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MINIREVIEW

Fur-Mediated Global Regulatory Circuits in Pathogenic *Neisseria* Species

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The ferric uptake regulator (Fur) protein has been shown to function as a repressor of transcription in a number of diverse microorganisms. However, recent studies have established that Fur can function at a global level as both an activator and a repressor of transcription through both direct and indirect mechanisms. Fur-mediated indirect activation occurs via the repression of additional repressor proteins, or small regulatory RNAs, thereby activating transcription of a previously silent gene. Fur mediates direct activation through binding of Fur to the promoter regions of genes. Whereas the repressive mechanism of Fur has been thoroughly investigated, emerging studies on direct and indirect Fur-mediated activation mechanisms have revealed novel global regulatory circuits.

Iron homeostasis is a highly regulated process in bacteria, as iron both is an essential nutrient and when in excess, can lead to toxicity via the production of hydroxyl or peroxide radicals. To maintain iron homeostasis, many bacteria utilize iron-binding transcriptional regulators, which, upon binding to free iron, are triggered to regulate transcription of genes involved in maintaining intracellular iron levels. Iron-binding proteins are members of a large class of metal ion-binding transcriptional regulators, includin MntR, DtxR, and Zur (58). These regulators act as environmental sensors of essential metals, including iron, and modulate gene expression accordingly. The master regulator of iron homeostasis is an iron-binding transcription factor termed the Ferric Uptake Regulator (Fur).

The first indication that bacterial iron homeostasis relied on a single central regulator was published by Ernst et al. in 1978 (31). Those studies showed that a *Salmonella enterica* serovar Typhimurium mutant lacked iron-responsive regulation of many genes, including those involved in iron-enterochelin and ferrochrome uptake. That mutant was termed an iron (Fe) uptake regulation (*fur*) mutant (31). A similar mutant was soon isolated in *Escherichia coli*, and the *E. coli fur* gene was subsequently cloned and sequenced (45, 84). Orthologues of *fur* have since been identified in numerous Gram-negative and Gram-positive species, and it has been shown that Fur proteins share a high degree of sequence homology between species (Fig. 1). In most organisms, Fur is present as a 15-to-17-kDa protein that forms dimers in the presence of iron (II) or other divalent cations (6, 24, 74, 91). As revealed by analysis of several crystal structures of the Fur protein from various pathogens, the amino terminus of Fur has been shown to bind to DNA whereas the carboxyl terminus is involved in dimer formation (26, 81, 91). Analysis of the crystal structure of Fur has also identified multiple metal-binding sites. These metal-binding sites contain a conserved histidine-histidine-aspartic acid-histidine (HHDH) motif (a specific region shown to be involved in cofactor binding), and mutagenesis studies have shown these four amino acids are crucial for Fur function (59, 83). As a repressor, the iron-bound Fur dimer binds to the −10 and −35 promoter regions to exclude binding of RNA polymerase, which results in the inhibition of transcriptional initiation (32) (Fig. 2A). The DNA sequence recognized by repressive Fur (designated a Fur box) was initially defined as a conserved 19-bp sequence, GATAA TGATAATCATTATC, in *E. coli* (24). Fur boxes determined in other Gram-positive or Gram-negative bacteria are similar to this consensus sequence (7, 24, 27, 37, 44, 69, 78, 80, 96, 98, 104, 105) (Table 1). Subsequently, the Fur box was interpreted as 9-1-9 inverted repeats (GATAATGAT-A-ATCATTATC), or a hexameric repetition of nATwAT (24, 33). The Fur boxes in *Bacillus subtilis* and *Helicobacter pylori* are represented by shorter inverted repeats of 7-1-7 (TGATAATnATTATCA and TAATAATnATTATTA, respectively) (37, 80). Based on these studies and the crystal structure of Fur, it is predicted that two Fur dimers simultaneously bind one Fur box and the two Fur dimers may occupy three to four hexameric repeats (81). This consensus sequence has been used to successfully predict novel Fur-regulated genes *in silico* (27, 44, 53).

Fur FUNCTIONS AS A GLOBAL REGULATORY PROTEIN

Recently, global analyses of iron- and/or Fur-responsive transcriptomes of diverse bacterial pathogens, such as *H. pylori*, *Pseudomonas syringae*, *Vibrio cholerae*, *Yersinia pestis*, *Haemophilus influenzae*, *S. enterica* serovar Typhimurium, and *Listeria monocytogenes*, have revealed a number of novel regulatory roles for Fur (12, 20, 40, 56, 68, 94, 101, 102, 107). First, Fur has been demonstrated to repress transcription even in the absence of iron, a process termed apo-Fur regulation (13) (Fig. 2B). apo-Fur repression has been primarily characterized in *H. pylori* and has not yet been well described in other bacteria (13, 72). Although iron may not be important for apo-Fur function, it is possible that other metal ions play a role in apo-Fur-mediated transcriptional control (24, 74, 91). Second, in addition to its role as a repressor, Fur can also function as an activator in both the iron-bound form and apo form. In *V. vulnificus*, apo-Fur-mediated activation was shown to positively regulate the *fur* gene itself (57) (Fig. 2C). Transcriptome analysis has also identified additional genes that are activated by iron-bound Fur (12, 56, 68, 101, 102, 107).

Theoretically, transcriptional activation through Fur can be fulfilled by several pathways, including both direct (Fig. 2D) and indirect (Fig. 2E) mechanisms. A few examples of Fur-mediated

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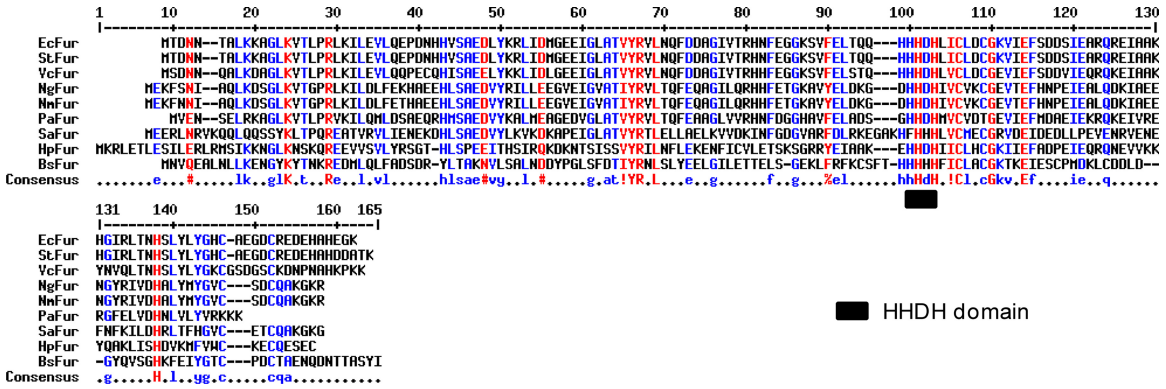

FIG 1 Alignment of Fur orthologues from both Gram-negative and Gram-positive bacteria.

indirect activation have been reported. Fur may activate transcription indirectly via repression of a small RNA (sRNA), such as RyhB of *E. coli* (5, 62, 99). As a result, the repressed targets of RyhB, consisting of *sdhCDAB*, *acnA*, *fumA*, and *sodB*, are activated when Fur is present (61–64). Fur-repressed sRNAs and their target genes have been identified in the Fur regulons of other bacteria, such as RyhB of *V. cholerae* (21), PrrF1 and PrrF2 of *P. aeruginosa* (103), and FsrA of *B. subtilis* (38), as well as NrrF in *Neisseria meningitidis* (65, 66). In addition to sRNAs, Fur may repress a proteinaceous repressor to activate downstream genes. For example, transcriptional activation of *hilA* in *S. enterica* serovar Typhimurium was demonstrated to result from the direct repression of a negative regulator of *hilA*, H-NS (histone-like nucleotide binding protein), by Fur (95). Fur has also been shown to directly bind to the promoter regions of Fur-activated genes. In *E. coli*, Fur and H-NS compete for overlapping binding sites within the promoter regions of *ftnA*, resulting in derepression of *ftnA* transcription (77). The transcription of the *V. cholerae* porin *ompT* gene was positively regulated by iron-bound Fur through the direct binding of Fur to the promoter region (17). However, in contrast to the wealth of studies describing Fur-mediated repression and despite the few previous examples, Fur-mediated direct activation via binding to defined promoter regions has been less studied.

In the remainder of this review, we discuss recent studies of Fur-mediated global regulatory circuits in the pathogenic *Neisseria* (*N. meningitidis* and *N. gonorrhoeae*). Since genomic analysis has revealed that there are fewer than 60 predicted regulatory proteins in the *Neisseria* genomes compared to ~200 in the *E. coli* genome (85), we propose that Fur-mediated regulation in these organisms may have more global and versatile consequences for gene expression and associated pathogenic mechanisms.

Fur REGULON OF *N. MENINGITIDIS*

An *N. meningitidis* global microarray analysis has identified 233 genes whose transcription levels were affected by growth under iron-replete versus -depleted conditions (44). Of these 233 genes, ~ 50% were predicted by *in silico* analysis to contain Fur boxes in their promoter regions (44). In addition, the majority of the predicted Fur binding promoter regions were experimentally demonstrated to bind Fur *in vitro* (44). A subsequent study examining iron regulation in a meningococcal *fur* mutant strain identified 83 genes whose iron-responsive regulation required Fur (22). Interestingly, 44 of those genes were repressed and 38 were activated, defining a new role for *Neisseria* Fur in activation of gene expression (22).

As demonstrated by electrophoretic mobility shift assay (EMSA) and/or DNase I footprinting results, genes and operons directly repressed by Fur in *N. meningitidis* encode proteins which can be classified into four major groups based on their functions: iron uptake and transport, energy metabolism and biosynthesis, toxin and stress responses, and regulation (Table 2) (22, 24, 44, 65, 66, 90). As expected, genes encoding iron uptake and transport proteins such as *tbpA*, *tbpB*, *lbpA*, and *lbpB* are repressed by Fur under iron-replete conditions, in agreement with the primary role of Fur as a maintainer of iron homeostasis (Table 2). The second group of Fur-repressed genes includes a large number of genes involved in energy metabolism and biosynthesis (Table 2). The protein products of these genes appear to enable bacterial growth, whereas their roles in pathogenesis have not yet been investigated. Interestingly, these genes have few homologs in *N. gonorrhoeae* (Table 2). The third group includes genes involved in virulence and bacterial adaption. It has been shown that FrpC-like proteins of *N. meningitidis* may play a role in pathogenesis (35, 79), and several *frpA- and frpC*-related gene loci, including NMB0364, NMB0584, NMB1405, and NMB1412 to -1414, were repressed by Fur directly (Table 2) (44). Several chaperone proteins and putative transposases, in addition to RecN, which are involved in DNA recombination and repair processes are also proposed to support bacterial adaption to the host environment and are directly repressed by Fur (Table 2) (44). The last group of Fur-repressed genes can be classified as regulators. A large percentage of Fur-dependent genes did not appear to be directly regulated by Fur, as demonstrated by the inability of Fur to bind to the promoter regions, which suggests the involvement of secondary regulators (22, 44). So far, only the small RNA NrrF has been identified as a transcriptional regulator directly controlled by Fur in *N. meningitidis* (Table 2) (65, 66). It is therefore logical to predict that additional, as-yet-uncharacterized Fur-controlled regulators could exist. Conversely, nine genes or operons directly activated by Fur fall into two major groups: iron storage and oxidative stress resistance genes and gene loci such as *sodB*, *kat*, *norB*, *aniA*, and NMB1438 to -1436 and energy metabolism loci such as the *nuo* complex (NMB0242 to -0244) (Table 2) (22, 44). In addition, a large number of hypothetical proteins under Fur regulation await further investigation (22, 44).

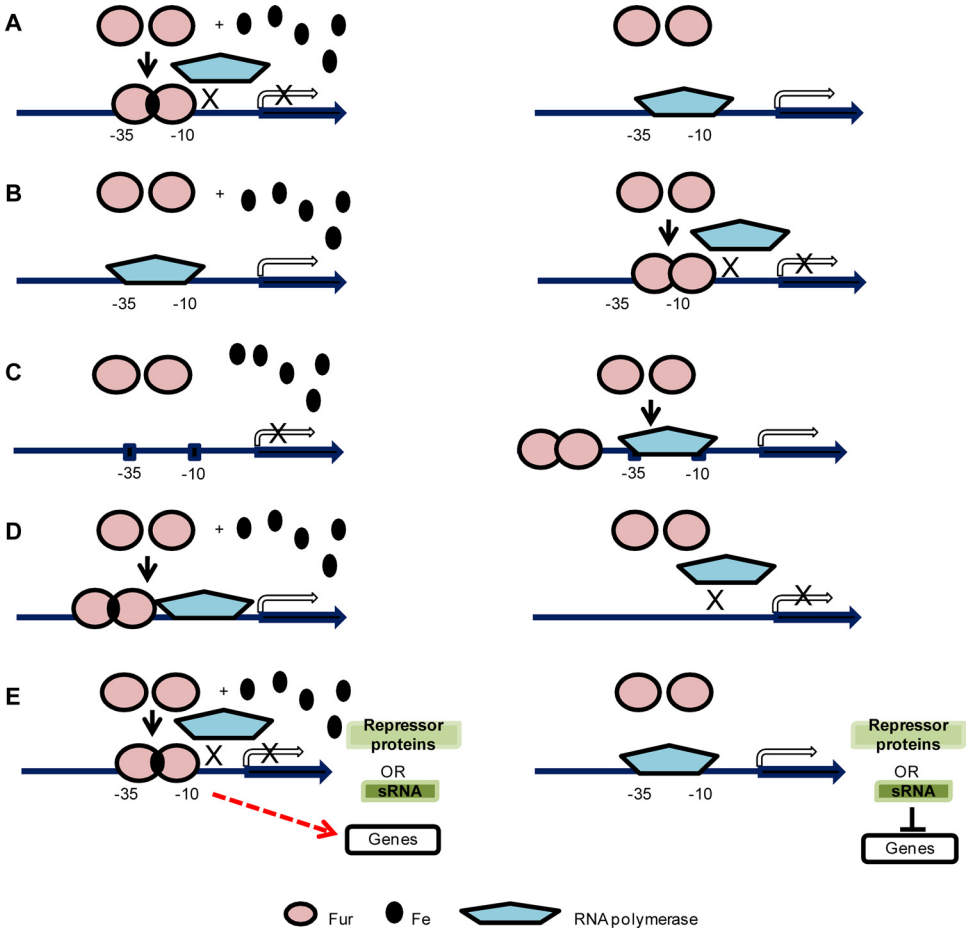

FIG 2 Mechanisms of Fur-mediated regulation. (A) Iron-bound Fur dimer binds to the −10 and −35 motifs in the promoter region, blocking binding of RNA polymerase and thus reducing transcription (left panel). Without iron, Fur does not bind to the promoter region, thereby allowing transcription via RNA polymerase (right panel). This mechanism has been well established by experimental evidence (32). (B) In the apo-Fur repression mechanism, when iron is present, Fur does not bind to the promoter region, resulting in transcription of the gene by RNA polymerase (left panel). Without iron, the apo-Fur dimer binds to the −10 and −35 motifs in the promoter region to inhibit binding of RNA polymerase and repress transcription (right panel). Apo-Fur repression was experimentally demonstrated in *H. pylori* (13, 72). (C) In the apo-Fur activation mechanism, Fur does not bind to the promoter region; thus, gene transcription is not active when iron is present (left panel). Without iron, the apo-Fur dimer binds to a site further upstream of the −10 and −35 motifs in the promoter region and upregulates transcription (right panel). This mechanism was first described in *V. vulnificus* (57). (D) Iron-bound Fur dimer binds to the promoter region and upregulates transcription (left panel). Without iron, Fur does not bind to the promoter region to initiate transcription (right panel). A few cases have been reported that indicate Fur-mediated direct activation through binding to defined promoter regions (50, 77, 105). (E) Iron-bound Fur dimer binds to −10 and −35 motifs in the promoter region and represses a negative regulator such as a protein repressor or a small RNA. Subsequently, genes that are repressed by the negative regulators are then transcribed and these genes show indirect Fur activation (left panel). Without iron and Fur repression, the negative regulators are transcribed and repress their target genes (right panel). Fur-repressed small RNAs have been reported in *E. coli* (61–64), *V. cholerae* (21), *P. aeruginosa* (103), and *B. subtilis* (38) as well as in the two pathogenic *Neisseria* species (28, 65, 66). Known Fur-repressed protein regulators include H-NS in *S. enterica* serovar Typhimurium (95) and MpeR in *N. gonorrhoeae* (53).

TABLE 1 Fur box consensus sequences of various Gram-positive or Gram-negative bacteria

Species	Consensus sequence of Fur box[a]	Reference(s)
Bacillus subtilis	TGATAATnATTATCA	7, 37
Staphylococcus aureus	GTTCATGATAATCATTATC	104
Escherichia coli	GATAATGATAATCATTATC	23
Pseudomonas aeruginosa	GATAATGATAATCATTATC	78
Salmonella enterica serovar Typhimurium	GATAATGATAATCATTATC	96
Vibrio cholerae	GATAATGATAATCATTATC	69
Helicobacter pylori	TAATAATnATTATTA	80, 98
Neisseria meningitidis	nATwATnATwATnATwATn	44
Neisseria gonorrhoeae	T-ATAAT-ATTATCA	27, 105

[a] See text for explanation of the significance of the hyphens and the uppercase and lowercase characters in the sequences.

Fur REGULON OF *N. GONORRHOEAE*

Microarray studies in *N. gonorrhoeae* determined that ~20% of the gonococcal genome is regulated in response to growth under iron-replete versus -depleted conditions (27, 53). When examined by *in silico* analysis, 92 genes or operons were predicted to contain a Fur box (27, 53). However, only a small percentage of these putative operator regions were demonstrated to bind Fur by a Fur titration assay (FurTa) (99), EMSA, and/or footprinting (Table 2) (25, 36, 41, 53, 105). Similar to *N. meningitidis*, gonococcal Fur-repressed genes include a large number involved in iron acquisition (Table 2). However, only three genes or loci encoding proteins for energy metabolism and biosynthesis (*fumC*, NGO0108, and NGO0114) are identified as being Fur repressed in *N. gonor-*

TABLE 2 Genes directly regulated by Fur in *N. meningitidis* and *N. gonorrhoeae*[a]

	N. meningitidis MC58				*N. gonorrhoeae* FA1090			
Category	Gene	Function	Expt(s)	Reference(s)	Gene	Function	Expt(s)	Reference(s)
Direct repression								
Iron acquisition	NMB0205	*fur*, ferric uptake regulator protein	EM, FP	44	NGO1779	*fur*	EM, FT	53, 86
	NMB0634	*fbpA*, iron binding protein	EM	44	NGO0215 to -0217	*fbpABC*	EM, FP, FT	25, 36, 53
	NMB1668	*hmbR*, hemoglobin receptor	FP	22	NGO1318	*hemO-hemR*, heme utilization protein	EM, FT	53, 86
	NMB0460	*tbp2*, transferrin binding protein B	EM	44	NGO1496	*tbpB*	FT	53
	NMB0461	*tbp1*, transferrin binding protein A	EM	44	NGO1495	*tbpA*	FT	53
	NMB1540	*lbpA*, lactoferrin binding protein A	EM	44		*lbpA*[b]	P[c]	9, 41
	NMB1541	*lbpB*, lactoferrin binding protein B	EM, FP	22, 44		*lbpB*[b]	P	8
	NMB1730	*tonB*, energy transducer	FP	22	NGO2176	*tonB*	EM	86
	NMB1728	*exbD*, biopolymer transport protein	EM	44				
	NMB0175	*zupT*, zinc transporter	FP	22				
	NMB1988	*frpB* (*fetA*), ferric enterobactin receptor	EM, FP	22, 44	NGO2093	*fetA*	FT	53
					NGO2092	*fetB*, ferric enterobactin periplasmic binding protein	EM, FT	53
					NGO0024	putative FetB2 protein	FT	53
					NGO0553	*tdfG*, putative TonB-dependent receptor	FT	53
					NGO2109	*hpuB*, hemoglobin-haptoglobin utilization protein B	FT	53
RTX toxin/virulence	NMB0364	FrpA/C-related protein	EM	44				
	NMB0584	FrpA/C-related protein	EM	44				
	NMB1405	FrpA/C-related protein	EM	44				
	NMB1412 to -1414	FrpA/C-related protein	EM	44				
					NGO0275	IgA1 protease	FT	53
						Opa (opacity-associated protein) A–K	EM	86
					NGO1822	*secY*	EM	86
Adaption/stress response					NGO0449	*sodB*	EM	86
					NGO0652	Thioredoxin I	FT	53
	NMB0544	*dnaK*, heat shock protein, chaperone	FP	22				
	NMB1472	*clpB*, chaperone	FP	22				
	NMB0740	*recN*, DNA repair protein	EM	44	NGO0318	*recN*	EM	86
	NMB0101	Putative transposase	EM	44				
	NMB1798	Putative transposase	EM	44				
Energy metabolism	NMB1395 and -1396	Alcohol dehydrogenase/*mutY*, A/G-specific adenine glycosylase	EM, FP	22, 44				
	NMB1377	*lldD*, L-lactate dehydrogenase	EM, FP	22, 44				
	NMB1458	*fumC*, fumarase C in TCA cycle	EM, FP	22, 44	NGO1029	*fumC*, fumarase C in TCA cycle	EM	86
					NGO0108	Putative oxidoreductase	FT	53
					NGO0114	Putative glutaredoxin	FT	53
Biosynthesis	NMB1898	*mlp*, lipoprotein	EM	44				
	NMB0317	7-cyano-7-deazaguanine reductase	EM	44				
	NMB0294	*dsbA-2*, disulfide interchange protein	EM	44				
	NMB0343	YciI-like protein	EM	44				
	NMB0394	*nadA*, quinolinate synthetase	EM	44				
	NMB0396	*nadC*, nicotinate-nucleotide pyrophosphorylase	EM	44				
	NMB1381	HesB/YadR/YfhF family protein	EM	44				
	NMB1380	*nifU*, nitrogen fixation	EM	44				
Regulators		*nrrF*, small RNA, transcriptional regulator	EM	65, 66		*nrrF*	P	28
					NGO0025	*mpeR*, AraC-like regulator	FT	53
Hypothetical	NMB0034 to -0036	Hypothetical protein	EM, FP	22, 44				
	NMB0744	Hypothetical protein	EM	44	NGO0322	Hypothetical protein	FT	53
	NMB0821	Hypothetical protein	EM	44				
	NMB0865 and -0864	Hypothetical protein	EM	44				

(Continued on following page)

TABLE 2 (Continued)

Category	*N. meningitidis* MC58				*N. gonorrhoeae* FA1090			
	Gene	Function	Expt(s)	Reference(s)	Gene	Function	Expt(s)	Reference(s)
	NMB1340	Hypothetical protein	EM	44				
	NMB1491	Hypothetical protein	EM	44				
	NMB1796	Hypothetical protein	FP	22				
	NMB1879 and -1880	Hypothetical protein	FP	22				
					NGO0554	Hypothetical protein	FT	53
Direct activation								
Iron acquisition/ storage					NGO1205	Putative TonB-dependent receptor	EM, FP	105
					NGO0794	*bfrA*, bacterioferritin	EM, FP, FT	53, 105
Energy metabolism	NMB1613	*fumB*, fumarate hydratase	FP	22				
	NMB0242 to -0244	*nuoB-nuoD*, NADH dehydrogenase subunits	EM, FP	23, 44	NGO1748 to -1751	*nuo* operon	EM, FP, FT	53, 105
					NGO0711	Alcohol dehydrogenase	EM, FP, FT	53, 105
					NGO2116	ATP-binding protein	EM, FP, FT	53, 105
					NGO0076	Putative phosphatase	EM, FP, FT	53, 105
Adaption/stress response	NMB0663	*nspA*, neisserial surface protein A	EM, FP	44, 90	NGO0233	*nspA*	EM, FP	105
	NMB1622	*norB*, nitric oxide reductase	FP	23	NGO1275	*norB*	EM, FP, FT	53, 105
	NMB1623	*aniA*, nitrite reductase	EM, FP	23, 44	NGO1276	*aniA*	EM, FP, FT	53, 105
	NMB0884	*sodB*, superoxide dismutase	EM	44				
	NMB0216	*kat*, catalase	EM	44				
	NMB1436 to -1438	Hypothetical proteins	EM, FP	22, 44	NGO0904 to -0906	Hypothetical Fe-S protein; hypothetical protein; Fe-S oxidoreductase	EM, FP, FT	53, 105
					NGO1317	Transposase	EM, FP, FT	53, 105
Transcription/ regulation					NGO0199	Transcription termination factor Rho	EM, FP	105
					NGO1851	DNA direct RNA polymerase subunit β	EM, FP	105
Hypothetical	NMB0298	Hypothetical protein	EM	44				
					NGO1207 to -1209	Excinuclease ABC subunit A; restriction endonuclease R.NgoMIII; DNA cytosine methyltransferase M.NgoMIII	EM, FP	105
					NGO1282	Hypothetical protein	EM, FP	105
					NGO1430	Hypothetical protein	EM, FP, FT	53, 105

[a] EM, electrophoresis mobility shift assay (EMSA): purified protein is incubated with radiolabeled probes (DNA or RNA) and subsequently run on a native polyacrylamide gel. Protein-bound probes show less mobility and shift up compared to free probes. FP, footprinting: DNase I is used to cut one end of labeled DNA, and the resulting patterns are analyzed by gel electrophoresis. The protein-bound site on the DNA is protected from cleavage and results in a clear area. FT (FurTa) (99), Fur titration assay: a bacterial genomic DNA library is constructed on a multicopy plasmid, such as puc18. The plasmids are transformed into an *E. coli* strain deficient in enterochelin synthesis and containing a fusion construct of the promoter of *fhuF*::*lacZ* in the chromosome. The promoter of *fhuF* has weak affinity to the $FurFe^{2+}$ repressor. If the multicopy plasmids do not contain a Fur box, $FurFe^{2+}$ represses the promoter region of *fhuF*::*lacZ* fusion construct so that the strain produces Lac (white) colonies on MacConkey plates supplemented with iron. In contrast, if the multicopy plasmids contain a Fur box, then the high number of these Fur boxes competes with the binding of $FurFe^{2+}$ to derepress the promoter region of *fhuF*::*lacZ* fusion construct, which results in Lac-positive colonies on MacConkey plates supplemented with iron.

[b] *lbpA* and *lbpB* genes are not present in *N. gonorrhoeae* strain FA1090 but were identified in strain FA19.

[c] Predicted to be directly regulated by Fur according to the presence of an *in silico* Fur box in the promoter region of the gene.

rhoeae (Table 2) (53, 86). The Fur-repressed virulence-associated genes and stress response genes are also different from those in *N. meningitidis* (Table 2). Genes encoding IgA1 protease, which cleaves human IgA on the mucosal surface (88), SecY, a putative preprotein translocase (86), and the opacity-associated Opa proteins (A to K) are directly repressed by Fur only in *N. gonorrhoeae* (Table 2), although *N. meningitidis* also contains these genes (18, 90). Interestingly, *sodB*, which is one of the Fur-activated genes in *N. meningitidis*, is repressed by Fur in the gonococcus (Table 2). In addition to *sodB*, only one gene and one locus, *recN* and NGO0652, are identified as stress response genes in *N. gonorrhoeae* (Table 2). NGO0652 encodes a putative thioredoxin pro-

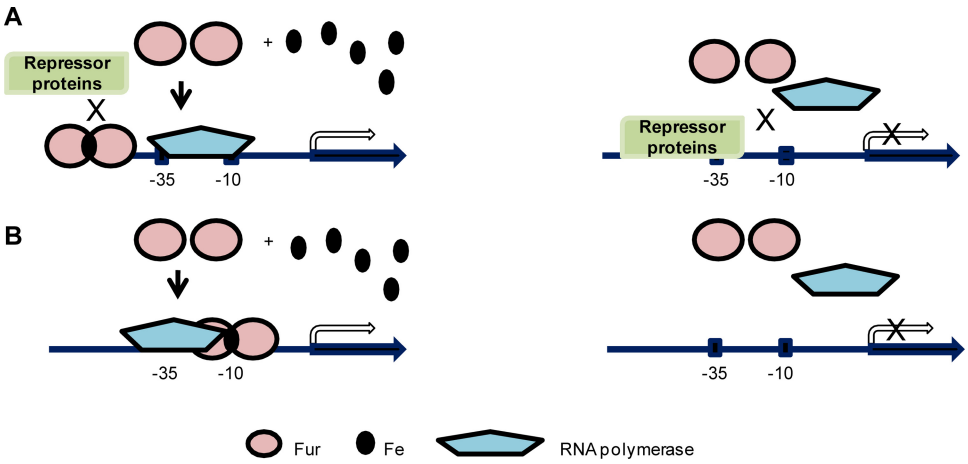

FIG 3 Fur mediates direct activation via variable mechanisms. (A) An iron-bound Fur dimer binds to a site further upstream of −10 and −35 motifs in the promoter region and out-competes binding of a repressor protein, thus activating transcription (left panel). Without iron, Fur does not function and the repressor protein binds to the promoter region to repress gene transcription (right panel). Three examples have been reported in *E. coli* and *N. gonorrhoeae* (50, 77). (B) An iron-bound Fur dimer binds to a site overlapping the −10 and −35 motifs in the promoter region in order to recruit RNA polymerase to activate transcription (left panel). Without iron, Fur does not bind to the promoter region and RNA polymerase is not recruited to initiate transcription (right panel). This mechanism is still a hypothesized model without experimental evidence (105).

tein (53), and a thioredoxin-like protein in the gonococcus has been suggested to play a role in defense against oxidative stress (1). More Fur-activated genes and operons have been identified in the gonococci than in the meningococci to date (Table 2). In addition to *nspA*, *norB*, *aniA*, and the *nuo* operon that have already been reported in the meningococcus, Fur-activated genes and loci in the gonococcus include those involved in iron storage and transport (NGO1205 and *bfrA*), transcription and regulation (NGO0199 and NGO1851), energy metabolism (NGO0711 and NGO2116), and adaption (NGO0076 and NGO1317) (Table 2) (53, 105). Their roles in gonococcal pathogenesis have yet to be investigated.

MECHANISMS OF Fur-MEDIATED DIRECT AND INDIRECT REGULATION IN PATHOGENIC *NEISSERIA*

Direct Fur repression. The repression mechanism of Fur, as well as the Fur box consensus sequence, is similar to that determined in other bacterial species (22, 25, 36, 43, 44, 86). Fur dimers bind to the −10 and −35 motifs in the promoter region to prevent the binding of RNA polymerase, which results in transcriptional repression of the gene. The apo-Fur repression mechanism may also be present in *Neisseria*, since several genes of *N. gonorrhoeae* have been shown to be repressed by Fur under iron-depleted conditions (105).

Direct Fur activation. The mechanism by which Fur functions to directly activate gene transcription is poorly understood. In general, proteins which function to activate transcription utilize one of the following three pathways: (i) the activator forces a repressor out of a potential binding site, allowing the initiation of transcription; (ii) the activator recruits RNA polymerase to enhance transcription; or (iii) the activator binding alters DNA morphology, allowing RNA polymerase binding (11). In addition, multiple mechanisms may be utilized simultaneously (11). Thus, in contrast to the simple mechanism of Fur-mediated repression, Fur-mediated direct activation may result from several different pathways.

Examples of the first pathway have been characterized in *Neisseria*. In *N. gonorrhoeae*, Fur-mediated activation of the *norB* gene is accomplished via the Fur box overlapping with the binding site of another repressor, ArsR. In this scenario, Fur binding to the promoter region of *norB* competes with ArsR and results in derepression of transcription (50). The position of the Fur box in the promoter region is at a distance further upstream from the −10 and −35 motifs than those in the genes repressed by Fur (Fig. 3A). Similar positioning of Fur boxes were reported in *aniA*, *norB*, and *nuoA* in *N. meningitidis* and in NGO0199, NGO1275 (*norB*), NGO1276 (*aniA*), and NGO1282 in *N. gonorrhoeae* (22, 105), suggesting that these genes could be activated by Fur via exclusion of a repressor. Interestingly, recent identifications of genes directly activated by Fur and characterization of their respective Fur boxes have shown that the Fur boxes of a majority of these genes in *N. gonorrhoeae* are in fact localized close to the −10 and −35 motifs, either overlapping both motifs or downstream of the −10 motif (105). This suggests an alternative mechanism utilized by Fur to directly initiate transcription. It is hypothesized that binding of Fur to a position close to the −10 and −35 motifs may recruit RNA polymerase binding in order to enhance transcription initiation (Fig. 3B) (105). Depending on the subtle differences of the positions of Fur box relative to the promoter motifs, Fur may interact with different subunits of RNA polymerase, although evidence for the direct interaction between Fur and subunits of RNA polymerase has not yet been established. However, we cannot rule out the possibility that Fur may utilize unidentified mechanisms other than ones discussed above to activate gene transcription.

Indirect Fur regulation. Fur-mediated indirect regulation in pathogenic *Neisseria* can occur via sRNA-mediated regulation or a regulator protein. Fur-mediated activation has been demonstrated to function indirectly through repressing a sRNA, NrrF, in *N. meningitidis*, which negatively regulates its target genes (65, 66). Nearly all sRNAs described in a variety of Gram-positive and Gram-negative bacteria share four common characteristics: (i) they are mainly localized in intergenic regions; (ii) the sequences are highly conserved among most genetically similar species; (iii)

the 3′ termini of sRNAs usually contain a Rho-independent terminator structure, which is composed of a stem-loop followed by a polyuridine region; and (iv) the lengths of sRNAs range from 50 to 300 nucleotides (100). Generally, sRNAs base pair to the ribosome-binding site on target mRNAs to interfere with ribosome binding and block the initiation of translation (100). The complementarity between a sRNA and mRNA requires a "seed" region of at least 8 to 9 continuous base pairs (100). Alternatively, sRNAs may lead to decreased stability of target mRNAs, resulting in reduced translation (100). In addition, many of the known sRNAs require a protein cofactor, Host Factor Qβ-phage (Hfq), to facilitate the binding of sRNAs to mRNA (42, 97).

The meningococcal sRNA NrrF (between loci NMB2073 and NMB2074) was identified by screening intergenic regions for Fur boxes upstream of Rho-independent terminators (65). One of 19 possible candidates, NrrF, was found to be iron regulated via Fur (65). NrrF is upregulated under iron-depleted conditions in a wild-type strain and derepressed in the *fur* mutant strain in a manner independent of the presence of iron, as determined by both Northern blot and reverse transcription-PCR (RT-PCR) experiments (65). The regulatory targets of NrrF were predicted using a bioinformatics approach which identified the *sdhA-sdhC* operon as a possible target (65). Subsequent experiments examining transcription of *sdhA-sdhC* in a wild-type strain and a *nrrF* mutant strain under iron-depleted or iron-replete conditions confirmed an NrrF-dependent repression pattern for *sdhA-sdhC* (65). Interestingly, unlike the Fur-regulated sRNAs in other bacteria, NrrF can function in the absence of Hfq. In an Δ*hfq* strain, the regulation of *sdhA-sdhC* in response to iron availability is unchanged, as is the stability of NrrF (66). NrrF is the first Fur-controlled negative regulator that has been discovered in pathogenic *Neisseria* (65, 66). Targets of NrrF, *sdhA-sdhC*, are in turn indirectly activated by Fur (65, 66). A NrrF homologue (between NGO2002 and NGO2004) has also been found in the *N. gonorrhoeae* genome (28).

To date, one *Neisseria* regulatory protein which is under Fur-mediated direct regulation has been reported. The *N. gonorrhoeae* Fur-repressed MpeR protein, an AraC-like regulator (53), activates *fetA*, an outer membrane transporter required for acquisition of xenosiderophore ferric enterobactin as an iron source (47) and represses MtrF and MtrR, which function in the *mtr* efflux pump and modulate antimicrobial resistance systems (34, 67, 89).

Fur GLOBAL REGULATORY CIRCUITS AND CROSS-TALK WITH NEWLY DEFINED REGULONS

With the discovery of increasing numbers of Fur-regulated genes, additional regulators have been found to cooperate with Fur to control the same gene or operon. For example, in *N. meningitidis*, Fur-regulated *hemO* and *hmbR* were shown to be positively regulated by the two-component system MisR/S under both iron-depleted and -replete conditions (106). Transcription of *N. meningitidis kat*, which is activated by Fur, is also repressed by OxyR in the absence of H_2O_2 and activated by OxyR with H_2O_2 (49). Perhaps the best-studied cooperative regulation has been shown for the gonococcal *aniA* and *norB* genes, involved in the anaerobic respiration pathway in *N. gonorrhoeae*. Several regulators are utilized for *aniA* and *norB* regulation, including Fur, NsrR, NarQ/P, FNR, and ArsR (50, 52). NsrR is a repressor containing a [2Fe-2S] cluster, which is involved in NO sensing (50, 52). In addition to iron limitation, the pathogenic *Neisseria* spp. may also encounter other stresses in the host environment, such as the simultaneous presence of NO, H_2O_2, and pH. The gonococcal Fur regulon has also been shown to overlap anaerobic and hydrogen peroxide regulons (51, 92). Thus, it is highly likely that Fur functions together with other regulators to enable *Neisseria* spp. to respond to complicated environmental conditions or stimuli within the human host.

We propose that the *Neisseria* Fur regulon encompasses a complicated network due to the ability of this protein to function as either a repressor or an activator in both direct and indirect pathways. To date, studies in the pathogenic *Neisseria* have examined only a small subset of Fur-regulated genes and have had limited value in deciphering a Fur-mediated global network. High-throughput bioinformatics techniques designed to assist in the analysis of the relationships among regulons of different regulators (19) should help to identify the entire *Neisseria* Fur regulon.

BIOLOGICAL ROLES OF Fur REGULATORY CIRCUITS

Although the genomes of *N. meningitidis* and *N. gonorrhoeae* are closely related, the Fur regulons of these two organisms are not completely overlapping (Table 2). These differences may relate to pathogen-specific requirements during human colonization and associated inflammatory pathologies.

N. gonorrhoeae mainly colonizes the human urethra, endocervix, fallopian tubes, and uterus. This pathogen causes urethritis in men, with obvious inflammatory symptoms such as a purulent discharge with an influx of polymorphonuclear leukocytes (PMN). In women, gonococcal infection presents as cervicitis, vaginitis, or a more serious pelvic inflammatory disease. Also, infection in women is typically asymptomatic and may lead to serious complications, including endometritis, salpingitis, and disseminated gonococcal infection (DGI) (30). *N. meningitidis* frequently colonizes the nasopharynx and causes meningitis or septicemia upon entering the cerebrospinal fluid or bloodstream, respectively. Generally, host niches for both organisms are iron-depleted environments, as free iron in the human host is scarce. Therefore, genes involved in iron acquisition, including *fur* itself, are upregulated in both pathogens during *N. meningitidis* infection in human blood and in the *N. gonorrhoeae* RNA isolated from cervical swab specimens from women with uncomplicated gonorrhea or urethral swab specimens from men with urethral infections (2, 3, 29). However, one of the differences between species-specific host niches is the iron source. In serum, transferrin is the major iron-carrying protein, while in the mucosal surfaces, lactoferrin is the major iron source (14). In addition, the concentration of lactoferrin can change with the menstrual cycle in human vaginal mucus (15). All *N. meningitidis* strains are able to utilize both transferrin and lactoferrin (70, 71), which may guarantee the survival of *N. meningitidis* in both types of host niches. In contrast, not all *N. gonorrhoeae* strains contain *lbpA* and *lbpB* genes, required for utilizing lactoferrin (8, 9, 41, 70). It has been proposed that gonococcal strains unable to utilize lactoferrin may be related to the asymptomatic infections often observed in women (10).

In *N. meningitidis*, the *aniA* (nitrite reductase), *kat* (catalase), and *nspA* (*Neisseria* surface protein A) genes are under direct Fur activation and have been demonstrated to be upregulated during *N. meningitidis* colonization in human blood (29). The *kat* and *aniA* genes have been postulated to play a role in *N. meningitidis* survival under conditions of stress from reactive oxygen and nitrogen species from neutrophils and macrophages (4, 87). Inter-

estingly, the *kat* gene is not under iron or Fur-mediated control in the gonococcus (53). The *nspA* gene encodes a human factor H binding protein that facilitates resistance to human complement, resulting in enhanced survival of *N. meningitidis* in blood (29, 60). Furthermore, genes under Fur-mediated direct activation, including *nspA* and *aniA*, were not detected in specimens isolated from women with gonococcal infection (3). Another significant difference between *N. meningitidis* and *N. gonorrhoeae* Fur regulons during infection may relate to the expression of Opa proteins, which are involved in bacterial adherence and invasion of human epithelial cells and neutrophils (18). *N. meningitidis* contains 3 to 4 Opa proteins, and at least one of them (NMB1636) is upregulated in human blood (29), although none of meningococcal *opa* genes are regulated via Fur (22, 44). In contrast, the putative promoter regions of all 11 gonococcal *opa* genes (A to K) have been shown to bind directly to Fur (86). Gonococcal Opa proteins have been shown to be expressed during natural infections as well as in experimentally infected volunteers (54, 55, 93). Furthermore, Opa–carcinoembryonic antigen-related cell adhesion molecule (CEACAM) interactions have been shown to promote gonococcal colonization in mouse models (16, 75, 76, 82). Above all, these results suggest that specific colonization niches and the associated pathogenic processes of the two pathogenic species serve to define the Fur regulons of these organisms and, in particular, those genes which are activated by Fur.

Moreover, the global effects resulting from Fur-mediated regulation determine the virulence of several bacterial pathogens. A large number of genes encoding iron-uptake protein homologues in the *Staphylococcus aureus* genome contain a predicted Fur box in their promoter regions, suggesting a major role of Fur in iron homeostasis (48). A *fur* mutant strain of *S. aureus* showed growth defects and higher sensitivity to H_2O_2 (48). These characteristics may have led to the reduced virulence (lower recovery of the *fur* mutant strain compared to the wild-type strain) seen in a murine skin abscess model (48). Fur of *Bacillus cereus*, an opportunistic human pathogen that causes food poisoning and endophthalmitis, may control at least 16 genes, according to the results of Fur-box prediction in the promoter regions of the genome (46). These genes include those involved in iron uptake and storage, secondary cellular metabolism, and virulence. The *fur* mutant strain of *B. cereus* also shows reduced virulence, with a 50% lethal dose (LD_{50}) value of 4,932 CFU compared to an LD_{50} value of 1,859 CFU of the wild-type strain in an insect model (46). Similarly, *V. cholerae* has been shown to have at least 65 Fur iron-repressed genes which are involved in iron acquisition and metabolism and two genes indirectly activated by Fur (69). In an infant mouse model of intestinal colonization, the *V. cholerae fur* mutant strain displayed significantly attenuated colonization when competing with the wild-type strain (69). Fur of the gastric pathogen *H. pylori* regulates genes critical for acid acclimation and oxidative stress (39, 80). The *fur* mutant strain of *H. pylori* displays a 100-fold-higher 50% infectious dose than the wild-type strain and lower colonization ability when competing with the wild-type strain in the Mongolian gerbil model (39, 73, 80). In addition, the *fur* mutant strain showed an attenuated ability to induce host inflammation and injury (73). All of the studies noted above emphasized the importance of the Fur regulon in bacterial pathogenesis.

CONCLUDING REMARKS

Recent studies have begun to define the Fur regulons of the pathogenic *Neisseria*. For both *N. meningitidis* and *N. gonorrhoeae*, global mechanisms of transcriptional control by Fur have been linked to the ability of these pathogens to cause disease and to respond to various stimuli within the human host. We predict that Fur-regulated circuits embrace broad components in the genomes and enable these organisms to respond to a variety of stress situations. Thus, understanding pathogenic aspects of Fur-mediated regulation is critical in revealing bacterial pathogenic mechanisms and will help to discover new therapeutic targets in these pathogens.

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