Supplementary Tables and Figures

Table S1. Strains and Plasmids

Strains	**Genotype/Description**	**Source/Reference**
***Salmonella* strains**		
14028	Wild-type Salmonella strain	Lab collection
QW165	14028 *ycgR*::KAN	Lab collection
QW108	14028 *yhjH*::KAN	Lab collection
VN55	14028 *yhjH ycgR*	Lab collection
JP1495	14028 *yhjH ycgR bcsA*	This work
E. coli strains		
AW405	Wild-type	1
HCB5	AW405 *fliC*	2
JP1442	AW405 *yhjH ycgR*	This work
JP1762	JP1442 *bcsA*	This work
JP1763	JP1442 *csgD*	This work
JP1764	JP1442 *fimA*	This work
JP1768	JP1442 *pgaC*	This work
JP1769	JP1442 *wcaD*	This work
JP1771	JP1442 *yjbE*	This work
JP1836	JP1442 suppressor flare isolate 1	This work
JP1837	JP1442 suppressor flare isolate 2	This work
JP1838	JP1442 suppressor flare isolate 3	This work
JP1839	JP1442 suppressor flare isolate 4	This work
JP1844	JP1442 suppressor flare isolate 5	This work
JP1852	JP1442 suppressor flare isolate 6	This work
JP1932	JP1442 *bcsA csgD pgaC fimA wcaD yjbE*	This work
JP1992	AW405 *rssA*	This work
JP2063	MG1655 *lacZ*::KAN	This work
JP2065	AW405 *lacZ*	This work
JP2066	JP1442 *lacZ*	This work

JP2173	JP1442 [a]pJP319	This work
JP2214	JP2066 *rssAB*::KAN	This work
JP2079	AW405 *rssB*	This work
JP2221	AW405 *rssAB*	This work
JP2222	JP1442 *rssAB*	This work
JP2347	AW405 *clpX*	This work
JP2348	JP1442 *clpX*	This work
JP2573	AW405 *rpoS*::KAN	This work
JP2574	JP1442 *rpoS*::KAN	This work
JP2586	AW405 *clpX rpoS*::KAN	This work
JP2587	JP1442 *rpoS*::KAN *clpX*	This work
JP2228	NM580 [b]PflhD::*lacZ*	This work
JP2234	JP2228 *rssAB*::KAN	This work
JP2241	JP2228 *ycgR yhjH*	This work
JP2248a	JP2228 *ycgR yhjH rssAB*	This work
JP2248b	JP2228 *clpX*	This work
JP2248c	JP2228 *ycgR yhjH clpX*	This work
VN133	AW405 *yhjH*	3
VN139	AW405 *bcsA*::KAN	4
VN140	AW405 *ycgR*	3
VN145	HCB5 *yhjH*	This work
VN147	HCB5 *yhjH ycgR*::KAN	This work
VN153	HCB5 *ycgR*::KAN	This work

Plasmid	**[c]Expressed Protein**	**Host Plasmid**	**Resistance**	**Induction**	**Reference**
pBAD24	P*ara*$_{BAD}$ Expression vector	-	Ampicillin	Arabinose	(3)
pCP20	FLP Recombinase	-	Ampicillin	Temperature	(2)
pSEVA224	Low-copy $lacI^{q}$-P*trc* expression vector	-	Kanamycin	IPTG	#
pTRC99a	$lacI^{q}$-P*trc* Expression vector		Ampicillin	IPTG	(1)
pDgcA	DgcA from *Caulobacter crescentus*	pTRC99a	Ampicillin	IPTG	(4)

pFD313	FliC^{sticky}	pTRC99a	Ampicillin	IPTG	(5)
pJP319	RssAB	pTRC99a	Ampicillin	IPTG	This work
pJP388	YcgR	pSEVA224	Kanamycin	IPTG	This work
pJP393	RssA	pBAD24	Ampicillin	Arabinose	This work
pJP394	RssB	pBAD24	Ampicillin	Arabinose	This work
pJP395	RssB^{D58E}	pBAD24	Ampicillin	Arabinose	This work
pJP396	RssB^{D58A}	pBAD24	Ampicillin	Arabinose	This work
pKD46	λ Red recombinase	-	Ampicillin	Arabinose	(2)
pPA114	Tsr	pKG116	Chloramphenicol	Salicylate	J.S.Parkinson
pRR53	Tsr	pBR322	Ampicillin/Tet	IPTG	J.S.Parkinson
pRS1551	*lac* reporter plasmid	-	Ampicillin	-	(9)
pRZ30	FRET reporter plasmid	-			J.S.Parkinson
pVN5	YcgR from *S. enterica*	pBAD24	Ampicillin	Arabinose	(6)
pVS88	FRET reporter plasmid	pTRC99a	Ampicillin	IPTG	J.S.Parkinson
pVS177	P*fliA*::*lacZ* reporter plasmid	pRS1551	Ampicillin	-	(10)
pVS182	P*flhD*::*lacZ* reporter plasmid	pRS1551	Ampicillin	-	(10)
pYhjH	YhjH from *S. enterica*	pTRC99a	Ampicillin	IPTG	Lab collection

[a] p=plasmid; [b] P=promoter; [c] All *E. coli* proteins, unless otherwise noted**;** **#** SEVA Resource http://seva.cnb.csic.es/

Table S2. Mutational changes in pseudorevertants of JP 2173 (ycgR yhjH/pRssAB)

Revertant Strain[a]	Mutant Genes[a]	Mutation (s)[b]	Gene Function[c]
JP2372	*pitA*	Δ635-636 nt	Phosphate transporter
	rssAB	Δ2-1734 nt	Regulator of RpoS
JP2373	*pitA*	A391T (GCG→ACG)	Phosphate transporter
JP2374	*sspA*	+295-303 nt	Stringent starvation protein A
	rssAB	Δ4-1687 nt	Regulator of RpoS
JP2375	*pitA*	G456E (GGG→GAG)	Phosphate transporter
JP2376	*pitA*	G456E (GGG→GAG)	Phosphate transporter
	rssAB	Δ1-1704 nt	Regulator of RpoS
JP2377	*cheB*	P193L (CCC→CTC)	Chemotaxis methylesterase
	pitA	Δ802-810 nt	Phosphate transporter
JP2378	*pitA*	G423E (GGG→GAG)	Phosphate transporter
	fliZ	Q124* (CAA→TAA)	Regulator of FliA
JP2379	*pitA*	R404L (CGT→CTT)	Phosphate transporter
	rssAB	Δ3-1745 nt	Regulator of RpoS
JP2380	*cheB*	G284S (GGC→AGC)	Chemotaxis methylesterase
	pitA	+IS1, +529-537 nt	Phosphate transporter
	rssAB	Δ13-1677nt	Regulator of RpoS
JP2381	*pitA*	W477* (TGG→TAG)	Phosphate transporter
	rssAB	Δ4-1709 nt	Regulator of RpoS
JP2382	*pitA*	W477* (TGG→TAG)	Phosphate transporter
	rssAB	Δ1-1707 nt	Regulator of RpoS
JP2383	*fliM*	N249Y (AAC→TAC)	Flagella motor switching component
	pitA	Δ391-395 nt	Phosphate transporter
	rssAB	Δ5-1717 nt	Regulator of RpoS
JP2384	*pitA*	Δ 391-395 nt	Phosphate transporter
JP2385	*cheB*	H233N (CAT→AAT)	Chemotaxis methylesterase
	pitA	Δ114-115 nt	Phosphate transporter
	rssAB	Δ1-1746 nt	Regulator of RpoS
JP2386	*pitA*	Δ1080-1180 bp	Phosphate transporter
	rssAB	Δ1-1694 nt	Regulator of RpoS
JP2387	*cheB*	R316C (CGC→TGC)	Chemotaxis methylesterase
	glrK	Δ130-1890 nt	Sensor kinase for glmY sRNA
	pitA	W112* (TGG→TAG)	Phosphate transporter
	rssAB	Δ3-1718 nt	Regulator of RpoS
JP2388	*fliM*	N249Y (AAC→TAC)	Flagella motor switching component

	pitA	+ 391-395 nt	Phosphate transporter
	rssAB	Δ1-1719 nt	Regulator of RpoS
JP2389	*cheB*	D37E (GAT→GAA)	Chemotaxis methylesterase
	pitA	+391-395 nt	Phosphate transporter
	rssAB	Δ7-1690 nt	Regulator of RpoS
JP2390	*cheB*	H233N (CAT→AAT)	Chemotaxis methylesterase
	pitA	Δ114-115nt	Phosphate transporter
	yahG	K129R (AAA→AGA)	DUF1116 family protein
	rssAB	Δ1-1697 nt	Regulator of RpoS
JP2391	*cheB*	T168A (ACT→GCT)	Chemotaxis methylesterase
	pitA	W181* (TGG→TAG)	Phosphate transporter
	rssAB	Δ1-1691 nt	Regulator of RpoS

[a]Mutational changes were identified by whole genome sequencing of twenty independent pseudorevertants using Breseq (19). [b]+, insertion; Δ, deletion; nt. Nucleotide; *, STOP codon; IS1, Insertion Sequence 1. [c]Gene product descriptions are from Genbank annotations. The *rssAB* deletions refer to nucleotides in the *rssAB* operon (1920 nt) rather than the individual genes. Full details of [d]JP strains found in Table S1.

Table S3. Statistical analysis of motility data presented in Fig. 5.

Strain Comparisons[a]	***p*-value**[b]
A)	
Wild-type vs. *ycgR yhjH*	<0.0001
Wild-type vs. *ycgR yhjH rssA*	<0.0001
Wild-type vs. *ycgR yhjH rssB*	<0.05
Wild-type vs. *ycgR yhjH rssAB*	NS
ycgR yhjH vs. *ycgR yhjH rssA*	NS
ycgR yhjH vs. *ycgR yhjH rssB*	<0.0001
ycgR yhjH vs. *ycgR yhjH rssAB*	<0.0001
ycgR yhjH rssA vs. *ycgR yhjH rssB*	<0.0001
ycgR yhjH rssA vs. *ycgR yhjH rssAB*	<0.0001
ycgR yhjH rssB vs. *ycgR yhjH rssAB*	NS
ycgR yhjH pCtrl vs. *ycgR yhjH* pRssA	NS
ycgR yhjH pCtrl vs. *ycgR yhjH* pRssB	<0.0001
ycgR yhjH pCtrl vs. *ycgR yhjH* $pRssB^{D58E}$	<0.0001
ycgR yhjH pCtrl vs. *ycgR yhjH* $pRssB^{D58A}$	NS
ycgR yhjH pRssA vs. *ycgR yhjH* pRssB	<0.0001
ycgR yhjH pRssA vs. *ycgR yhjH* $pRssB^{D58E}$	<0.0001
ycgR yhjH pRssA vs. *ycgR yhjH* $pRssB^{D58A}$	NS
ycgR yhjH pRssB vs. *ycgR yhjH* $pRssB^{D58E}$	<0.01
ycgR yhjH pRssB vs. *ycgR yhjH* $pRssB^{D58A}$	<0.0001
ycgR yhjH $pRssB^{D58E}$ vs. *ycgR yhjH* $pRssB^{D58A}$	<0.0001
B)	
Wild-type vs. *ycgR yhjH*	<0.0001
Wild-type vs. *rpoS*	<0.01
Wild-type vs. *ycgR yhjH rpoS*	<0.0001
Wild-type vs. *clpX*	<0.001
Wild-type vs. *ycgR yhjH clpX*	<0.0001
Wild-type vs. *ycgR yhjH clpX rssAB*	<0.0001
Wild-type vs. *ycgR yhjH clpX rpoS*	<0.0001

Wild-type vs. *ycgR yhjH clpX rpoS rssAB*	<0.0001
ycgR yhjH vs. *rpoS*	<0.0001
ycgR yhjH vs. *ycgR yhjH rpoS*	NS
ycgR yhjH vs. *clpX*	<0.0001
ycgR yhjH vs. *ycgR yhjH clpX*	<0.0001
ycgR yhjH vs. *ycgR yhjH clpX rssAB*	NS
ycgR yhjH vs. *ycgR yhjH clpX rpoS*	NS
ycgR yhjH vs. *ycgR yhjH clpX rpoS rssAB*	NS
rpoS vs. *ycgR yhjH rpoS*	<0.0001
rpoS vs. *clpX*	NS
rpoS vs. *ycgR yhjH clpX*	<0.0001
rpoS vs. *ycgR yhjH clpX rssAB*	<0.0001
rpoS vs. *ycgR yhjH clpX rpoS*	<0.0001
rpoS vs. *ycgR yhjH clpX rpoS rssAB*	<0.0001
ycgR yhjH rpoS vs. *clpX*	<0.0001
ycgR yhjH rpoS vs. *ycgR yhjH clpX*	<0.0001
ycgR yhjH rpoS vs. *ycgR yhjH clpX rssAB*	NS
ycgR yhjH rpoS vs. *ycgR yhjH clpX rpoS*	NS
ycgR yhjH rpoS vs. *ycgR yhjH clpX rpoS rssAB*	NS
clpX vs. *ycgR yhjH clpX*	<0.0001
clpX vs. *ycgR yhjH clpX rssAB*	<0.0001
clpX vs. *ycgR yhjH clpX rpoS*	<0.0001
clpX vs. *ycgR yhjH clpX rpoS rssAB*	<0.0001
ycgR yhjH clpX vs. *ycgR yhjH clpX rssAB*	<0.0001
ycgR yhjH clpX vs. *ycgR yhjH clpX rpoS*	<0.0001
ycgR yhjH clpX vs. *ycgR yhjH clpX rpoS rssAB*	<0.0001
ycgR yhjH clpX rssAB vs. *ycgR yhjH clpX rpoS*	NS
ycgR yhjH clpX rssAB vs. *ycgR yhjH clpX rpoS rssAB*	NS
ycgR yhjH clpX rpoS vs. *ycgR yhjH clpX rpoS rssAB*	NS

[a]A and B refer to strains tested in Fig. 5A and B. [b]Data were processed using One way ANOVA (Tukey's Comparison) using Graphpad Prism 6, with calculated *p*-values shown.

Fig. S1

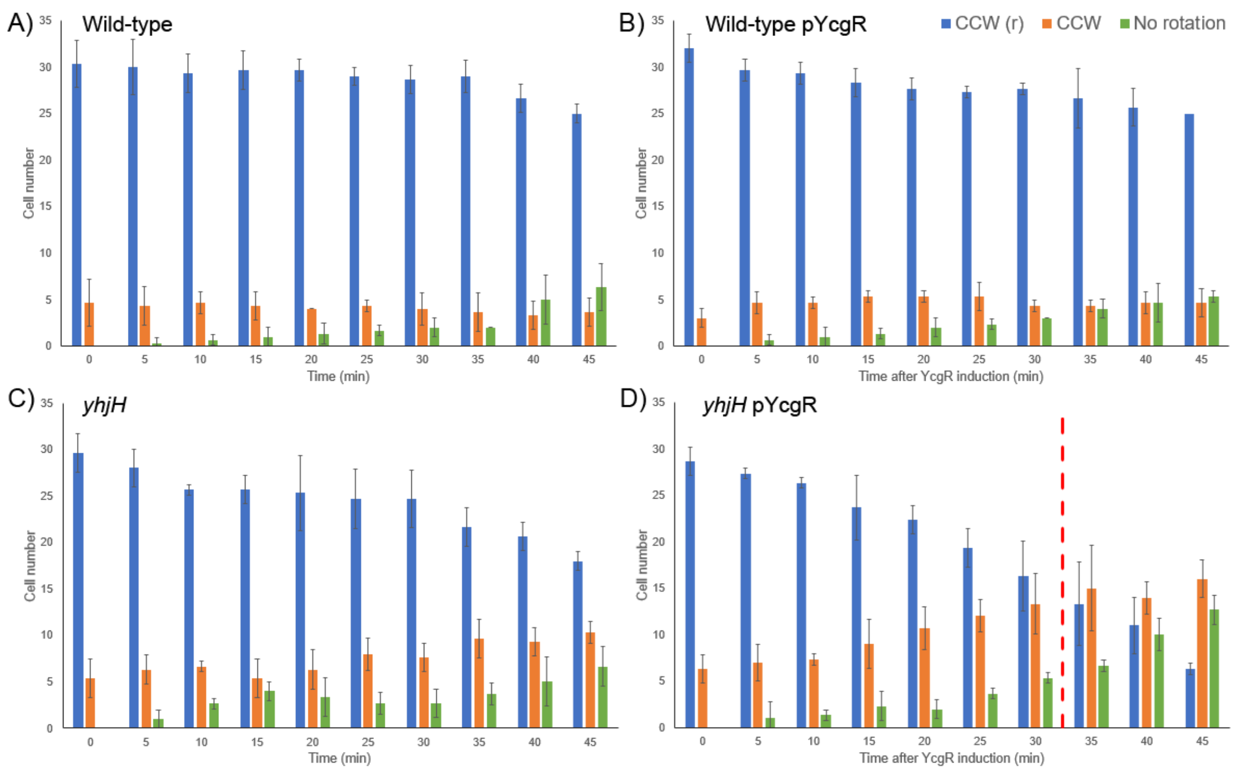

Figure S1. Time course monitoring motor speed and bias in response to YcgR induction using a whole-cell tethering assay in *Salmonella*. Strains monitored are A) WT *S. enterica* (14028), B) WT with pYcgR, C) Isogenic *yhjH* deletion strain, D) *yhjH* with pYcgR (pVN5). In each experiment, performed thrice independently, 35 tethered cells were recorded for 45 min and analyzed during playback at 5 min intervals, with or without induction of YcgR from a plasmid with 0.2% arabinose inducer. Motor behavior was scored for the three indicated categories: CCWr, CCW, and Stopped. The basal motor behavior of WT was CCW with reversals (CCWr) (A). This behavior was fairly constant, with a small increase in the number of stopped motors by the end of the observation period. For an isogenic *yhjH* strain (C), where a chromosomal copy of *ycgR* is still present, the expectation was that the motors would be more CCW biased as observed with *E. coli* motors (Fig. 1). However, the rotation bias was being determined visually, and a small change in bias from the WT might have been missed due to the inherently smooth (i.e. CCW-biased) nature of the motors with (7). The CCW population increased slightly over the course of the experiment, as did the fraction of stopped motors (C). Motor speeds were not computed because they are low to begin with in this assay (~150 revolutions per min; (8)). When a plasmid expressing YcgR was introduced in both strains, and the inducer arabinose was added, the pattern of motor behavior in the WT strain remained unchanged (compare B with A). In the *yhjH* strain, however, CCWr population began to steadily decrease upon addition of the inducer, with a reciprocal increase in the CCW population (D). These two trends merged at 35 min, and at this time (dotted red line), greater than 50% of the cell population exhibited visibly slower speeds compared to the same population at the start of the experiment. The “stopped motor” population began to increase around this time point as well. Despite the subjective nature of this experiment, the overall trend of the data in D clearly showed that upon YcgR induction, the shift in motor bias was observed first and arrest of motor rotation occurred later, implying that changes at the rotor preceded those at the stators.

Figure S2

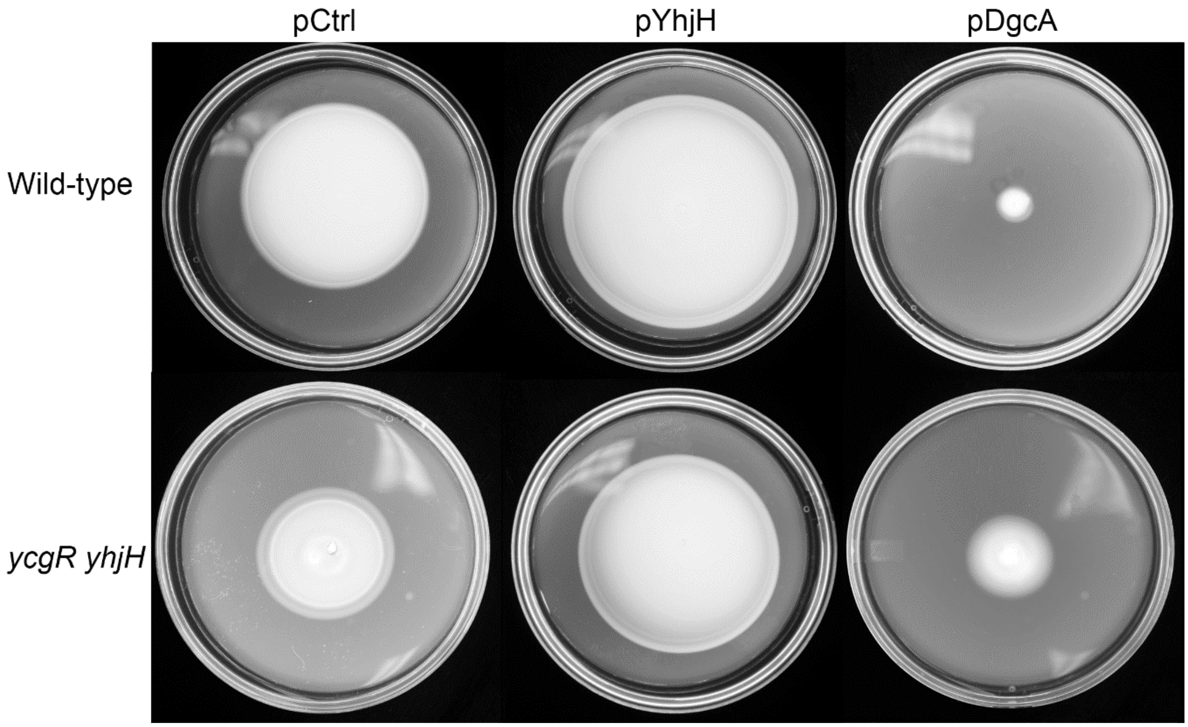

Figure S2. Effect of overexpression of YhjH and DgcA on *E. coli* motility. Wild-type *E. coli* and its *ycgR yhjH* mutant derivative were transformed with a control plasmid (empty vector), pYhjH or pDgcA plasmids, before inoculation at the center of 0.3% LB swim agar plates supplemented with ampillicin (plasmid selection), and 0.2% arabinose (inducer for expression of cloned genes) and incubated at 30°C for 8 h.

References

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