Supporting Information

Duigou *et al.* 10.1073/pnas.0803904105

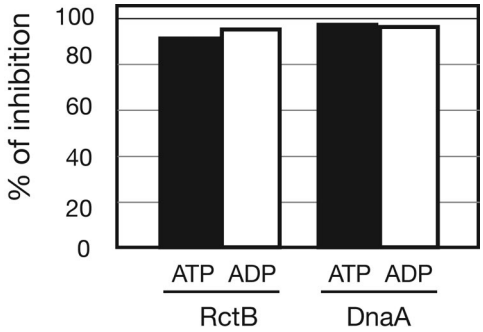

Fig. S1. Competitive ATP and ADP binding by RctB and DnaA. A total of 50 nM P^{32}-αATP plus either 5 mM of unlabeled ATP or ADP were mixed with 80 nM of DnaA (*Right*) or 80 nM of RctB (*Left*), then the bound labeled ATP was detected by using the filter binding assay. The percent inhibition of binding of labeled ATP was calculated by dividing the amount of radioactive ATP bound in the presence of the unlabeled competitor by the amount of radioactive ATP bound in the absence of the competitor and then subtracting this value from 1. The values presented are an average from two experiments

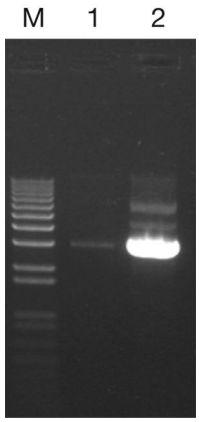

Fig. S2. Effect of R269S RctB on the copy number of an *oriCIIvc*-based plasmid. Ethidium bromide-stained *oriCIIvc*-based plasmid DNAs are shown. Plasmid DNA was extracted from overnight cultures and electrophoresed on an agarose gel. (lane 1) pYB289, an *oriCIIvc*-based plasmid containing WT *rctB*, (lane 2) pYB286, and an *oriCIIvc*-based plasmid containing *rctB* R269S, (lane M) 1 kb plus ladder. We used real-time qPCR to quantitatively compare the copy number of *oriCIIvc*-based plasmids in *E. coli*. Real-time qPCR was performed as described (1) except that primers to amplify the *kan* gene (for plasmid DNA; 5′-TTCTTTCGCCTGATCGTCAG-3′ and 5′-ATGAAAAGGTCGGCCTGCA-3′) and *narW* (for *E. coli* chromosomal DNA; 5′-GGCAGGATCTCCTGTCATC-3′ and 5′- GGTC-GAATGGGCAGGTAG -3′) were used.

1. Burrus V, Waldor MK (2003) Control of SXT integration and excision. *J Bacteriol* 185:5045–5054.

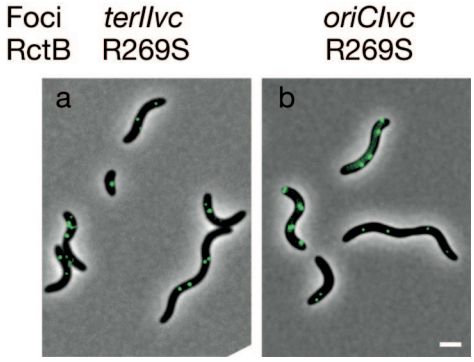

Fig. S3. *In vivo* characterization of *V. cholerae* overexpressing R269S RctB. Detection by fluorescent microscopy of *terIIvc* (*a*) and *oriCIvc* (*b*) using TetR-YFP in N16961 derivatives that contain *tet* operator arrays near these loci. Representative fields after 1 h of induction of TetR-YFP and RctB are shown. (Bar = 2 μm.)

Table S1. Plasmids and *V. cholerae* strains used in this study

	Relevant characteristics/description	Ref./source
Plasmid		
pBR	pBR322	NE BioLabs
pET-RctB	pET28B *rctB*	This study
pET-RctBR269S	pET28B *rctB R269S*	This study
pGZ119EH	IPTG-inducible expression vector	1
pLAU53	Arabinose-inducible *lacI-cfp* and *tetR-yfp*	2
pOriI	pBR322 *oriCIvc*	This study
pOriII	pBR322 *oriCIIvc*	This study
pYB285	pGZ119EH *rctB*	This study
pYB286	*rctB R269S oriCIIvc rctA kan*	This study
pYB289	*rctB oriCIIvc kan*	This study
Strain		
N16961	Sequenced El Tor *V. cholerae* strain	3
YBB008	N16961 VCA0008::*tetOP*	4
YBB022	N16961 VCA0596::*tetOP*	This study
YBB025	N16961 VC0018::*tetOP*	5
YBB682	N16961 / pYB285	This study
YBB688	N16961 / pYB291	This study
YBB703	N16961 / pGZ119	This study
YBB727	N16961 VCA0008::*tetOP*/pLAU53 pGZ119	This study
YBB728	N16961 VCA0008::*tetOP*/pLAU53 pYB285	This study
YBB729	N16961 VCA0008::*tetOP*/pLAU53 pYB291	This study
YBB732	N16961 VCA0596::*tetOP*/pLAU53 pYB291	This study
YBB733	N16961 VC0018::*tetOP*/pLAU53 pYB291	This study

1. Lessl M, *et al.* (1992) Dissection of IncP conjugative plasmid transfer: Definition of the transfer region Tra2 by mobilization of the Tra1 region in trans. *J Bacteriol* 174:2493–2500.
2. Lau IF, *et al.* (2003) Spatial and temporal organization of replicating *Escherichia coli* chromosomes. *Mol Microbiol* 49:731–743.
3. Heidelberg JF *et al.* (2000) DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* 406:477–483.
4. Yamaichi Y, Fogel MA, McLeod SM, Hui MP, Waldor MK (2007) Distinct centromere-like *parS* sites on the two chromosomes of *Vibrio* spp. *J Bacteriol* 189:5314–5324.
5. Fogel MA, Waldor MK (2006) A dynamic, mitotic-like mechanism for bacterial chromosome segregation. *Genes Dev* 20:3269–3282.