTAg with a 1338 bp amplicon containing the coding sequence of *ftsA* from *N. gonorrrhoeae* CH811	This study; unpublished data
pAR2	pTAg with a 1324 bp amplicon containing the coding sequence of *ftsZ* from *N. gonorrrhoeae* CH811	Radia and Dillon, unpublished data
pLES94	pUC18 based vector containing a promoterless *lacZ* gene for studying promoter activity	Silver and Clark, 1995
pLESE14	pLES94 with a 466 bp amplicon containing the promoters upstream of *ftsE*	This study
pLESQ6	pLES94 with a 790 bp amplicon containing the promoter upstream of *ftsQ*	This study
pLESZ25	pLES94 with a 606 bp amplicon containing the promoters upstream of *ftsZ*	This study

2.3 OLIGONUCLEOTIDES

All oligonucleotides were designed using the Primer Designer software (Scientific and Education Software). Oligonucleotides were synthesized at the University of Ottawa's Biotechnology Research Institute (Ottawa, Ont.) or by Operon Technologies Inc. (Alameda, CA), and are listed in Table 2.

2.4 CLONING OF PROMOTER REGIONS OF *FTSE*, *FTSQ*, AND *FTSA* IN pLES94

The vector pLES94 (Silver and Clark, 1995) was used to clone upstream regions of genes *ftsE*, *ftsQ*, and *ftsZ* so that the promoter activities of these genes could be studied under different environmental conditions. pLES94 contains a promoterless *lacZ* gene with a unique *Bam*HI site at its 5' end so that gonococcal promoters can be cloned upstream of this gene, and promoter activity can be measured using β-galactosidase assays (Figure 4). Three fragments were generated by PCR for cloning into pLES94, each containing *Bam*HI restriction endonuclease sites at each end: a 466 bp amplicon comprising the upstream region of the *ftsE* gene (primers FB43 and FB44; Table 2), a 606 bp amplicon comprising the upstream region of the *ftsZ* gene (primers FB45 and FB46; Table 2), and a 790 bp amplicon comprising the upstream region of the *ftsQ* gene (FB47 and FB48; Table 2). PCR amplicons were purified using the QIAquick PCR purification kit (QIAGEN; Chatsworth, CA). The plasmid pLES94 was prepared using the QIAfilter Plasmid Midi Kit of QIAGEN. The plasmid and inserts were subsequently digested with *Bam*HI (NEB) and were purified using QIAGEN's Plasmid Mini Kit and PCR purification kit respectively. These were ligated overnight at 16°C in a stationary water bath using T4 DNA ligase (NEB) and were

Table 2. Sequences of the oligonucleotide primers used for PCR, RT-PCR, and primer extension.

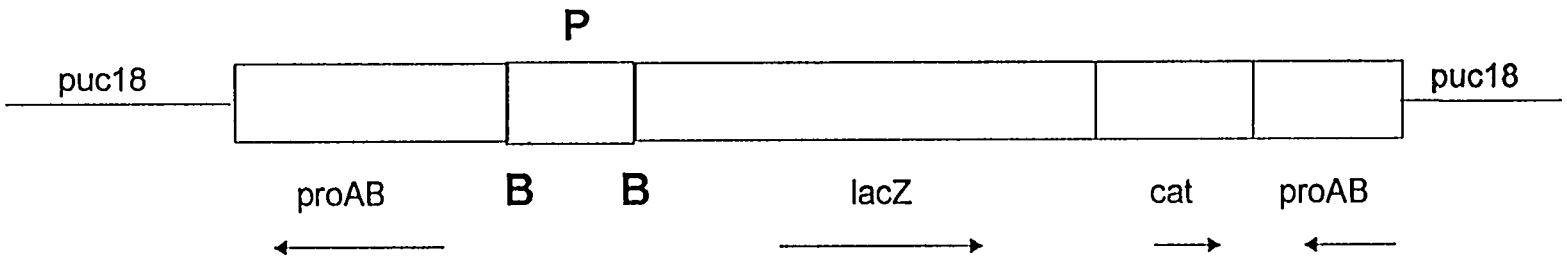
Oligonucleotide	5'-Sequence-3'	Gene of Interest
1900-10	AATCCACCGTCCTAAGCTGA	*ftsE*
ftsEA	GGATCGTCCAATTCTCGTCC (c)	*ftsE*
ftsXA	CAGAGCAGGTGTTGTGTGGT (c)	*ftsX*
ftsXB	GCATCATCCACTACTTCTCG	*ftsX*
ar10	CTCCGTCCAAGGTTACATCG (c)	*ftsZ*
ar13	GGGTGAAGACGTAACTATGC	*ftsZ*
ar19	GCCGTCTGAA[a]-TGGTTGCCAACAATGTG	*ftsZ*
ar20	GCCGTCTGAA[a]-CATCTTCGCTCATGGTC (c)	*ftsZ*
FB35	CGCACATTGTTGGCAACCAT (c)	*ftsZ*
FB40	GATCAGTGCGAGGACTTTAG (c)	*ftsA*
FB41	CTTGACGGGCAGATGATTCG (c)	*ftsQ*
FB42	CGCCGGGATAGGTTTTGGAA (c)	*ftsE*
FB43	GATTCACGGATCC[a]-GGCATCGCGCTCGACACAT	*ftsE*
FB44	GGTCTACGGATCC[a]-CGGATCATAGGAGGTCCTGT (c)	*ftsE*
FB45	GATCTACGGATCC[a]-TGGCATCATGCGATACGGAAG	*ftsZ*
FB46	GGCGCGCGGATCC[a]-ATTCCATTCAAAAACTCCTGA (c)	*ftsZ*
FB47	ATGCTACGGATCC[a]-TCACGGTACTTACGGCGAAGA	*ftsQ*
FB48	ATGCTACGGATCC[a]-TCGCCATCATGACAAGCAGCC (c)	*ftsQ*
CVA3	TTACGGCCTGGCGTCTGAAA	*ftsA*
CVA4	TTCTCACCTGTCAAACGGCA (c)	*ftsA*
CVQ1	CATGTGGGATAATGCCGAAG	*ftsQ*
CVQ2	CTGTTCCATTGTTCGTCTGC (c)	*ftsQ*
CVH22	CATACCATAGGCGGCACGTC (c)	*hyp1*
CVC20	GTGTCCGTAATCGTCCACCA (c)	*murC*
CVC21	AACCAACCGGAACGCAACAT	*murC*
CVG26	GGAATACGCGGCAACGGCAT	*murG*

CVG27	TCAACTCGGCAATCGTCAGC (c)	*murG*
CVW24	GTTGCGCAGCATGGAAAGTT	*ftsW*
CVW25	GCGGCGGTAATCATCAGCAC (c)	*ftsW*
CVD16	GCATCGACGCCGATCAGGAA (c)	*murD*
CVD17	GCGTGGCGCAAATCGGTAAG	*murD*
CVY15	CGGTGCAGAATATAGCGACT (c)	*mraY*
CVY23	CGCACATTTCAGCAACTGGT	*mraY*
CVME38	CACATCCATACCATCCTGCT	*minE*
CVMC39	GCTGGTCAAATTGGGCAAGA	*minC*
CVMC40	GCCTGCATGGAGTGGATAAA	*minC*
CVRA41	TACTGTTGGTGGTGTTCAGG	*rpoA*

a) 5' extensions

(c) represents primers located on the complementary strand.

Figure 4. Schematic diagram of the pLES94 vector (adapted from Silver and Clark, 1995). pLES94 is a pUC18 based vector that contains a promoterless *lacZ* gene. At the 5' end of this *lacZ* gene is a unique *Bam*HI restriction site for cloning (B), and upstream of this cloning site is a terminator of transcription so that no read-through from upstream promoters can occur. Downstream of *lacZ* is a *cat* gene with a *ptac* promoter for selection upon transformation into the gonococcus. Flanking this entire region are two fragments from the gonococcal *proAB* genes so that the entire region can recombine into the *proAB* locus when transformed into the gonococcus.


P
puc18
puc18
proAB
B
B
lacZ
cat
proAB

transformed into *E. coli* MC1061 using the calcium chloride method (Sambrook *et al.*, 1989). Clones were selected based on ampicillin resistance and by the selection of blue colonies on LB plates containing X-gal, since the successful cloning of a promoter would cause expression from the *lacZ* gene. Clones were screened initially by a quick lysis method (Sambrook *et al.*, 1989), and were verified by PCR with one of the primers annealing to the insert (FB43, FB47, and FB45 for *ftsE*, *ftsQ*, and *ftsZ* respectively; Table 2) while the second annealed to the *lacZ* gene of the vector (FBlac). The quick lysis method was performed by resuspending one colony in 50 µL of 10 mM EDTA, followed by the addition of 50 µL lysis solution (0.2 N NaOH, 0.5% SDS, 20% sucrose) and vortexing for 30 s. These cells were incubated at 70°C for 5 min., cooled to room temperature, and were mixed with 1.5 µL of 4 M KCl and 1 µL of bromophenol blue. The suspensions were vortexed again for 30 sec., chilled on ice for 5 min., and centrifuged for 3 min. at 4°C at 13,000 rpm in a microcentrifuge, and 10-15 µL of the supernatant was loaded on an agarose gel.

2.5 RNA AND DNA MANIPULATIONS

Total RNA for use in Northern hybridizations and RT-PCR experiments was isolated from overnight cultures of *N. gonorrhoeae* CH811 and FA1090, grown both aerobically and anaerobically, using QIAGEN's RNeasy Total RNA kit. RNA for primer extension was isolated from overnight cultures of *N. gonorrhoeae* CH811 grown aerobically and anaerobically using TRIzol™ Reagent (Gibco BRL, Gaithersberg, MD) according to the instructions of the manufacturer. TRIzol™ was used for the primer extension experiments because much greater quantities of RNA could be isolated using this system. RNA was

harvested quickly on the benchtop for both the aerobic and anaerobic cultures. Approximately one third of a 10 μL loopful was used for each QIAGEN preparation, or for each 1 mL of TRIzol™ reagent used. Approximately 4 plates of anaerobically grown *N. gonorrhoeae* was required for every one plate of aerobically grown culture due to the differences in amount of growth. The RNA was digested with RQ1 RNase-Free DNase (Promega, Fisher Scientific, Nepean, Ont.) according to the manufacturer's instructions. All RNA preparations were stored at -70°C in RNase free water. RNase free water was prepared by adding 0.1% diethyl pyrocarbonate (DEPC; ICN Biomedicals, Aurora, OH) to water, incubating overnight at 37°C, and autoclaving for 45 min. in order to inactivate the DEPC.

Chromosomal DNA from *N. gonorrhoeae* CH811 was extracted according to Dillon *et al.* (1985) and purified by caesium chloride density gradient ultracentrifugation in a Beckman Type 65 rotor in a L8-70M ultracentrifuge (Sambrook *et al.*, 1989). The plasmid pLES94 (Silver and Clark, 1995) was prepared using the QIAfilter Plasmid Midi Kit of QIAGEN; all other plasmids were isolated using the Quantum Prep Plasmid Miniprep Kit (BIO-RAD, Hercules, CA) or QIAGEN's Plasmid Mini Kit. All DNA preparations were stored in water at -20°C.

All restriction endonuclease digests were performed using enzymes from New England Biolabs (NEB; Beverly, MA) or Pharmacia Biotech (Baie D'Urfé, Quebec) according to the instructions of the manufacturer.

DNA and RNA concentrations were determined spectrophotometrically using the GeneQuant DNA/RNA Calculator (Pharmacia Biotech). DNA concentration is equal to the optical density at λ_{260} × 50 ng/μL × dilution factor; RNA concentration is equal to the optical

density at λ_{260} × 40 ng/μL × dilution factor (Sambrook *et al.*, 1989).

2.6 GENOMIC ANALYSIS OF THE CELL DIVISION AND *MIN* CLUSTERS FROM *N. GONORRHOEAE* FA1090

The nucleotide sequence of *ftsZ* from *N. gonorrhoeae* CH811 (Radia and Dillon, unpublished data) and of the *min* genes from *E. coli* were sent to the *N. gonorrhoeae* Sequence Database BLAST at http://dna1.chem.uoknor.edu/gono_blast.html which contains the raw data from the genome project of *N. gonorrhoeae* FA1090. The unverified sequence surrounding *ftsZ* and the *min* cluster was retrieved and the 5' end of the contiguous sequence (contig) containing *ftsZ* was sent back to the gonococcal BLAST database to retrieve a second contig. These were assembled manually and were analyzed for open reading frames (ORFs) using the sequence analysis software PCGene (Intelligenetics Inc., Geneva, Switzerland). The deduced protein sequences of the ORFs were then sent to the BLAST database at the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov/BLAST/ in order to identify the gene homologues. The complete cell division and *min* clusters were analyzed using PCGene and the Genetics Computer Group (GCG) software package (version 7, Madison, WI) for repetitive sequences, Neisserial uptake sequences, and transcriptional terminators.

2.7 ELECTROPHORESIS

All PCR amplicons, restriction enzyme digests, and plasmids were separated on 1% or 2% agarose (Pharmacia Biotech) gels in 1X Tris-acetate EDTA buffer at a constant

voltage of 50 V (Sambrook *et al.*, 1989). The gels were stained in 1 mg/L of ethidium bromide, and DNA was visualized under ultra-violet light (Fotodyne Inc., New Berlin, WI). Gels were photographed using the BioPhotonics GelPrint 2000i digital imaging system (BIO/CAN, Mississauga, Ont.).

2.8 POLYMERASE CHAIN REACTION (PCR)

Probes for Northern hybridizations were generated using PCR techniques. The 282 bp *ftsE* probe was synthesized using primers 1900-10 and ftsEA, the 895 bp *ftsX* probe was synthesized with primers ftsXA and ftsXB, and the 256 bp *ftsZ* probe was generated with primers ar10 and ar13 (see Table 1 for primer sequences). The PCR Core Kit (Boehringer Mannheim Canada, Laval, Québec) was used for all reactions with the following conditions: 1X PCR buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM of the appropriate oligonucleotides, and 2.5 U of *Taq* DNA polymerase per 100 μL reaction. All amplifications were done using the Perkin Elmer GeneAmp PCR System 9600 Thermocycler (Perkin Elmer Corp., Norwalk, CT) with the following profile: 3 min. at 94°C; 35 cycles of 15 sec. at 94°C, 15 sec. at 50°C, 1 min. at 72°C; 5 min. at 72°C, and a final hold at 4°C.

2.9 NORTHERN HYBRIDIZATIONS

Northern hybridizations with *ftsE*, *ftsX*, and *ftsZ*- specific probes were performed. Approximately 5 μg (5 μL) of total RNA isolated from *N. gonorrhoeae* CH811 and FA1090 grown either aerobically or anaerobically was mixed with 4 volumes (20 μL) of sample preparation buffer and denatured at 65°C for 10 min. The sample preparation buffer

contained 250 µl of formamide, 83 µl of formaldehyde, 50 µl 10X MOPS (200mM 4-morpholinopropanesulfonic acid, 50 mM sodium acetate, 10 mM EDTA, pH 7), 8 µl of 0.08 g/mL bromophenol blue, and 9 µL of 10 mg/mL ethidium bromide and was prepared directly before use. RNA was separated on 1% agarose gels (Sigma) prepared with 1X MOPS, and containing 6% formaldehyde (Sambrook *et al.*, 1989). The gels were subsequently washed 4 times for 15 min. each in RNase free water. The RNA was transferred to positively charged nylon membranes (Boehringer Mannheim) for 2.5 h. using a Model 785 Vacuum Blotter (BIO-RAD). Probes were prepared using PCR techniques. The 282 bp *ftsE* probe (Probe E) was synthesized using primers 1900-10 and ftsEA such that the ATP binding site of *ftsE* was excluded; the 895 bp *ftsX* probe (Probe X) was synthesized with primers ftsXA and ftsXB, and the 256 bp *ftsZ* probe (Probe Z) was generated with primers ar10 and ar13 such that the GTP binding site of *ftsZ* was excluded (see Table 1 for primer sequences). The probes were electrophoresed on 1% low melting point agarose gels and the appropriate fragments were excised from the gel. The agarose was trimmed to approximately 0.04 g and 100 µL of water was added, and was incubated at 95-100°C for 10 min. From this, 30 µL was transferred to tubes for labelling with 50 µCi of Redivue [α-^{32}P]dCTP (Amersham Canada Limited; Oakville, Ont.) using the Rediprime DNA labelling system of Amersham Canada Limited, following the instructions for labelling DNA in agarose. These probes were hybridized at 65°C for 2 h. using Rapid-hyb buffer (Amersham Canada) as per the manufacturer's instructions, with the exception that the prehybridization time was extended to 1 h. The membrane was washed for 20 min. in 2X SSC, 0.1% SDS at room temperature, and twice for 15 min. in 1X SSC, 0.1% SDS at 65°C. Blots were exposed to film for

autoradiography (Dupont, Mississauga, Ont.) and were developed using a Cordell MXR developer (Cordell Engineering, Peabody, MA).

All Northern hybridizations were performed at least twice.

2.10 REVERSE TRANSCRIPTASE - POLYMERASE CHAIN REACTION (RT-PCR)

Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to determine whether the genes found in the cell division clusters were co-transcribed. Appropriate oligonucleotide primers (Table 1) were used to make cDNA using 1 μg of total RNA from *N. gonorrhoeae* CH811 as a template in a reverse transcriptase reaction using either the Expand™ Reverse Transcriptase (Boehringer Mannheim) or Superscript™II (Gibco BRL) according to the instructions of the manufacturer. When the Expand™ system was used, the RNA was annealed to 3 pM of an appropriate gene-specific oligonucleotide at 65°C for 10 min. and was chilled on ice. Reverse transcriptase reactions were performed with the following reaction conditions: 1X Expand™ reverse transcriptase buffer, 10 mM DTT, 1 mM each deoxynucleotide (dNTP), and 50 U of Expand™ reverse transcriptase for 1 h. at 42°C. When Superscript™II was used, the RNA and primers were annealed at 70°C, and the final reaction conditions were 1X First Strand buffer, 10 mM DTT, 1mM each dNTP, and reactions conditions were also at 42°C for 1h. The cDNA was subsequently used as a template for PCR using appropriate primers. Aliquots of 2 μL of the RT reactions were used as a DNA template for PCR in a Perkin-Elmer 9600 GeneAmp PCR System. The PCR Core Kit was used with the following reaction conditions: 1X PCR buffer containing 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 μM primers, and 1.25 U of Taq DNA polymerase in a 50 μL

reaction. The thermal profile consisted of: 1 cycle: 94°C for 3 min.; 30 cycles: 94°C for 15 sec., 55°C for 15 sec., 72°C for 1.5 min.; 1 cycle: 72°C 5 min. Negative controls consisted of identical reactions to which no reverse transcriptase was added. In addition, PCR reactions were performed directly on the RNA sample to ensure no DNA was present in the RNA preparations. All reactions were performed at least twice.

2.11 PRIMER EXTENSION ANALYSIS

The transcriptional start sites of *ftsE*, *ftsQ*, *ftsA*, and *ftsZ* were determined by primer extension analysis. The oligonucleotide FB42 was used to map the promoter of *ftsE*, FB41 was used for *ftsQ*, FB40 was used for *ftsA*, and FB35 was used for *ftsZ* (Table 1). These primers, which are complementary to the respective RNA strands, were end-labelled with [γ-^{32}P]dATP (Amersham Canada) using T4 polynucleotide kinase (Pharmacia Biotech). The radiolabelled primer was subsequently cleaned with the QIAquick nucleotide removal kit (QIAGEN). Primer extension reactions consisted of 3 pmol of radiolabelled primer, 20-50 μg total RNA, and 200 U Superscript™ II reverse transcriptase (Gibco BRL) and reactions were performed as per the manufacturer's instructions. Briefly, the primer was annealed to the RNA at 70°C for 10 min. and chilled on ice. To this was added 4 μL 5X First Strand buffer, 2 μL 0.1 M DTT, and 1 μL 10 mM dNTP mix and this reaction was incubated at 42°C for 2 min. The reverse transcriptase was subsequently added and incubations were at 42°C for 1 h. Completed reactions were precipitated with ethanol (Sambrook *et al.*, 1989) and resuspended in 3 μL water, and the entire reaction was separated on a 6% polyacrylamide sequencing gel alongside a sequencing reaction generated using the same primer. For the

clones pCV11 which contains the *ftsQ* gene, pCV21 which contains *ftsA*, and pAR2 which contains *ftsZ*, the entire promoter region was not contained on the cloned fragment. Therefore while the primer annealed to the cloned fragment, some of the sequence generated in the sequencing reactions was actually of the vector sequence. By determining the number of bases on the vector that were sequenced and extrapolating to the known DNA sequence of the genes of interest, the exact transcriptional start site was determined for each of these genes. The TaqTrack™ DNA sequencing kit (Promega, Fisher Scientific, Nepean, Ontario) was used for these sequencing reactions, and reactions were electrophoresed using the Model S2 sequencing gel apparatus of Gibco BRL. The sequencing gels were dried using the Model 583 Gel Dryer from BIO-RAD, and the gels were exposed to film (Dupont) for autoradiography which were developed using a Cordell MXR developer (Cordell Engineering). Each primer extension reaction was performed at least twice using two different RNA preparations.

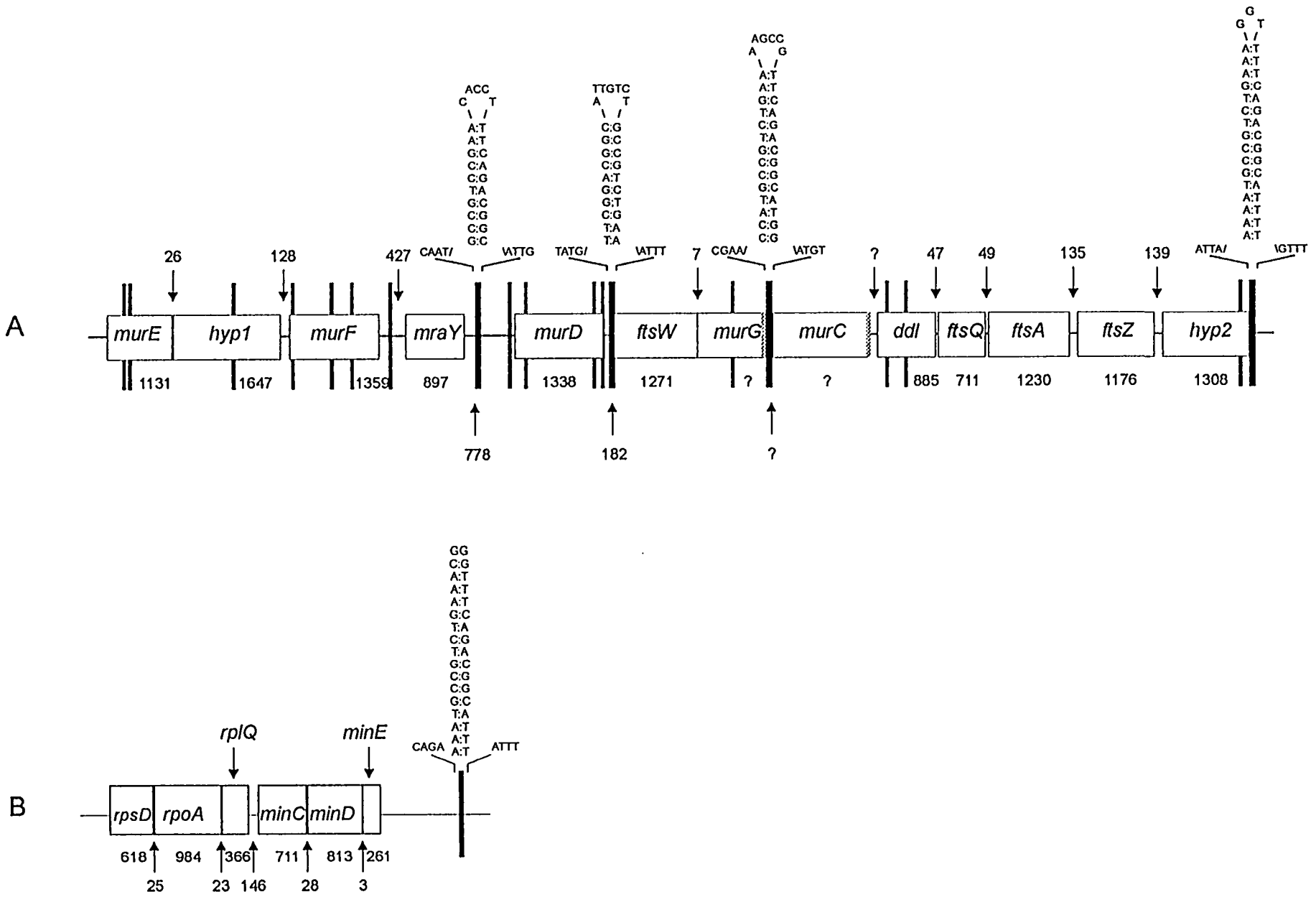
3. RESULTS

3.1 THE CELL DIVISION AND *MIN* CLUSTERS OF *N. GONORRHOEAE* FA1090

After *ftsZ* from *N. gonorrhoeae* CH811 had been cloned and sequenced in our laboratory (Radia and Dillon, unpublished data), the raw data from the Gonococcal Genome Sequencing Project became available on the Internet in the form of a searchable database. Using the DNA sequence of our *ftsZ* sequence, the genome project of *N. gonorrhoeae* FA1090 was then searched in order to determine the organization and type of genes surrounding *ftsZ* in the gonococcus. Initially, the *N. gonorrhoeae* CH811 *ftsZ* sequence was compared to that of strain FA1090, and four mismatches were identified between the two coding regions. However the FA1090 sequence was not verified at that time and could have contained sequence errors. A contiguous region comprising almost 18.7 kb was assembled using the genome project database (Figure 5A).

The gonococcal *ftsZ* lies near the 3' end of a large cluster of cell growth and division gene homologues in the order 5'-*murE-hyp1-murF-mraY-murD-ftsW-murG-murC-ddl-ftsQ-ftsA-ftsZ-hyp2*-3' (Figure 5A). Ten putative gonococcal genes homologous to *E. coli* cell growth and division genes were identified: *murE*, *murF*, *murD*, *ftsW*, *murG*, *murC*, *ddl*, *ftsQ*, and *ftsA* (Lutkenhaus and Mukherjee, 1996); while two hypothetical genes not found in the *E. coli* cell division cluster were also identified in this region (*hyp1* and *hyp2*). The gonococcal cell division cluster was found to be less compact than that of *E. coli*, with intergenic regions ranging from 7 bp to 778 bp, and no overlapping genes were contained within the cluster. A notable difference was the absence of the *envA* gene downstream of the gonococcal *ftsZ* gene. A homologue of *envA* was identified on the gonococcal chromosome

Figure 5. Schematic representation of the cell division clusters of *N. gonorrhoeae*. A. The *mur-fts* cell division cluster of *N. gonorrhoeae* FA1090 determined using the raw data from the Gonococcal Genome Project (Roe *et al.*, 1997). Question marks indicate that due to errors in the genome project, the exact stop codons could not be identified and therefore the exact gene sizes are uncertain. Gene sizes and intergenic spaces are indicated in bp. **B.** The *min* cluster from *N. gonorrhoeae* FA1090, also determined using the Gonococcal Genome Project. Gene and intergenic sizes are indicated in bp. Black vertical lines represent Neisserial uptake sequences, and when they occur in pairs to form transcriptional terminators, the DNA sequence is shown. Gene sizes and intergenic spaces are indicated in bp.


A
ACC
C T
A:T
A:T
G:C
C:A
C:G
T:A
G:C
C:G
C:G
G:C
TTGTC
A T
C:G
G:C
G:C
C:G
A:T
G:C
G:T
C:G
T:A
T:A
AGCC
A G
A:T
A:T
G:C
T:A
C:G
T:A
G:C
C:G
C:G
G:C
T:A
A:T
C:G
C:G
G
G T
A:T
A:T
A:T
G:C
T:A
C:G
T:A
G:C
C:G
C:G
G:C
T:A
A:T
A:T
A:T
A:T
26
128
427
CAAT/
\ATTG
TATG/
\ATTT
7
CGAA/
\ATGT
?
47
49
135
139
ATTA/
\GTTT
murE
hyp1
murF
mraY
murD
ftsW
murG
murC
ddl
ftsQ
ftsA
ftsZ
hyp2
1131
1647
1359
897
1338
1271
?
?
885
711
1230
1176
1308
778
182
?
B
GG
C:G
A:T
A:T
A:T
G:C
T:A
C:G
T:A
G:C
C:G
C:G
G:C
T:A
A:T
A:T
A:T
rplQ
minE
CAGA
ATTT
rpsD
rpoA
minC
minD
618
984
366
711
813
261
25
23
146
28
3

by searching the genome sequence database, but it was not located within the cell division cluster. Primary sequence analysis revealed the presence of 23 Neisserial uptake sequences (US) within the cluster (Figure 5A); nine of these contained 1 mismatch from the consensus sequence, and eight of these were involved in forming four stem-loop structures representing putative transcriptional terminators. These terminators were found to lie between *mraY* and *murD*, *murD* and *ftsW*, *murG* and *murC*, and at the end of the cluster after *hyp2* (Figure 5A).

A second cluster of genes involved in cell division is the *min* cluster which was also identified from the genome project of *N. gonorrhoeae* FA1090 (Figure 5B). This cluster contains genes *minC*, *minD*, and *minE* which are involved in localization of septation (Lutkenhaus and Mukherjee, 1996). *rplQ*, *rpoA*, and *rpsD* were identified upstream of *minC*. A putative terminator of transcription consisting of a stem-loop structure containing Neisserial uptake sequences was identified downstream of *minE*. This gene organization is notably different from that of *E. coli*, where the *min* genes are located at the 26 min region of the *E. coli* chromosome, while the *rplQ*, *rpoA*, and *rpsD* genes which are involved in DNA transcription/translation are found at the 72 min region.

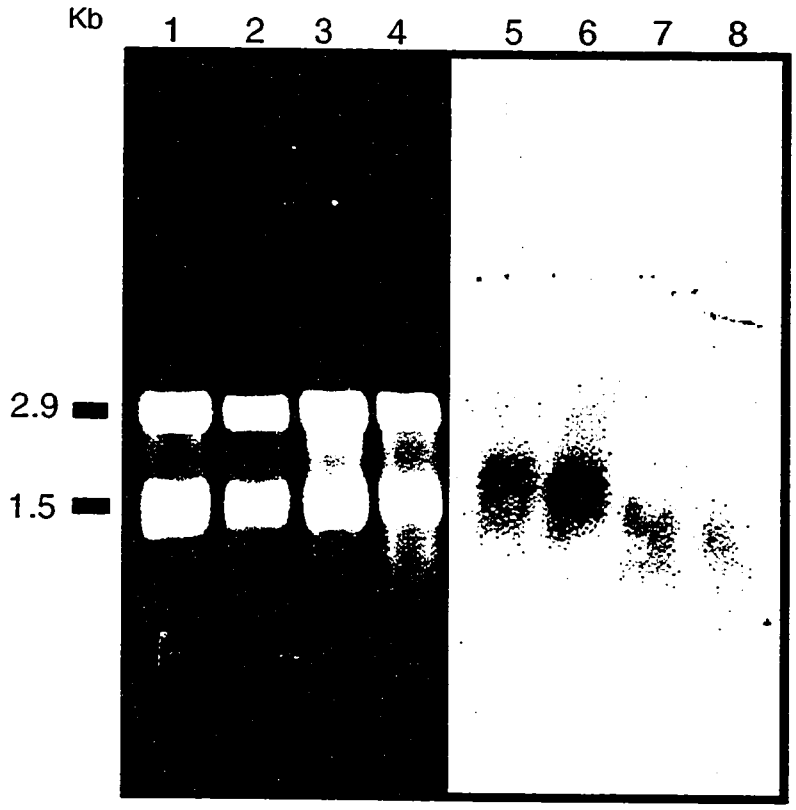
3.2 Northern hybridizations indicate differences in expression of cell division genes from aerobically and anaerobically grown gonococci.

It was proposed that *ftsE* and *ftsX* were co-transcribed in *N. gonorrhoeae* because these genes were shown to overlap by 4 bp (Bernatchez and Dillon, unpublished data). *In vitro* transcription/translation also showed that *ftsX* does not have its own promoter because FtsX was synthesized only when provided with an upstream vector-borne promoter

(Bernatchez and Dillon, unpublished data). In order to confirm that *ftsE* and *ftsX* are co-transcribed, Northern hybridizations were performed using internal gene probes of *ftsE* and *ftsX* to RNA isolated from *N. gonorrhoeae* strains CH811 and FA1090 grown both aerobically and anaerobically. The 282 bp *ftsE* internal gene probe was amplified from chromosomal DNA from *N. gonorrhoeae* CH811 by PCR using primers 1900-10 and ftsEA (Figure 3). This probe was named Probe E and it excluded the putative ATP binding site of *ftsE* so that the probe did not hybridize to transcripts of other ATP binding proteins. This probe hybridized to a prominent transcript of approximately 1.7 to 2.0 nt in size from the RNA isolated from the aerobically grown *N. gonorrhoeae* (Figure 6, Lanes 5 and 6). The size of this hybridizing RNA corresponds well to the size of a single transcript containing both *ftsE* and *ftsX*, which overlap and are 1,569 nt in length. This sizing was estimated from the ribosomal RNA species which run at 1.5 and 2.9 nt. A single RNA species slightly smaller than the 1.7 to 2.0 nt band hybridized to the same probe when RNA isolated from anaerobically grown cells was used, however this band was less prominent than for RNA isolated from aerobically grown cells (Figure 6, Lanes 7 and 8). The sizes of the RNA that hybridized to the *ftsE* probe when cells were grown under aerobic and anaerobic conditions were slightly different in size, indicating that there may be a difference between the expression of *ftsEX* in aerobically and anaerobically grown *N. gonorrhoeae*.

The 895 bp *ftsX* internal gene probe was synthesized by PCR using primers ftsXA and ftsXB and the resulting fragment was named Probe X (Figure 3). This probe hybridized to a band from RNA isolated from aerobically grown cells which was approximately 1.6 to 2 nt in size, which was expected if *ftsE* and *ftsX* were indeed co-transcribed as was suggested

Figure 6. Northern hybridization of Probe E containing the partial *ftsE* gene to total RNA isolated from *N. gonorrhoeae* CH811 and FA1090 grown under aerobic and anaerobic conditions. Lane 1, Total RNA from CH811 (aerobic); Lane 2, Total RNA from FA1090 (aerobic); Lane 3, Total RNA from CH811 (anaerobic); Lane 4, Total RNA from FA1090 (anaerobic); Lane 5, Probe E hybridized to CH811 RNA (aerobic); Lane 6, Probe E hybridized to FA1090 RNA (aerobic); Lane 7, Probe E hybridized to CH811 (anaerobic); Lane 8, Probe E hybridized to FA1090 (anaerobic).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by the Northern hybridization of *ftsE* (Figure 7, Lanes 5 and 6). The size of the hybridizing RNA again corresponded to a co-transcribed *ftsEX* transcript. No distinct band was identified from anaerobically grown *N. gonorrhoeae* when *ftsX* was used as a probe, indicating that the stability of the mRNA was decreased under anaerobic growth conditions, and that this RNA degradation may be occurring at the 3' end of the transcript because the 3' gene *ftsX* was more affected than the upstream *ftsE* (Figure 7, Lanes 7 and 8). The hybridization pattern visibel in Figure 7, Lane 8 is likely due to non-specific hybridization.

Multiple promoters have been identified for *ftsQ*, *ftsA*, and *ftsZ* in *E. coli* (Aldea, 1990). In order to determine whether transcription of *ftsZ* is also highly complex in *N. gonorrhoeae*, analysis of transcription of *ftsZ* was undertaken using Northern analysis. A 256 bp internal gene probe of *ftsZ* was generated by PCR using primers ar10 and ar13 such that the GTP binding site of *ftsZ* was excluded. This fragment was named Probe Z and was hybridized to total gonococcal RNA which was isolated from cells grown under both aerobic and anaerobic conditions. The *ftsZ* probe hybridized to a broad range of RNA species from the RNA isolated from aerobically grown cells, from approximately 1 to 3 nt in size (Figure 8, Lanes 5 and 6); this probe also hybridized to a range of RNA isolated from anaerobically grown cells, but in this case the sizes varied from approximately 1 to 1.5 nt (Figure 8, Lanes 7 and 8). The *ftsZ* gene is almost 1.2 kb in size, while *ftsA* and *ftsZ* together are approximately 2.5 kb, and *ftsQ*, *ftsA* and *ftsZ* together are approximately 3.3 kb. These data suggest that under aerobic growth conditions transcripts of *ftsZ* alone, *ftsA* and *ftsZ* together or *ftsZ* and *hyp2* exist, however a transcript containing *ftsQAZ* may not be produced, or may be produced at very low levels under aerobic growth conditions. Under anaerobic

Figure 7. Northern hybridization of Probe X hybridized to total RNA isolated from *N. gonorrhoeae* CH811 and FA1090 grown under aerobic and anaerobic conditions. Lane 1, Total RNA from CH811 (aerobic); Lane 2, Total RNA from FA1090 (aerobic); Lane 3, Total RNA from CH811 (anaerobic); Lane 4, Total RNA from FA1090 (anaerobic); Lane 5, Probe X hybridized to CH811 RNA (aerobic); Lane 6, Probe X hybridized to FA1090 RNA (aerobic); Lane 7, Probe X hybridized to CH811 (anaerobic); Lane 8, Probe X hybridized to FA1090 (anaerobic).

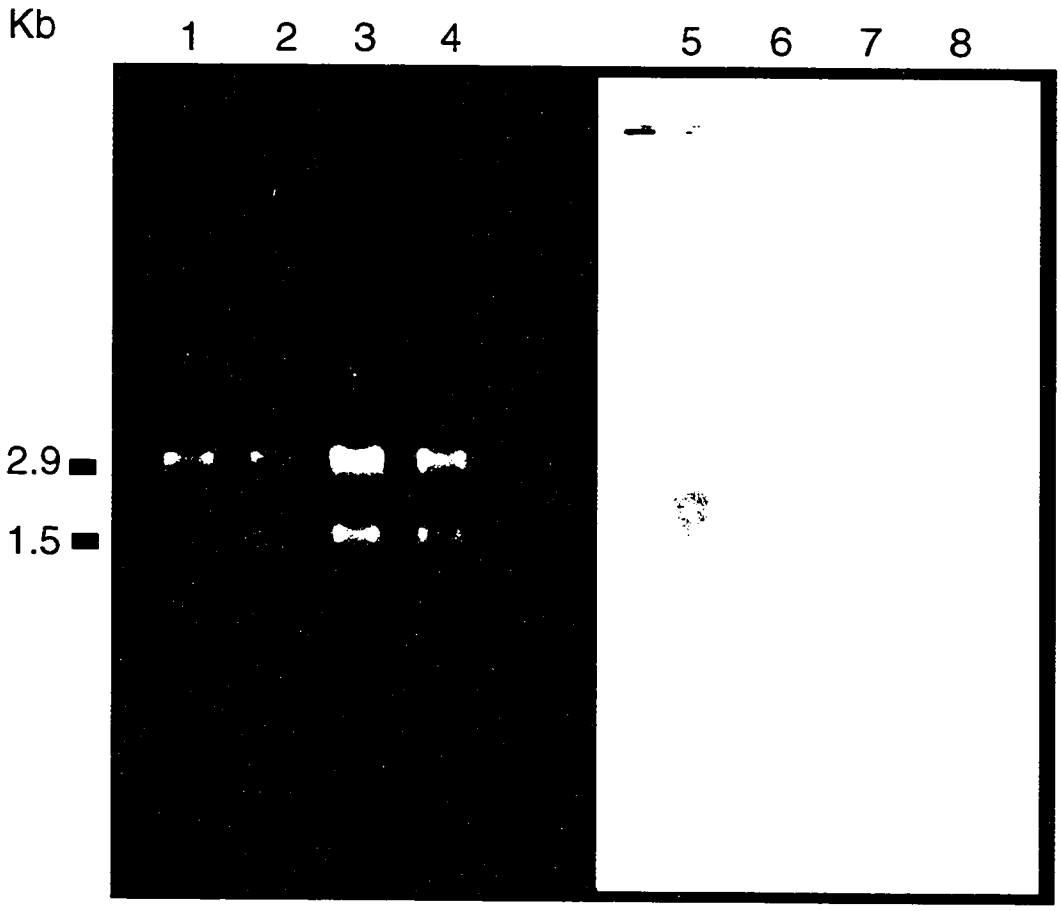
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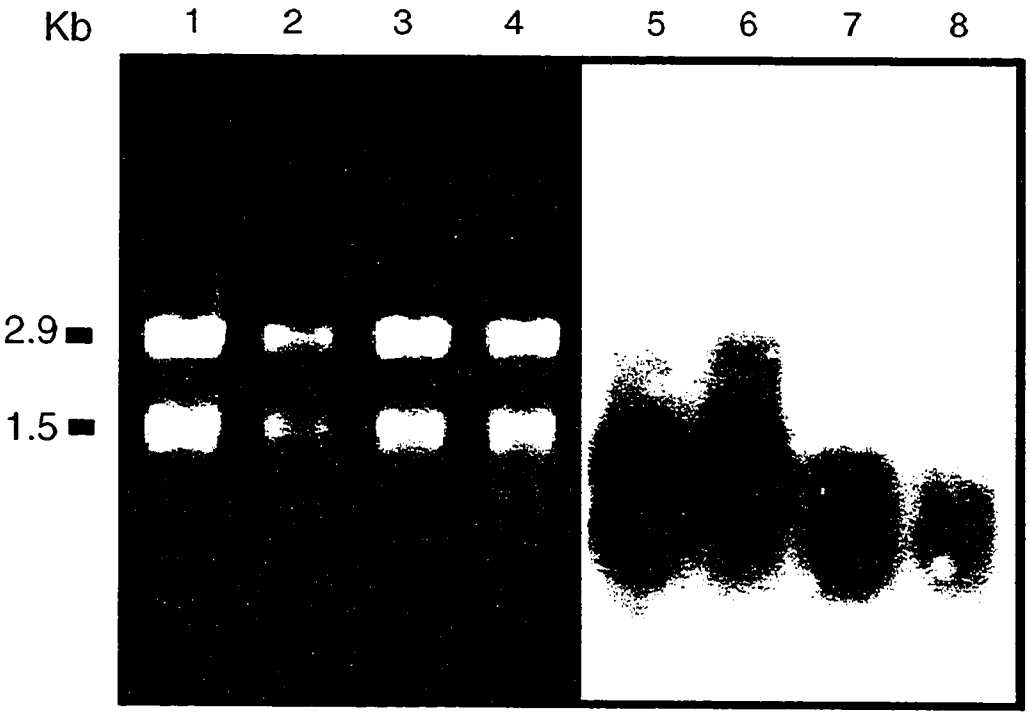
Figure 8. Northern hybridization of *ftsZ* to total RNA isolated from *N. gonorrhoeae* CH811 and FA1090 grown under aerobic and anaerobic conditions. Lane 1, Total RNA from CH811 (aerobic); Lane 2, Total RNA from FA1090 (aerobic); Lane 3, Total RNA from CH811 (anaerobic); Lane 4, Total RNA from FA1090 (anaerobic); Lane 5, Probe Z hybridized to CH811 RNA (aerobic); Lane 6, Probe Z hybridized to FA1090 RNA (aerobic); Lane 7, Probe Z hybridized to CH811 (anaerobic); Lane 8, Probe Z hybridized to FA1090 (anaerobic).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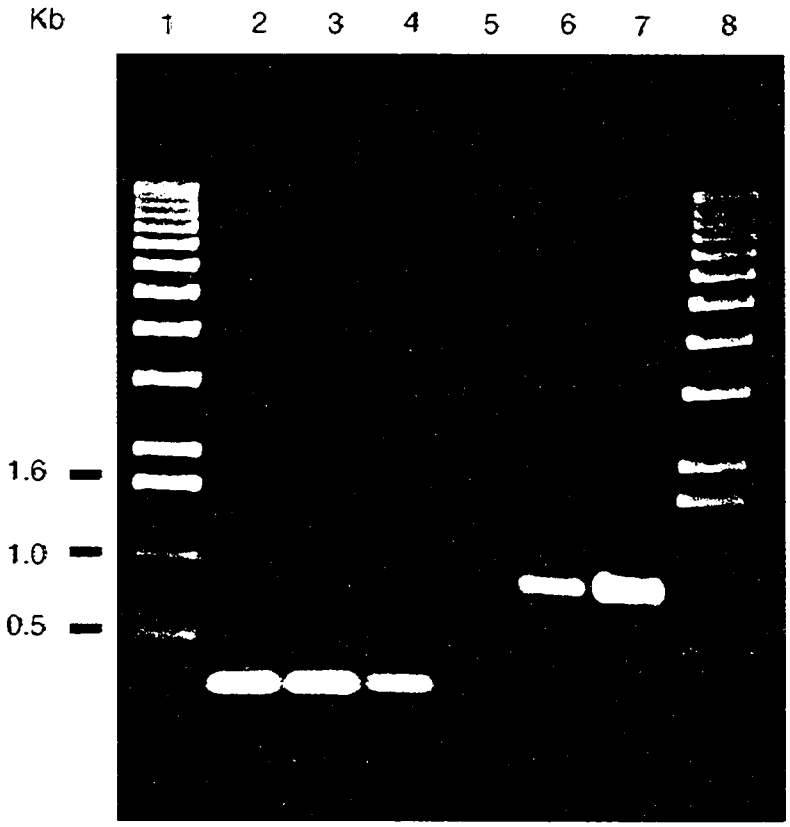
conditions, *ftsZ* does not seem to be transcribed with the upstream or downstream genes. These data also indicate that multiple promoters may exist upstream of the cell division genes *ftsQ*, *ftsA*, and *ftsZ*.

3.3 *FTSEX*, *FTSQAZ*, AND *MINCDE* ARE CO-TRANSCRIBED AS SHOWN BY RT-PCR

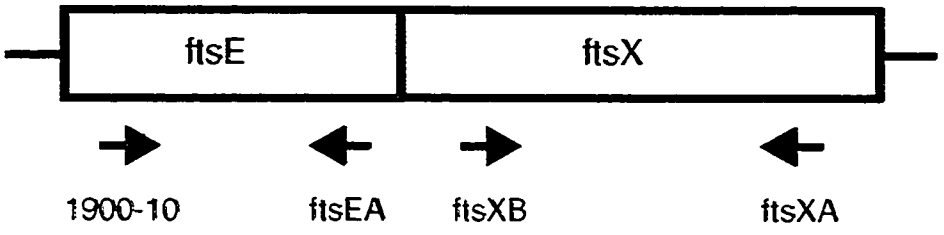
Due to the differences of the Northern hybridizations with RNA isolated from cells grown under aerobic and anaerobic conditions, RT-PCR studies were used to verify that *ftsEX*, and *ftsQAZ* were indeed co-transcribed under both growth conditions. This technique is more sensitive than a Northern blot due to the amplification step in the PCR reaction. In these experiments, a downstream primer was used to generate a cDNA copy of the mRNA of interest using reverse transcriptase. If two or more genes shared the same promoter, then a cDNA containing these genes would be synthesized, and subsequently each gene could be amplified by PCR. For *ftsEX*, cDNA was generated from total RNA isolated from both aerobically and anaerobically grown *N. gonorrhoeae* CH811 using primer ftsXA which anneals close to the 3' end of *ftsX* (Table 1; Figure 3). If *ftsE* and *ftsX* were co-transcribed, the resulting cDNA would contain both genes, however if they were not co-transcribed, only *ftsX* would be present on this cDNA fragment. PCR was performed using the cDNA as a template. Primers ftsXA and ftsXB were used as a positive control to amplify the *ftsX* gene, while primers ftsEA and 1900-10 were used to determine if *ftsE* was also present on the reverse transcript. For the *ftsX* PCR reaction, amplicons of the expected size of 894 bp were obtained from RT-PCR reactions generated from both aerobic and anaerobic RNA (Figure 9, Lanes 5 and 6), although the band representing the amplicon in Lane 5 was less intense

Figure 9. RT-PCR shows that *ftsE* and *ftsX* are co-transcribed in *N. gonorrhoeae* CH811 when grown both aerobically and anaerobically. A. A reverse transcriptase reaction was performed at the 3' end of *ftsX*, and PCR was subsequently performed on these reactions. Lane 1, 1 kb ladder; Lane 2, RT-PCR of *ftsE* from CH811 (aerobic); Lane 3, RT-PCR of *ftsE* from CH811 (anaerobic); Lane 4, PCR of *ftsE* from CH811 chromosomal DNA (positive control); Lane 5, RT-PCR of *ftsX* from CH811 (aerobic); Lane 6, RT-PCR of *ftsX* from CH811 (anaerobic); Lane 7, PCR of *ftsX* from CH811 chromosomal DNA (positive control); Lane 8, 1 kb ladder. **B.** Primer locations of the primers used for the RT-PCR experiment.

A



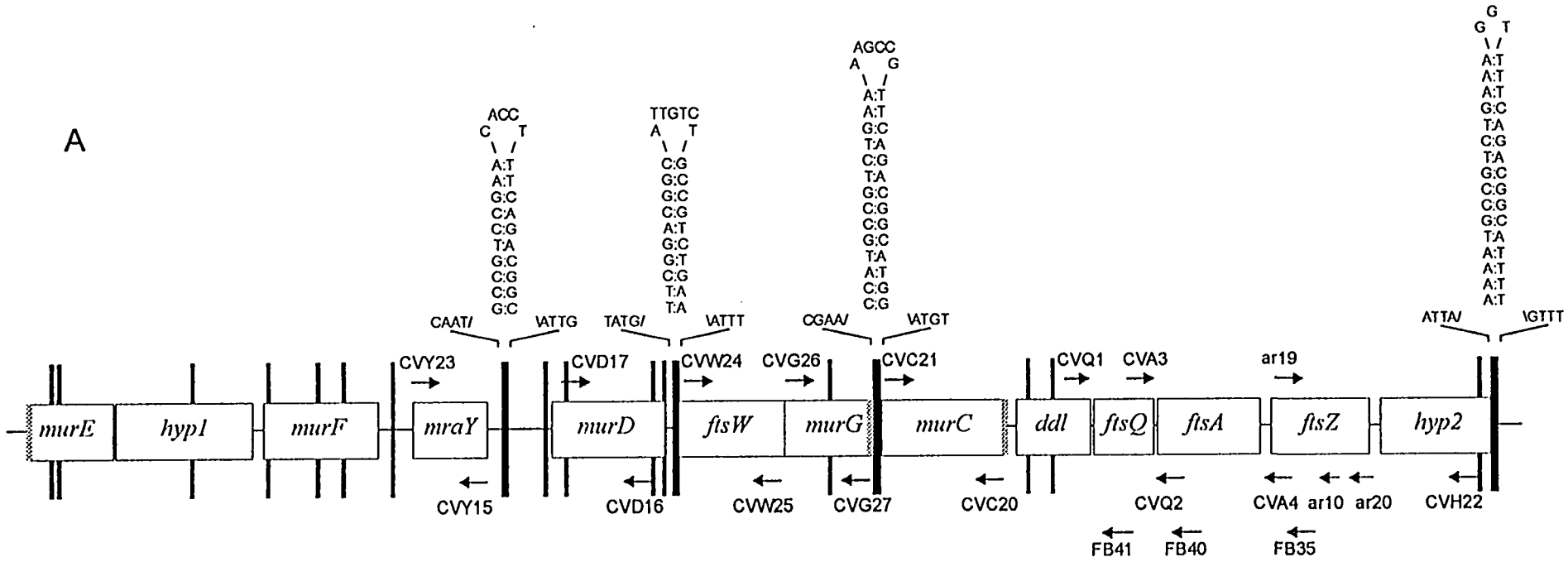
B



than that from Lane 6. The reason for this difference is unknown. Similarly for *ftsE*, amplicons of the expected size of 282 bp were obtained using cDNA prepared from RNA isolated from aerobically and anaerobically grown cells (Figure 9, Lanes 2 and 3). In addition, PCR using primers 1900-10 and ftsXA produced amplicons of the expected size when cDNA produced from RNA from aerobically and anaerobically grown cells was used as a template (data not shown). Negative controls which were performed identically to the experimental reactions with the exception that the reverse transcriptase was not added did not produce RT-PCR products. Therefore, *ftsE* and *ftsX* are co-transcribed under both aerobic and anaerobic growth conditions.

Similar experiments were performed in order to show that *ftsQ*, *ftsA*, and *ftsZ* were also co-transcribed. Primer ar20 (Table 1; Figures 10 and 11) which anneals at the 3' end of *ftsZ* was used to generate cDNA from total RNA isolated from both aerobically and anaerobically grown gonococci. PCR was performed using primers ar19 and ar10 which were specific for *ftsZ*, and an amplicon of the expected size of 683 bp was produced from the cDNA generated from RNA isolated from both aerobically and anaerobically grown cells (Figure 11, Lanes 8 and 9). Primers CVA3 and CVA4 which bind just upstream and downstream of *ftsA* were used for PCR with the RT reaction in order to determine if *ftsA* was transcribed with *ftsZ*. An amplicon of the expected size of 1338 bp was produced from both aerobic and anaerobic RNA, indicating that *ftsA* and *ftsZ* are co-transcribed under both growth conditions (Figure 11, Lanes 5 and 6). The amplicon from Lane 5 (aerobic RNA) was less intense from the amplicon in Lane 6 (anaerobic RNA), but the reason for this difference is unknown. Primers CVQ1 and CVQ2 which were specific for *ftsQ* were also

Figure 10. Primers used for studies of the cell division cluster and the *min* cluster of *N. gonorrhoeae*. **A.** Locations of primers used for studies of the *mur-fts* cell division cluster. **B.** Locations of primers used for studies of the *min* cluster.


A
ACC
C T
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A:T
G:C
C:A
C:G
T:A
G:C
C:G
C:G
G:C
TTGTC
A T
C:G
G:C
G:C
C:G
A:T
G:C
G:T
C:G
T:A
T:A
AGCC
A G
A:T
A:T
G:C
T:A
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CAAT/
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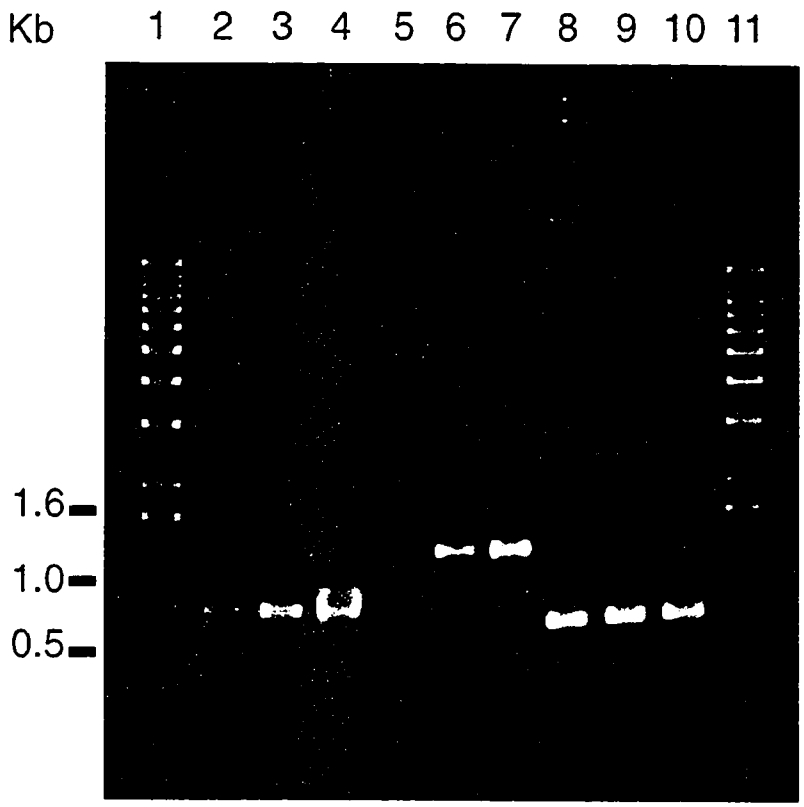
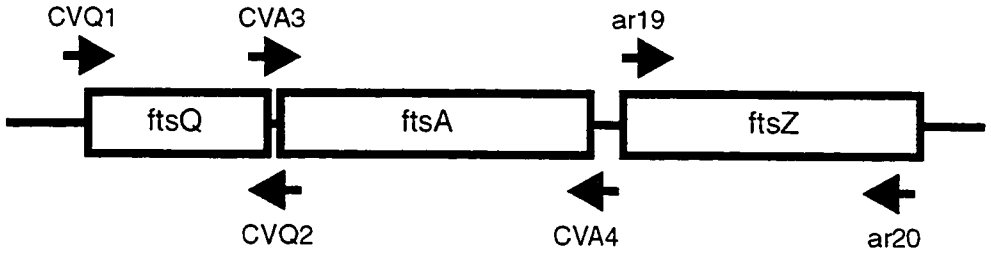
TATG/
\ATTT
CGAA/
\ATGT
ATTA/
\GTTT
CVY23
CVD17
CVW24
CVG26
CVC21
CVQ1
CVA3
ar19
murE
hyp1
murF
mraY
murD
ftsW
murG
murC
ddl
ftsQ
ftsA
ftsZ
hyp2
CVY15
CVD16
CVW25
CVG27
CVC20
CVQ2
CVA4
ar10
ar20
CVH22
FB41
FB40
FB35
B
GG
C:G
A:T
A:T
A:T
G:C
T:A
C:G
T:A
G:C
C:G
C:G
G:C
T:A
A:T
A:T
A:T
CAGA
ATTT
CVRA41
rpsD
rpoA
rplQ
minC
minD
CVRA42
CVME38

Figure 11. RT-PCR shows that *ftsQ*, *ftsA*, and *ftsZ* are co-transcribed in *N. gonorrhoeae* CH811 when grown both aerobically and anaerobically. A. A reverse transcriptase reaction was performed at the 3' end of *ftsZ*, and PCR was subsequently performed on these reactions. Lane 1, 1 kb ladder; Lane 2, RT-PCR of *ftsQ* from CH811 (aerobic); Lane 3, RT-PCR of *ftsQ* from CH811 (anaerobic); Lane 4, PCR of *ftsQ* from CH811 chromosomal DNA (positive control); Lane 5, RT-PCR of *ftsA* from CH811 (aerobic); Lane 6, RT-PCR of *ftsA* from CH811 (anaerobic); Lane 7, PCR of *ftsA* from CH811 chromosomal DNA (positive control); Lane 8, RT-PCR of *ftsZ* from CH811 (aerobic); Lane 9, RT-PCR of *ftsZ* from CH811 (anaerobic); Lane 10, PCR of *ftsZ* from CH811 chromosomal DNA (positive control) Lane 11, 1 kb ladder. **B.** Locations of the primers used for the RT-PCR experiment are shown.

A


Kb
1 2 3 4 5 6 7 8 9 10 11
1.6
1.0
0.5

B


CVQ1
CVA3
ar19
ftsQ
ftsA
ftsZ
CVQ2
CVA4
ar20

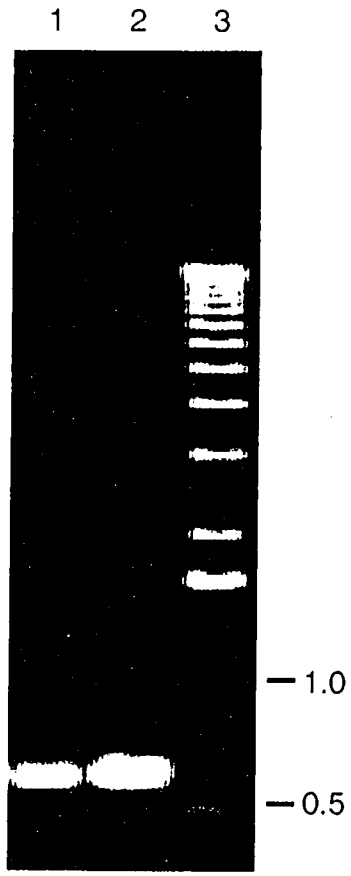
used for PCR with the same RT reactions. Amplicons of the expected size of 822 bp were also produced from both types of RNA, indicating that a transcript consisting of *ftsQ*, *ftsA*, and *ftsZ* exists under aerobic and anaerobic growth conditions (Figure 11, Lanes 2 and 3). In addition, PCR reactions were performed using primers CVQ1 and ar10 as an additional confirmation that the *ftsQAZ* transcript exists (data not shown). This does not preclude the possibility that additional promoters also exist upstream of *ftsA* and *ftsZ* such that smaller mRNA transcripts are also produced.

A similar experiment was also performed to determine if genes from the *min* cluster were co-transcribed. Primer CVME38 was used to generate cDNA from total RNA isolated from *N. gonorrhoeae* CH811 grown aerobically. This cDNA was used as a template for PCR using primers CVRA41 and CVRA42 which hybridize internally to the upstream gene *rpoA*. An amplicon of the expected size of 657 bp was produced from this RT reaction which could only occur if an mRNA transcript containing *rpoA* to *minE* is synthesized (Figure 12, Lane 1). Therefore, the genes *rpoA*, *rplQ*, *minC*, *minD*, and *minE* are all co-transcribed (Figure 12).

3.4 MULTIPLE TRANSCRIPTS AND TERMINATORS EXIST IN THE CELL DIVISION CLUSTER OF *N. GONORRHOEAE* CH811

Because of the novel putative terminators found within the cell growth and division cluster of *N. gonorrhoeae*, we hypothesized that the genes in this cluster were regulated differently from those of the *E. coli* cluster, which does not contain any terminators and is believed to be transcribed as a single operon (Donachie, 1993). RT-PCR was used in similar

Figure 12. RT-PCR of the *min* cluster. A reverse transcriptase reaction was performed at the 3' end of *minE*, and PCR was subsequently performed on these reactions. Lane 1, RT-PCR of *rpoA* from CH811 RNA (aerobic); Lane 2, RT-PCR of *rpoA* from CH811 chromosomal DNA (positive control); Lane 3, 1 kb ladder.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experiments as those described above to determine if the putative transcriptional terminators found within the novel cell growth and division cluster of *N. gonorrhoeae* CH811 were functional. In these experiments, a downstream primer was used to generate a cDNA copy of the mRNA of interest using reverse transcriptase. If two or more genes shared the same promoter, then a cDNA containing these genes would be synthesized, and subsequently each gene could be amplified by PCR. Primer CVH22 which annealed to the 3' end of the *hyp2* gene was used to generate a cDNA copy from the RNA. Primers ar19 and ar10 which annealed to the upstream gene *ftsZ*, and primers CVC20 and CVC21 which annealed to the *murC* gene further upstream were used to PCR amplify genes from this RT reaction. In both cases, amplicons were produced (Figure 13A, Lanes 2 and 4), indicating that one long transcript which contains *murC-ddl-ftsQ-ftsA-ftsZ-hyp2* is produced. Primer CVC20 which annealed to the downstream end of *murC* was then used for a RT reaction in order to determine if the stem-loop structure between *murC* and *murG* acted as a transcriptional terminator. Primers CVC20 and CVC21 were used to PCR amplify the *murC* gene as a positive control for the RT reaction (Figure 13A, Lane 6), but when primers CVG26 and CVG27 and CVW24 and CVW25 were used to PCR amplify *murG* and *ftsW* respectively (Figure 13A, Lanes 7 and Figure 13B, Lane 2), no amplicons were produced, indicating that *murG* and *ftsW* are not transcribed with *murC*. These two genes were successfully amplified from *N. gonorrhoeae* CH811 chromosomal DNA as a positive control.

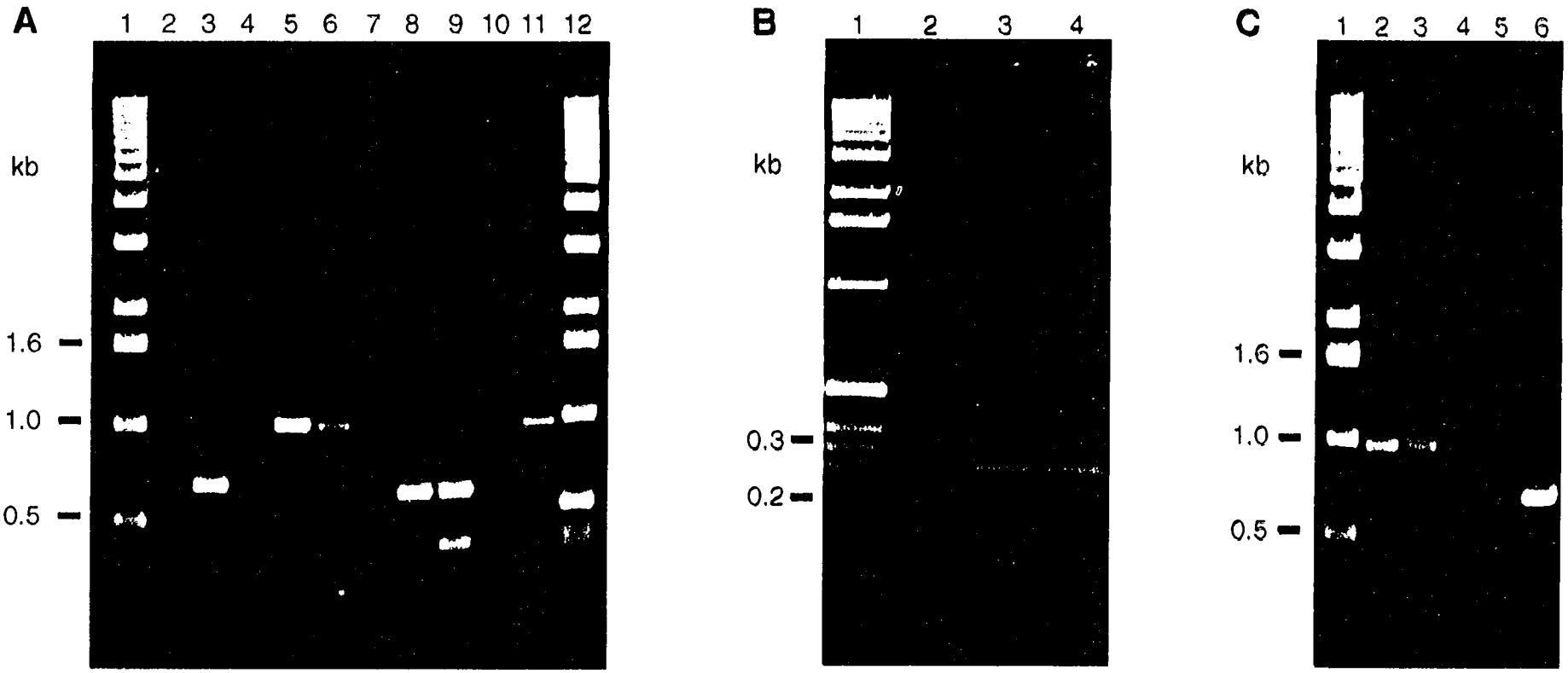
The next stem-loop structure which separates *murD* from *ftsW* and *murG* was investigated by amplifying *murG* (primers CVG26 and CVG27), *ftsW* (primers CVW24, CVW25), and *murD* (primers CVD16, CVD17) from a RT reaction generated from *murG*

Figure 13. RT-PCR of the *mur-fts* cluster from *N. gonorrhoeae* FA1090.

A. RT-PCR of gonococcal *hyp2*, *ftsZ*, *murC*, *murG*, and *murD* in the cell division and murein biosynthesis gene cluster of *N. gonorrhoeae*. Lane 1, 1 kb ladder; Lane 2, *ftsZ* amplified from *hyp2* RT reaction; Lane 3, *ftsZ* from CH811 chromosomal DNA; Lane 4, *murC* from *hyp2* RT reaction; Lane 5, *murC* from CH811; Lane 6, *murC* from *murC* RT reaction; Lane 7, *murG* from *murC* RT; Lane 8, *murG* from CH811; Lane 9, *murG* from *murG* RT; Lane 10, *murD* from *murG* RT; Lane 11, *murD* from CH811; Lane 12, 1 kb ladder.

B. 2% agarose gel of RT-PCR of *ftsW* in the cell division cluster. Lane 1, 1 kb ladder; Lane 2, *ftsW* amplified from *murC* RT reaction; Lane 3, *ftsW* from *murG* RT reaction; Lane 4, *ftsW* from CH811 chromosomal DNA.

C. RT-PCR of *murD* and *mraY* genes in the gonococcal cell division cluster. Lane 1, 1 kb ladder; Lane 2, *murD* amplified from *murD* RT reaction; Lane 3, *murD* from CH811 chromosomal DNA; Lane 4, *mraY* from *murD* RT; Lane 5, *mraY* from CH811 chromosomal DNA; Lane 6, *mraY* from *mraY* RT.


A
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kb
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0.5
B
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kb
0.3
0.2
C
1 2 3 4 5 6
kb
1.6
1.0
0.5

(primer CVG27). As expected, amplicons of the correct size were produced from both *murG* and *ftsW* (Figure 13A, Lane 9 and 13B, Lane 3) indicating that they are transcribed together, while no amplicon was produced when primers specific to *murD* were used for PCR from this same RT reaction (Figure 13A, Lane 10). A second product was consistently obtained from the RT-PCR of *murG*, however the major band produced migrated to the same position (628 bp) as *murG* amplified from CH811 chromosomal DNA. Therefore, *murD* is not co-transcribed with *ftsW* and *murG*.

The last putative terminator that was examined separates *mraY* from *murD*. When PCR was attempted on *mraY* (primers CVY15, CVY23) and *murD* (primers CVD16, CVD17) after an RT reaction generated from *murD* (primer CVD16), a PCR amplicon was obtained from *murD* only (Figure 13C, Lane 2); no amplicon of *mraY* was obtained (Figure 13C, Lane 4), indicating that *mraY* and *murD* are separately transcribed.

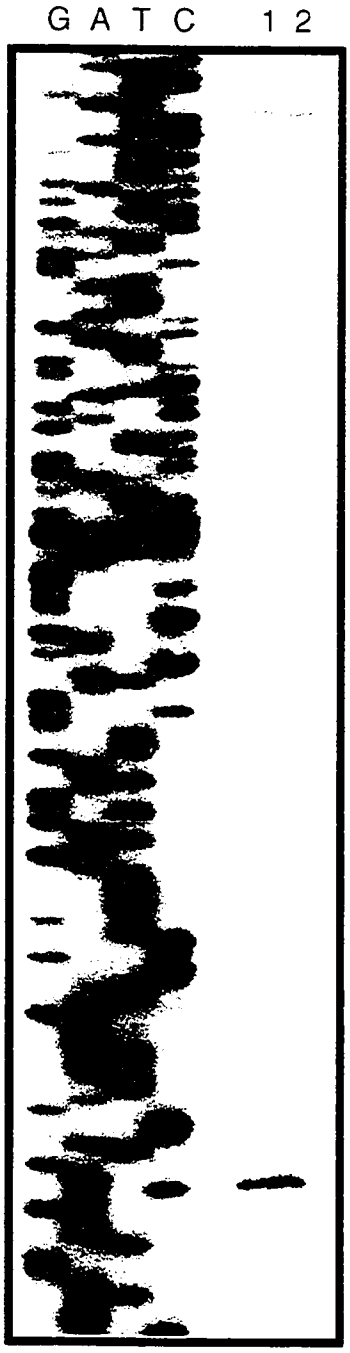
From these RT-PCR studies, it is evident that the genes of the gonococcal cell division cluster are not all co-transcribed, and that three transcriptional terminators exist within the cluster, with a fourth one at the end of the cluster.

3.5 TWO PROMOTERS REGULATE THE EXPRESSION OF THE *FTSEX* OPERON IN *N. GONORRHOEAE* CH811

Primer extension analysis was performed in order to identify promoters upstream of *ftsEX* in aerobically and anaerobically grown *N. gonorrhoeae*. Primer FB42 which anneals 21 nt downstream from the start of the *ftsE* gene was used to generate a reverse transcript of the upstream region of the *ftsE* gene, corresponding to the size between the transcription start

Figure 14. Primer extension analysis of *ftsE*. **A.** Lanes labelled G, A, T, C represent the sequencing reaction from pSB1900. Lane 1, Primer extension reaction using RNA isolated from aerobically grown *N. gonorrhoeae*; Lane 2, Primer extension reaction using RNA from anaerobically grown *N. gonorrhoeae*. **B.** The DNA sequence surrounding the promoters is indicated. Nucleotides in red indicate transcriptional start sites; blue text indicates the -10 consensus sequences, and green text indicates -35 consensus sequences. Bold text indicates the start site of the *ftsE* gene.

A



B

CAATGTCGGC GTACTGCCCT TTACCG**TCGT CG**AAGCCCCG AAATGCGGA**T**
GTTACAGCCG CATGACGGGA AATGGCAGCA GCTTCGGGGC TTTACGCCTA

P_E2

ACAGGCAGAC CATTA**CA**GGA GAGTTGAACG AGAAAAGCCT GACCGAAGCC
TGTCCGTCTG GTAATGTCCT CTCAACTTGC TCTTTTCGGA CTGGCTTCGG

GTCAAACTCG CCCATTCAAA ATGCCGTTAA ACGCCGGATG CCGTCCGAAA
CAGTTTGAGC GGGTAAGTTT TACGGCAATT TGCGGCCTAC GGCAGGCTTT

CCGCTTCAGA CGGCATTTTC CCGCCCGGCC TTCGGTATCC GCCAAACTTA
GGCGAAGTCT GCCGTAAAAG GGCGGGCCGG AAGCCATAGG CGGTTTGAAT

P_E1

TCCACTA**TCT AAA**AACAGGC GGAATCTT**TA TAAT**CGGTAC T**G**TCTTACCT
AGGTGATAGA TTTTTGTCCG CCTTAGAAAT ATTAGCCATG ACAGAATGGA

ATTGTTCAGA CGGCATATCC CTGCGGACGC AACCGCCCGA AACGATATGC
TAACAAGTCT GCCGTATAGG GACGCCTGCG TTGGCGGGCT TTGCTATACG

CGCCCTTCCT TACAGGACCT CCT**ATG**ATCC GTTTCGAACA AGTTTCCAAA
GCGGGAAGGA ATGTCCTGGA GGATACTAGG CAAAGCTTGT TCAAAGGTTT

ACCTATCCCG
TGGATAGGGC

site and the site of primer annealing. Two transcriptional start sites were identified and were observed to correspond to two different promoters (P_E1 and P_E2; Figure 14). The first of these promoters (P_E1) had a transcriptional start site 82 nt upstream of the start codon of *ftsE* in the intergenic region between *tlpA* and *ftsE*. A perfect -10 consensus sequence for a σ^{70} promoter (TATAAT) was identified 8 bp upstream of this start site, and a sequence (**TCTAAA**) sharing 50% identity (3/6 nt) with the consensus -35 sequence (TTGACA) was located 16 nt upstream of this putative -10 sequence. A second promoter (P_E2) was identified within the *tlpA* gene, 258 nt upstream of the *ftsE* start codon when RNA from aerobically grown cells was used as a template. However, when RNA from anaerobically grown cells was used, the transcription initiation site was only 257 nt upstream of the *ftsE* start codon. This one nucleotide difference was found consistently when different preparations of RNA were used. Just upstream of this start of transcription was the sequence **TACAGG** which shares 3 of 6 bp with a consensus -10 sequence, and at the -35 position wa the sequence **TCGTCG** which shares 3 of 6 nt with a consensus -35. No consensus sequences for other promoter types such as gearbox or σ^N were identified in the sequences preceding the two *ftsE* transcriptional start sites identified. From the consensus sequences determined, the promoters likely belong to the σ^{70} family of promoters. The intensity of the band representing the start site of transcription for P_E1 was greater than that for P_E2, indicating that the former may be a stronger promoter, however the decreased intensity of P_E2 may be due to pausing by the reverse transcriptase at the gonococcal uptake sequences that form the transcriptional terminator downstream of *tlpA*. This pausing at the site of the terminator is visible as a band in Lanes 1 and 2. Because it was shown that *ftsE* and *ftsX* are

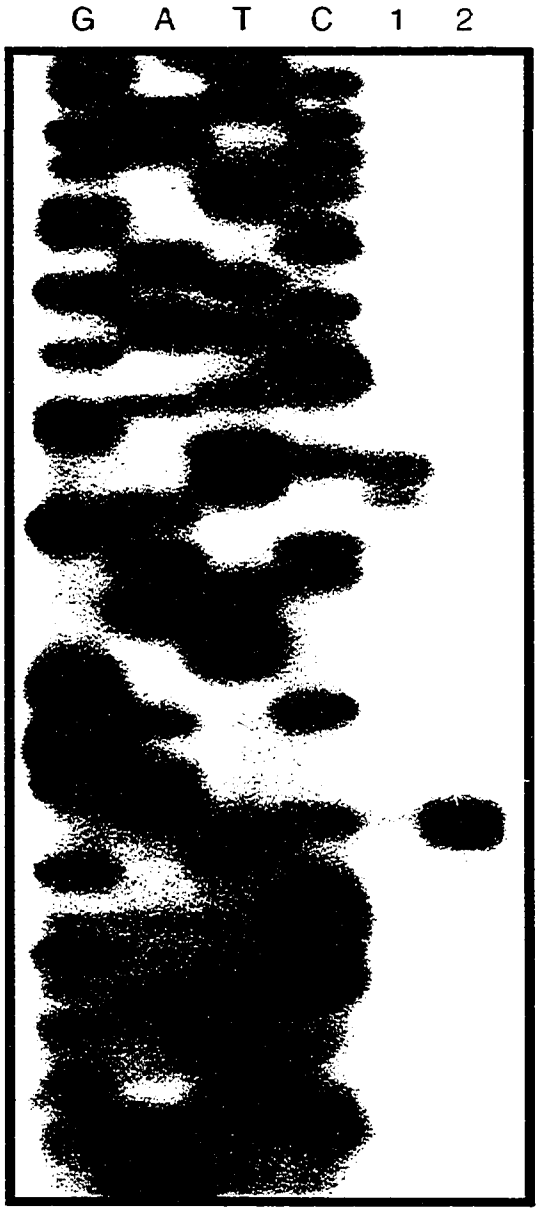
co-transcribed, the two promoters identified upstream of *ftsE* regulate transcription of both *ftsE* and *ftsX* genes.

3.6 MULTIPLE PROMOTERS REGULATE EXPRESSION OF *FTSQ*, *FTSA*, AND *FTSZ* IN *N. GONORRHOEAE* CH811

Primer extension was also used to identify the transcriptional start site of *ftsZ*. Primer FB35 which anneals 97 nt downstream from the start of the *ftsZ* gene was radiolabelled and used for primer extension as described above. Three transcriptional start sites were identified (Figure 15). The first of these promoters (P_Z1) caused transcription to occur from two start sites located 24 and 25 nt upstream of the *ftsZ* start codon, and these two bands were much more intense when RNA from anaerobically grown cells was used as a template as compared to RNA from aerobically grown cells, though both were present under both growth conditions. This difference in intensity was consistently seen when different preparations of RNA were used. A highly AT rich region of 28 nucleotides was present upstream of this site at the -10 position, which could account for the two primer extension products identified since the RNA polymerase might bind in more than one position. Further upstream at the -35 position, the sequence **TT**CTT**A** was found, which contains 3/6 nt of a consensus σ^{70} -35 promoter sequence. The second transcriptional start site initiated by promoter P_Z2 was located 44 and 47 nt upstream of the *ftsZ* start codon, and these two fragments were more intense from the aerobically isolated RNA, though it was also present under both growth conditions. When this experiment was repeated using different RNA preparations, there was

Figure 15. Primer extension reaction of *ftsZ*. **A.** The sequencing reaction using pAR2 as a template is shown in lanes labelled G, A, T, C. The primer extension reactions using aerobically and anaerobically isolated RNA are shown in lanes 1 and 2 respectively. **B.** The sequence surrounding the promoters is indicated. Red text marks the transcriptional start sites. Blue text denotes the -10 consensus sequences; green text denotes the -35 consensus sequence, and bold text indicates the start codon of *ftsZ*.

A



B

GAAAACAACC TCTGAACAGT TGGA**TTGCCG TTTGACA**GGT GAGAAG**TATT**
CTTTTGTTGG AGACTTGTCA ACCTAACGGC AAACTGTCCA CTCTTCATAA

P_a3 P_a2
TTGCCAGCAG TA**A**GATAC**TT CTTATATAAT** AAATAA**T**AA**T** T**TATTTA**AAC
AACGGTCGTC ATTCTATGAA GAATATATTA TTTATTATTA AATAAATTTG

P_a1
CGTCCTCT**GA** ACGGGGCGAT CAGGAGTTTT TGA**ATG**GAAT
GCAGGAGACT TGCCCCGCTA GTCCTCAAAA ACTTACCTTA

no difference between the RNA isolated from aerobically and anaerobically grown *N. gonorrhoeae*; both promoters were used equally. Again a highly AT rich region was present at the -10 region which contained a perfect -10 consensus sequence of **TATAAT**, and at the -35 position was the sequence **TTGCCA** which shares 5/6 nt with a consensus σ^{70} -35 promoter sequence. The third start site was much less prominent than the first and second, and was located 71 nt upstream of the *ftsZ* start codon. This band was equally intense when either aerobic or anaerobic RNA was used. At the -10 position was the sequence **TATTTT** and at approximately the -35 position was found the sequence **TTGACA** which is a perfect -35 consensus sequence for a σ^{70} promoter.

Primer extension was also used to identify the transcriptional start site of *ftsA* using primer FB40 which anneals 46 nt downstream of the *ftsA* start codon. Two putative transcriptional start sites were identified (Figure 16). The first of these (P_A1) was located 139 nt upstream of the *ftsA* start codon, and seemed to be used equally under both aerobic and anaerobic growth conditions. At the -10 position was found the σ^{70} sequence **TATAA**G, but no discernible -35 consensus sequence was identified. The second start site (promoter P_A2) was located 159 nt upstream of the *ftsA* start codon, and the bands also looked to be of equal intensity when RNA from cells grown aerobically and anaerobically were compared. At the -10 position was the sequence **TATCCT**, and upstream of that was the sequence **TTG**CGT, indicating that this is also a σ^{70} promoter.

Primer extension was also used to identify the transcriptional start site of *ftsQ* using primer FB41 which anneals 47 nt downstream of the start codon of *ftsQ*. The site identified was located 145 nt upstream of the *ftsQ* start codon and appeared to be used equally under

Figure 16. Primer extension reaction of *ftsA*. The sequence surrounding the promoters *ftsA* is indicated. Red text indicates the transcriptional start points; blue text denotes the -10 consensus sequences; green text indicates -35 consensus sequences, and bold text indicates the start codon of *ftsA*.

P_A2

CGTGGCAGCA TCTG**TTGCGT** AAGAATAAAA ATCGG**TTATC CT**ATGTG**G**AT
GCACCGTCGT AGACAACGCA TTCTTATTTT TAGCCAATAG GATACACCTA

P_A1

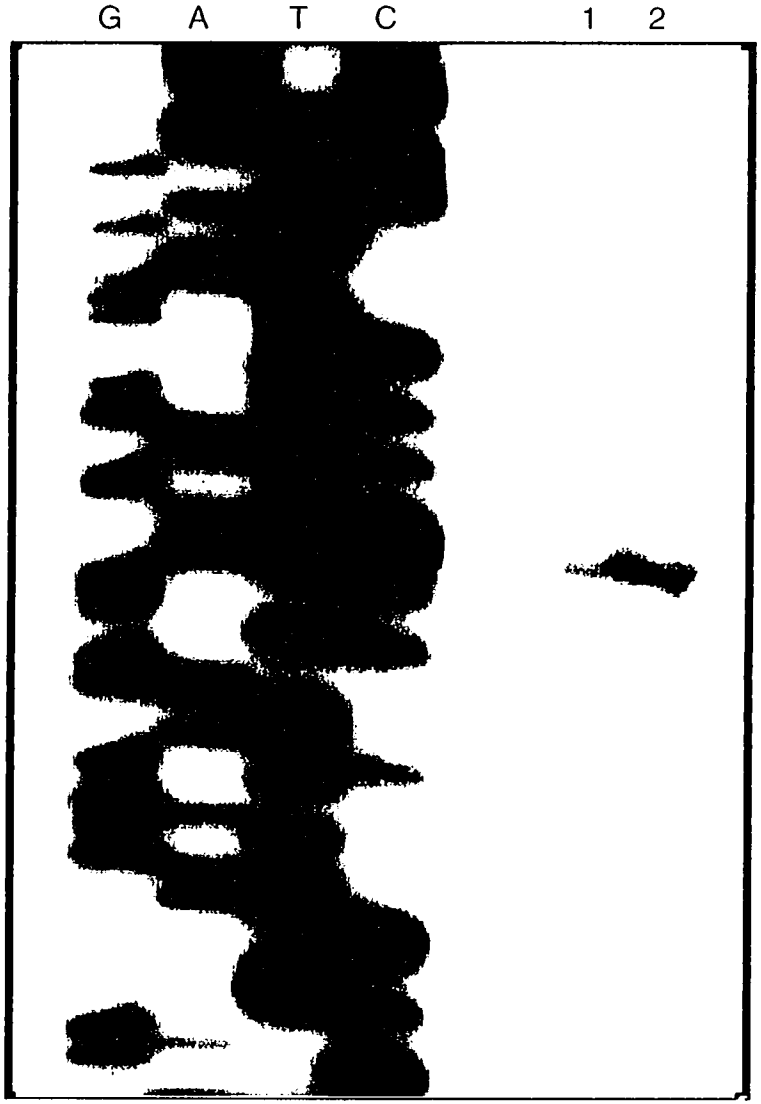
ATGAGG**TATA AG**GACGG**A**TT TTCAGTCCGC CATGCTCCCG ACGGTTTACC
TACTCCATAT TCCTGCCTAA AAGTCAGGCG GTACGAGGGC TGCCAAATGG

CGAAAAAGAA TCCGAAGAAT ATTGGGAACA GGTGTTGGAC ATATTACGGC
GCTTTTTCTT AGGCTTCTTA TAACCCTTGT CCACAACCTG TATAATGCCG

CTGGCGTCTG AAACGGTTCG ACGCAAATTT CAATCAGTTT TAAGGGCAGA
GACCGCAGAC TTTGCCAAGC TGCGTTTAAA GTTAGTCAAA ATTCCCGTCT

CGAACA**ATG**G AACAGCAGAA
GCTTGTTACC TTGTCGTCTT

Figure 17. Primer extension reaction of *ftsQ*. A. Lanes labelled G, A, T, C represent the sequencing reaction from pCV21. The primer extension reactions using RNA from cells grown aerobically and anaerobically are shown in lanes 1 and 2 respectively. **B.** The sequence surrounding the promoters is indicated. Red text indicates transcriptional start sites; blue text denotes a potential -10 consensus sequence; green text indicates a potential -35 consensus sequence, and bold text indicates the start codon of *ftsQ*.


G A T C
1 2

B

GCGTCGATTT CCTCAAAGAT A**CCGACG**GCA AACTCTATCT GTTGG**AAATC**
CGCAGCTAAA GGAGTTTCTA TGGCTGCCGT TTGAGATAGA CAACCTTTAG

P$_{2}$1
AACACCCTGC CC**G**GTATGAC CGGCCATAGT TTAGTACCGA AATCCGCTGC
TTGTGGGACG GGCCATACTG GCCGGTATCA AATCATGGCT TTAGGCGACG

CGTTACGGGC GTGGGTTTTG CCGATTTATG TATTGAAATT TTGAAGGCCG
GCAATGCCCG CACCCAAAAC GGCTAAATAC ATAACTTTAA AACTTCCGGC

CACATGTGGG ATAATGCCGA AGCGATGGAA CGGCTGACGC GCTGGCTGCT
GTGTACACCC TATTACGGCT TCGCTACCTT GCCGACTGCG CGACCGACGA

TGTCATG**ATG** GCGATGCTGC TTGCTGCGTC CGGGCTGGTT TGGTTTTACA
ACAGTACTAC CGCTACGACG AACGACGCAG GCCCGACCAA ACCAAAATGT

both aerobic and anaerobic growth conditions (Figure 17A). Very weak -10 and -35 consensus sequences were identified upstream of this transcriptional start point, and no consensus sequences for any other type of sigma factor were present (Figure 17B). Although the band representing this initiation site was quite prominent, 50 μg of RNA was required in order to see the band, therefore indicating that P_Q1 is not a strong promoter.

3.7 CLONING OF PROMOTER REGIONS IN pLES94 FOR STUDYING PROMOTER ACTIVITY UNDER DIFFERENT GROWTH CONDITIONS

In order to further elucidate the effects of different environmental conditions on the activities of promoters controlling transcription of cell division genes, experiments were begun in which promoter regions were cloned upstream of a promoterless *lacZ* gene in the vector pLES94 using a unique *Bam*HI restriction site (Silver and Clark, 1995). Three fragments were generated by PCR for cloning into pLES94 using primers that contained *Bam*HI restriction sites at their 5' ends. These were a 466 bp amplicon comprising the upstream region of *ftsE*, a 606 bp amplicon comprising the upstream region of *ftsZ*, and a 790 bp amplicon comprising the upstream region of *ftsQ*. All three fragments were cloned into pLES94. After initial quick screening of blue colonies by the cracking method, plasmids were purified using minipreps as described in Methods and were verified by PCR using one primer that annealed to the insert (Primer FB43, FB47, and FB45 for *ftsE*, *ftsQ*, and *ftsZ* respectively) and the second primer which annealed to the vector within the *lacZ* gene (FBlac) in order to confirm the presence and orientation of each of the inserts (Figure 18). Amplicons of the expected sizes of 903 bp, 1227 bp, and 1043 bp for *ftsE*, *ftsQ*, and *ftsZ*

respectively were obtained (Figure 18, Lanes 2, 3, and 4). Therefore three clones were confirmed: pLESE14 contains the *ftsE* promoter region, pLESQ6 contains the *ftsQ* promoter region, and pLES25 contains the *ftsZ* promoter region. Figure 19 illustrates the fragments that were cloned as well as the promoter locations within each of these fragments.

Figure 18. Confirmation of pLES94 clones containing the promoter region of *ftsE*, *ftsQ*, and *ftsZ*. Lane 1, 1 kb ladder; Lane 2, pLESE14; Lane 3, pLESQ6; Lane 4, pLESZ25

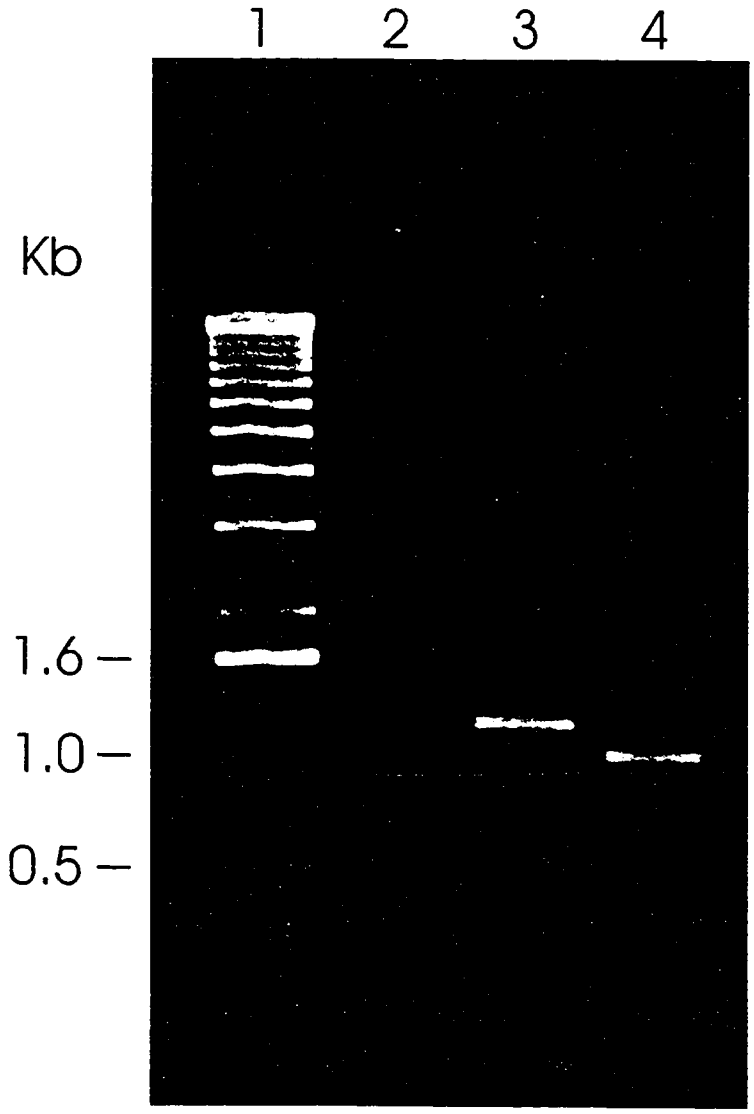
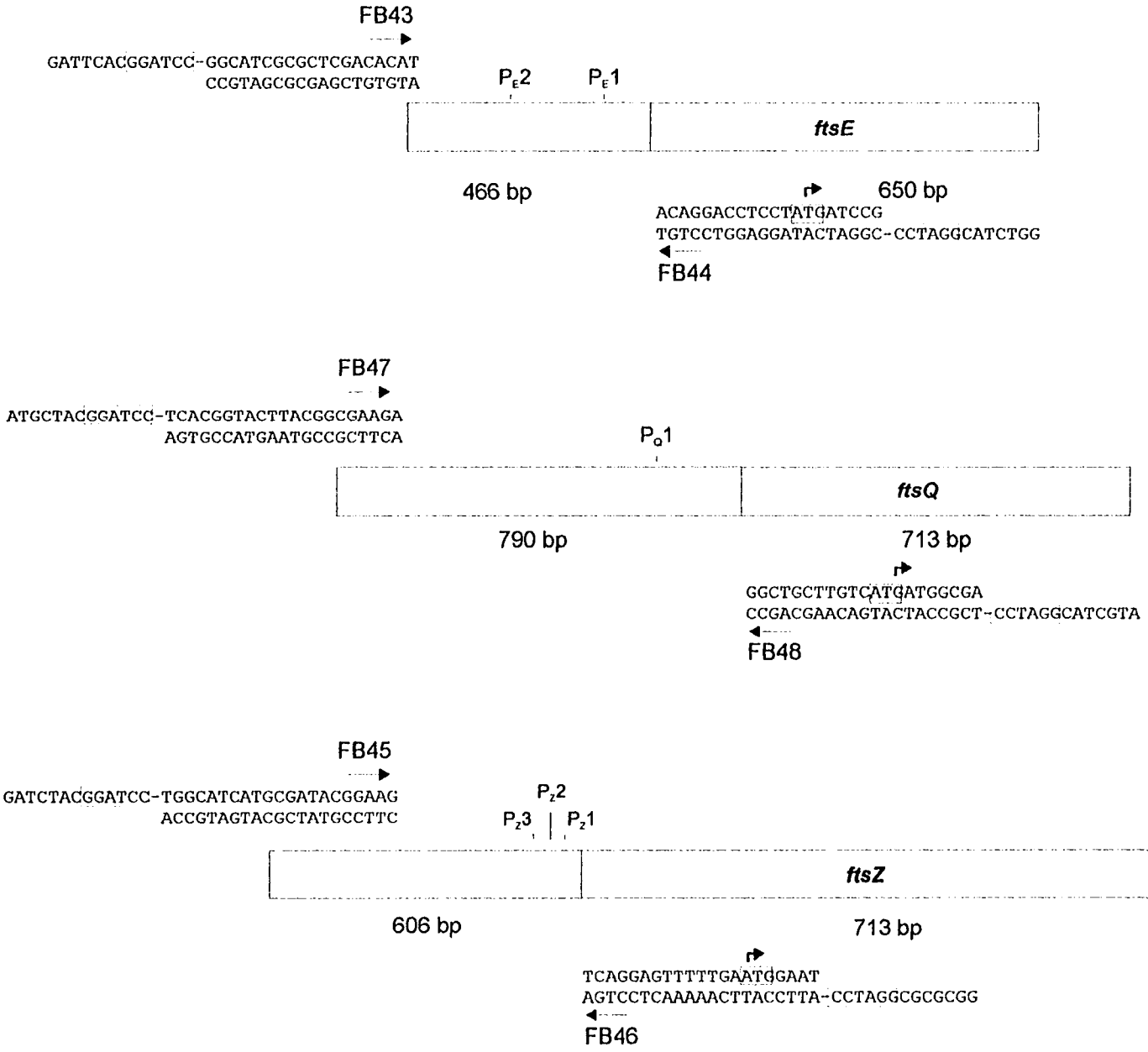

1
2
3
4
Kb
1.6
1.0
0.5

Figure 19. Promoters cloned into the pLES94 vector. The primers used to amplify the promoter regions upstream of *ftsE*, *ftsQ*, and *ftsZ* are shown. The blue boxes indicate the *Bam*HI sites within the primer sequences. The ATG start codons of the genes are indicated by red boxes. Locations of promoters determined by primer extension are indicated (P).


FB43
GATTCACGGATCC-GGCATCGCGCTCGACACAT
CCGTAGCGCGAGCTGTGTA
P_E2
P_E1
ftsE
466 bp
650 bp
ACAGGACCTCCTATGATCCG
TGTCCTGGAGGATACTAGGC-CCTAGGCATCTGG
FB44
FB47
ATGCTACGGATCC-TCACGGTACTTACGGCGAAGA
AGTGCCATGAATGCCGCTTCA
P_o1
ftsQ
790 bp
713 bp
GGCTGCTTGTCATGATGGCGA
CCGACGAACAGTACTACCGCT-CCTAGGCATCGTA
FB48
FB45
GATCTACGGATCC-TGGCATCATGCGATACGGAAG
ACCGTAGTACGCTATGCCTTC
P_z2
P_z3
P_z1
ftsZ
606 bp
713 bp
TCAGGAGTTTTTGAATGGAAT
AGTCCTCAAAAACTTACCTTA-CCTAGGCGCGCGG
FB46

4. DISCUSSION

This work represents the first studies of the genomic and transcriptional analysis of cell division genes in the Gram negative coccus *N. gonorrhoeae*.

A contiguous region of almost 18.7 kb of sequence surrounding the pivotal cell division gene *ftsZ* from *N. gonorrhoeae* FA1090 was assembled using the raw, unassembled data from the Gonococcal Genome Project. The gonococcal cluster contained 13 genes, most of which are involved in cell growth and division, and although the gene organization of this cluster was similar to that found in the Gram negative *E. coli* and *H. influenzae*, some novel features of gene organization, intergenic spacing, and gene expression were identified. While *E. coli* and *H. influenzae* contain all of the same homologues, *N. gonorrhoeae* is lacking the *envA* gene downstream of *ftsZ* at the 3' terminus of the cluster, although *envA* was found to be present elsewhere on the gonococcal chromosome by searching the genome project data base. In the place of *envA* is a gene of unknown function homologous to a hypothetical protein in *E. coli* that we have designated *hyp2*. Another hypothetical gene (*hyp1*) is located between *murE* and *murF* in *N. gonorrhoeae* whereas in *E. coli* and *H. influenzae* *murE* and *murF* are adjacent to each other. The presence of these two hypothetical genes within the cell division cluster suggests that they may play a role in cell growth or division in the gonococcus since *hyp2* is co-transcribed with *ftsZ*, and *hyp1* may be co-transcribed with *murE*, *murF*, and *mraY*. The gonococcal cluster also differs from that of *E. coli* as it has large intergenic regions ranging in size from 7 bp to 778 bp with no overlapping genes, while the *E. coli* genes are tightly organized with numerous overlaps. Putative transcriptional terminators were found within some of these intergenic spaces,

which indicates that the gonococcal cluster differs with respect to gene expression from the similar cluster in *E. coli*. These terminators were located downstream of *mraY*, *murD*, *murG*, and *hyp2* and their presence indicated that the regulation of this cluster consisted of several discrete transcriptional units. This type of regulation within the cluster is not present in the homologous *E. coli* cluster, as no transcriptional terminators were identified downstream of any of those genes.

The cell division clusters from the Gram negative bacteria *N. gonorrhoeae*, *E. coli*, and *H. influenzae* are highly conserved with respect to gene organization (Figure 20). The organization of the gonococcal cluster was: 5'-*murE-hyp1-murF-mraY-murD-ftsW-murG-murC-ddl-ftsQ-ftsA-ftsZ-hyp2*-3', while the similar clusters from *E. coli* and *H. influenzae* had identical gene organizations: 5'-*ftsL-ftsI-murE-murF-mraY-murD-ftsW-murG-murC-ddl-ftsQ-ftsA-ftsZ-envA*-3' (Lutkenhaus and Mukherjee, 1996; Fleischmann *et al.*, 1995). These latter two clusters differed from the gonococcal cluster because they were much more compact with overlapping genes. Similar cell division clusters were also identifed in three other Gram negative organisms: *H. pylori*, *P. aeruginosa*, and *C. crescentus*. *H. pylori* had only *ftsA* and *ftsZ* clustered together on the chromosome however (Tomb *et al.*, 1997), while *P. aeruginosa* had a cluster containing 5'-*ddl-ftsQ-ftsA-ftsZ-envA*-3' (Sanschagrin *et al.*, 1997), and *C. crescentus* had a cluster containing 5'-*orfB-divA*-(10 kb)-*divB*-(10 kb)-*ftsA-ftsZ*-3' (Ohta *et al.*, 1997). This region from *C. crescentus* corresponds in size to the similar cluster from *E. coli*, and therefore the unknown sequences within this *C. crescentus* cluster may well contain other cell division and biosynthesis gene homologues.

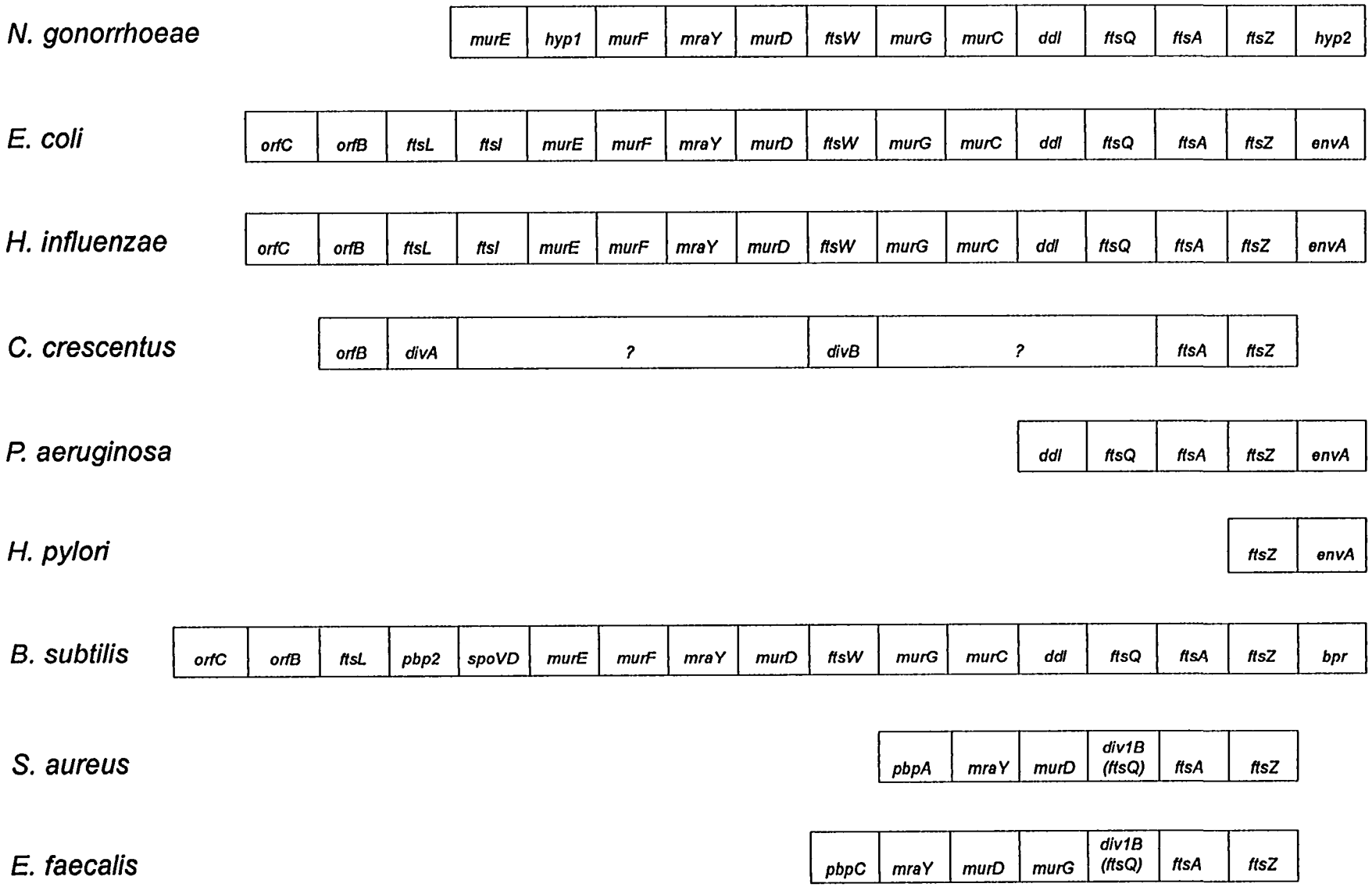
Similar clusters were also identified in numerous Gram positive organisms. In

Bacillus subtilis this cluster contained: 5'-*ftsL-pbp2-spoVD-murE-murF-mraY-murD-ftsW-murG-murC-ddl-ftsQ-ftsA-ftsZ*-3' (Biaudet *et al.*, 1996). Clusters are also present in Gram positive cocci such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* (Pucci *et al.*, 1997). The organization of the clusters from *S. aureus* and *E. faecalis* were: 5'- *pbpA-mraY-murD-divIB(ftsQ)-ftsA-ftsZ*-3', and 5'-*pbpC-mraY-murD-murG-divIB(ftsQ)-ftsA-ftsZ*-3' respectively (Pucci *et al.*, 1997). The Gram positive clusters contained larger intergenic spaces than were found in *E. coli*, with spaces greater than 100 bp not uncommon, however there are at present no reports of transcriptional terminators within these intergenic spaces. A summary of the organization of the above clusters is shown in Figure 20.

From this comparison of the cell division clusters from *N. gonorrhoeae* and various other Gram negative and Gram positive bacteria, the hypothesis that a cluster containing numerous cell growth and cell division genes is present in *N. gonorrhoeae* was confirmed. This cluster was very similar in terms of gene organization to the homologous cluster from *E. coli*.

ftsZ was also conserved in *Mycoplasma genitalium* and the *Archaea Methanococcus jannaschii* and *Haloferax volcanii* despite the fact that these organisms do not contain cell walls (Lutkenhaus and Addinall, 1997). From these genomic studies, it is evident that *ftsZ* is ubiquitous throughout the *Bacteria* and the *Archaea*, and in many cases this critical gene is clustered with other genes important in cell division. Clusters may be advantageous for maintaining control over regulation of cell division as multiple promoters and cumulative expression are likely important in the expression of these genes (Lutkenhaus and Mukherjee,

Figure 20. The genetic organization of the cell division clusters from various bacteria. The question marks in the *C. crescentus* cluster indicate that the genes have not yet been identified. This figure shows the genetic organization of the clusters, and therefore is not to scale.


N. gonorrhoeae
murE hyp1 murF mraY murD ftsW murG murC ddl ftsQ ftsA ftsZ hyp2
E. coli
orfC orfB ftsL ftsI murE murF mraY murD ftsW murG murC ddl ftsQ ftsA ftsZ envA
H. influenzae
orfC orfB ftsL ftsI murE murF mraY murD ftsW murG murC ddl ftsQ ftsA ftsZ envA
C. crescentus
orfB divA ? divB ? ftsA ftsZ
P. aeruginosa
ddl ftsQ ftsA ftsZ envA
H. pylori
ftsZ envA
B. subtilis
orfC orfB ftsL pbp2 spoVD murE murF mraY murD ftsW murG murC ddl ftsQ ftsA ftsZ bpr
S. aureus
pbpA mraY murD div1B (ftsQ) ftsA ftsZ
E. faecalis
pbpC mraY murD murG div1B (ftsQ) ftsA ftsZ

1996). Studies of co-transcription of the cell division clusters have been limited. The first nine genes from the *mra* operon of *E. coli* (*orfC* to *ftsW*) have been shown to be co-transcribed, and it is likely that this transcript proceeds to include *envA* since no transcriptional terminators are present. No other studies of co-transcription from any of the other clusters have been performed, and there are no reports of transcriptional terminators within these clusters from any other organisms. Therefore the presence of transcriptional terminators within the gonococcal cluster is a novel and interesting feature.

Traditionally the gonococcus has been studied aerobically *in vitro*, however this type of growth may not truly reflect the growth conditions of this organism which almost certainly has an anaerobic component *in vivo* (Clark *et al.*, 1988). In order to investigate whether the regulation of some of the cell division genes being studied in our laboratory is affected by aerobic and anaerobic growth, Northern hybridizations were performed for *ftsE*, *ftsX*, and *ftsZ*. These studies used RNA from both aerobically and anaerobically grown cells to elucidate the effects of anaerobic growth in the gonococcus. RNA from aerobically and anaerobically grown cells from two different strains of *N. gonorrhoeae* (CH811 and FA1090) was probed with Probe E, a 282 bp fragment comprising a portion of *ftsE*. These two gonococcal strains were selected because CH811 is the strain that we have used in our laboratory for all of our cloning and sequencing studies, while FA1090 is the strain used for the gonococcal genome project. A prominent band which corresponds closely to the expected size of an mRNA transcript containing both *ftsE* and *ftsX* hybridized to Probe E, suggesting that the hypothesis that these two genes are co-transcribed is true. The RNAs from anaerobically grown cells that hybridized to Probe E were slightly smaller and less

prominent than the RNAs from aerobically grown cells indicating differences in the expression of *ftsE* under the two different growth conditions. The differences suggest that a more proximal promoter was being used under anaerobic growth conditions, or that the stability of the mRNA transcript was decreased anaerobically.

Similar results were obtained when Northern blots were performed using an *ftsX*-specific probe (Probe X). Probe X, an 895 bp fragment containing a portion of *ftsX*, hybridized to a band from RNA isolated from aerobically grown cells which corresponded in size with the RNA that hybridized to the *ftsE* probe, and corresponded well to the size of *ftsE* and *ftsX* together. Therefore this was further evidence that *ftsE* and *ftsX* are co-transcribed. When aerobic RNA was hybridized to Probe X, the resulting band was not as sharp as that which hybridized to the *ftsE* probe. In addition, no fragment hybridized to the *ftsX* probe when anaerobic RNA was examined. I propose that the difference between the expression of *ftsE* and *ftsX* under aerobic and anaerobic conditions is due to more rapid turnover of the mRNA anaerobically. RNA degradation is an important mechanism of gene regulation, and it occurs by both endoribonucleolytic and exoribonucleolytic cleavage (Harwood, 1992). *E. coli* has two main endonucleases: RNase III and RNase E, and two main exonucleases: RNase II and polynucleotide phosphorylase, both of which degrade RNA at the 3' end. To date no 5' exonucleases have been identified in *E. coli* (Harwood, 1992). I believe that the *ftsEX* transcript is degraded more rapidly under anaerobic growth conditions and that this degradation occurs from the 3' end. This theory explains why the bands were less distinct when aerobic and anaerobic RNA were compared for each gene, and it also explains why the hybridization pattern was less distinct when *ftsX* was compared to *ftsE*

because *ftsX* is at the 3' end of the transcript. In addition, primer extension experiments indicated that the 5' ends of the transcripts maintained their integrity under both aerobic and anaerobic growth conditions since the amounts of the 5' termini detected were similar under both growth conditions.

Northern hybridizations indicated that multiple promoters are likely responsible for transcription of *ftsZ* in *N. gonorrhoeae*, as was shown in *E. coli* because the bands that hybridized to Probe Z were broad, which could be a result of multiple transcripts hybridizing to the probe. These promoters may be differentially regulated under aerobic and anaerobic growth conditions because the longer transcripts were not seen in Northern blots of RNA from anaerobically grown cells. A second possible explanation for this difference is that the stability of the longer transcripts in anaerobically grown cells may be decreased as predicted for *ftsEX*. The sizes of the bands correspond to transcripts containing *ftsZ* alone and *ftsAZ* together. There was no evidence of a transcript containing all three genes *ftsQAZ* together. Because Probe Z hybridized to the larger transcripts only when RNA from aerobically grown cells was used, there is a difference in the expression of *ftsZ* under aerobic and anaerobic growth conditions. The *ftsZ* transcripts however are clearly produced both aerobically and anaerobically.

In order to confirm that the mRNA turnover is greater under anaerobic conditions, experiments to determine RNA stability under the two different growth conditions would be interesting. These experiments involve measuring the chemical half-life of the mRNA of interest by growing the cells, adding rifampin to halt transcription, and isolating RNA at different time intervals. The degradation of the mRNA of interest could then be measured

by Northern hybridizations (Kushner, 1996). This technique is difficult however, with measurements varying substantially between and even within laboratories (Kushner, 1996). Measuring the functional half-lives of the mRNA might provide more accurate results. In these experiments, the length of time an mRNA remains intact and able to direct the synthesis of a protein is measured using either immunological or enzymatic assays (Kushner, 1996). However, because *N. gonorrheae* is difficult to grow in broth, these assays may be inaccurate because the growth phase of the cells could not be determined using solid media.

The results of the Northern hybridizations suggested that *ftsE* and *ftsX* are co-transcribed in *N. gonorrhoeae*, and that *ftsA* and *ftsZ* are co-transcribed. RT-PCR was used to confirm definitively that *ftsE* and *ftsX* are co-transcribed under both aerobic and anaerobic growth conditions. This result was not unexpected because the two genes overlap and because *in vitro* transcription/translation experiments showed that a FtsX protein was not synthesized without a vector-borne promoter (Bernatchez and Dillon, unpublished data).

A similar experiment using RT-PCR also showed that *ftsQ*, *ftsA*, and *ftsZ* are also co-transcribed under both aerobic and anaerobic growth conditions. The fact that Northern hybridizations did not detect a transcript containing all three of these genes may be because the promoter upstream of *ftsQ* is weak, as shown by primer extension, and therefore not a lot of the *ftsQAX* transcript is synthesized. Detection by RT-PCR is possible however due to the amplification step in the PCR reaction. Therefore even a small amount of transcript may be detected using this technique.

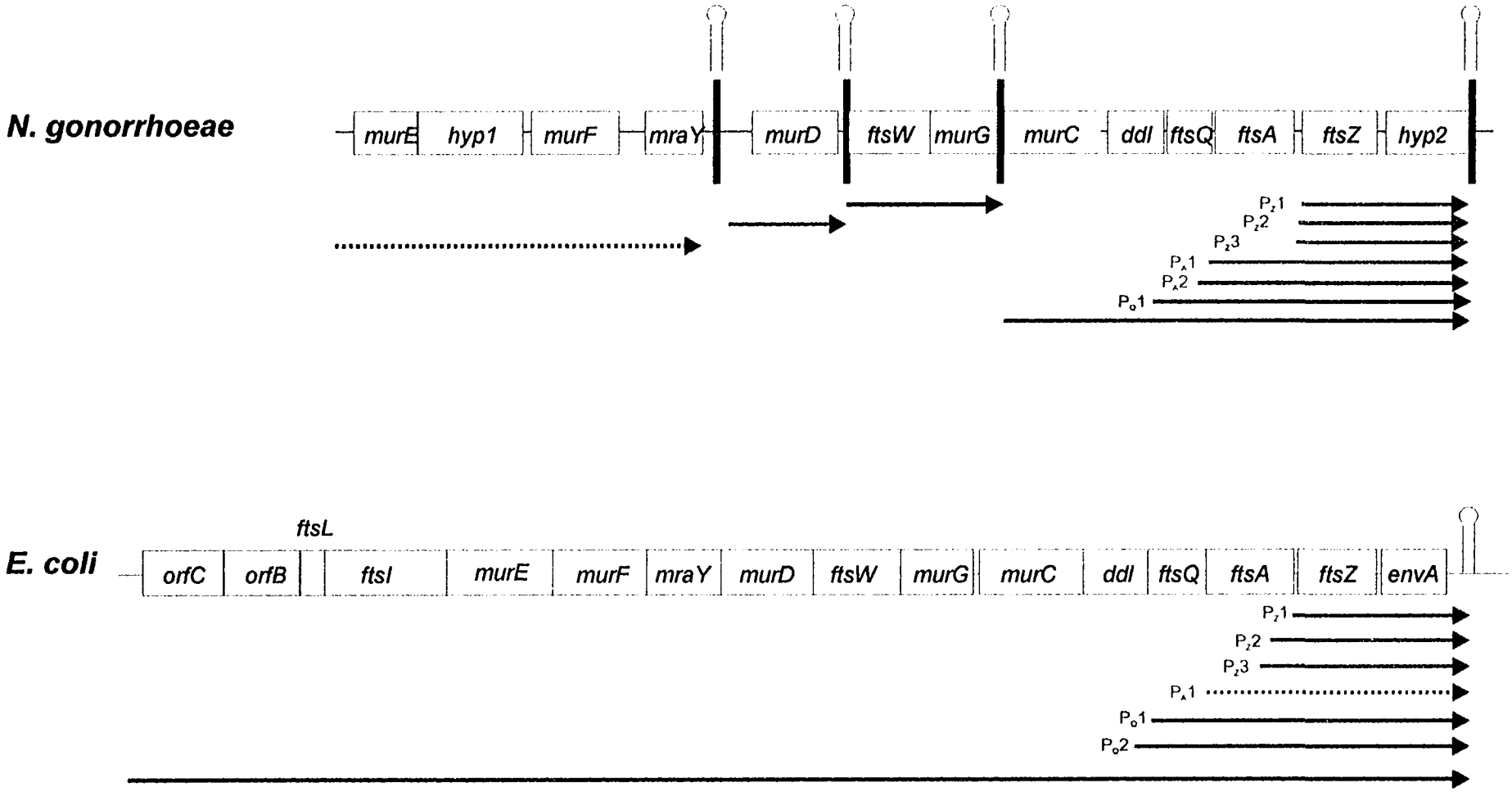
RT-PCR experiments were also performed in the cell growth and division cluster to determine which genes were co-transcribed, and to confirm that the putative transcriptional

terminators in that cluster were functional. The results of these studies showed that multiple transcripts and terminators existed as predicted by the sequence analysis, with genes separated by terminators being separately transcribed, and those not separated by terminators being co-transcribed. This regulation of the gonococcal *mur-fts* cluster is different than the proposed model for gene expression of the *E. coli* cluster. In *E. coli* it is believed that a single transcriptional terminator at the downstream end of the *E. coli mra* operon is an essential factor in the regulation of cell division and murein biosynthesis gene products (Beall and Lutkenhaus, 1987). In *E. coli* there are no terminators downstream of any of the genes of the cluster except for the final gene (*envA*). It is therefore believed that all these genes are co-transcribed, and that the more highly transcribed genes are located at the downstream end of the cluster and are therefore transcribed from the upstream promoters as well as from their own promoters, resulting in a cumulative effect on gene expression (Lutkenhaus and Mukherjee, 1996). This is supported by the fact that the ratios of FtsQ:FtsA:FtsZ must be strictly maintained at 25:150:5000-20,000 in *E. coli* in order for proper cell morphology to occur (Dewar *et al.*, 1992).

A summary of the different transcripts identified by RT-PCR for the *fts-mur* cluster is shown in Figure 21. The same figure also shows the transcripts identified by primer extension. It must be noted that RT-PCR does not identify all of the transcripts; smaller transcripts may also be present. In order to identify these smaller transcripts, primer extension would have to be performed for each of the genes of interest.

A possible explanation for the presence of transcriptional terminators in the middle of the gonococcal *mur-fts* cluster is due to the "plasticity" of the *N. gonorrhoeae* genome

Figure 21. Summary of the mRNA transcripts identified in *N. gonorrhoeae* and *E. coli.* Transcripts identified by primer extension are labelled with the promoter names and are in colour (red: *ftsZ*; blue: *ftsA*; green: *ftsQ*). Dotted lines indicated uncertainty of the location of the transcriptional start site.


N. gonorrhoeae
murE
hyp1
murF
mraY
murD
ftsW
murG
murC
ddl
ftsQ
ftsA
ftsZ
hyp2
Pz1
Pz2
Pz3
PA1
PA2
Po1
E. coli
ftsL
orfC
orfB
ftsI
murE
murF
mraY
murD
ftsW
murG
murC
ddl
ftsQ
ftsA
ftsZ
envA
Pz1
Pz2
Pz3
PA1
Po1
Po2

(Gibbs and Meyer, 1996). Because *N. gonorrhoeae* is naturally competent for DNA uptake (Sparling, 1966) and has a series of mechanisms for recombination and horizontal transfer (Biswas *et al.*, 1989), it may be adaptive for the organism to have fewer functional operons in favour of more discrete, independent transcriptional units. Lawson *et al.* (1995; and unpublished data) have shown that *carA* and *carB* genes of *N. gonorrhoeae* are separately transcribed unlike all other previously characterized prokaryotic *carA* and *carB* genes, and this different expression may also reflect the heterogeneity of the *N. gonorrhoeae* genome.

From the above RT-PCR data, the hypothesis that gonococcal cell division genes are regulated differently from those of *E. coli* was confirmed. In *E. coli*, all of the genes from the *mur-fts* gene cluster are thought to be co-transcribed because no transcriptional terminators are present within this cluster. The homologous gonococcal cluster contained four different terminators however, resulting in at least four separate operons.

The *min* cluster represents an ususual operon. Although *minC*, *minD*, and *minE* are co-transcribed as was expected, the fact that they are co-transcribed with unrelated upstream genes *rpoA* and *rplQ* was surprising. In *E. coli*, the genes of the *min* cluster are located at the 26 min region of the chromosome, while *rpoA*, *rplQ*, and *rpsD* are at the 72 min region. Genes of the *min* cluster are involved in the localization of septum formation, while *rpoA*, *rplQ* and *rpsD* are involved transcription and translation. *rpoA* encodes the α subunit of RNA polymerase, and only one copy of this gene exists on the chromosomes of both *E. coli* and *N. gonorrhoeae*. *rplQ* and *rpsD* encode ribosomal proteins L17 which is found with the large ribosomal subunit (50S), and S4 which is found with the small (30S) subunit, respectively (Record *et al.*, 1996). These two genes are also present as one copy per

chromosome. In *E. coli*, the α operon consists of 4 ribosomal proteins as well as the α subunit of RNA polymerase. The translation of the 4 ribosomal proteins in *E. coli* is regulated by S4, the product of *rpsD* (Record *et al.*, 1996). The explanation of why these two sets of seemingly unrelated genes are co-transcribed remains unclear. The *min* genes may also be regulated by S4, or perhaps their co-transcription in *N. gonorrhoeae* is simply a result of a recombination event that created this unlikely operon.

Primer extension experiments were used to identify promoters responsible for the expression of cell division genes *ftsE*, *ftsZ*, *ftsQ*, and *ftsA*. We had noted differences in the expression of *ftsE* and *ftsZ* under aerobic and anaerobic growth conditions, and believed these differences were due to a difference in the stability of the mRNA transcripts, but primer extension experiments were required to ensure that the differences were not due to alternate promoter usage. Two promoters that regulate the expression of *ftsEX* in *N. gonorrhoeae* were identified. One of these promoters is located in the intergenic region between *ftsE* and *tlpA* (P_E1), while the second is further upstream within the *tlpA* gene (P_E2). Just upstream of P_E1, a perfect -10 consensus sequence was identified, however the -35 region had only 3/6 bp identical to a consensus -35 sequence. P_E2 did not have upstream regions very similar to any promoter consensus sequences. The -10 region had only 3/6 bp identical to a consensus -10 sequence, and no discernible -35 sequence was identified. No other consensus sequences for any other promoter types were identified within the region upstream of P_E2. This lack of consensus sequences indicates that P_E2 may be a weaker promoter than P_E1, which is supported by the fact that the band representing P_E2 in the primer extension reaction is less intense than that representing P_E1. It must also be noted however that this reduced intensity

may be due in part to the fact that the reverse transcriptase paused at the region of the Neisserial uptake sequences which form the transcriptional terminator downstream of *tlpA*. Reverse transcriptase is known to pause at sites of secondary structure. A faint band is visible in the primer extension reaction corresponding to that section of high secondary structure of the RNA strand.

In *E. coli*, two promoters allow expression of genes of the *ftsYEX* operon (Gill and Salmond, 1990). The first of these promoters (P_Y) was identified upstream of *ftsY*, while the second promoter (P_E) was found upstream of *ftsE* within the *ftsY* gene (Gill and Salmond, 1990). No promoter was identified directly upstream of *ftsX* in this organism (Gill and Salmond, 1990). A promoter was found directly upstream of *ftsX* within the *ftsE* gene in *M. tuberculosis* however, even though the organization of *ftsEX* was similar to that of *E. coli*.

In general, the closer a promoter is to the consensus sequence, the stronger is the promoter (Busby and Ebright, 1994); however this is an oversimplification of the effects of promoter sequence on promoter strength. Additional regions upstream (the upstream element, or UP) and downstream (the downstream regions, or DSR) of the promoter also affect promoter strength, as do DNA binding proteins such as activators and repressors (Knaus and Bujard, 1990). Some σ^{70} promoters such as *gal* P1 from *E. coli* have no obvious -35 sequences, however the sequence directly upstream of the -10 sequence is important for the activity of this promoter (Record *et al.*, 1996). In *N. gonorrhoeae*, the P1 promoter upstream of *pilE* described in the introduction is very efficient, and there is no evidence of a -35 consensus sequence for that promoter (Fyfe *et al.*, 1995). There are likely other unknown elements involved in the strength of gonococcal promoters.

The transcriptional start site of P_E2 varied by one nucleotide under aerobic and anaerobic growth conditions, with the anaerobic start site being more proximal to the start codon of *ftsE*. This unusual difference could possibly be due to the presence of a DNA binding protein under one of these growth conditions resulting in an altered position of RNA polymerase binding, or perhaps by a difference in the secondary structure of the DNA at the promoter site, which could also result in an altered binding of the polymerase.

From the promoter consensus sequences identified upstream of *ftsE*, *ftsQ*, *ftsA*, and *ftsZ*, all of the promoters involved in controlling expression of these cell division genes are likely to be from σ^{70} or σ^{70}-like promoters. There are at least 6 different sigma factors in *E. coli* and each of these allows the RNA polymerase to initiate transcription at a different promoter consensus sequence. The majority of genes involved in most cell processes such as metabolism, biosynthesis and other functions during exponential growth are regulated by σ^{70} promoters. The other sigma factors are used to regulate more specialized sets of genes, for example genes involved in the heat shock response, nitrogen assimilation, flagellar formation, and stationary phase growth (Record *et al.*, 1996). In *E. coli*, one of the promoters upstream of *ftsQ* was a gearbox promoter which is induced upon entry into stationary phase, however there was no evidence that the single promoter upstream of *ftsQ* in *N. gonorrhoeae* was a gearbox promoter, and it is likely to be a σ^{70} promoter.

It is possible that one or more of the transcriptional start sites identified in this work are actually RNase E cleavage sites as was the case for the most proximal site upstream of *ftsZ* in *E. coli*. RNase E plays an important role in mRNA decay, and therefore in gene regulation. The site of RNase E cleavage has been controversial. Recently it was suggested

that the primary structure of the RNA is not very important except that the enzyme prefers to cut 5' to an AU sequence; therefore to determine whether any of the promoters that I have identified are in fact RNase E cleavage sites would have to be determined experimentally *in vitro* using RNase E mutants. Primer extension could be repeated using these mutants and if the same sites were identified then they would likely be true transcriptional start sites.

From the Northern blot and primer extension experiments, I propose that the stability of the mRNA of *ftsE* is decreased under anaerobic growth, and that the transcript is affected at the 3' end because primer extension showed that the integrity of the 5' end was similar under both aerobic and anaerobic conditions.

The promoters upstream of the *ftsQAZ* genes were also identified using primer extension. These genes were highly regulated with the same promoters used under both aerobic and anaerobic conditions although the degree of usage varied. Six promoters were identified; three directly upstream of *ftsZ*, two upstream of *ftsA*, and one weaker promoter upstream of *ftsQ*. This regulation is similar to that of *E. coli*, in which the numerous promoters are believed to be important for the strict regulation of these cell division genes, which is critical for normal cell division to occur (Lutkenhaus and Mukherjee, 1996). Differences were observed in the utilization of two of the three promoters directly upstream of *ftsZ* under aerobic and anaerobic growth conditions. The most proximal promoter (P_Z1) was preferentially used under anaerobic conditions, though a faint band indicated that it was also used under aerobic conditions. The next promoter (P_Z2) was preferentially used aerobically in one experiment, but when this was repeated, expression appeared to be similar under the two growth conditions. The third promoter (P_Z3) was not as strong a promoter as

the first two, indicated by a weaker band on the sequencing gel, and its use appeared to be similar under aerobic and anaerobic conditions. The differential promoter usage, particularly by P_Z1 could be a result of DNA binding proteins or of differential bending of the DNA under the different growth conditions, both of which could result in alternate promoter usage. *ftsZ* transcripts may be produced in greater numbers anaerobically since P_Z1 is more highly utilized under anaerobic growth, however this increased production may be counter-acted by increased turnover of this RNA as indicated by the Northern hybridizations. Quantifying the amount of FtsZ in the gonococcus throughout the cell cycle would determine if the differences in transcription of *ftsZ* result in different amounts of protein in the cell. In addition, studies quantifying the growth rate of *N. gonorrhoeae* under aerobic and anaerobic conditions would be useful to understand how cell division is affected. Comparing the sizes and morphologies of cells could also indicate the amount of FtsZ within the cell since studies in *E. coli* showed that increased amounts of this protein results in smaller cells, and reduced amounts results in longer cells or even filaments (Lutkenhaus and Mukherjee, 1996).

The two promoters identified upstream of *ftsA* both seemed to be used equally under aerobic and anaerobic growth conditions as indicated by similar intensities of the bands in the primer extension gels. A promoter upstream of *ftsA* was not identified in *E. coli* when S1 nuclease assays were used, although later studies using *lacZ* fusions showed that at least one promoter does exist usptream of *ftsA* (Flardh et al., 1997). Therefore the S1 nuclease assay was not sensitive enough to detect this promoter, or conditions were not optimal to detect it. Similarly the single promoter upstream of *ftsQ* appeared to be utilized equally under the two growth conditions. This promoter is likely to be a very weak promoter, since

50 μg of RNA was required in order to identify the transcriptional start site using primer extension. The other reactions for *ftsE*, *ftsZ*, and *ftsA* required only 10 to 20 μg. There was a lack of a strong promoter consensus sequence upstream of the start site of transcription from *ftsQ* which further indicates that this promoter is relatively weak. This organization can be related to the amounts of each protein produced in the cell. In *E. coli*, FtsZ is the most abundant protein, with 5000-20,000 molecules per cell; the level of FtsA is approximately 150 molecules per cell, while FtsQ is present in only approximately 25 copies per cell (Donachie, 1993). These amounts are likely similar in *N. gonorrhoeae*, though these have not been quantified. Therefore the most highly produced protein, FtsZ has at least six promoters regulating its expression; FtsA which is the second highest has three promoters, and FtsQ which is not abundant in the cell has one weak promoter.

The Northern blot and primer extension experiments confirmed the hypothesis that cell division genes in *N. gonorrhoeae* are regulated differently under aerobic and anaerobic growth conditions. These differences in the regulation of cell division genes under the two different growth conditions were somewhat surprising. We know that *N. gonorrhoeae* must grow and undergo cell division in both cases, so we would not necessarily expect differences in the regulation of cell division genes under these two environmental conditions. Few studies on the kinetics of growth of *N. gonorrhoeae* under anaerobic conditions exist. Knapp and Clark in 1984 found that the growth curves for gonococci grown aerobically and anaerobically were very similar as described in the introduction of this work, though more comprehensive studies still need to be done. Differences in growth may exist, especially if anaerobiosis results in differences in colonization or pathogenesis *in vivo*. The cell sizes

may be smaller anaerobically which would likely be a result of increased expression of *ftsZ*. Examining the cells under a light microscope did not reveal size differences, however a more powerful microscope might show a difference. Regulation of gene expression is important for optimizing growth and survival of organisms, therefore minor differences may result in more optimal efficiency of growth.

Fragments containing the promoters of the cell division genes *ftsQ*, *ftsZ*, and *ftsE* which have been cloned into pLES94 may provide further information regarding the transcriptional regulation under both aerobic and anaerobic conditions, as well as under other environmental conditions. β-galactosidase assays could be used to monitor the expression of these cell division genes under different conditions. At present, each of the three clones contains all of the promoters upstream of each gene, however new clones could be generated containing subsets of the promoters which would give additional information with respect to the importance of each individual promoter.

Studies of bacterial cell division are important because it is such a fundamental process which is critical for bacterial propagation and survival. In addition, proteins involved in cell division may provide targets for the development of novel antibacterial agents. If the cell division process could be arrested by a compound that interferes with the activity of FtsZ or another critical cell division gene, this chemical could be a candidate for the development of newer and more effective drugs (Desnottes, 1996).

In conclusion, this work is the first study of the regulation of cell division genes in a Gram negative coccus. In addition, it is the first study comparing the regulation of genes which are not directly involved in metabolic processes under aerobic and anaerobic growth

conditions. A large cluster of cell growth and division genes was present in *N. gonorrhoeae* FA1090, and this cluster contained multiple transcripts and terminators indicating that the regulation of this cluster was different from the homologous cluster in *E. coli* in which all of the genes are believed to be co-transcribed. Another smaller cluster which contained the *minC*, *minD*, and *minE* genes was also present on the gonococcal chromosome, and these genes were found to be co-transcribed with unrelated upstream genes involved in DNA transcription and translation. *ftsE* and *ftsX* were also shown to be co-transcribed as predicted by sequence analysis and *in vitro* transcription/translation experiments. Northern hybridizations coupled with primer extension experiments suggested that the stability of the mRNA of *ftsE*, *ftsX*, and *ftsZ* is decreased under anaerobic growth conditions, and that this degradation is likely occurring from the 3' end of the transcripts. Primer extension studies showed that the cell division genes are highly regulated by multiple promoters which indicates that the cell division process must be tightly controlled for normal division to occur.

From the above work, three hypotheses were confirmed. First, it was proposed that a cluster of cell growth and division genes similer to the cluster found at the 2 min region of the *E. coli* chromosome was present on the *N. gonorrhoeae* chromosome. This was indeed the case as was shown using the genome project database from *N. gonorrhoeae* CH811. Secondly, it was hypothesized that the regulation of the gonococcal cell division genes was different from *E. coli* in which the genes of the cluster are thought to be co-transcribed. RT-PCR confirmed that at least four separate operons are present within the *mur-fts* cluster from *N. gonorrhoeae*. Lastly, we hypothesized that the regulation of gonococcal cell division

genes differed under aerobic and anaerobic growth conditions. Northern hybridizations showed a difference in the mRNA stability under aerobic and anaerobic conditions, and primer extension showed that promoter usage differed under these two growth conditions. Therefore, gonococcal cell division genes are regulated differently under aerobic and anaerobic conditions.

Numerous questions remain to be answered. A global study comparing the stability of gonococcal mRNA under aerobic and anaerobic conditions would be interesting to determine if this decreased stability is a general effect on gene expression in the gonococcus. The mechanisms of this increased RNA turnover could also be determined. Additional studies of regulation would also be of interest in order to determine if activators, repressors, antisense RNA, and RNase E cleavage also play a role in the expression of cell division genes in *N. gonorrhoeae*.

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COLLABORATIONS

All of the experimental manipulations described in this thesis were performed by Finola Francis with the exception of the assembly of the gene clusters using the Gonococcal Genome project, and the RT-PCR studies of the *mur-fts* and the *min* clusters. These experiments were performed by Charles Victor, who was an Honours student working under the direct supervision of Finola Francis.

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EDUCATION

1995 - 1998: M.Sc. (Biochemistry, Microbiology and Immunology), University of Ottawa.
Thesis title: Transcriptional analysis of cell division genes in *Neisseria gonorrhoeae*
Thesis supervisor: Dr. J. R. Dillon

1990 - 1994: B.Sc. (Biology) with Biotechnology option (Magna Cum Laude) University of Ottawa
Honours project: The intervening sequence between *carA* and *carB* in *Neisseria gonorrhoeae* and other *Neisseria* species is highly variable
Thesis supervisor: Dr. J. R. Dillon

June, 1990 French certificate (Earl of March Secondary School)

WORK EXPERIENCE

1994 - 1995
Laboratory technician
J. R. Dillon, Professor and Chair, University of Ottawa

- Performed molecular studies of arginine biosynthesis genes from the bacterium *Neisseria gonorrhoeae*.
- Typed, tested and analyzed the antimicrobial susceptibilities of bacterial isolates from North and South America as a part of an ongoing World Health Organization project.
- Managed the daily running of the laboratory including ordering all materials and reagents.
- Supervised and trained students including a high school co-operative education student, a technician trainee, and a university summer student.

Summer 1992 and 1993 (Full time); Fall/Winter 1992 - 1993 (Part-time)
Research technician
i-STAT Canada Limited

- Assisted in the development of prototype electrodes for blood analysis products in the research and development department.
- Operated manual test cell and analyzer for evaluating prototype devices. Learned specialized software.

RELATED WORK EXPERIENCE

October of 1995, 1996, 1997
Laboratory demonstrator
University of Ottawa, Faculty of Medicine

- Taught microbiological laboratory concepts and skills to first year medical students.
- Contributed to the set-up and dismantling of the equipment and experiments.

OTHER SKILLS

- ***MICROBIOLOGY***: culture, isolation and identification of pathogenic bacteria, preparation of culture media
- ***MOLECULAR BIOLOGY***: DNA and RNA isolation and purification, Polymerase Chain Reaction, DNA and RNA gel electrophoresis, DNA sequencing, Northern and Southern hybridizations, primer extension
- ***COMPUTER***: Windows 95, Word Perfect, Word, Power Point, Corel Draw, Netscape Navigator, Specialized software for molecular biology (PCGene and Primer Designer)

PEER-REVIEWED PUBLICATIONS

Lawson, F. S., **F. M. Billowes**, and J. R. Dillon. 1995. Organization of carbamoyl-phosphate synthase genes in *Neisseria gonorrhoeae* includes a large, variable intergenic sequence which is also present in other *Neisseria* species. Microbiology. **141**:1183-1191.

IN PREPARATION

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F. M. Francis and J. R. Dillon. 1998. Cell division genes in *Neisseria gonorrhoeae* have different transcriptional and environmental controls. Graduate students Poster presentation, Department of Biochemistry, Microbiology and Immunology.
Awarded First Prize in M.Sc. category.