A transcriptional response to replication status mediated by the conserved bacterial replication protein DnaA

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Organisms respond to perturbations in DNA replication. We characterized the global transcriptional response to inhibition of DNA replication in Bacillus subtilis. We focused on changes that were independent of the known recA-dependent global DNA damage (SOS) response. We found that overlapping sets of genes are affected by perturbations in replication elongation or initiation and that this transcriptional response serves to inhibit cell division and maintain cell viability. Approximately 20 of the operons (>50 genes) affected have potential DnaA-binding sites and are probably regulated directly by DnaA, the highly conserved replication initiation protein and transcription factor. Many of these genes have homologues and recognizable DnaA-binding sites in other bacteria, indicating that a DnaA-mediated response, elicited by changes in DNA replication status, may be conserved.

Materials and Methods

Strains and Alleles. Standard procedures were used for strain construction and growth conditions (21). Relevant strain genotypes are indicated in the text and legends, and detailed descriptions of the B. subtilis strains used are in Supporting Materials and Methods and Table 1, which are published as supporting information on the PNAS web site.

Media and Growth Conditions. Cells were grown in defined minimal medium (see Supporting Materials and Methods) supplemented with glucose (1%), glutamate (0.1%), and required amino acids.

For experiments in which replication elongation was arrested with 6-hydroxy-phenylazo-uracil (HPUra), cells were grown at 37°C, and HPUra (38 µg/ml final concentration) was added during midexponential growth. For experiments using temperature-sensitive mutants, cells were grown at 32°C and shifted to 46°C during midexponential growth. For microarray analysis, 7.5-ml samples were taken from treated and untreated cultures grown in parallel before and 15, 30, and 60 min after addition of HPUra or 30, 60, and 90 min after shift to nonpermissive temperature.

DNA Microarrays. DNA microarrays were prepared by using PCR products from >99% of the annotated B. subtilis ORFs spotted onto Corning GAPS II slides, essentially as described in ref. 22. A reference sample was added to each experimental sample for normalization and comparisons. Briefly, all microarray experiments were done at least three times and evaluated by using statistical analysis of microarrays (SAM) (23). For all experiments, a change in mRNA levels between one condition and another is considered statistically significant if there is <1% probability that this change occurred by chance (false positive discovery rate <1%). Details are described in Supporting Materials and Methods.

Abbreviations: HPUra, 6-hydroxy-phenylazo-uracil; SOS, global DNA damage.

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Chromatin Immunoprecipitations. DNA fragments bound to DnaA were immunoprecipitated as described in ref. 24, except that extracts were successively incubated with 1:10,000 diluted chicken anti-DnaA, 1:500 diluted donkey anti-chicken, and 3% protein-A Sepharose beads, all for 1 h at room temperature. Serial dilutions of total DNA and immunoprecipitated DNA were analyzed by PCR. Equal volumes (10 μl) were loaded onto a 2% agarose gel stained with ethidium bromide. Similar results were obtained with DNA samples from parallel cell cultures, untreated or treated with HPURA, were taken at various times for a direct comparison. Data presented are from 60 min after treatment with HPURA, except for results for the Fur and PerR regulons, which were maximally affected 15 min after replication-fork arrest with HPURA. (C–E) For experiments with temperature-sensitive mutants, cells were grown at permissive temperature (32°C) to midexponential phase then were shifted to nonpermissive temperature (46°C); samples taken at various times after shift. Data from 90 min after temperature shift are presented. (C) Temperature-sensitive helicase mutant dnaCts (strain KI365), compared with wild type (strain AG174). (D) Temperature-sensitive initiation mutant dnaDts recA (strain KI1365) compared with dnaB+ recA (strain KI1365). (E) Temperature-sensitive initiation mutant dnaDts (strain KPL73), compared with wild type (AG174). (F) Presence of potential DnaA-binding sites. A black cell indicates the presence of at least two potential DnaA-binding sites (allowing one mismatch from consensus) within the 500 bp upstream of the first gene of the operon.

To inhibit replication elongation, we treated cell cultures with HPURa, which binds to the catalytic (alpha) subunit of DNA polymerase, encoded by polC (27). In the recA sda double mutant, treatment with HPURa affected (99% confidence) the mRNA levels of 108 genes in 55 operons that were also affected in wild-type cells (Fig. 1; and see also Table 2, which is published as supporting information on the PNAS web site). The 55 operons contain a total of 131 genes, but the changes in 23 of the genes did not pass our statistical criteria (see Materials and Methods). Fifteen of the operons had increased and 40 had decreased mRNA levels after inhibition of replication elongation. In 43 of the 55 operons, the magnitude of these effects was 2- to 7-fold less in recA mutant cells than in recA+ cells (see Discussion).

We also arrested replication elongation using a temperature-sensitive mutation (dnaC30ts) in the gene for the replicative DNA helicase. Upon shift of the helicase mutant (dnaC30ts, recA+, sda+) to nonpermissive temperature, replication elongation rapidly stops (28). We measured changes in mRNA levels in the helicase mutant at various times after the shift to nonpermissive temperature and found that the response was remarkably similar to that caused by replication arrest due to treatment with HPURa. Of the 131 genes in the 55 operons that were affected by HPURa in the recA sda double mutant, 107 were also affected by temperature shift in the helicase mutant (Fig. 1 and Table 2), including peroxide-inducible recA-dependent SOS response (3).

In B. subtilis, RecA affects expression of ≥300 genes (A.I.G., E. Kuester-Shoeck, J. D. Wang, and A.D.G., unpublished work). In addition, Sda, which is induced by replication arrest (12), indirectly affects the expression of >100 genes involved in sporulation and other stationary phase phenomena, all controlled by Spo0A (12, 26). To identify regulatory responses that are independent of recA and sda, some of our analyses were done in recA sda double mutants.

Fig. 1. Changes in mRNA levels in response to inhibition of replication. The relative mRNA levels in cells blocked for replication was determined by using cDNA microarrays. Results are shown for genes in the 55 operons significantly affected by blocking replication elongation with HPURa in both wild type and the recA sda double mutant. (A–E) Brightest green represents ≥3.5-fold decrease, and brightest red represents ≥3.5-fold increase in relative mRNA levels, in response to a block in replication. Gray cells indicate no data for the particular gene in the particular experiment. Genes are organized by operon and functional categories and are presented in the same order as in Table 2. Arrows next to operon names or descriptions indicate the direction of the effect. (A and B) Inhibition of replication elongation with HPURa. (A) A wild type (strain AG174). (B) recA sda double mutant (strain AIG9). Samples from parallel cell cultures, untreated or treated with HPURa, were taken at various times for a direct comparison. Data presented are from 60 min after treatment with HPURa, except for results for the Fur and PerR regulons, which were maximally affected 15 min after replication-fork arrest with HPURa. (C–E) For experiments with temperature-sensitive mutants, cells were grown at permissive temperature (32°C) to midexponential phase then were shifted to nonpermissive temperature (46°C); samples taken at various times after shift. Data from 90 min after temperature shift are presented. (C) Temperature-sensitive helicase mutant dnaCts (strain KI365), compared with wild type (strain AG174). (D) Temperature-sensitive initiation mutant dnaDts recA (strain KI1365) compared with dnaB+ recA (strain KI1365). (E) Temperature-sensitive initiation mutant dnaDts (strain KPL73), compared with wild type (AG174). (F) Presence of potential DnaA-binding sites. A black cell indicates the presence of at least two potential DnaA-binding sites (allowing one mismatch from consensus) within the 500 bp upstream of the first gene of the operon.

Results

Inhibiting Elongation of DNA Replication Affects Multiple Genes Independently of RecA and Sda. Production of stalled replication forks by arresting replication elongation is known to induce the recA-dependent SOS response (3). In B. subtilis, RecA affects expression of ≥300 genes (A.I.G., E. Kuester-Shoeck, J. D. Wang, and A.D.G., unpublished work). In addition, Sda, which is induced by replication arrest (12), indirectly affects the expression of >100 genes involved in sporulation and other stationary phase phenomena, all controlled by Spo0A (12, 26). To identify regulatory responses that are independent of recA and sda, some of our analyses were done in recA sda double mutants.
genes, iron-regulated genes, and genes involved in nucleotide biosynthesis, DNA replication, and cell division (see below). The extent of the effects was similar to that observed in wild-type cells treated with HPUra (Fig. 1 and Table 2). Thus, the vast majority of the effects of HPUra on mRNA levels are likely caused by the effects of HPUra on replication elongation and not by some other effect on cell physiology. These results indicate that two different mechanisms of inhibiting replication elongation induce a common transcriptional program that is independent of the well characterized recA-dependent SOS response.

Many Genes Respond to Inhibition of Either Elongation or Initiation of DNA Replication. The changes in gene expression caused by arresting replication elongation might be due to the presence of a stalled replication fork, and/or to the absence of active replication. To distinguish between these possibilities, we arrested replication in the absence of significant fork stalling, inhibiting replication initiation by shifting a temperature-sensitive dnaB (dnaBts) mutant to the nonpermissive temperature. B. subtilis dnaB encodes a protein required for replication initiation, and inactivation of this protein prevents initiation of replication but allows elongation to continue (29, 30). Many genes (~84 in 26 operons) affected by inhibiting replication elongation in cells lacking recA and sda were also affected by inhibiting replication initiation in dnaBts, recA mutant (Fig. 1 and Table 2). Similar results were observed by inhibiting replication initiation by using a temperature-sensitive mutation in dnaD (Fig. 1 and Table 2).

Because a set of genes responds to perturbations in both initiation and elongation of DNA replication, it appears that the mechanism(s) regulating expression of these genes does not require the detection of stalled replication forks.

Affected Genes Are Involved in Many Essential Cellular Processes. The genes affected by perturbations in replication initiation and elongation (independently of recA and sda) are involved in many aspects of bacterial physiology (Fig. 1 and Table 2). The genes are involved in replication (dnaA, dnaN, and dnaB), cell division (yibB-ylcA-fsl-lycA-ywlC), amino acid and nucleotide biosynthesis (lysC, proBA, pur, and pyr operons, and nrdEF), and perhaps translation and ribosome assembly (yqehL and ywLC) and other unknown functions.

Other operons are affected only by inhibiting replication elongation. One of the earliest and strongest effects of blocking replication elongation was the induction of the PerR regulon (peroxide-inducible) and repression of the Fur regulon (iron-responsive). These responses are known to help cells survive oxidizing conditions (31).

The Regulatory Response to Replication Status Is Mediated, in Part, by DnaA. One of the most interesting effects of replication status is an expression of genes known to be controlled by DnaA. In B. subtilis, DnaA, the essential and highly conserved replication initiation protein and transcription factor, is known to repress expression of dnaA and dnaN (20) and to activate expression of sda (12). Consistent with previous findings (12, 32), we observed that the expression of dnaA and dnaN was decreased and that of sda was increased by inhibition of either replication initiation or elongation (Fig. 1 and Table 2).

We searched for potential DnaA-binding sites in the regulatory regions upstream (within 500 bp upstream of the start codon) of operons containing genes affected in response to inhibition of replication elongation in the recA sda mutant. By using a strict consensus sequence for the DnaA-binding site TTT(a/t)NCACA (33) and allowing only one mismatch, we found that, of the 55 operons affected by inhibition of replication elongation, 34 (71 genes) contained at least two potential DnaA-binding sites (Figs. 1 and 2A and Table 2). Eighteen of these 34 operons were also significantly affected by inhibition of replication initiation (Fig. 1 and Table 2). An additional 15 operons (53 genes) contained one potential DnaA-binding site (Fig. 1 and Table 2).

Assuming a Poisson distribution of DnaA-binding sites across the genome, it is expected that <1 of every 10 random operons will have two or more DnaA-binding sites (8.5% chance of finding >1 DnaA-binding site within a 500-bp region, based on ~42% guanosine and cytosine content of B. subtilis). The total number of operons in the B. subtilis genome with consensus binding sites or >1 site with one mismatch agrees with this estimate. In contrast, 62% of the 55 identified operons had more than one potential DnaA-binding site. There are operons with more than one potential binding site that were not affected under the conditions analyzed. These potential sites might not be properly positioned to affect transcription. There might also be additional regulatory factors affecting expression of these operons, or we might not fully understand what constitutes a DnaA-binding site.

Binding of DnaA to Putative Regulatory Regions in Vivo. To test whether DnaA is associated with some of the regulatory regions of the affected operons, we used chromatin immunoprecipitation (ChIP) with anti-DnaA antibodies (Fig. 2B). The six promoters with putative DnaA-binding sites tested (dnaA, sda, ylbB (fsl), dnaB, ywlC, and yydA (Fig. 2B)). Based on these findings, we propose that, in B. subtilis, DnaA controls a global regulatory response to perturbations in replication. In addition, we suspect that DnaA also binds to potential sites upstream of other identified operons. Based on this finding, we suggest that at least 19 operons (52 genes) are regulated directly by DnaA.

Repression of an Essential Cell Division Gene Couples Replication Status to Cell Division in a recA-Independent Manner. One of the most important and conserved aspects of the cellular response to DNA damage and perturbations in replication is the inhibition of cell division. The characterized mechanisms for inhibiting cell division in E. coli and B. subtilis involve the RecA-dependent induction of division inhibitors (3, 4). There are also RecA-independent mechanisms for inhibiting cell division (10, 11), although the genes responsible for this inhibition have not been identified.

Perturbations in replication caused a recA-independent decrease in expression of an operon that contains genes required for cell division. mRNA levels for ylbB, ylcA, fslL, and pbpB decreased in the absence of ongoing replication (Fig. 1 and Table 2). Both fslL and pbpB are essential for cell division (34). FslL is an unstable protein (35, 36); a decrease in fslL mRNA levels quickly causes a decrease in FslL protein and inhibits cell division (37). Conversely, PbpB is likely to be a stable protein. Depleting PbpB alone in B. subtilis results in very slow inhibition of cell division (data not shown), and the PbpB homologue in E. coli, FtsL, has been reported to be a stable protein (38, 39).

If a decrease in the expression of this operon contributes significantly to the inhibition of cell division in the absence of replication, then continued expression of these genes under conditions of replication-fork arrest should allow cell division to continue. To test this hypothesis, we constructed a strain that has two copies of fslL, one under its normal regulation and one controlled by the LacI-repressible-IPTG-inducible promoter Pspac and a single copy of pbpB under control of Pspac. This construct rendered the cells dependent on inducer (IPTG) for growth, because expression of pbpB was IPTG-dependent. In this strain, in the presence of IPTG, the mRNA levels of fslL after replication arrest are approximately equal to mRNA levels in the untreated wild-type strain, as shown.
There was a significant difference in the size distribution between after replication-fork arrest induced by the addition of HPUra. Division (4).

Gene responsible for much of the in the ymaA ORF, shown as a dashed line. The relative location and direction of potential DnaA-binding sites (arrowheads) in the regions 500 bp upstream of the first gene in the operon are shown. Filled arrowheads represent consensus DnaA-binding sites, and open arrowheads represent single mismatch sites. The bar at the top indicates the distance, in base pairs, from the start of the gene indicated at the right. Published reports summarizing RNA-polymerase-binding sites were used to label the transcription start sites, indicated by an arrow and +1 (12, 65). DnaA binds in vivo to the promoters of genes affected by perturbations in replication. Cultures of dnaA (AIG174) or dnaA (AIG200) strains growing exponentially were treated with formaldehyde and collected for chromatin immunoprecipitation analysis. DnaA and cross-linked DNA was immunoprecipitated with an anti-DnaA antibody. Total DNA was collected from the same samples before the addition of antibody. The cross-links were reversed, and the precipitated DNA was analyzed by PCR, with primers targeting the promoter regions of the genes listed, except for the dnaA primers that amplify the oric region between dnaA and dnaN. We obtained similar results with primers for the DnaA promoter as well (not shown). ysl and yxb are genes with no putative DnaA bindings sites (negative controls). In the dnaA strain, the oric region is deleted, and no PCR product is detected. This strain initiates replication from an ectopic origin orin that does not require DnaA (66).

by microarrays (data not shown). To focus on the effects of fisL and pppB in the recA-independent response, we also deleted yneA, the gene responsible for much of the recA-dependent inhibition of cell division (4).

We measured cell length (an indicator of cell-division frequency) after replication-fork arrest induced by the addition of HPUra. There was a significant difference in the size distribution between fisL and Pspac-fisL-pppB cells (Fig. 3A). The average length of fisL cells 150 min after replication-fork arrest was 5.0 ± 2.8 μm (average cell length ± SD), compared with 3.2 ± 1.6 μm for the Pspac-fisL-pppB cells (P < 0.05). In contrast, before replication arrest, the average cell lengths were virtually indistinguishable, 1.8 ± 0.42 μm and 2.0 ± 0.53 μm for fisL and Pspac-fisL-pppB cells, respectively (Fig. 3B).

In yneA cells, the effects were similar (data not shown), but the average cell length was longer because of the contribution of YneA to inhibiting cell division. Similar results were obtained by placing the whole operon (ysl-yneA-fisL-pppB) under the control of an inducible promoter (data not shown).

In addition to effects on cell division, constitutive expression of fisL-pppB during replication-fork arrest caused an increase in cell lysis (Fig. 3C) and a decrease in cell viability (Fig. 3D). Even though there is a general loss of viability after prolonged exposure to HPuA, the Psps-pppB strain experienced a four- to eightfold greater loss in viability 120 min after replication-fork arrest, as compared with the identically treated fisL strain (Fig. 3D). These results indicate that regulation of the fisL operon in response to replication status is important for the proper control of cell division and maintenance of cell viability.

Even under conditions where both the recA-dependent and recA-independent effects on cell division were bypassed by expression of fisL in a yneA-null mutant, there was a partial inhibition of cell division after replication-fork arrest (compare 2.0 μm before and 3.2 μm after replication arrest in strain AIG107). We suspect...
Conservation of potential DnaA-binding sites in other bacteria. Shown are the first genes of the 31 operons in B. subtilis that are affected by inhibition of replication elongation, have more than one potential DnaA binding site, and have homologues in the genomes of the other bacteria. The yllB operon contains ftsL, and the ymaA operon contains nde-F. Seven different genomes were obtained from GenBank and were searched for homologues of the B. subtilis (Bs) operons. Filled boxes, more than one putative DnaA-binding site with one mismatch or two or more DnaA-binding sites with one mismatch; empty boxes with diagonal line, no binding sites matching the consensus or with one mismatch. In all examined genomes, the number of homologous genes that have a consensus site in their operon structure in B. subtilis was not conserved within the given genome. For each identified homologue, we searched 500 bp upstream of the start site of the first ORF in the operon for the presence of consensus DnaA-binding sites, allowing for zero or one mismatch. Filled boxes, more than one putative DnaA-binding site with one mismatch or at least one consensus DnaA-binding site; shaded boxes, single binding site with one mismatch; empty boxes with diagonal line, no binding sites matching the consensus or with one mismatch. In all examined genomes, the number of homologous genes that have a consensus site in two or more DnaA-binding sites with one mismatch was higher than expected by chance. The chance of finding sites in similar locations in multiple genomes is considerably lower than that for an individual genome. The numerical data for this figure are presented in Table 3.

Conservation of Genes and Potential DnaA-Binding Sites Among Bacterial Species. Because DnaA is found in virtually all bacteria, and several of the genes affected in B. subtilis have homologues in E. coli that are also regulated by DnaA, we thought that DnaA might be controlling a similar set of genes in other organisms. We first searched several bacterial genomes for homologues of the genes that are affected by replication status and have putative DnaA binding sites in B. subtilis. We then searched for potential DnaA-binding sites in the regions upstream of these conserved genes. Strikingly, many of the homologous genes have putative DnaA-binding sites (Fig. 4; see also Table 3, which is published as supporting information on the PNAS web site). In all bacterial genomes that we searched, the number of identified homologous operons with more than one DnaA binding site is higher than expected by chance (Fig. 4 and Table 3). These findings indicate that these homologous operons with potential DnaA-binding sites are likely to be regulated directly by DnaA in several different bacterial species.

Discussion

Perturbations in DNA replication are fairly common, even during normal growth (2), and cells respond to these perturbations by altering gene expression and cell physiology to increase their chances of survival. We analyzed the global transcriptional response elicited by inhibiting different steps of DNA replication in the bacterium B. subtilis and found that expression of >100 genes is altered independently of the well-characterized recA-dependent SOS response. This response alters the expression of genes involved in several physiological processes, including DNA replication and cell division. In addition, our results show that there are mechanisms for detecting the absence of replication that are independent of sensing stalled replication forks.

In recA-null cells, the transcriptional effect on most genes was less than that in wild-type cells (Fig. 1 and Table 2). The vast majority of these operons have no discernible LexA-binding sites and are not known to be repressed by LexA. Therefore, the differences caused by the presence or absence of RecA are likely due to the ability of hypercell division. In addition, our results show that there are mechanisms for detecting the absence of replication that are independent of sensing stalled replication forks.

Conserved Regulation by DnaA. A significant portion of the recA-independent transcriptional response appears to be mediated directly by the conserved replication-initiation protein and transcription factor DnaA. Many of the genes affected have potential DnaA-binding sites in their regulatory regions, and DnaA is associated with at least several of these regions in vivo. In addition, some of the genes affected in B. subtilis have homologues in other species with potential DnaA-binding sites upstream of the ORFs, indicating that the regulatory response may be highly conserved in bacteria.

Altogether, we propose that at least 20 operons (56 genes) are likely to be regulated directly by DnaA (Fig. 2). We used stringent criteria for identifying potential DnaA binding sites and regulated genes (>1 binding site with zero or one mismatch upstream of the operon affected). There are examples in E. coli where one site with one mismatch and another more degenerate site are sufficient for DnaA-dependent regulation. Additionally, some genes controlled by DnaA have binding sites downstream of the promoter regions, sometimes within an ORF (47, 48). These cases would have been missed by our analysis. Additional DnaA targets might include operons like soj-spo0J (which has a consensus site within the spo0J ORF), yqmA-sipW-tasA (which has >15 sites with two mismatches), and yeeA (which has one site with one mismatch). These three operons responded to both inhibition of initiation and elongation of DNA replication. Thus, it seems likely that the DnaA regulon includes more genes than those identified here.

Inhibiting Transcription of an Essential Cell-Division Gene Contributes to Coupling Cell Division to DNA Replication. We observed that expression of the essential cell-division gene ftsL is inhibited by perturbations in replication and that this inhibition is important for maintaining cell viability in B. subtilis. Many bacteria have FtsL homologues that are likely unstable and DnaA regulated (Fig. 4 and Table 3) (49). Therefore, the inhibition of cell division by DnaA-mediated inhibition of ftsL may be conserved. Decreased synthesis of an unstable protein is an effective way to regulate gene expression and, in the case of ftsL, inhibit cell division.
The transcriptional response to replication perturbations couples replication to development, metabolism, and, perhaps, translation. The transcriptional response to perturbations in replication induces expression of \( sda \) in a DnaA-dependent manner (12, 15). Sda couples sporation to DNA replication status (12). If this coordination is lost, cells that experience DNA damage continue to develop and lose viability (12). Thus, at least two of the operons regulated by this global response (\( sda \) and \( f5L \)) affect cell viability.

Increased transcription of \( ndfEFR \), which encodes nucleotide reductase needed for deoxyribonucleotide biosynthesis, is a common response to DNA damage and replication-fork arrest in several organisms (50, 51). The increase in ribonucleotide reductase in \( Saccharomyces \) \( cerevisiae \) is regulated by this global response (12). Thus, at least two of the operons regulated by this global response affect cell viability.

Expression of the \( ymaA-nrdE-nrdF-ymaB \) operon is positively affected by inhibition of either replication initiation or elongation in \( B. \) \( subtilis \). There is one potential DnaA-binding site with one mismatch within the \( ymaA \) promoter region and two one-mismatch and one consensus site within \( ymaA \) (Fig. 24). Based on these observations, we suggest that the \( ymaA \) operon in \( B. \) \( subtilis \) is regulated by DnaA.

Many of the genes affected by perturbations in replication have no known function but have been shown to be essential. Two genes, \( ywIC \) and \( yqH \), may be involved in translation, judging by their homology to translation factors. Thus, the transcriptional response to perturbations in replication may adapt cellular physiology by modulating the translational machinery.

Accumulation of the ATP-Bound Form of DnaA May Affect Gene Expression. We suspect that variations in the amount of DnaA bound to ATP affect transcription in response to perturbations in replication. The ATP-bound form of DnaA is active in replication initiation and in modulating transcription (55–56). In \( E. \) \( coli \), the amount of ATP-DnaA is regulated during the cell cycle by ongoing replication through an intermediary regulatory protein Hda (57, 58). B. \( subtilis \) lacks an obvious orthologue of Hda but may still regulate ATP hydrolysis by DnaA (59). Arrest of replication elongation leads to an accumulation of ATP-DnaA in \( E. \) \( coli \) (57). We suggest that arrest of replication elongation or initiation allows high levels of ATP-DnaA to accumulate and persist which alters the transcription of many genes.

Because the levels of ATP-DnaA fluctuate from 20% to 80% of total DnaA during a normal \( E. \) \( coli \) cell cycle, the expression of at least some of the DnaA-regulated genes fluctuates as well (57, 60–62). The fluctuation of ATP-DnaA levels is one of the mechanisms that regulates the timing of initiation of DNA replication (57, 63, 64). We suggest that cycling of ATP-DnaA levels couples replication and other physiological processes, including cell division and development. This DnaA-mediated effect on gene expression has at least two roles: (i) to time and regulate cell-cycle gene expression and (ii) to induce a response to perturbations in replication that help the cells survive.

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