Supplementary Information

The genes that encode the gonococcal transferrin binding proteins, *tbpB* and *tbpA*, are differentially regulated by MisR under iron-replete and iron-depleted conditions

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Running title: MisR regulates the *tbpBA* operon in *N. gonorrhoeae*

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Supplemental Table S1
RNA-Seq analysis of the *misR* regulon in *N. gonorrhoeae* strain FA19

MisR-repressed genes

Gene	**ORF** (FA1090 designation)	[a]**Fold Change** (*misR*$^-$/WT)	[b]**Bonferroni value**	[c]**Function**
nadC	NGO0377†	10.42	0.00E+00	putative NadC family sodium/dicarboxylate symporter
lctP$_{trunc}$	NGO1361†	9.54	1.60E-11	putative L-lactate permease (truncated)
marR2	NGO1244†	5.90	2.04E-05	MarR family protein (regulator of multiple antibiotic resistance)
tbpB	NGO1496†	5.76	0.00E+00	transferrin-binding protein, TbpB
[d]*tdf*$_{trunc}$	NGO1362†	5.65	1.90E-04	putative TonB-dependent iron siderophore receptor (truncated)
ppk2	NGO2113	5.05	0.00E+00	putative polyphosphate kinase 2 (catalyzes the transfer of inorganic phosphate from poly P to GDP to create GTP, an important signalling molecule)
clpB	NGO1046	5.01	0.00E+00	ClpB molecular chaperone
	NGO1546	4.15	3.33E-11	putative S-adenosyl-L-methionine-dependent methyltransferase
opdA	NGO1770	4.03	4.45E-13	oligopeptidase A
	NGO1245†	4.02	4.07E-06	putative integral membrane protein (multidomain homology to an ABC-type multidrug transport system—ATPase and permease components)
	NGO0651	3.32	0.00E+00	S-adenosylmethionine-dependent methyltransferase
	NGO1287	3.15	0.00E+00	Ras-like GTPase
tbpA	NGO1495†	3.05	0.00E+00	transferrin-binding protein TbpA

GAPDH	NGO1776	3.04	0.00E+00	glyceraldehyde-3-phosphate dehydrogenase
opcA	NGO0868	2.84	1.33E-05	outer membrane protein (adhesion and invasion)
lon	NGO0775	2.79	0.00E+00	Lon serine protease—hydrolyses ATP to degrade protein substrates
	NGO1588	2.78	0.00E+00	uncharacterized conserved neisserial protein
grx	NGO0114	2.69	2.87E-09	glutaredoxin 3 [GRX is a glutathione (GSH) dependent reductase, catalyzing the disulfide reduction of target proteins]
pitB	NGO1581	2.66	0.00E+00	predicted phosphate transport permease
	NGO2014†	2.64	0.00E+00	predicted periplasmic binding protein; ABC transporter probably involved in glutamine transport
ppiB	NGO0376†	2.64	0.00E+00	peptidyl-prolyl cis-trans isomerase B
grpE	NGO1422	2.62	0.00E+00	GrpE co-chaperone; GrpE is the adenine nucleotide exchange factor of DnaK (Hsp70)-type ATPases
mpeR	NGO0025	2.55	2.17E-02	Mtr protein efflux regulator
rpe	NGO0758	2.55	0.00E+00	ribulose-5-phosphate 3-epimerase
scpA	NGO0961	2.49	5.29E-02	segregation and condensation protein A; involved in chromosomal partitioning during cell division
recN	NGO0318	2.41	0.00E+00	ATP-binding DNA repair protein
glnA	NGO1600	2.40	7.02E-03	glutamine synthetase
putP	NGO1552	2.37	0.00E+00	highly conserved bacterial sodium/proline symporter

glnQ	NGO2013†	2.35	2.81E-10	GlnQ ATP-binding component of the bacterial periplasmic glutamine permease
	NGO0834	2.32	4.18E-09	CsgG-family lipoprotein (CsgG is involved in curli formation in *E. coli*)
thiO	NGO2008†	2.27	2.75E-06	putative glycine oxidase with minimal identity to ThiO
cytX	NGO2009†	2.24	0.00E+00	putative hydroxymethyl-pyrimidine transporter CytX involved in thiamine production
rusA	NGO0489	2.23	3.24E-02	putative endodeoxyribonuclease/ Holliday junction resolvase RusA
sdhD	NGO0922†	2.23	1.49E-02	succinate:quinone oxidoreductase
kdtA	NGO1915	2.23	2.18E-05	putative KdtA (3-deoxy-D-manno-octulosonic-acid transferase a.k.a. kdotransferase)
acnB	NGO1231	2.23	0.00E+00	aconitate hydratase B (catalyses the formation of cis-aconitate from citrate as part of the TCA cycle)
dnaK	NGO1429	2.20	0.00E+00	DnaK (a.k.a. Hsp70) chaperone
potD	NGO1494	2.19	1.60E-11	PotD (spermidine/putrescine-binding periplasmic protein)
	NGO0165	2.18	2.86E-02	conserved hypothetical protein of unknown function
	NGO0115	2.18	3.33E-10	hypothetical protein of unknown function
	NGO1582†	2.18	2.97E-06	conserved hypothetical protein of unknown function
mafA3	NGO1584†	2.17	1.69E-07	putative adhesin MafA3
thiC	NGO2041	2.14	1.11E-03	ThiC; participates in the formation of 4-amino-5-hydroxymethyl-2-methylpyrimidine from AIR, an

				intermediate in de novo pyrimidine biosynthesis
tmp/tenI	NGO2007†	2.10	1.38E-06	TMP thiamine monophosphate synthase; catalyzes an important step in the thiamine biosynthesis pathway
mafA2	NGO1393	2.08	2.22E-12	putative adhesin MafA2
gltA	NGO0918	2.07	0.00E+00	GltA-like citrate synthase (CS)
shmt	NGO0866†	2.06	4.89E-12	serine/glycine hydroxymethyl-transferase
	NGO0865†	2.02	1.07E-11	hypothetical protein of unknown function
rpoH	NGO0288	2.01	0.00E+00	alternative sigma factor RpoH; sigma-32, sigma factor H, heat shock sigma factor
yojH	NGO1980	2.00	0.00E+00	YojH malate:quinone-oxidoreductase
tnaB	NGO2073	2.00	4.45E-13	TnaB tryptophan permease
sdhA	NGO0921†	1.99	1.78E-12	SdhA succinate dehydrogenase/ fumarate reductase
trxI	NGO0652	1.99	1.59E-06	TRX(I) thioredoxin I
	NGO1864	1.97	8.78E-04	conserved bacterial protein of unknown function
hsp33	NGO1189	1.86	0.00E+00	Hsp33

MisR-activated genes

nlpC	NGO1190†	-2.02	6.54E-05	NlpC/P60 family cell envelope protein
	NGO0420	-2.03	3.14E-07	COG3471, predicted periplasmic/secreted protein
ftsL	NGO1543†	-2.04	2.62E-07	FtsL cell division protein
dca	NGO1540†	-2.08	1.69E-23	Dca putative membrane associated sulfatase

clpA	NGO0408	-2.10	1.21E-14	ClpA ATP-binding subunit of the Clp protease
rpmB	NGO1680	-2.23	1.19E-09	RpmB 50S ribosomal protein L28
	NGO1282	-2.23	2.72E-02	hypothetical protein of unknown function (3’ of *serC* sequence and 5' of *lptA* sequence)
exoIII	NGO1561	-2.24	1.28E-12	highly conserved DNA exonuclease III (exodeoxyribonuclease III)
pbp2	NGO1542†	-2.31	6.84E-59	PBP2 penicillin-binding protein 2 peptidoglycan transpeptidase
nrdA	NGO0614†	-2.32	1.29E-30	NrdA ribonucleotide-diphosphate reductase subunit alpha
	NGO0867	-2.34	3.94E-22	predicted transcriptional regulator (Helix-turn-helix XRE-family like protein)
tatB	NGO0182†	-2.40	7.49E-77	twin arginine-targeting protein translocase TatB (Sec-independent protein translocase)
	NGO1556	-2.45	1.95E-02	conserved hypothetical protein (100% homologous duplication of NGO1462)
arsB/nhaD	NGO1411	-2.46	1.97E-36	predicted anion permease ArsB/NhaD; these permeases have been shown to translocate sodium, arsenate, antimonite, sulfate and organic anions across biological membranes
mraW	NGO1544†	-2.46	4.98E-19	MraW S-adenosyl-methyltransferase
	NGO0722	-2.46	1.10E-05	putative phage associated protein of unknown function
	NGO0618	-2.57	1.66E-24	conserved hypothetical protein of unknown function
	NGO1191†	-2.58	2.88E-14	conserved hypothetical protein of unknown function

macA	NGO1440†	-2.69	1.68E-49	MacA (membrane fusion component of the MacAB macrolide efflux pump)
nrdB	NGO0615†	-2.69	2.73E-53	NrdB ribonucleoside-diphosphate reductase
	NGO1462	-2.77	9.06E-03	hypothetical protein of unknown function
mraZ	NGO1545†	-2.83	6.04E-04	MraZ cell division protein
rpmE	NGO2126	-3.07	6.17E-29	RpmE 50S ribosomal subunit protein L31
bfrA	NGO0794†	-3.26	2.98E-05	BfrA bacterioferritin
macB	NGO1439†	-3.31	5.07E-38	MacB (ATP-binding permease protein component of the MacAB macrolide efflux pump)
	NGO1412	-3.46	3.00E-02	IS1016 transposase
	NGO1861	-3.51	1.76E-15	conserved gonococcal hypothetical protein
rplL	NGO1852	-3.59	3.44E-18	L7/L12 ribosomal protein
laz	NGO0994	-3.76	4.81E-03	Laz azurin (a copper-binding protein in the plastocyanin/azurin family)
bfrB	NGO0795†	-3.79	8.18E-80	BfrB bacterioferritin
htpX	NGO0399	-3.84	1.04E-05	HtpX heat shock protein that endoproteolytically cleaves misfolded membrane proteins
	NGO1215	-5.65	6.64E-03	COG2847; putative copper (I) binding envelope protein
	NGO1981	-6.00	3.71E-13	hypothetical protein of unknown function
	NGO0179†	-6.90	6.42E-03	hypothetical protein of unknown function
hlyIII	NGO1289	-7.76	1.69E-08	conserved protein homologous to the HlyIII channel-forming cytolysin

dsbD	NGO0978	-9.21	1.89E-42	DsbD (thiol:disulfide interchange protein)
maf	NGO0180†	-11.90	7.11E-10	predicted Maf family nucleotide binding protein involved in septation
ompA	NGO1559	-17.48	4.56E-03	OmpA family protein
imm22	NGO1590	-18.50	7.72E-11	putative immunity/antitoxin protein (encoded next to a putative toxin gene encoded by NGO1589)
tatC	NGO0181†	-24.34	2.71E-57	TatC Sec-independent protein translocase component

†genes that are adjacent to and encoded in the same orientation as other MisR regulatory targets (i.e., possibly coregulated by MisR as part of an operon).
Repressed groups: NGO0376/NGO0377 (*ppiB*/*nadC*); NGO0865/NGO0866 (hypo/*shmt*); NGO0921/NGO0922 (*sdhA*/*sdhD*); NGO1244/NGO1245 (*marR2*/hypo ATPase and permease); NGO1361/NGO1362 (*lctP*$_{trunc}$/*tdf*$_{trunc}$); NGO1495/NGO1496 (*tbpA*/*tbpB*); NGO1582/NGO1584 (hypo/*mafA3*); NGO2007/NGO2008/NGO2009 (*tmp*/*thiO*/*cytX*); NGO2013/NGO2014 (*glnQ*/amino acid transporter).
Activated groups: NGO0179/NGO0180/NGO0181/NGO0182 (hypo/*maf*/*tatC*/*tatB*); NGO0614/NGO0615 (*nrdA*/*nrdB*); NGO0794/NGO0795 (*bfrA*/*bfrB*); NGO1190/NGO1191 (*nlpC*/hypo); NGO1439/NGO1440 (*macB*/*macA*); NGO1540/NGO1542/NGO1543/NGO1544/NGO1545 (*dca*/*pbp2*/*ftsL*/*mraW*/*mraZ*)

[a] Fold change is calculated as the ratio of reads-per-kilobase-per-million-reads (RPKM) values for each gene in the FA19 *misR::kan* cells/FA19 WT cells. These data are representative of three independent comparisons.

[b] The Bonferroni Correction (http://mathworld.wolfram.com/BonferroniCorrection.html), which is a multiple-comparison correction used when several dependent or independent statistical tests are being performed simultaneously, was performed as a more stringent method of determining whether or not fold change values show statistical significance. Fold changes with Bonferroni values >0.05 were considered non-significant.

[c] Protein functions were determined using a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) on the NGO designation of the gene, followed by a characterization of any conserved domains present using the Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi).

[d] "*tdf*" refers to the "TonB-dependent function" naming scheme.
See Cornelissen and Hollander, 2011, TonB-dependent transporters expressed by *N. gonorrhoeae*, Front. Microbio. 2 (117):1-13.

Supplemental Figure S1

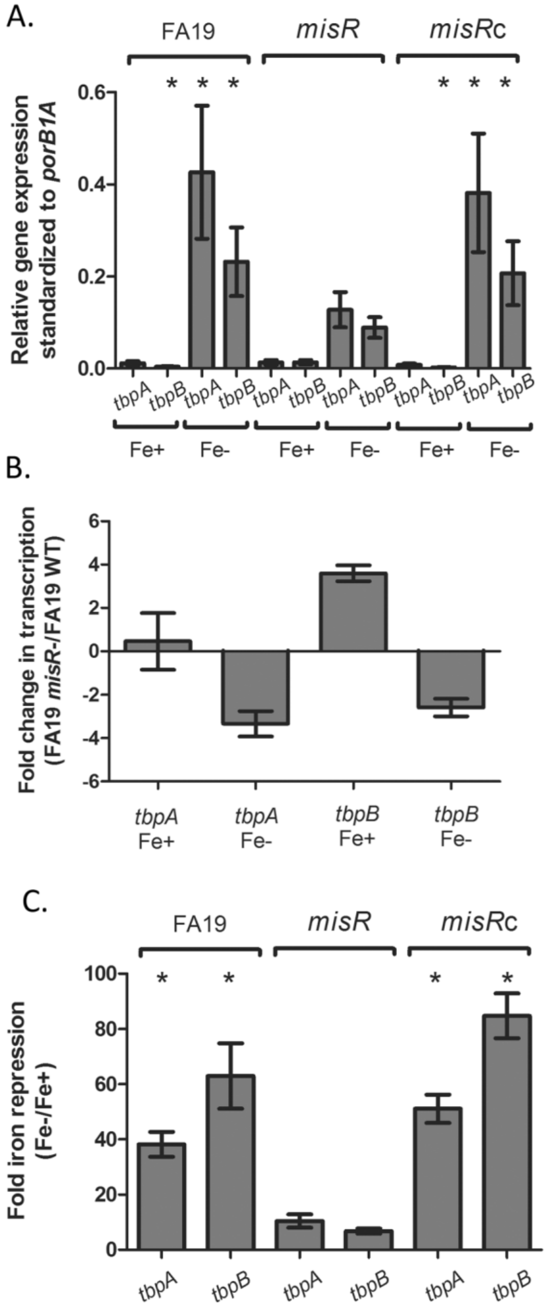

Fig S1. qRT-PCR demonstrates differential expression of *tbp* genes depending on iron status. Cultures were grown in GCB medium (Fe+ or Fe-) with 10 mM supplemental Mg^{2+} as employed for the RNA-seq experiment in Fig. 1. **Panel A.** Relative gene expression for *tbpA* and *tbpB* genes under iron-replete and iron-depleted conditions for WT, *misR* and *misR*c strains. Shown are the average relative gene expression values (standardized to the *porB1A* gene); error bars represent the standard deviation from the mean. Asterisks demonstrate those values that were significantly different ($P < 0.05$) as compared to the *misR* mutant in pairwise comparisons using an unpaired Student's *t*-test. **Panel B.** The average fold change in expression of the *tbp* genes is shown on the Y-axis and represents the ratio of FA19 *misR*-/FA19 WT gene expression (as shown in Fig. 1). Error bars represent standard deviation from the mean for each gene and condition. **Panel C.** Fold iron repression is shown on the Y-axis and represents the relative expression of *tbp* genes under iron-depleted/iron-replete conditions.

The error bars represent the standard deviation from the mean. Asterisks demonstrate those values that were significantly different ($P < 0.01$) as compared to the *misR* mutant in pairwise comparisons using an unpaired Student’s *t*-test.