Dynamic association of the replication initiator and transcription factor DnaA with the Bacillus subtilis chromosome during replication stress

Citation: Breier, Adam M., Grossman, Alan D. Dynamic Association of the Replication Initiator and Transcription Factor DnaA with the Bacillus subtilis Chromosome during Replication Stress J. Bacteriol. 2009 191: 486-493

As Published: http://dx.doi.org/10.1128/jb.01294-08

Publisher: American Society for Microbiology

Persistent URL: http://hdl.handle.net/1721.1/57484

Version: Author's final manuscript: final author's manuscript post peer review, without publisher's formatting or copy editing

Dynamic association of the replication initiator and transcription factor DnaA with the *Bacillus subtilis* chromosome during replication stress

Adam M. Breier and Alan D. Grossman*

Department of Biology

Massachusetts Institute of Technology

Cambridge, MA 02139

running title: Dynamics of *B. subtilis* DnaA binding to DNA in vivo

Key words: *Bacillus subtilis*; DnaA; replication stress; transcription

*Corresponding author:
Department of Biology
Building 68-530
Massachusetts Institute of Technology
Cambridge, MA 02139
phone: 617-253-1515
fax: 617-253-2643
E-mail: adg@mit.edu

Abstract

DnaA functions as both a transcription factor and the replication initiator in bacteria. We characterized the DNA binding dynamics of DnaA on a genomic level. Based on crosslinking and immunoprecipitation (ChIP) data, DnaA binds at least 17 loci, 15 of which are regulated transcriptionally in response to inhibition of replication (replication stress). Six loci, each of which has a cluster of at least 9 potential DnaA binding sites, had significant increases in binding by DnaA when replication was inhibited, indicating that the association of DnaA with at least some of its target sites is altered after replication stress. When replication resumed from *oriC* after inhibition of replication initiation, these high levels of binding decreased rapidly at origin-proximal and origin-distal regions, well before a replication fork could pass through each of the regulated regions. These findings indicate that there is rapid signaling to decrease activation of DnaA during replication and that interaction between DnaA bound at each site and the replication machinery is not required for regulation of DnaA activity in response to replication stress.

Introduction

Cells use multiple mechanisms to detect and respond to perturbations in replication. In bacteria, the well-characterized RecA-dependent SOS response affects expression of many genes after disruptions in replication elongation and DNA damage (13). Additionally, there are *recA*-independent mechanisms that affect gene expression in response to alterations in replication. A significant part of the *recA*-independent response to perturbations in replication (replication stress) appears to be mediated directly by DnaA (15).

DnaA is widely conserved in bacteria and is best known as the replication initiator {reviewed in (23, 33, 40)}. It binds to a 9 bp site that appears multiple times in the origin of replication in bacterial chromosomes. Binding sites are also found upstream of many genes and throughout the chromosome. At replication origins, DnaA mediates melting of DNA (open complex formation) and helps to recruit proteins required for replication. DnaA is a member of the AAA+ family of ATPases and the ATP-bound form (DnaA-ATP) is required for replication initiation (36). Newly synthesized DnaA is predominantly in the ATP-bound form and nucleotide hydrolysis converts DnaA-ATP to the inactive DnaA-ADP after replication initiation. In *E. coli*, the levels of DnaA-ATP and DnaA-ADP are linked to replication status and there can be high levels of DnaA-ATP in cells that are unable to replicate (27).

DnaA is also a transcription factor. DnaA activates or represses transcription of various genes, including its own (1, 3, 26, 34, 39). It is thought that, as is the case for replication initiation, DnaA-ATP is the active form for transcriptional regulation {reviewed in (23, 37, 40)}, and its ability to function as a transcription factor appears to increase when replication initiation or elongation is inhibited (14, 15). Thus, accumulation of DnaA-ATP during replication stress

63 may result in activation of the transcriptional regulatory activity of DnaA as well as its initiator
64 activity.

65 In *Bacillus subtilis*, DnaA appears to control expression of >50 genes in ~20 operons in
66 response to perturbations in replication (15). Transcription of these operons changes in response
67 to inhibition of either replication elongation or replication initiation, independently of the well-
68 characterized RecA-mediated SOS response. Furthermore, these operons contain putative DnaA
69 binding sites in their regulatory regions and DnaA appears to associate with at least six of these
70 in vivo based on results from chromatin immunoprecipitation (ChIP) or affinity purification
71 (ChAP-chip) experiments (15, 20). Alterations in DnaA levels during exponential growth also
72 affect expression of several genes and a genome-wide analysis of DnaA binding to chromosomal
73 DNA demonstrated strong binding to eight intergenic regions, five of which appear to regulate
74 gene expression (20).

75 In this study, we used ChIP-chip and ChIP-PCR to monitor binding of *B. subtilis* DnaA to
76 DNA in vivo during exponential growth and after inhibition of replication. We detected
77 association of DnaA with at least 17 chromosomal regions, 15 of which correspond to operons
78 whose expression responds to perturbations in DNA replication. Following inhibition of
79 replication elongation or initiation, DnaA binding increased significantly at 6 chromosomal
80 regions each of which contains at least 9 potential DnaA binding sites. We found that passage of
81 a replication fork through a regulatory region was not required for release of bound DnaA from
82 that region following the resumption of replication. Our results support the notion that DnaA
83 regulates a global response to perturbations in replication and indicate that alterations in DnaA
84 binding to target promoters contributes to the changes in gene expression following inhibition of
85 replication.

Materials and Methods

Media and growth conditions

For all experiments, cells were grown in $S7_{50}$ defined minimal medium (18, 21) with 0.1% glutamate and supplemented with required amino acids (typically trp and phe). Glucose (1%) was the carbon source except for strains bearing Pxyl-*dnaN*, for which arabinose (1%, together with xylose at 0.1% as an inducer) was used. Cells were typically grown at 32°C and shifted to 47°C for temperature sensitive mutants.

Strains and alleles

Strains used in this study were the lab wild type, AG174 (*trp, phe*; a.k.a., JH642); KPL69 {*dnaB134ts-zhb83*::Tn*917*, *trp, phe* (29, 30, 38)}; KPL73 {*dnaD23ts chr*::Tn917ΩHU151(*mls*), *trp, phe* (29)}; and AIG200 {Δ(*dnaA-oriC-dnaN*)::*spc, amyE*::(PxylA-*dnaN, cat*)*, spoIIIJ*::(*oriN, repN, kan*)*, phe, trp*$^+$ (15)}.

dnaD23ts and *dnaB134ts* are temperature sensitive mutations that prevent initiation of replication at nonpermissive temperature (7, 8, 10, 24, 30).

dnaA is normally essential, but it can be deleted in strains capable of initiating chromosomal replication from a heterologous origin (2, 19, 22, 32). Therefore, in strain AIG200, the plasmid origin of replication, *oriN*, along with the plasmid initiator gene *repN*, was integrated into the chromosome at *spoIIIJ*, near *oriC*. This strain also contains a deletion-insertion that removes *oriC* and the *dnaA-dnaN* operon, and inserts *spc*. *dnaN*, the essential gene encoding the ß-processivity clamp of DNA polymerase that is normally in an operon with *dnaA*, is expressed at a heterologous locus from the xylose-inducible promoter Pxyl (Pxyl-*dnaN*) (2, 15).

Chromatin immunoprecipitation

Chromatin immunoprecipitation of DNA bound to DnaA was done essentially as described (15), with minor modifications. Briefly, protein and DNA were crosslinked with formaldehyde, lysed and sheared with 1 mg/ml lysozyme and sonication, and immunoprecipitated with chicken anti-DnaA primary antibodies, donkey anti-chicken secondary antibodies, and protein A-sepharose beads. Samples were washed and crosslinks reversed and DNA eluted followed by further washing of the beads with 50 mM Tris pH 8, 10 mM EDTA, 0.8% SDS for 5 min at 37°C. The eluate and wash were combined, treated with proteinase K (0.1 mg/ml, 30 minutes, 37ºC), extracted with phenol-chloroform, precipitated with ethanol, and resuspended in 15 µl ddH_2O. Samples of total DNA were taken prior to incubation with primary antibodies and treated with proteinase K (0.1 mg/ml, 30 minutes, 37ºC) in the presence of 0.8% SDS, then subjected to crosslink reversal, phenol-chloroform extraction and ethanol precipitation in parallel with the immunoprecipitates. There were at least 3 biological replicates for each experiment.

ChIP-PCR

For ChIP-PCR, samples were diluted (typically between 1:10 and 1:150) to give an appropriate level of signal. PCRs were done for 28 cycles and products were run on 1.5% agarose gels containing ethidium-bromide and analyzed and quantified with AlphaEaseFC software v4.0 (Alpha Innotech). The locus *yabM* was used as a negative control site for background reference. Enrichment at this locus was at background levels in ChIP-chip data (Fig. 1) and it behaved similarly in ChIP-PCR experiments to other loci where DnaA was not expected to bind (not shown). Despite not having specific binding by DnaA, there was a backgroung signal and this signal was used for normalization. Enrichment was calculated as a ratio of ratios in order to normalize to the background level, as represented by *yabM*: $(IP_i/Total_i)$ /

130 (IP_{yabM}/$Total_{yabM}$) where IP_i and $Total_i$ represent the dilution-adjusted band intensities at the locus
131 of interest.

132 *ChIP-chip*

133 To generate sufficient signal in ChIP-chip experiments, we typically took samples of 100 ml
134 of mid-exponential phase culture (grown in $S7_{50}$ defined minimal medium). DNA samples were
135 prepared for microarray analysis by labeling samples of the resuspended immunoprecipitated
136 DNA or total DNA with aminoallyl-dUTP using 13 U Sequenase (USB Corp.) and 5 µg random
137 nonamer as primers. There was not an amplification step. The labeled samples were purified
138 and conjugated to Cy3 or Cy5. We used DNA microarrays containing PCR products
139 representing >95% of the open reading frames in the *B. subtilis* genome and nearly all of the 295
140 intergenic regions that are >364 bp in length, as described (4, 5, 16). Microarray hybridization
141 and data acquisition were performed as described (4).

142 Enrichment of DnaA at each genomic locus represented on the microarrays was calculated as
143 the relative amount of DNA in the immunoprecipitate divided by the amount of total DNA for
144 that locus without immunoprecipitation. This value was normalized to the background signal
145 calculated as the median value for all chromosomal loci of the amount of DNA in the
146 immunoprecipitate divided by the amount of total DNA. Where presented, enrichment data are
147 plotted according to chromosome position.

148

149 **Results**

150 **Binding of DnaA to several chromosomal regions increases following replication stress**

151 Previously, using ChIP-PCR, we found that DnaA is associated with several putative target
152 operons whose mRNA levels change in response to perturbations in replication and that contain

potential DnaA binding sites (15). Since blocking replication elongation or preventing replication initiation (while allowing ongoing replication to finish) elicits transcriptional responses controlled by DnaA (15), we postulated that binding of DnaA to some targets might increase when replication is inhibited. To test this hypothesis, we monitored DnaA binding at three regulatory targets, *dnaA*, *sda*, and *ywlC*, using ChIP-PCR during replication and after inhibition of replication. Binding was quantified by measuring the enrichment of a given DNA region in the crosslinked and precipitated material relative to that of other chromosomal regions (see Experimental Procedures). We inhibited replication elongation by treating cells with HPUra, a DNA Polymerase III inhibitor (6). We inhibited replication initiation by shifting the temperature sensitive replication initiation mutants *dnaBts* and *dnaDts* to non-permissive temperature and allowing ongoing rounds of replication to finish.

During replication in wild type and the temperature sensitive mutants at permissive temperature, there was low but detectable enrichment for each of the three regions (*dnaA*, *sda*, and *ywlC*) in the DnaA immunoprecipitates, relative to other chromosomal regions (Table 1). Sixty minutes after treatment with HPUra to block replication elongation, there was a significant increase in enrichment of each of these three regions, indicating that association of DnaA with these regions was increased (Table 1). There was also a significant increase in enrichment of these regions 90 min after shifting the *dnaBts* and *dnaDts* mutants to nonpermissive (47°) temperature (Table 1) and the *dnaDts* typically gave higher enrichment levels than the *dnaBts* or treatment with HPUra. There was no increase in enrichment of these regions in wild-type cells incubated at 47°C (Table 1), indicating that the increased binding by DnaA to DNA in the *dnaBts* and *dnaDts* mutants at non-permissive temperature (47°C) was not due to the temperature shift per se.

Analysis of genome-wide DnaA binding

We used ChIP-chip to identify additional chromosomal regions bound by DnaA in vivo. Based on the increase in association of DnaA with *dnaA, sda,* and *ywlC* after replication stress, we analyzed DnaA binding by ChIP-chip under these conditions as well as during exponential growth. We detected high levels of DnaA binding (~10-70-fold enrichment) at 6 chromosomal regions following arrest of either replication elongation or initiation (Figs. 1, 2). The regions include *dnaA, sda, ywlC, yydA*, and the intergenic regions between the 3' ends of *gcp* and *ydiF*, and the 5' ends of *ywcI* and *vpr*. All six of these regions contained at least nine instances of the DnaA box sequence (allowing one mismatch) clustered within a 500 bp region (Fig. 3), and we refer to these as "clustered" sites. Four of the regions with high binding (*dnaA, sda, ywlC, yydA*) are associated with operons whose transcription changes in response to perturbations in replication {Fig. 3 and (15)}. We postulate that the increased binding of DnaA following inhibition of replication could directly affect transcription of these target operons.

DnaA was also bound at the six clustered sites in exponentially growing wild-type cells (Figs. 1C, 2), and in *dnaBts* and *dnaDts* cells at permissive temperature (not shown). However, the enrichment values of ~2-8-fold over background indicated that DnaA binding was significantly lower than that following replication inhibition (~10-70-fold). As above, binding did not increase in wild-type cells incubated at 47°C (not shown).

The increase in DnaA binding at clustered sites after replication was blocked was not due to an increase in the intracellular concentration of DnaA. The increase in binding occurred despite transcriptional auto-repression of *dnaA* (15, 34), which should decrease DnaA levels in response to replication inhibition. As expected, we found that levels of DnaA, as a fraction of total protein, had decreased by approximately 35-50% 60 min after inhibition of replication

199 elongation with HPUra (data not shown). Thus, the increase in DNA binding by DnaA is not
200 due to increased levels of DnaA, but rather is likely due to increased activity of DnaA in
201 response to replication inhibition.
202 To verify that the signal in the ChIP-chip experiments was specific to DnaA, we did similar
203 experiments in the presence and absence of HPUra in a *dnaA* null mutant, in which DnaA has
204 been rendered nonessential by the substitution of *oriC* with the plasmid replication origin *oriN*
205 (2, 15, 19, 22, 32). There was no detectable enrichment of the six clustered loci in the absence of
206 DnaA (Figs. 1E, 2). There was enrichment detected from *yutF* in both *dnaA*$^+$ and *dnaA* null cells
207 (Fig. 1E), indicating that this signal is not due to specific immunoprecipitation of DnaA and is
208 perhaps due to a cross-reacting protein. The enrichment value appeared higher in *dnaA* null
209 cells, probably due to the reduced background signal throughout the chromosome caused by the
210 absence of DnaA and the subsequent lack of non-specific binding throughout the genome. We
211 have excluded *yutF* from further analyses and discussion.
212 In addition to the six strong DnaA binding regions identified above, the ChIP-chip data
213 indicated that there were other regions that were weakly and/or inconsistently enriched.
214 Comparison of ChIP-chip data from three conditions, exponentially growing cells, after
215 inhibition of replication initiation, and after inhibition of replication elongation, indicated that
216 only two additional regions had enrichment levels >2-fold (relative to both the genomic
217 background and the ChIP-chip data from the *dnaA* null mutant) under at least two of the three
218 conditions. One region, near *yrhC*, is in the middle of an operon and its transcription does not
219 appear to change in response to replication stress and is not analyzed further. The other, near
220 *spo0J*, does respond transcriptionally to replication stress (15) and is discussed further below. An
221 independent genome-wide analysis of DnaA binding in untreated cells also identified the six

clustered sites plus *dnaN* and *thdF* (20). We have included *dnaN* in the analysis of *dnaA* as the two genes constitute an operon and the sites are also part of *oriC*. *thdF* did not qualify as significantly enriched in our analysis due to variability, although it was 2.05-fold enriched in arrested *dnaBts* cells. It is possible that our microarray probe for *thdF* was poorly placed to detect bound DnaA.

Analysis of DnaA binding to regions affected by inhibition of replication by ChIP-PCR

Several chromosomal regions previously found to be enriched in DnaA immunoprecipitates in ChIP-PCR experiments (15) were not identified in our ChIP-chip experiments nor in chromatin affinity precipitation (ChAP-chip) experiments (20). This discrepancy indicates that the ChIP-PCR experiments are probably more sensitive than the ChIP-chip or ChAP-chip experiments. Previously we had observed a difference in sensitivity between ChIP-PCR and ChIP-chip for the DNA binding protein Spo0J (4). If the difference in sensitivity between ChIP-chip and ChIP-PCR exists for DnaA, as we expect, then there are likely to be chromosomal regions bound by DnaA that were not detected by the ChIP-chip and ChAP-chip approaches.

Using ChIP-PCR, we tested for DnaA binding at almost all of the operons previously postulated to be regulated by DnaA in response to inhibition of replication (15) for which ChIP-chip results were negative. mRNA levels from these operons change in response to inhibition of replication in a RecA-independent manner, and the regulatory regions have at least two matches to the DnaA box consensus (allowing for one mismatch per box) within 500 bp of the start codon of the first gene (15). We found that nine of the 14 loci tested had statistically significant enrichment in the DnaA immunoprecipitates during replication and/or after inhibition of replication elongation (Table 2). These regions include: *citZ, lysC, yurY, yclN, dnaB, ypvA, kdgR,* and *ykuN* (Table 2). Additionally, *spo0J*, which responds to perturbation of replication

stress but has only one DnaA box within 500 bp of the beginning of its operon (15), was found to bind DnaA in the ChIP-chip experiments (Table 2 and see above). The coding sequence of the *spo0J* operon contains three DnaA boxes.

DnaA binding changed notably at only three of these nine regions following inhibition of replication elongation. The *lysC* region was reproducibly increased in enrichment after inhibition of replication elongation (Table 2). In contrast, the enrichment in the *dnaB* and *yurY* regions significantly decreased after inhibition of replication elongation and was quite high during ongoing replication. This decrease might indicate modulation of DnaA by factors that do not affect binding at other regions. Alternatively, it might indicate different binding specificities for the two different nucleotide bound forms of DnaA, DnaA-ATP and DnaA-ADP.

In contrast to the chromosomal regions with clustered DnaA boxes that had significant increases in DnaA binding after inhibition of replication, most of these regions (Table 2) did not consistently show significant changes in levels of DnaA binding following treatment with HPUra. However, given the uncertainty in the data, we cannot distinguish whether DnaA binding is relatively constant regardless of replication status, or whether increases occur that are not detectable with this approach.

The ChIP-PCR results for the *dnaB* and *yurY* regions differed from the ChIP-chip results. Enrichment levels for these regions were approximately 20-fold in exponentially growing cells in the ChIP-PCR experiments, but neither was found to be significantly enriched in the ChIP-chip experiments; DnaA enrichment at *dnaB* was generally ~2-fold, but it was not statistically significant due to variability, and no enrichment was detected at *yurY*. It is possible that these loci had unusually large differences in sensitivity between the two techniques for reasons such as the location of the probes relative to DnaA binding sites, as the ChIP-PCR primers targeted the

upstream region containing binding sites, and the array probes corresponded to ORFs. The hybridization intensity for the *dnaB* spot on the microarrays was also below average and not of high quality, perhaps contributing to the unreliability of the data.

Five of the 14 loci tested gave inconclusive results. They had enrichment levels at least 1.6-fold above background, but the levels were not statistically significant (Table 2).

Rapid changes in DnaA binding upon resumption of replication

In *E. coli*, Hda is required for the regulatory inactivation of DnaA (RIDA) during replication elongation {(23, 27) and references therein}. It is not known if RIDA activity is completely distributive or requires passage of a replication fork through chromosomal regions bound by DnaA-ATP. We suspected that the increases in DnaA binding and activity that occur when replication is blocked in *B. subtilis* were likely to be reversible. We directly tested binding of DnaA after resumption of replication and found that DnaA was released from DNA and that release occurred in a distributive manner at all the regions tested before passage of a replication fork through each region.

We monitored binding of DnaA to five regions with clusters of binding sites—*dnaA, yydA, ywlC, ywcI,* and *sda*—using ChIP and quantitative PCR while replication was blocked and when replication resumed. Samples were taken: 1) during exponential growth, 2) after inhibition of replication initiation, and 3) at various times following release of the replication block during a synchronized round of replication. If replication forks must interact with DnaA bound at the various regions to facilitate release of DnaA, then DnaA binding should decrease at regions closer to the replication origin before decreasing at distal regions. Alternatively, if DnaA bound at these regions is able to exchange rapidly in response to the presence of active replication

forks, then DnaA binding should decline similarly at all regions, before the replication fork reaches each region.

To block replication, we shifted exponentially growing *dnaDts* cells to the restrictive temperature and waited for 60 minutes to allow most ongoing rounds of replication to finish. As expected, enrichment of the five tested regions in the DnaA immunoprecipitates increased 60 min after inhibition of replication initiation (Fig. 4). Replication was allowed to re-initiate from *oriC* by shifting the *dnaDts* cells back to permissive temperature (32°C). By five minutes after the temperature shift-down, binding of DnaA to the chromosomal regions had already decreased by about 50% or more. After 15 min at permissive temperature, binding of DnaA at each region was near its original level (Fig. 4). The positions of replication forks were estimated based on previous work with synchronized *B. subtilis* cultures (38). The rate of replication elongation is ~0.5 kb/s or ~2.6°/min. A fully synchronous round of bidirectional replication initiating at *oriC* (0°/360°) would result in fork positions at approximately 13° and 347° at 5 minutes, 39° and 321° at 15 minutes, and 104° and 256° at 40 minutes after replication initiation (Fig. 4, inset). However, cells do not actually initiate in a fully synchronous manner. Rather, initiation occurs over a period of 5-10 min after release. Thus, in the cell population, replication forks are spread over the approximately 15°-20° trailing the positions given above.

The similar behavior of these five sites irrespective of chromosomal location indicates that direct interaction between the replication machinery and the DnaA-DNA nucleoprotein complex at each site along the chromosome is not required to control the level of DnaA binding to DNA at each region. Rather, it seems likely that DnaA can exchange rapidly in vivo and respond globally to the presence of an active replication fork.

Discussion

We found that DnaA associates with multiple regions of chromosomal DNA in vivo and that association at the regions that have clustered DnaA boxes increases in response to inhibition of replication. We readily detected binding at the clustered regions, and most were detected previously using ChIP-PCR (15) and ChAP-chip (20). DnaA association with other regions was more difficult to detect with the genomic approaches (ChIP-chip and ChAP-chip) but was detected using ChIP-PCR. We did not detect significant changes in the level of DnaA association with most of these regions in response to perturbations in replication. Clearly, the current techniques are limited in their ability to detect many of the DnaA binding sites in vivo. Nonetheless, there is useful information from analysis of the available data from in vivo experiments.

DnaA binding sites

A combination of in vivo and in vitro analyses demonstrated that the DnaA binding site is a somewhat degenerate 9 bp sequence, $TT^{A}/_{T}TNCACA$ (23, 31), found in multiple copies in bacterial origins of chromosomal replication. Allowing for one mismatch in any of the 9 positions, there are ~12,000 potential binding sites throughout the *B. subtilis* genome. Comparative DNA sequence analyses have been used to estimate the conservation, and hence significance, of some of these sequences (9, 15, 20). In addition, studies of physical association of DnaA with chromosomal DNA revealed binding to several regions that contain multiple potential binding sites (15, 20). The set of DnaA-bound sites that we observed by ChIP-chip and ChIP-PCR very clearly fell into two groups, distinguished on both a functional and sequence level (Fig. 3).

335 *Clustered DnaA binding sites*

336 Clustered DnaA binding sites, which had greater than 9 potential DnaA binding sites with at
337 most 1 mismatch, behaved differently with respect to DnaA binding than regions with only a few
338 potential DnaA binding sites. Enrichment of the regions with clustered DnaA binding sites
339 increased dramatically in response to inhibition of replication. Increased enrichment of these
340 regions with clusters of sites indicates that either the number of bound DnaA molecules
341 increased, or that the crosslinking efficiency of individual molecules increased, perhaps due to a
342 conformational change. These possibilities are not mutually exclusive. DnaA appears to form a
343 polymeric nucleoprotein filament (12), and it is quite possible that this type of structure allows
344 for more efficient binding and/or crosslinking to DNA. Such filamentation was originally
345 proposed for DnaA at *oriC*, but it is reasonable to extend this notion to other chromosomal
346 regions with clusters of DnaA binding sites, analogous to the number of sites in *oriC*. (Other
347 than *oriC*, the regions with clustered sites do not function as replication origins). Both increased
348 binding and a conformational change could be affected by the nucleotide-bound state of DnaA
349 (see below), and changes in binding and conformation are not mutually exclusive; we suspect
350 that both occur.

351 *Non-clustered DnaA binding sites*

352 The other targets of DnaA that we analyzed have fewer potential binding sites, less than nine
353 in a 500-bp region (Fig. 3). We focused on those regions upstream of genes whose expression
354 changes in response to replication stress (15). In most of these regions, there was little or no
355 detectable change in DnaA binding following inhibition of replication. Because the levels of
356 DnaA binding at these regions were generally low relative to the detection limit of the technique,
357 we cannot conclude that the level of DnaA binding did not change. A change of around 50% or

less would be very difficult to detect by ChIP because of the variability between replicates, but potentially could cause a significant change in gene expression. If the amount of binding does not actually change at these regions, then another mechanism must be involved in controlling the genes downstream from these regulatory regions. For example, the nucleotide-bound state of DnaA could change and affect gene expression perhaps via interactions with the transcriptional machinery or alterations in the DNA. Alternatively, there could be other regulatory proteins involved in expression of some of the target operons.

The 9-bp DnaA binding site consensus sequence is quite common when one mismatch is allowed (11,983 occurrences in the genome), and thus there are many potential binding sites throughout the *B. subtilis* genome. A 500 bp region has a 5.5% chance (Poisson statistics) of containing at least four matches. In contrast, the chance of a cluster of sites with 9 or more boxes in a 500 bp region is 0.0018%. Thus, throughout the genome, there are many collections of potential binding sites that are similar in sequence and number to those in the lower part of Figure 3. Given the limited sensitivity of the ChAP-chip (20) and ChIP-chip data, it is likely that some (perhaps many) of these sites are bound by DnaA in vivo. For many transcription factors, degenerate binding sequences are more common than demonstrable regulatory activity (see, e.g., (11, 17, 28)), and DnaA is no different. If these sites are in fact bound by DnaA and serve any function, then it is possible that regulation of expression of nearby genes occurs under specific conditions that have not been tested. Alternatively, these sites may play a more general role in some aspect of DnaA function such as titrating the levels of DnaA available to bind sites in regulatory regions that directly influence gene expression or replication.

Nucleotide binding and regulation of DnaA

DnaA is a member of the AAA+ family of ATPases (25) and, based on work with *E. coli* DnaA, changes in the nucleotide binding state of DnaA serve as a regulatory switch; the ATP bound form is fully active, and the ADP bound form is not, although it binds DNA (23, 37, 40). Applying this model to transcriptional regulatory sites leads to the notion that inhibition of replication causes the accumulation of DnaA in its active, ATP bound form, thereby causing an increase in binding and/or a conformational change resulting in changes in transcription of target operons.

When replication resumes, association of DnaA with chromosomal regions with clusters of sites rapidly returns to normal levels, well before a replication fork has had time to traverse each of the clustered binding regions. These findings indicate that either there is a diffusible signal that controls the activity of DnaA in response to ongoing replication, and/or that DnaA rapidly associates with and dissociates from DNA in vivo. The off rate of DnaA dissociation from its binding site in vitro is a few minutes (35), consistent with the possibility of this type of regulation. For example, if the nucleotide bound state of DnaA is affected by passage of a replication fork through sites to which DnaA is bound, then there would be local conversion of DnaA-ATP to DnaA-ADP. Rapid association and dissociation in vivo could allow for such local changes in DnaA to quickly equilibrate with the pool of DnaA throughout the cell and affect sites far from the replication fork. We are not aware of any measurements of the in vivo exchange of DnaA on and off DNA.

Whereas much is known in *E. coli* about regulation of the activity of DnaA at *oriC* and the regulatory factors involved {(23, 40) and references therein}, many of these factors are not present in *B. subtilis* and other Gram-positives, nor in many Gram-negatives (40). Our findings

indicate that the association of DnaA with some of its target sites significantly changes in response to replication stress in *B. subtilis*. One of the present challenges is to determine how the activity of DnaA is modulated in response to replication stress.

Acknowledgements

This work was supported by NIH grant GM41934 to A.D.G. and NIH Kirschstein NRSA 5 F32 G-076950 fellowship to A.M.B. We thank Stephen P. Bell, Catherine A. Lee, Wiep Klaas Smits, Richard B. Weart, and Lyle A. Simmons for critical reading of the manuscript, Alexi I. Goranov and Melanie M. Berkmen for helpful conversations, and Hannah Blitzblau for technical advice.

References

1. **Atlung, T., E. S. Clausen, and F. G. Hansen.** 1985. Autoregulation of the dnaA gene of Escherichia coli K12. Mol Gen Genet **200:**442-450.
2. **Berkmen, M. B., and A. D. Grossman.** 2007. Subcellular positioning of the origin region of the Bacillus subtilis chromosome is independent of sequences within oriC, the site of replication initiation, and the replication initiator DnaA. Mol Microbiol **63:**150-165.
3. **Braun, R. E., K. O'Day, and A. Wright.** 1985. Autoregulation of the DNA replication gene dnaA in E. coli K-12. Cell **40:**159-169.
4. **Breier, A. M., and A. D. Grossman.** 2007. Whole-genome analysis of the chromosome partitioning and sporulation protein Spo0J (ParB) reveals spreading and origin-distal sites on the Bacillus subtilis chromosome. Mol Microbiol **64:**703-718.
5. **Britton, R. A., P. Eichenberger, J. E. Gonzalez-Pastor, P. Fawcett, R. Monson, R. Losick, and A. D. Grossman.** 2002. Genome-wide analysis of the stationary-phase sigma factor (sigma-H) regulon of *Bacillus subtilis*. J Bacteriol **184:**4881-4890.
6. **Brown, N. C.** 1970. 6-(p-hydroxyphenylazo)-uracil: a selective inhibitor of host DNA replication in phage-infected Bacillus subtilis. Proc Natl Acad Sci U S A **67:**1454-1461.
7. **Bruand, C., A. Sorokin, P. Serror, and S. D. Ehrlich.** 1995. Nucleotide sequence of the Bacillus subtilis dnaD gene. Microbiology **141 (Pt 2):**321-322.
8. **Bruand, C., M. Velten, S. McGovern, S. Marsin, C. Serena, S. D. Ehrlich, and P. Polard.** 2005. Functional interplay between the Bacillus subtilis DnaD and DnaB proteins essential for initiation and re-initiation of DNA replication. Mol Microbiol **55:**1138-1150.
9. **Burkholder, W. F., I. Kurtser, and A. D. Grossman.** 2001. Replication initiation proteins regulate a developmental checkpoint in Bacillus subtilis. Cell **104:**269-279.

10. **Callister, H., S. Le Mesurier, and R. G. Wake.** 1977. Initiation of deoxyribonucleic acid replication in germinating spores of Bacillus subtilis 168 carrying the dnaB (Ts)134 mutation. J Bacteriol **130:**1030-1037.
11. **Comella, N., and A. D. Grossman.** 2005. Conservation of genes and processes controlled by the quorum response in bacteria: characterization of genes controlled by the quorum-sensing transcription factor ComA in Bacillus subtilis. Mol Microbiol **57:**1159-1174.
12. **Erzberger, J. P., M. L. Mott, and J. M. Berger.** 2006. Structural basis for ATP-dependent DnaA assembly and replication-origin remodeling. Nat Struct Mol Biol **13:**676-683.
13. **Friedberg, E., G. Walker, W. Siede, R. Wood, R. Schultz, and T. Ellenberger.** 2005. DNA Repair and Mutagenesis, second edition ed. American Society for Microbiology, Washington, D.C. .
14. **Gon, S., J. E. Camara, H. K. Klungsoyr, E. Crooke, K. Skarstad, and J. Beckwith.** 2006. A novel regulatory mechanism couples deoxyribonucleotide synthesis and DNA replication in Escherichia coli. Embo J **25:**1137-1147.
15. **Goranov, A. I., L. Katz, A. M. Breier, C. B. Burge, and A. D. Grossman.** 2005. A transcriptional response to replication status mediated by the conserved bacterial replication protein DnaA. Proc Natl Acad Sci U S A **102:**12932-12937.
16. **Goranov, A. I., E. Kuester-Schoeck, J. D. Wang, and A. D. Grossman.** 2006. Characterization of the global transcriptional responses to different types of DNA damage and disruption of replication in Bacillus subtilis. J Bacteriol **188:**5595-5605.
17. **Hamoen, L. W., W. K. Smits, A. de Jong, S. Holsappel, and O. P. Kuipers.** 2002. Improving the predictive value of the competence transcription factor (ComK) binding site in Bacillus subtilis using a genomic approach. Nucleic Acids Res **30:**5517-5528.
18. **Harwood, C. R., and S. M. Cutting.** 1990. Molecular biological methods for *Bacillus*. John Wiley & Sons, Chichester, England.
19. **Hassan, A. K., S. Moriya, M. Ogura, T. Tanaka, F. Kawamura, and N. Ogasawara.** 1997. Suppression of initiation defects of chromosome replication in Bacillus subtilis dnaA and oriC-deleted mutants by integration of a plasmid replicon into the chromosomes. J Bacteriol **179:**2494-2502.
20. **Ishikawa, S., Y. Ogura, M. Yoshimura, H. Okumura, E. Cho, Y. Kawai, K. Kurokawa, T. Oshima, and N. Ogasawara.** 2007. Distribution of Stable DnaA-Binding Sites on the Bacillus Subtilis Genome Detected using a Modified ChIP-chip Method. DNA Res **14:**155-168.
21. **Jaacks, K. J., J. Healy, R. Losick, and A. D. Grossman.** 1989. Identification and characterization of genes controlled by the sporulation-regulatory gene spo0H in Bacillus subtilis. J Bacteriol **171:**4121-4129.
22. **Kadoya, R., A. K. Hassan, Y. Kasahara, N. Ogasawara, and S. Moriya.** 2002. Two separate DNA sequences within oriC participate in accurate chromosome segregation in Bacillus subtilis. Mol Microbiol **45:**73-87.
23. **Kaguni, J. M.** 2006. DnaA: controlling the initiation of bacterial DNA replication and more. Annu Rev Microbiol **60:**351-375.
24. **Karamata, D., and J. D. Gross.** 1970. Isolation and genetic analysis of temperature-sensitive mutants of B. subtilis defective in DNA synthesis. Mol Gen Genet **108:**277-287.
25. **Katayama, T.** 2008. Roles for the AAA+ motifs of DnaA in the initiation of DNA replication. Biochem Soc Trans **36:**78-82.

26. **Kucherer, C., H. Lother, R. Kolling, M. A. Schauzu, and W. Messer.** 1986. Regulation of transcription of the chromosomal dnaA gene of Escherichia coli. Mol Gen Genet **205:**115-121.
27. **Kurokawa, K., S. Nishida, A. Emoto, K. Sekimizu, and T. Katayama.** 1999. Replication cycle-coordinated change of the adenine nucleotide-bound forms of DnaA protein in Escherichia coli. Embo J **18:**6642-6652.
28. **Lazazzera, B., T. Palmer, J. Quisel, and A. D. Grossman.** 1999. Cell density control of gene expression and development in *Bacillus subtilis*, p. 27-46. *In* G. M. Dunny and S. C. Winans (ed.), Cell-Cell Signaling in Bacteria. ASM Press, Washington DC.
29. **Lemon, K. P., I. Kurtser, J. Wu, and A. D. Grossman.** 2000. Control of initiation of sporulation by replication initiation genes in Bacillus subtilis. J Bacteriol **182:**2989-2991.
30. **Mendelson, N. H., and J. D. Gross.** 1967. Characterization of a temperature-sensitive mutant of Bacillus subtilis defective in deoxyribonucleic acid replication. J Bacteriol **94:**1603-1608.
31. **Messer, W.** 2002. The bacterial replication initiator DnaA. DnaA and oriC, the bacterial mode to initiate DNA replication. FEMS Microbiol Rev **26:**355-374.
32. **Moriya, S., A. K. Hassan, R. Kadoya, and N. Ogasawara.** 1997. Mechanism of anucleate cell production in the oriC-deleted mutants of Bacillus subtilis. DNA Res **4:**115-126.
33. **Mott, M. L., and J. M. Berger.** 2007. DNA replication initiation: mechanisms and regulation in bacteria. Nat Rev Microbiol **5:**343-354.
34. **Ogura, Y., Y. Imai, N. Ogasawara, and S. Moriya.** 2001. Autoregulation of the dnaA-dnaN operon and effects of DnaA protein levels on replication initiation in Bacillus subtilis. J Bacteriol **183:**3833-3841.
35. **Schaper, S., and W. Messer.** 1995. Interaction of the initiator protein DnaA of Escherichia coli with its DNA target. J Biol Chem **270:**17622-17626.
36. **Sekimizu, K., D. Bramhill, and A. Kornberg.** 1987. ATP activates dnaA protein in initiating replication of plasmids bearing the origin of the E. coli chromosome. Cell **50:**259-265.
37. **Speck, C., C. Weigel, and W. Messer.** 1999. ATP- and ADP-dnaA protein, a molecular switch in gene regulation. Embo J **18:**6169-6176.
38. **Wang, J. D., G. M. Sanders, and A. D. Grossman.** 2007. Nutritional Control of Elongation of DNA Replication by (p)ppGpp. Cell **128:**865-875.
39. **Wang, Q. P., and J. M. Kaguni.** 1987. Transcriptional repression of the dnaA gene of Escherichia coli by dnaA protein. Mol Gen Genet **209:**518-525.
40. **Zakrzewska-Czerwinska, J., D. Jakimowicz, A. Zawilak-Pawlik, and W. Messer.** 2007. Regulation of the initiation of chromosomal replication in bacteria. FEMS Microbiol Rev **31:**378-387.

Table 1. DnaA binding during exponential growth and replication stress.

	Enrichment value ± standard error (fold-change)[1]							
	dnaBts		*dnaDts*		HPUra		wt	
locus	32°	47°	32°	47°	-	+	32°	47°
dnaA	2.2 ± 2.3	42.2 ± 9.5 (18.8)	8.8 ± 2.4	180 ± 130 (20.4)	4.8 ± 0.9	61.4 ± 1.9 (12.7)	6.4 ± 4.0	6.3 ± 3.4 (1.0)
sda	1.7 ± 0.4	6.8 ± 1.0 (4.0)	2.2 ± 0.5	15.7 ± 4.2 (7.1)	2.0 ± 0.5	4.4 ± 0.1 (2.2)	1.3 ± 0.04	1.5 ± 0.4 (1.2)
ywlC	2.3 ± 0.3	9.7 ± 0.8 (4.3)	3.4 ± 1.2	70.0 ± 3.1 (20.7)	2.8 ± 0.4	14.4 ± 5.9 (5.1)	1.7 ± 0.38	1.6 ± 0.1 (0.9)

[1] Values presented are the mean (from three independent biological replicates) enrichments of the indicated DNA regions (*dnaA, sda, ywlC*) measured by ChIP-PCR analysis ± the standard error. Data are normalized to the *yabM* locus, which does not bind DnaA specifically (Fig.1 and data not shown). Values in parentheses are the fold-changes for the indicated pair. Strains used were KPL69 (*dnaBts*), KPL73 (*dnaDts*), and AG174 (wt; ±HPUra). Cells were grown at 32°C and treated with HPUra for 60 min. Temperature shifts were to 47° and samples were taken after 90 min at that temperature.

Table 2. ChIP-PCR analysis of DnaA binding at putative regulatory targets[1]

	- HPUra		+ HPUra		+/-HPUra
locus[2]	enrichment ± SE	*p*-value	enrichment ± SE	*p*-value	*p*-value
citZ	1.3 ± 0.8	(0.72)	2.5 ± 0.4	(0.03)	0.40
dnaB	26.4 ± 9.2	(0.01)	0.9 ± 0.4	(0.72)	0.002
flgB	2.0 ± 1.1	(0.32)	1.0 ± 0.3	(0.97)	0.31
kdgR	1.8 ± 0.1	(0.01)	3.3 ± 1.6	(0.17)	0.34
lysC	1.7 ± 0.4	(0.16)	4.7 ± 1.1	(0.02)	0.05
nrdI (*nrdEF ymaB*)	1.8 ± 0.3	(0.07)	2.2 ± 1.1	(0.18)	0.69
pyrP	2.6 ± 0.6	(0.04)	3.5 ± 2.4	(0.13)	0.63
spo0J[3] (*soj*)	3.9 ± 0.4	(0.001)	6.3 ± 1.7	(0.02)	0.62
sunA (*sunT*)	4.4 ± 1.7	(0.06)	8.6 ± 4.8	(0.08)	0.42
yclN (*yclOPQ*)	5.4 ± 1.6	(0.03)	6.8 ± 2.1	(0.03)	0.62
ykuN (*ykuOP*)	5.4 ± 1.2	(0.02)	4.8 ± 3.3	(0.10)	0.85
yllB (*ylxA ftsL pbpB*)	1.8 ±0.5	(0.18)	1.7 ± 0.9	(0.35)	0.91
ypvA	3.1 ± 0.7	(0.05)	2.7 ± 0.3	(0.02)	0.65
yurY (*yurX csd yurVU*)	21.7 ± 5.2	(0.01)	5.2 ± 1.1	(0.02)	0.01
ywzC (*ywfO ywgA*)	4.2 ± 1.7	(0.05)	3.2 ± 1.6	(0.12)	0.62

[1] Wild-type cells (AG174) untreated (-HPUra) and treated with HPUra (+HPUra) for 60 min were harvested and analyzed by ChIP-PCR for DnaA binding at the indicated loci. Values presented are the mean enrichments ± standard error from at least three independent biological replicates, normalized to the *yabM* locus, which is not specifically bound by DnaA. *p*-values are in parentheses (*t*-test). *p*-values comparing the results with (+) and without (-) HPUra are indicated in the last column.

[2] Operons that respond transcriptionally to replication stress and have at least 2 potential DnaA binding sites were chosen for analysis. Loci are sorted alphabetically by the first gene. Additional genes in a given operon that are also affected by replication stress are indicated in parentheses.

[3] Data for *spo0J* are taken from the ChIP-chip results (Fig. 1).

544 **Figure Legends**

545 **Figure 1. Genome-wide DnaA ChIP-chip profiles in arrested, replicating, and *dnaA* null**
546 **cells**. The enrichment of each chromosomal region in the DnaA ChIP relative to total DNA is
547 plotted versus chromosomal position relative to *oriC* at 0°, in the center of the graphs. For each
548 data set, at least three biological replicates were collected, and the median for each locus on the
549 arrays is shown.

550 A. Wild-type cells (AG174) in exponential growth were treated with HPUra for 60 min at
551 32°C, crosslinked, and harvested for ChIP-chip.

552 B. *dnaDts* (KPL73) cells in exponential growth at 32°C were shifted to the restrictive
553 temperature (47°C) for 90 minutes, crosslinked, and harvested for ChIP-chip.

554 C. *dnaBts* (KPL69) cells in exponential growth at 32°C were shifted to the restrictive
555 temperature (47°C) for 90 minutes, crosslinked, and harvested for ChIP-chip.

556 D. Wild-type cells (AG174) in exponential growth at 32°C were crosslinked and harvested
557 for ChIP-chip.

558 E. *dnaA* null mutant cells (AIG200) in exponential growth at 32°C were crosslinked and
559 harvested for ChIP-chip. The signal at 283° (-77° in the figure) is *yutF*; there appears to be a
560 protein bound here that interacts nonspecifically with the anti-DnaA antibody (see text).

561

562

562 **Figure 2. DnaA binding at loci with clustered DnaA boxes.** Data are shown for specific
563 regions from Fig. 1, plotted for ~8 kb regions centered around clustered DnaA boxes where
564 DnaA binding increased substantially upon inhibition of replication. Chromosomal positions are
565 listed as distance (in degrees) from *oriC* and enrichment is as described in Fig. 1. DnaA box
566 locations are indicated by diamonds below the data traces; filled and open diamonds represent
567 perfect and single-mismatch boxes, respectively. Gene locations and orientations are indicated
568 with arrows. Expression of genes with asterisks is altered after inhibition of replication (15).
569 Expression of all of the genes indicated here with asterisks decreases after replication inhibition,
570 except for that of *sda*, which increases. Data are shown in the vicinities of *dnaA* (A), *ywlC* (B),
571 *ywcI* (C), *yydA* (D), *gcp* (E), and *sda* (F), respectively. Filled squares with solid lines represent
572 wild type cells (AG174) 60 min after treatment with HPUra; filled circles with dotted lines
573 represent untreated wild type cells (AG174); gray open circles and lines represent *dnaA* null
574 mutant cells (AIG200); and filled triangles with solid lines represent *dnaDts* cells (KPL73) after
575 90 min at 47°C.
576
577

Figure 3. Distribution of DnaA boxes at sites bound by DnaA. Shown are locations of perfect (solid circles) and single mismatch (triangles) DnaA boxes occurring from 750 bp upstream to 375 bp downstream of the start codon of genes where DnaA binding was detected, either in ChIP-chip or ChIP-PCR experiments. The first six regions indicated (above the thick line) all have at least 9 potential DnaA binding sites with ≤1 mismatch from consensus and have increased enrichment after inhibition of replication (Table 1; Figs. 1, 2). Regions indicated below the thick line are those with fewer than 9 potential DnaA binding sites. Enrichment at these regions did not consistently increase when replication was inhibited, and DnaA enrichment at *flgB, nrdI, sunA,* and *yllB* was greater than at the background locus *yabM* but not statistically significant (Table 2). At right are the transcriptional changes in each of these genes due to inhibition of elongation with HPUra and inhibition of initiation by high-temperature incubation of *dnaDts* cells. Listed in parentheses are genes in the same operon that also responded transcriptionally. Data are from (15). Bracketed values are not statistically significant.

Functions of the genes potentially regulated by DnaA have been summarized previously (15, 20) and include genes involved in: DNA replication (*dnaA, dnaN, dnaB*, and perhaps *ypvA*), cell division (*ftsL, pbpB*), nucleotide metabolism (*nrdI, nrdE, F*), pyrimidine biosynthesis (*pyrP*), lantibiotic biosynthesis (*sunA, T*), sporulation (*sda, spo0J*), and several unknowns ("y" genes).

†*sda* and the *yqeGH-aroD-yqeIJK* operon are divergently transcribed and both respond to inhibition of replication; the *yqeG* operon decreases ~2-3 fold.

*The region shown for *gcp* is downstream of its 3' end.

**Gene expression values are listed for *ywfO*; *ywzC* did not respond to replication inhibition.

599 **Figure 4. Release of DnaA binding after resumption of replication.** *dnaDts* cells
600 (KPL73) in exponential growth were shifted to restrictive temperature for 60 minutes and then
601 shifted back to permissive temperature in order to generate a synchronous round of replication
602 initiation. Samples were taken for ChIP-PCR analysis immediately before and at the end of the
603 incubation at restrictive temperature, and 2, 5, 10, 15, and 40 minutes after the shift back to
604 permissive temperature. DnaA binding was analyzed at *oriC/dnaA* (solid diamonds, black lines);
605 *yydA* (gray asterisks, gray dashed lines); *ywcI* (solid triangles, black lines); *ywlC* (black X
606 characters, black dashed lines); and *sda* (filled gray squares, solid gray lines). Data were
607 normalized according to the basal and maximal level of signal detected to facilitate comparison
608 of binding kinetics at sites with different levels of DnaA binding (see, for example, levels at
609 *dnaA/oriC* and *sda* in Fig. 2). The inset shows the positions of each analyzed site on the
610 chromosome and the approximate extent of replication at 5, 15, and 40 minutes after release.
611

Fig. 1

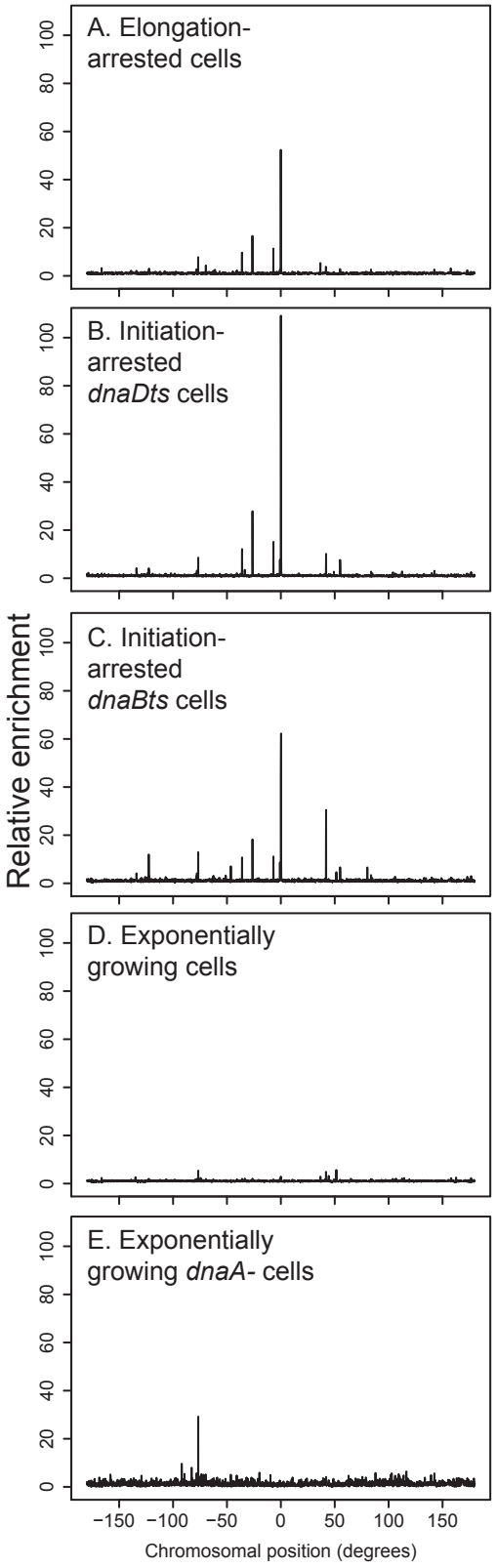

A. Elongation-arrested cells
B. Initiation-arrested *dnaDts* cells
C. Initiation-arrested *dnaBts* cells
D. Exponentially growing cells
E. Exponentially growing *dnaA*- cells
Relative enrichment
0
20
40
60
80
100
−150
−100
−50
0
50
100
150
Chromosomal position (degrees)

Fig. 2

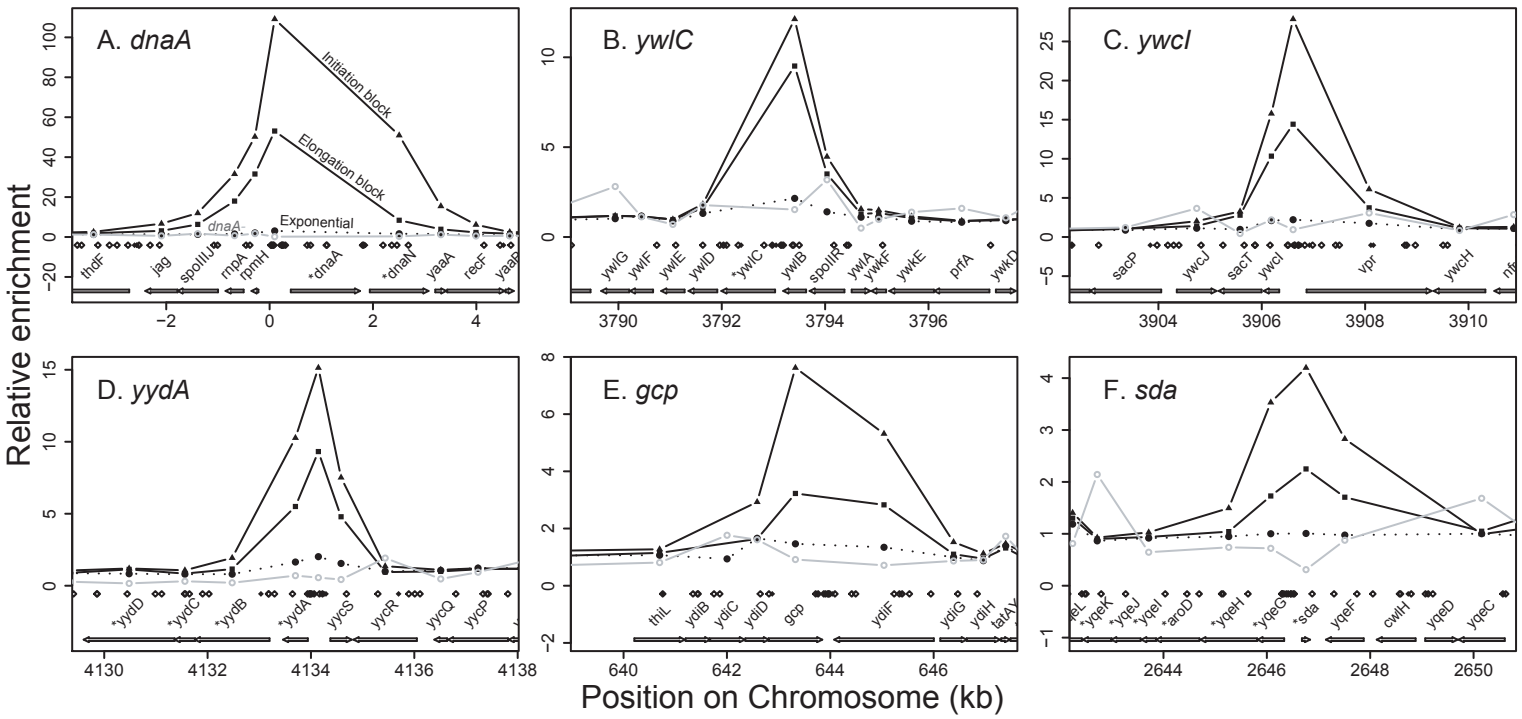
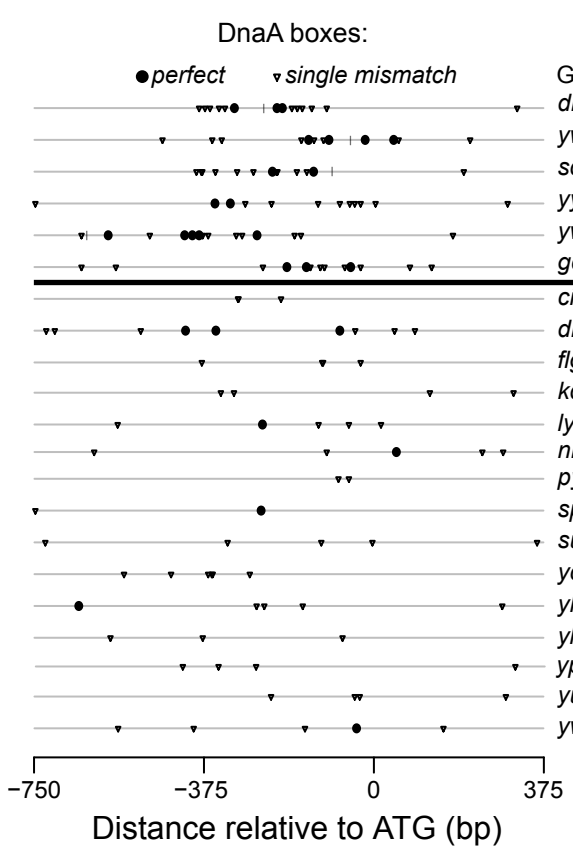
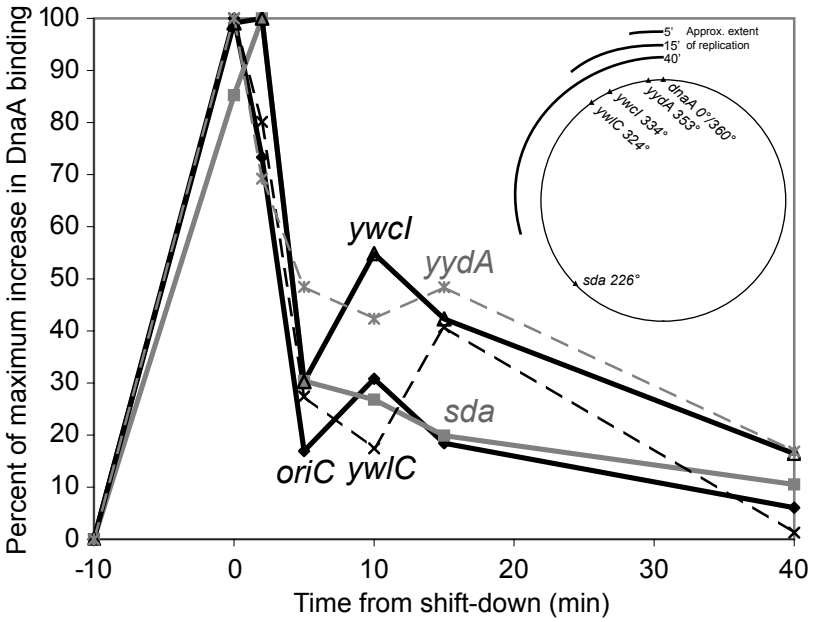

A. dnaA
B. ywlC
C. ywcI
D. yydA
E. gcp
F. sda
Initiation block
Elongation block
Exponential
dnaA
Relative enrichment
Position on Chromosome (kb)

Fig. 3



Gene	Fold change in gene expn. HPUra	*dnaDts*
dnaA (dnaN)	-7.7	-7.4
ywlC	-3.2	-3.2
sda†	2.0	2.0
yydA (yydBCD)	[-1.5]	-1.6
ywcI	[-1.2]	[1.1]
*gcp**	[1.2]	[1.1]
citZ	2.1	1.8
dnaB	-2.4	N/A
flgB	-2.3	[-1.1]
kdgR	-2.3	-1.8
lysC	-5.6	-4.3
nrdI (nrdEF ymaB)	[1.5]	2.8
pyrP	-3.0	[-1.5]
spo0J (soj)	[-1.4]	-1.9
sunA (sunT)	-3.2	-2.0
yclN (yclOPQ)	-3.8	-1.8
ykuN (ykuOP)	-16.4	[1.1]
yllB (ylxA pbpB ftsL)	-2.5	-3.3
ypvA	1.8	1.7
yurY (yurX csd yurVU)	2.3	[1.7]
*ywzC (ywfO ywgA)***	2.8	2.3

Fig. 4


Percent of maximum increase in DnaA binding
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
Time from shift-down (min)
ywcI
yydA
sda
oriC
ywlC
5'
15'
40'
Approx. extent of replication
dnaA 0°/360°
yydA 353°
ywcI 334°
ywlC 324°
sda 226°