Supporting Information

Natrajan et al. 10.1073/pnas.0908966106

SI Text

Cloning, Expression, and Purification of the HobA/DnaA$^{I–II}$ Complex. HobA gene was amplified by PCR using the forward 5′-act**tcatga**aaaatttctacgattggatc and reverse primer 5′-tg**gagctc**t-tcacagagtaaccttattgtatagc introducing the *Pag*I and SacI restriction sites (indicated in boldface type), respectively. The digested fragment was introduced between the NcoI and SacI restriction sites of the pRSF-Duet vector. The resulting pRSF-HobA plasmid was co-transformed with pET151-Nterm (expressing the domain I and II, i.e., residues 1–112 of DnaA fused to a N-terminal His-6-tag) into BL21(DE3) *E. coli* cells. For protein production, 10 ml of an overnight culture were diluted into 1 L of LB media containing ampicillin (100 μg/ml) and kanamycin (100 μg/ml), and grown at 37 °C till an OD of 0.7 was reached, whereupon protein expression was induced by adding 1 mM IPTG (final concentration) for 16 h at 20 °C. Cells were resuspended in lysis buffer (50 mM Hepes, pH 7.5, 400 mM NaCl, 5% glycerol, 1 mM MgCl2, 10 mM β-mercaptoethanol, protease inhibitor mixture tablets, lysozyme, and Dnase-I), and disrupted using sonication. The lysate was cleared by centrifugation at 31,000 g, and applied immediately on a batch purification column containing Ni-NTA resin preequilibrated with buffer A (50 mM Hepes, pH 7.5, 400 mM NaCl, 5% glycerol, 10 mM β-mercaptoethanol). After this, the Ni-NTA beads were washed thoroughly with 10% buffer B (buffer A with 500 mM imidazole) and eluted with buffer B.

The eluted complex was then subjected to TEV protease cleavage to remove the His-6-tag from the DnaA$^{I–II}$ fragment. After buffer exchange to buffer A, the complex was once again loaded on to Ni-NTA beads to remove the TEV protease and other impurities. The final, pure complex obtained was concentrated to 5 mg/ml after first adding additional glycerol to a final concentration of 10%.

Protein Expression and Purification. Full-length DnaA (flDnaA), DnaA$^{I–II}$, His-6-DnaA$^{I–II}$, HobA, and His-6-HobA proteins were purified for pull-down assays and/or isothermal titration calorimetry (ITC) experiments. The purifications of flDnaA, His-6-HobA, and HobA have been described elsewhere (1, 2). DnaA$^{I–II}$ expression using the pET151-Nterm and BL21 cells was induced in 1 L *E. coli* cells for 16 h at 20°. Harvested cells were resuspended in lysis buffer (50 mM Tris-HCl, pH 8, 300 mM NaCl, 5% glycerol) and disrupted by sonication. The supernatant obtained from a 20-min centrifugation at 40,000 *g* was then loaded on a Ni-NTA gravity column. After a wash in buffer A (50 mM Tris-HCl, pH 8, 300 mM NaCl, 5% glycerol), the protein was washed with 15 ml buffer A + 10 mM imidazole. Pure His-6-DnaA$^{I–II}$ was eluted with buffer A + 500 mM imidazole and dialyzed against buffer A. To obtain DnaA$^{I–II}$, His-6-DnaA$^{I–II}$ was subjected to TEV cleavage at 4 °C overnight. The nondigested protein was separated from DnaA$^{I–II}$ by reloading the solution on the Ni-NTA column and collecting the flow-through. Pure DnaA$^{I–II}$ was then concentrated in buffer A.

Crystallization, Structure Determination, and Refinement. Crystals of the HobA/DnaA$^{I–II}$ were grown by vapor diffusion in hanging drops by mixing 1 μl protein complex (at 5 mg/ml) to 1 μl of a reservoir solution containing 19–21% (wt/vol) polyethylene glycol (PEG) 3350, 200 mM potassium acetate, and 50 mM Tris-HCl pH 8.0. Typically, small plate-shaped crystals grew after 3 to 5 days with dimensions of 100 μm × 100 μm × 10 μm. Crystals were transferred into a cryo-solution (21% [wt/vol] PEG 3350, 200 mM potassium acetate, and 20% glycerol [wt/vol]), and a dataset was collected at the European Synchrotron Radiation Facility beamline ID14EH4, at a temperature of 100K and a wavelength of 0.93 Å. Intensities were integrated with MOSFLM (3) and scaled with SCALA (4). Crystals of HobA/DnaA$^{I–II}$ belonged to the spacegroup C2, with cell dimensions of a = 127.6 Å b = 55.9 Å and c = 96.3 Å, $\alpha = \gamma = 90.00$ and $\beta = 97.9$ (Table S1). Crystals diffracted to a resolution of 2.6 Å and contained two molecules of HobA and two molecules of DnaA in the asymmetric unit. Molecular replacement was done with HobA monomer and a DnaA model (generated by the PHYRE server http://www.sbg.bio.ic.ac.uk/phyre/) using *E. coli* DnaA as template. The program PHASER (5) was used, which led to an initial set of phases of excellent quality. However, only a small fraction of the DnaA model was in agreement with the density. Thus the two DnaA molecules had to be rebuilt, with iterative rounds of refinement using PHENIX (6). The final model contained two molecules of HobA (chains A and B) and two molecules of DnaA, including residues 6–91 of Chain C and residues 5–91 of chain D (Fig. S6). The final model was refined to good geometry and had more than 98% of the residues in the allowed region of the Ramachandran plot. A great improvement in Rfree was seen upon using NCS restraints between the chains A, B and C, D as determined automatically by PHENIX. TLS refinement using the individual chains as rigid bodies was also very useful in lowering the Rfree. The crystallographic and refinement parameters can be seen in Table S1.

Isothermal Titration Calorimetry Experiments. The HobA and DnaA$^{I–II}$ proteins, purified separately, were dialyzed into the ITC buffer (50 mM Tris-Hcl, pH 7.5, 250 mM NaCl, 10% glycerol) overnight. The ITC experiment was done using a MicroCal VP-ITC Calorimeter. The HobA protein, at a concentration of 9 μM was placed in the sample cell of the ITC, and DnaA$^{I–II}$ (the ligand) at a concentration of 77 μM was injected into the cell. Injections (10-μl) of the ligand were performed until the curve reached saturation, and the data were fitted using MicroCal Origin (OriginLab). The experiment was carried out at room temperature (23 °C). The data generated by the isothermal titration calorimetry (ITC) experiment was analyzed using the Origin software provided with the ITC instrument. The peaks generated upon injection of the ligand into the protein (raw data in μcal/s) were integrated using the program, after first setting their baseline (determined previously by injecting the same amount of ligand into buffer). The curve obtained (ΔH values in kcal/mole versus the molar ratio of the ligand to protein) was used for model building. It was noticed that the model with the best fit was a single site binding model, having as parameters the stoichiometry (N), the association constant (Ka), and the change in enthalpy (ΔH). The model fitting was done using the nonlinear least-square fit method, available as a part of the Origin software.

Site-Directed Mutagenesis. HobA mutants were generated by site directed mutagenesis using the quick change protocol (Stratagene) and pPHp1230 as DNA template (1). The purifications of HobA mutants were carried out using the same protocol that was used for the wild-type HobA.

Pulldown Assays. The His-6-tag on the flDnaA or DnaA$^{I–II}$ was used for pull-down assays with untagged HobA or HobA mutants obtained by cleaving the His-6-tag from His$_6$-HobA with

the TEV protease as described in (1). The TEV cleavage was monitored SDS/PAGE. Complex formation was performed by incubating 12.5 μg of each protein in binding buffer (50 mM Tris, pH 7.5, 250 mM NaCl, 10% glycerol, 10 mM imidazole). The solution was loaded onto spin columns containing 100 μl of Talon resin, pre-equilibrated in 50 mM Tris, pH 7.5, 250 mM NaCl, 10% glycerol. The protein mixture was incubated with the resin for half an hour at 4 °C. After this, the columns were extensively washed with the binding buffer. The complex was finally eluted with binding buffer containing 500 mM imidazole. Samples from elutions were loaded on a 15% SDS/PAGE gel; 4 μg of each protein was loaded onto the gel as input as controls.

Size-Exclusion Chromatography. Purified HobA, DnaA$^{I–II}$ and HobA/DnaA$^{I–II}$ complexes were subjected to size exclusion chromatography on a Superdex 200 column equilibrated in 50 mM Tris–HCl, pH 7.5, 200 mM NaCl, and 5 mM β-mercaptoethanol. In all cases, 500 μg of protein were injected, and the purification was run at 0.5 ml/min at room temperature.

Chemical Cross-Linking. For cross-linking assays with DnaA$^{I–II}$ and HobA, proteins were first dialyzed against cross-linking buffer (50 mM Hepes, pH 7.5, 200 mM NaCl, 10% glycerol). Crosslinking agents glutaraldehyde (0.01% final concentration), EGS, and BS3 (both to a final concentration of 1 mM) were added to 10 μg of the protein. The mixture was then incubated at room temperature for 20 min, and the reaction was quenched with 2 μl of 1M Tris-HCl, pH 7.5. The samples were run on a 15% SDS/PAGE gel for analysis. The cross-linking assays to study complex formation were performed by incubating 5 μg of HobA or its mutants with an equal amount of DnaA$^{I–II}$ for 20 min in the presence of EGS (1 mM) at room temperature in the cross-linking buffer. Complex formation was assessed on gradient (10%–15%) SDS/PAGE gels stained with Coomassie blue. Controls for each sample were also performed with 5 μg of HobA or HobA mutants using the same conditions.

Random Mutagenesis and Selection for HobA Interaction–Defective Mutations in the Yeast 2HB. Mutational mapping of HobA interacting surface was performed using a recently developed strategy based on a GAL4-derived yeast, two-hybrid assay (7). HobA coding sequence was subjected to random mutagenesis by error-prone PCR favoring single mutational events as already described (7). Because both the BD and the AD-HobA fusions self-interact and interact with DnaA in yeast 2HB (Fig. S2), two independent libraries of the mutated HobA ORF, fused with either AD or BD functional domains of GAL4, were established in yeast by the gap repair procedure and arrayed in the 96-well plate format. An array of 1,500 colonies per library, expressing potential mutant HobA proteins, was mated with strains expressing HobA or DnaA in fusion with the complementary GAL4 functional domain. Diploids were monitored for the expression phenotypes, i.e., for their ability to grow on selective media lacking uracil, leucine, histidine, or adenine (–LUH and –LUA). Diploids colonies that specifically failed to express the interaction phenotypes between HobA and DnaA while keeping the HobA–HobA self-interaction were screened. The corresponding haploid clones harboring the HobA mutant derivatives (from either the pGAD or pGBDU-HobA mutant library) were pooled and tested again for the loss of interaction phenotypes. The corresponding mutations within *hobA* were identified by sequencing.

Site-directed mutagenesis of HobA was carried out as described above for L174A, ERP, A101E, and Y175E using BD-HobA as a template. BD-HobA mutants were tested for their ability to interact with AD-wt-HobA and AD-DnaA following the same procedure as just described.

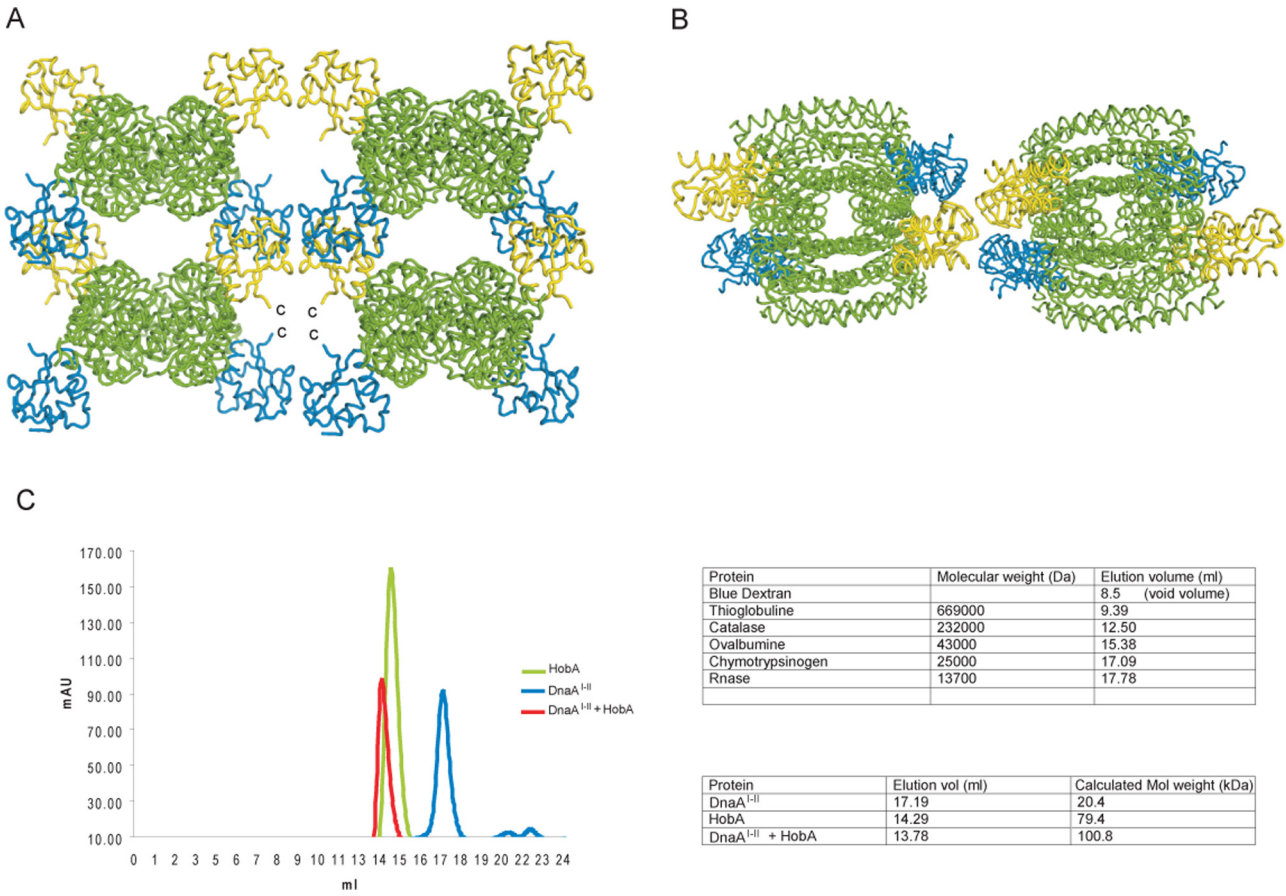
Yeast Three-Hybrid Assay. Each of the haploid strains harboring pGBDU empty vector, pGBDU-HobA, or pGBDU-DnaA vectors were transformed with either p3HB vector (8) or p3HB carrying the *hobA* gene placed under the control of the constitutive ADH promoter (p3HB-HobA). The six resulting haploid (a) strains containing a combination of the pGBDU and p3HB vectors were selected on -UW media and organized in a small matrix. This matrix was mated with haploid α strain carrying the pGAD-DnaA vector. Diploïds were selected on -LUW media and the interaction phenotypes were monitored on -LUWH media (lacking leucine, uracil, and tryptophane) as described elsewhere (8). A ternary interaction is revealed when the interaction phenotype is expressed conditionally to the presence of a third protein partner (here HobA).

***H. pylori* and Culture Conditions.** *H. pylori* strain 26695 was cultivated at 37 °C under microaerobic conditions. *H. pylori* plate cultures were grown on blood agar base medium (BA) supplemented with 10% defibrinated equine blood. The liquid cultures were prepared in brain heart infusion (BHI) containing 10% FCS. All *H. pylori* cultures were supplemented with an antibiotic mix (9). Plate cultures were incubated for 24 h, and the growth of liquid cultures was monitored by measuring the optical density at 600 nm. For selection of *H. pylori* transformants (3–5 days of growth), kanamycin was added to the medium to concentrations of 15 $\mu g \cdot ml^{-1}$.

Construction of *H. pylori* Mutants. HobA mutated genes were amplified from pPHp1230 expression vector by PCR (using 5′-cg**ggatcc**ggagggacatatgaaaaatttctacgattggatc-3′and 5′-cg**ggatcc**tcacagagtaaccttattgtatag-3′ as forward and reverse primers, respectively) and transferred in to a pUC18 vector using BamHI (boldface) restriction sites. Mutated *hobA* genes were excised from pUC18*hobA*mt (where mt corresponds to A101E, L174A, L80R, ERP, and Y175E) with BamHI and inserted into a suicide plasmid pILL2283 containing a *aph*-3 nonpolar cassette (10) digested with the same restriction enzyme giving p*hobA*mt. *H. pylori* cells were transformed with purified plasmid DNA by natural transformation (11) where p*hobA*mt was used as a suicide vector to introduce the desired mutations into *hobA* on the *H. pylori* 26695 chromosome (Fig. S4). After transformation with p*hobA*mt the *H. pylori* kanamycin-resistant mutants were isolated and analyzed by PCR analysis using 1229_fw (5′-gtatcgcattaacgtggatatgatc-3′) and 1231_rev (5′-cataaatgtcttcaaatccagcttg-3′) primers. To assess the presence of *mthobA and aph*-3 cassette, BamHI restriction analysis of the PCR product was first performed (Fig. S4*B*). Mutations in chromosomal *hobA* were then identified by sequencing of the PCR product with a primer N1230_KpnI (5′-gggtaccatgaaaaatttctacgattggatc-3′) (Fig. S4*C*).

1. Natrajan G, Hall DR, Thompson AC, Gutsche I, Terradot L (2007) Structural similarity between the DnaA-binding proteins HobA (HP1230) from *Helicobacter pylori* and DiaA from *Escherichia coli*. *Mol Microbiol* 65:995–1005.
2. Zawilak-Pawlik AM, Kois A, Zakrzewska-Czerwinska J (2006) A simplified method for purification of recombinant soluble DnaA proteins. *Protein Expr Purif* 48:126–133.
3. Leslie AGW (1992) Recent changes to the MOSFLM package for processing film and image plate data. Joint CCP4 and ESF-EACMB Newsletter on Protein Crystallography No26 Daresbury Laboratory (Warrington, UK).
4. Collaborative Computational Project-4 (1994) The CCP4 suite: Programs for protein crystallography. *Acta Crystallogr D* 50:760–763.
5. McCoy AJ, et al. (2007) Phaser crystallographic software. *J Appl Crystallogr* 40:658–674.
6. Adams PD, et al. (2002) PHENIX: Building new software for automated crystallographic structure determination. *Acta Crystallogr D Biol Crystallogr* 58:1948–1954.
7. Noirot-Gros MF, et al. (2006) Functional dissection of YabA, a negative regulator of DNA replication initiation in *Bacillus subtilis*. *Proc Natl Acad Sci USA* 103:2368–2373.

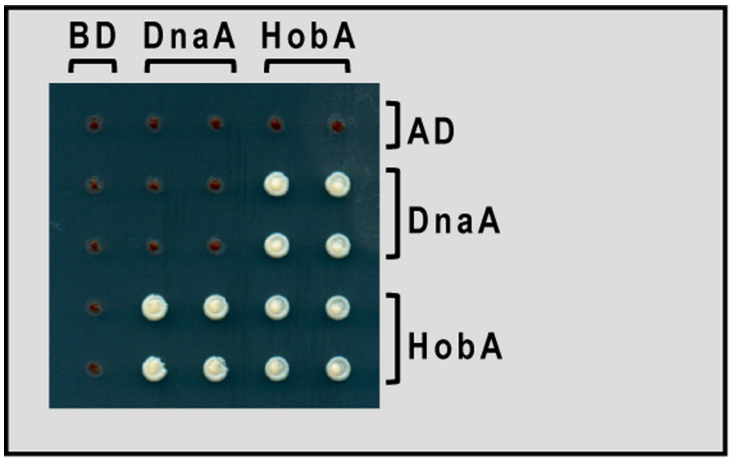
8. Soppa J, et al. (2002) Discovery of two novel families of proteins that are proposed to interact with prokaryotic SMC proteins, and characterization of the *Bacillus subtilis* family members ScpA and ScpB. *Mol Microbiol* 45:59–71.
9. Contreras M, Thiberge JM, Mandrand-Berthelot MA, Labigne A (2003) Characterization of the roles of NikR, a nickel-responsive pleiotropic autoregulator of *Helicobacter pylori. Mol Microbiol* 49:947–963.
10. Zawilak-Pawlik A, et al. (2007) HobA—a novel protein involved in initiation of chromosomal replication in *Helicobacter pylori. Mol Microbiol* 65:979–994.
11. Chevalier C, Thiberge JM, Ferrero RL, Labigne A (1999) Essential role of *Helicobacter pylori* gamma-glutamyltranspeptidase for the colonization of the gastric mucosa of mice. *Mol Microbiol* 31:1359–1372.



Protein	Molecular weight (Da)	Elution volume (ml)
Blue Dextran		8.5 (void volume)
Thioglobuline	669000	9.39
Catalase	232000	12.50
Ovalbumine	43000	15.38
Chymotrypsinogen	25000	17.09
Rnase	13700	17.78

Protein	Elution vol (ml)	Calculated Mol weight (kDa)
DnaA^{I-II}	17.19	20.4
HobA	14.29	79.4
DnaA^{I-II} + HobA	13.78	100.8

Fig. S1. Stoichiometry and crystal packing of the HobA/DnaA complex. (*A*) View of the crystal packing toward the a axis, showing the contacts made by DnaA molecules. Molecules are displayed in worm representation and colored according to their chains. HobA molecules are colored in green, DnaA chain C in blue, and chain D in yellow. C-terminal ends of the DnaA polypeptide point toward each other, suggesting that they may establish contact and play a role in the crystal packing. (*B*) View of the packing toward the b axis. This view clearly shows how the tetramers of HobA are formed in the crystal and how the four DnaA molecules bind to one tetramer of HobA. (*C*) Analytical gel filtration analysis of the complex formation. Chromatograms obtained with the injections of DnaA (blue), HobA (green), and the HobA/DnaA$^{I–II}$ complex (red) are shown on the left side. On the right are shown the exact elution volumes and molecular weights corresponding to the calibration samples (*top*) and samples tested (*bottom*). HobA alone elutes as a tetramer (79 kDa) and the complex HobA/DnaA$^{I–II}$ elutes at a smaller volume, suggesting that the HobA tetramer is not disrupted by the binding of one or several DnaA$^{I–II}$ molecules. According to the same profile however, the stochiometry of the complex seems different from 1:1 (DnaA/HobA) observed in the pull-down assays and the ITC, with putatively less than 4 molecules of DnaA$^{I–II}$ bound to HobA. DnaA$^{I–II}$ elutes at a volume of 17.19 ml (corresponding to a molecular weight of 20kDa) suggesting that the protein is a monomer in solution.



Mutational screen efficiency

	Nbr	%
Clones screened	2100	-
interaction phenotypes	73	3,5
Single mutational events	65	3,1
Single aa substitution	42	2
1 bp deletion/insertion or stop	23	1,1
Double events or more	8	0,4

DnaA- HobA-
DnaA- HobA+
Intermediate phenotype
(no DnaA+ HobA- obtained)

Single Substitutions	Mutated target	
	AD-HobA	BD-HobA
I41T	X	
H43R (x4)	X	X
V44A	X	
L45P	X	
L51S (x2)		X
L51F	X	
D55G	X	
F61C	X	
S67P (x3)	X	X
L69P		X
R77C	X	
V82A	X	
I83M	X	
S88P		X
S99P	X	
Y107H	X	
M111V	X	
F112S	X	
V115D	X	
I118T	X	
E119G	X	
L127P		X
L128P	X	
L137P		X
F138L	X	
F138S (x2)	X	X
L141P	X	
L146S (x2)	X	X
L162P	X	
F166S		X
A169V	X	
L170P	X	
L174P (x2)	X	X
K177*	X	

Fig. S2. Characterization of HobA/DnaA loss-of-interaction mutations. Gray box: yeast two-hybrid assays (2HB). Initial screen for interactions between HobA/HobA, DnaA/DnaA, and HobA/DnaA. Each interaction has been tested with two replicates. The interacting phenotypes were monitored by the ability to grow onto selective media (-LUA). No self-interaction was detected for DnaA in a 2HB assay, whereas a HobA–HobA interaction was observed. The matrix also shows that DnaA and HobA interact on both combinations, i.e., BD-HobA/AD-DnaA and AD-HobA/BD-DnaA. (*Table*, *right*). Two mutational screens for HobA loss-of-interaction (LOI) mutants with HobA and DnaA have been made using both AD-HobA and BD-HobA fusions mutated libraries as described in the *SI Text*. A summary of the single amino acid substitutions obtained is given, and the corresponding interaction phenotypes are specified by colors. Some substitutions have been obtained more than once, and some residues have been diversely substituted, suggesting a good saturation of the screens. HobA mutations that prevent self-interaction as well as interaction with DnaA are marked in green. These mutations most likely affect the overall structure of the complex. ''Intermediate phenotypes'' (purple) refers to HobA mutant derivatives expressing weak interaction phenotypes, suggesting that they may have retained some interaction with DnaA although diminished. The mutations affecting the ability of HobA to interact with DnaA while retaining self-interaction (light orange) are indicated in red. These mutations are expected to affect residues positioned at the interface with DnaA. One of them, L174P, has been obtained in both screens. Minor variations of phenotypes could be observed for some HobA mutants whether they are fused to AD or BD domains, most probably reflecting a slight conformational difference between the two fusions.

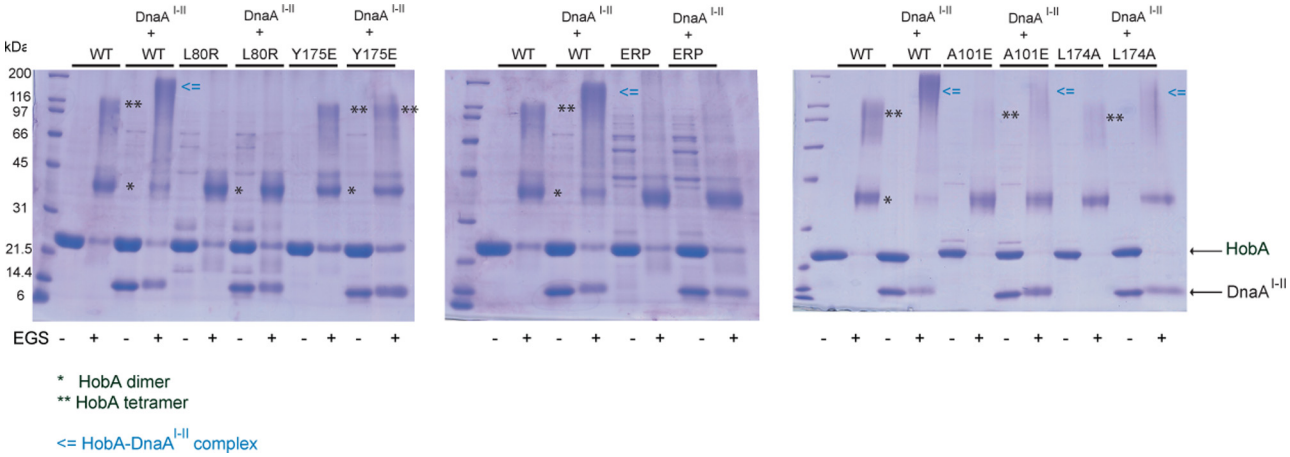
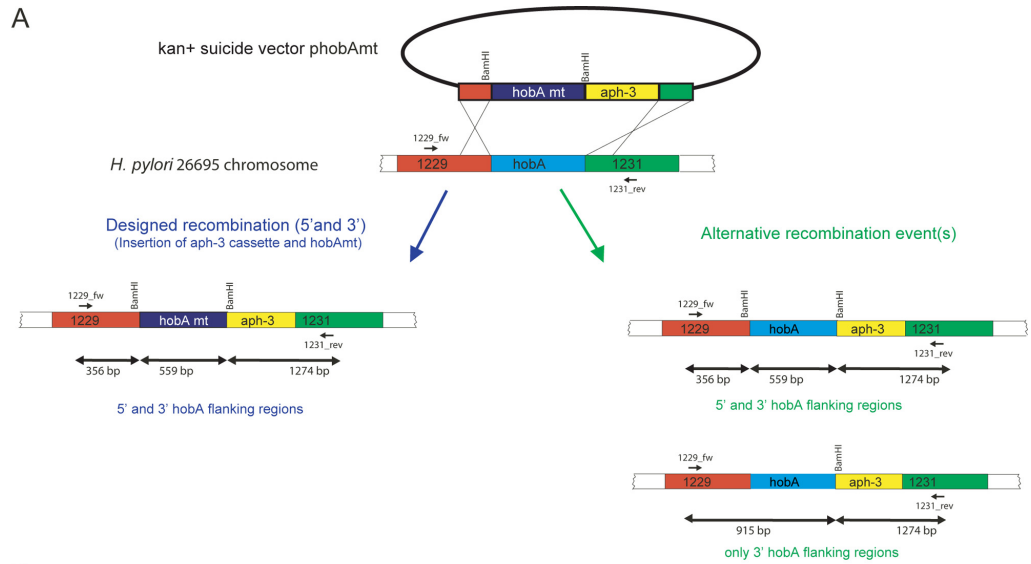
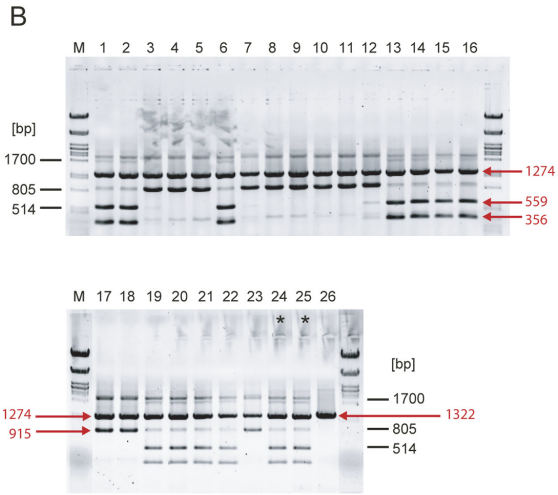


Fig. S3. Cross-linking assay for complex formation between DnaA$^{I–II}$ and HobA mutants. Formation of HobA tetramer and of HobA-DnaA$^{I–II}$ complexes in the absence and presence of EGS crosslinking agent. Blue arrow indicates complexes formed by HobA or HobA mutants with DnaA$^{I–II}$. Single stars indicate the HobA dimer; double stars indicate the HobA tetramer. This assay shows that HobA mutants are defective in forming the complex, except for A101E and L174A, for which some complex could be detected.





	PCR (1229_fw-1231_rev)	*BamHI* restriction fragment(s)
wt	1322 bp	1322 bp
5′ and 3′ recombined	2189 bp	356, 559, 1274 bp
only 3′ recombined	2189 bp	915, 1274 bp

C

phobAmt recombination plasmid	observed recombination at the locus hobA	expected mutations in chromosomal hobA	mutations introduced in chromosomal hobA
A101E	5′ and 3′	C302A, C303G	C302A, C303G
L174A	5′ and 3′	C520G, T521C	C520G, T521C
L80R	5′ and 3′	T238C, T239G	none
	3′		
ERP	5′ and 3′	A227C, C229G, G230C, C232G	none
	3′		
Y175E	5′ and 3′	T523G, C525G	none
	3′		

Fig. S4. Schematic representation of the genetic complementation approach used to study HobA mutants. (*A*) Scheme representing the recombination strategy. The suicide vector pmthobA was used as a donor to introduce simultaneously *hobA* mutants (*hobAmt*) and the *aph-3* cassette (resistance to kanamycin), by recombination between hp1229 (5′) and hp1231 (3′) chromosome regions. Successful 5′-3′recombination leads to the introduction of the *hobAmt* gene and *aph-3* cassette (*left*, blue arrow). In some cases, alternative recombination events occurred and resulted in the introduction of partial fragments at the 3′end or both 5′ and 3′ ends of wild-type *hobA* (*right*, green arrow). Discrimination between these recombinant *H. pylori* colonies is achieved by PCR amplification using 1229_fw and 1231_rev primers followed by BamHI restriction analysis of the PCR products and sequencing. (*B*) Agarose gel showing the results of BamHI digestion analysis of PCR fragments obtained from genomic DNA of 26695 mutants using 1229_fw and 1231_rev primers. Length of PCR fragments obtained with 1229_fw and 1231_rev primers and the corresponding BamHI restriction fragments (colored in red) are presented in the table on the *right.* M, DNA marker (λ/PstI); 1–8, L80R; 9–16, ERP; 17–23, Y175; 24, A101E, 25, L174A, 26, 26695 wt. Red labels correspond to the BamHI fragments lengths in base pairs. Mutants (A101E and L174A) with a desired sequence confirmed by sequencing analysis are marked by star. (*C*) Table summarizing analyzed *H. pylori* mutants. HobA A101E and L174A mutations could be obtained using the designed 5′-3′recombination. In the case of ERP, L80R, and Y175E mutants, both 5′- 3′and 3′ alternative recombinants were observed; in all cases, HobA sequences were that of the wild-type.

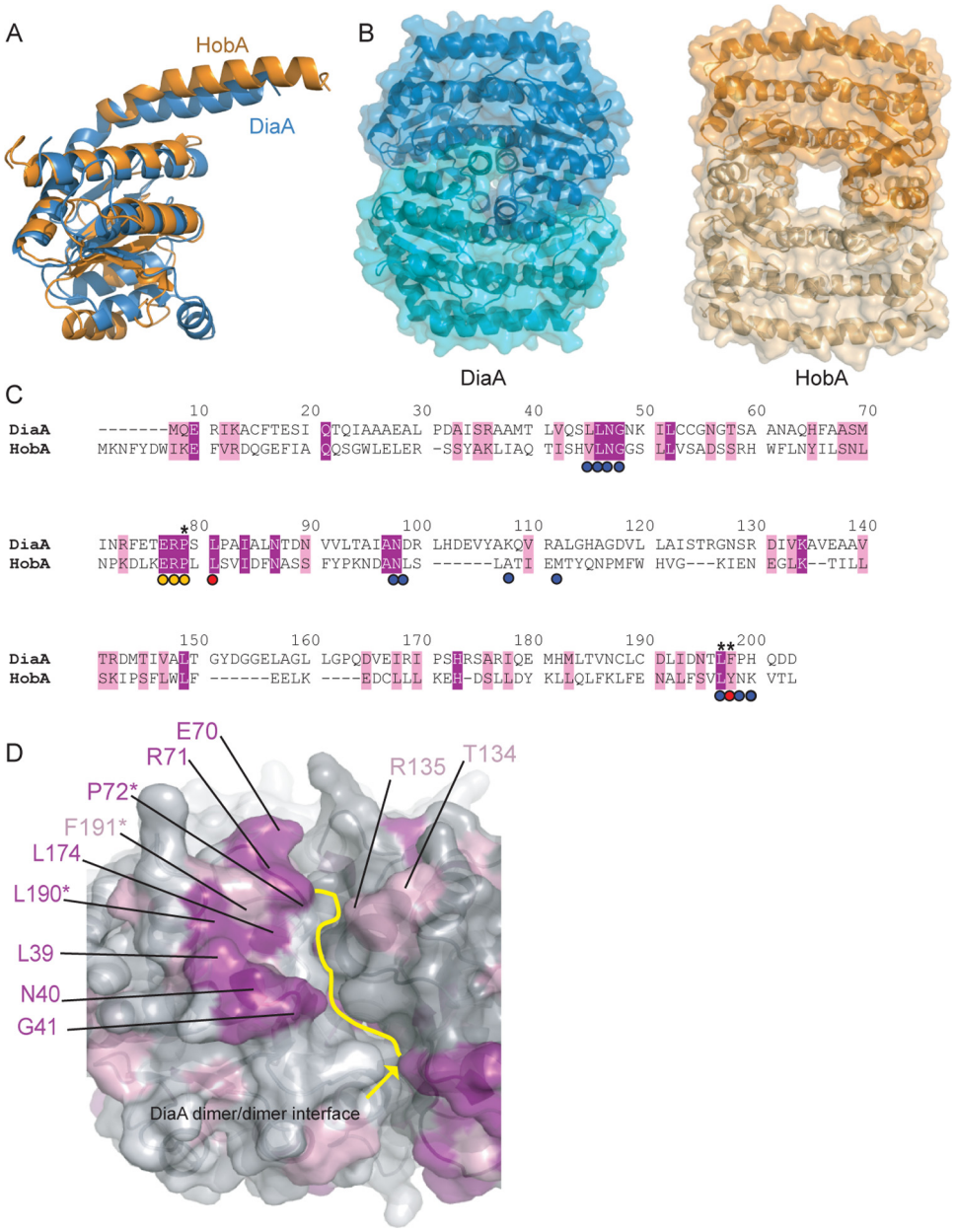


Fig. S5. Structural conservation of the DnaA binding site of HobA in *E. coli* DiaA. (*A*) Ribbon diagram showing the structural superimposition of HobA monomer (orange, pdb code 2UVP) with DiaA monomer (blue, pdb code 2YVA). HobA and DiaA share the same Sugar ISomerase (SIS) fold as proposed by Natrajan et al. (1). (*B*) Comparison between the structures of the DiaA tetramer (*left*) with dimers A/B and C/D colored in blue and light blue, respectively, and the HobA tetramer (*right*). Both proteins share a similar oligomeric organization as dimer of dimers. Although the center of the DiaA tetramer structure is plugged by the arrangement of an extra helix, the HobA tetramer has a solvent channel, as previously observed (1). (*C*) Structure-based sequence alignment of HobA and DiaA. Identical residues are indicated in purple; conserved residues are shown in pink. Blue dots below HobA sequence indicate residues that participate in the interface in the HobA/DnaA complex crystal structure; red dots indicate HobA point mutations resulting in complex formation defect; and yellow dots indicate the ERP motif. Stars indicate residues that were shown to be important in DiaA for DnaA binding. (*D*) Surface representation of DiaA tetramer interface (indicated by yellow line) colored according to the residue conservation as in (*C*). Equivalent residues of DiaA participating in the HobA/DnaA interface are labeled. Stars indicate residues necessary for interaction of DiaA with DnaA as in (*C*).

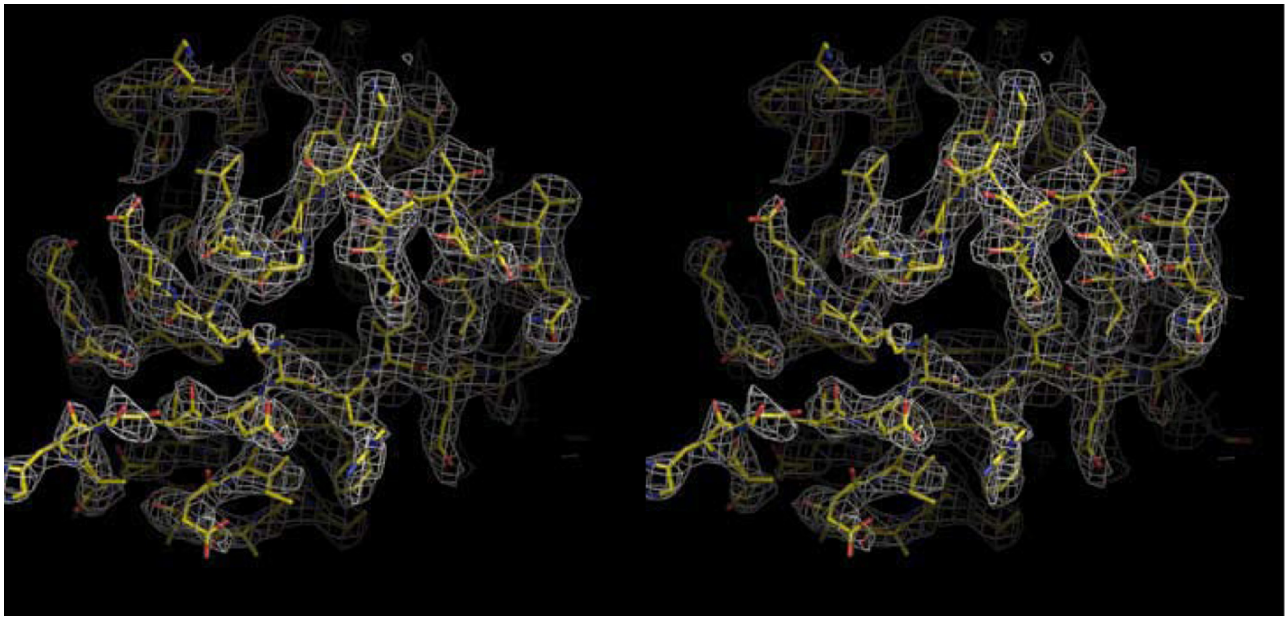


Fig. S6. Representative experimental electron density map. Stereoview of the 2mF0DFc map, contoured at 1σ level.

Table S1. Data collection and refinement statistics

Statistical data	
Cell parameters	
a, b, c (Å)	127.6, 55.9, 96.3
α, β, γ (°)	90, 97.9, 90
Resolution range (Å)	50.0–2.67 (2.80–2.67)
Completeness (%)	93.4 (94.7)
I/σ(I)	6.8 (1.7)
Rmerge	0.116 (0.547)
Redundancy	2.6 (2.5)
No. of reflections	45247 (6558)
Unique reflections	17498(2578)
Refinement statistics	
R	0.2204
R_{free}	0.2647
RMSD (bond) (Å)	0.011
RMSD (angle)(°)	1.448
No. of atoms	4316
No. of waters	156

Data in parentheses belong to the highest-resolution shell.