Regulation of the replication cycle: conserved and diverse regulatory systems for DnaA and *oriC*

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Abstract | Chromosomal replication must be limited to once and only once per cell cycle. This is accomplished by multiple regulatory pathways that govern initiator proteins and replication origins. A principal feature of DNA replication is the coupling of the replication reaction to negative-feedback regulation. Some of the factors that are important in this process have been discovered, including the clamp (DNA polymerase III subunit-β (DnaN)), the *datA* locus, SeqA, DnaA homologue protein (Hda) and YabA, as well as factors that are involved at other stages of the regulatory mechanism, such as DnaA initiator-associating protein (DiaA), the DnaA-reactivating sequence (DARS) loci and Soj. Here, we describe the regulation of DnaA, one of the central proteins involved in bacterial DNA replication, by these factors in *Escherichia coli*, *Bacillus subtilis* and *Caulobacter crescentus*.

Chromosomal replication must be highly regulated to ensure that the number of chromosomes in the cell remains constant. This means that chromosomal DNA must be replicated only once per cell cycle. To ensure that this is the case, bacterial, archaeal and eukaryotic cells have evolved sophisticated regulatory mechanisms that control the initiation reaction of chromosomal replication.

Initiation of DNA replication in all three domains of life requires the formation of highly organized nucleoprotein complexes on the chromosomal replication origin (*oriC*) in the DNA[1–6] (FIG. 1). The formation of these multimeric complexes is induced by specific proteins, including DnaA in bacteria and ORC (origin recognition complex) proteins in eukaryotes. To initiate DNA replication, the nucleoprotein complex is activated and undergoes a conformational change that leads to local unwinding of the duplex DNA[1,5,6] (FIGS 1;2).

Subsequently, in *Escherichia coli* the replicative DNA helicase (DnaB), which forms a complex with the helicase-loader DnaC in solution, is loaded onto the single-stranded region in a manner mediated by DnaC and *oriC*-bound DnaA, resulting in the formation of the prepriming complex[6] (FIG. 2). DnaB then migrates along the DNA, expanding the region of single-stranded DNA[6]. DNA primase (DnaG) and the DNA polymerase III holoenzyme (which consists of the Pol III* subassembly and the clamp, DNA polymerase III subunit-β (DnaN)) are subsequently loaded onto the DNA to form the replisome and mediate the synthesis of primer RNA and complementary DNA, respectively[1,6,7] (FIG. 2). Pol III* contains the catalytic centre and the activity required for loading the clamp, which is a ring-shaped DNA-binding protein that encircles the DNA and tethers Pol III* to the DNA[6–8]. After the synthesis of Okazaki fragments, the clamp remains loaded on the nascent DNA (FIG. 2) while Pol III* loads another clamp and mediates the synthesis of the next Okazaki fragment[7].

After the initiation reaction, the initiation proteins and the origin DNA are inactivated to prevent re-initiation in the same round of the cell cycle[4,5,9] (FIG. 1). This process is coupled with the progress of chromosomal replication and other cell cycle events. In *E. coli* and *Bacillus subtilis*, the main regulatory mechanism of replication initiation is a negative-feedback loop that inhibits the replication reaction, largely by inhibiting the activity of DnaA[9] (FIG. 1).

Similar principles operate in eukaryotic cells. In addition to the replication-coupled feedback system (FIG. 1), in these cells cyclin-dependent kinase (CDK)-dependent regulatory cascades control the assembly of proteins at the origin region, the timely activation of the origin complexes at S phase entry and the inactivation of the initiation proteins during S phase, through changes in CDK activities at various stages throughout the cell cycle[10–13]. This is known as a cascade-type system (FIG. 1).

In this Review, we describe different levels of regulation of DNA replication in bacteria, with a focus on the regulation of the activity of DnaA in *E. coli*, *B. subtilis* and *Caulobacter crescentus*.

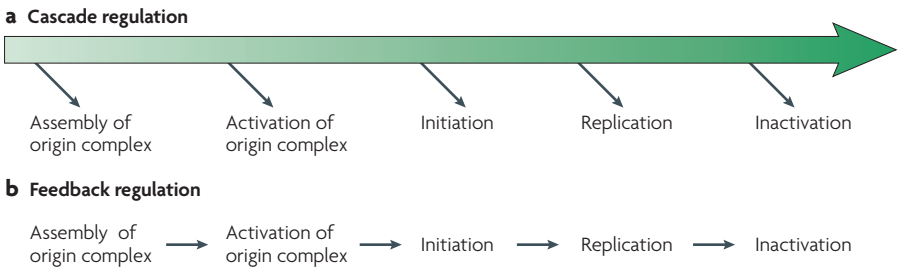

Figure 1 | **Regulatory systems for replication initiation in bacteria and eukaryotes. a** | In the cascade type of regulation, the ordered assembly of proteins at the replication origin and the downstream events are regulated by a mechanism that is itself controlled by cell cycle progression. The activated complex must be inactivated in a timely manner so as to repress extra initiation reactions. **b** | In the feedback type of regulation, the ordered progression of initiation and inactivation is ensured by replication-coupled negative-feedback regulation.

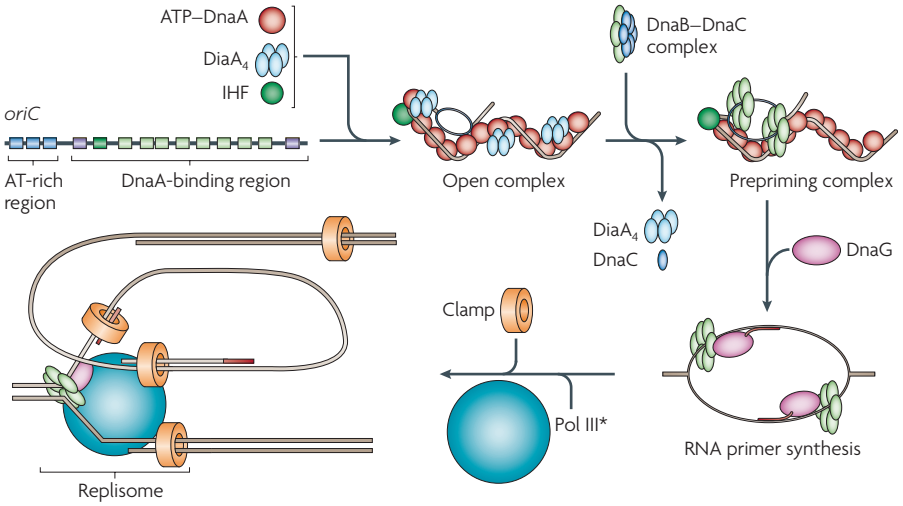

Figure 2 | **Model mechanism of initiation at the origin of replication in *Escherichia coli*.** The chromosomal replication origin (*oriC*) region contains AT-rich repeats, each 13 residues long, and the DnaA-binding region, which carries high-affinity sites (purple boxes) at each end as well as low-affinity sites (light-green boxes). The integration host factor (IHF)-binding site (dark green box) is also located in this region. ATP–DnaA molecules bind the DnaA-binding region with the aid of DnaA initiator-associating protein (DiaA) tetramers and IHF, which results in multimerization of ATP–DnaA and local duplex unwinding of the AT-rich region. Replicative DNA helicase (DnaB) in the DnaB–DnaC complex is loaded onto this single-stranded region in a manner that is dependent on its interaction with DnaA. During this process, DnaC and DiaA are released from the *oriC* complex. DnaB expands the single-stranded region, and DNA primase (DnaG) is loaded and synthesizes primers. DNA polymerase III holoenzyme, which consists of the Pol III* subassembly and the clamp (DNA polymerase III subunit-β (DnaN)), is loaded onto the primed sites and synthesizes the complementary DNA strands. After the synthesis of the Okazaki fragment, the clamp remains loaded on the DNA, and Pol III* loads another clamp molecule onto the newly primed site to synthesize the next Okazaki fragment. In this figure, only a single replication fork is shown and single-stranded DNA-binding protein is omitted for simplicity.

Replication initiation in *E. coli*

The initiation of chromosomal replication has been best studied in *E. coli*, both *in vivo* and *in vitro*. The *E. coli* replication origin, *oriC*, has a conserved AT-rich region and a region carrying multiple DnaA boxes, which are 9-nucleotide-long sequences that function as specific binding sites for DnaA[1,6] (FIG. 2). DnaA is a member of the AAA+ (ATPase associated with diverse cellular activities) protein family and is highly conserved in bacterial species[1–3]. In *E. coli*, DnaA forms a tight complex with ATP or ADP, but only ATP–DnaA can initiate replication[1–3,6]. The cellular level of ATP–DnaA fluctuates during the replication cycle, with a peak at the time of initiation, and this fluctuation is used as a means of regulating the correct timing of DNA replication[14]. Initiation of replication from *oriC in vivo*[6] requires DnaA, DnaB and DnaC; DnaB and DnaC are also required for the elongation process of *in vivo* replication[6]. Integration host factor (IHF), a histone-like protein, and DnaA initiator-associating protein (DiaA) stimulate timely initiation in a cell cycle-coordinated manner *in vivo*[1,6,15–17] (FIG. 2).

Biochemical analysis using reconstituted systems with purified proteins has revealed that 10 to 20 ATP–DnaA molecules bind cooperatively to the DnaA boxes in *oriC*[1,3,6,17] (FIG. 2). The resultant DnaA multimer is suggested to take on a spiral shape, like those of some well-analysed AAA+ proteins[18,19]. ATP–DnaA and ADP–DnaA bind with similar affinity to two DnaA boxes (called R1 and R4) at the edges of the DnaA-binding region in *oriC*[1,17,19–22]. However, ATP–DnaA binds more efficiently than ADP–DnaA to additional DnaA boxes (designated R2, R3 and R5) and derivative DnaA-binding sites (called I1–I3 and τ1–τ2; these sites reside between the R1 and R4 boxes), even though these sites have a lower affinity for ATP–DnaA than R1 and R4[3,17,19–22]. ATP–DnaA binding to these low-affinity sites is crucial for conformational activation of the resultant complex and for unwinding of the AT-rich region in *oriC*, which results in formation of the open complex[19–21] (FIG. 2).

IHF assists in the formation of DnaA multimers by binding to a specific site in *oriC*, leading to bending of the DNA and enhancing ATP–DnaA binding to the I1–I3 sites[22,23] (FIG. 2). In addition, DiaA enhances ATP–DnaA binding to all low-affinity sites[16,17,24] (FIG. 2). DiaA forms homotetramers in solution that bind multiple DnaA molecules and stimulate DnaA assembly on *oriC*[17]. In the initiation complex containing the ATP–DnaA multimer and DiaA, duplex DNA is unwound at an AT-rich region in *oriC*[17,24].

DnaA has four functional domains that are involved in protein–protein and protein–DNA interactions[1,3] (FIG. 3). In *E. coli*, DnaA domain I mediates inter-DnaA interactions, DiaA binding and loading of DnaB onto *oriC*–DnaA complexes[24–26]. Domain II is an unfolded, flexible linker region[25]. Domain III carries several AAA+ motifs (including a typical Walker motif and unique ATP-interacting motifs) and the sites that bind to single-stranded DNA[3,19,21,27–30]. The carboxy-terminal domain IV contains a helix–turn–helix fold that binds directly and specifically to DnaA box sequences[1,3,31,32].

Regulation of initiation in *E. coli*

Negative regulation of* oriC*: binding of SeqA. The *E. coli oriC* contains 11 copies of the sequence GATC. SeqA binding to *oriC* and the subsequent inhibition of replication initiation (FIG. 4a) requires methylation of the A residues of these motifs by DNA adenine methylase (Dam)[1,6,33–37]. Semi-conservative replication results in hemimethylated GATC sites, which are the preferential binding sites for SeqA[33,34] (TABLE 1). The SeqA-binding region overlaps with the low-affinity DnaA-binding sites, and SeqA binding to the hemimethylated DNA inhibits ATP–DnaA binding[33,38,39]. In this way, a replication-coupled negative-feedback loop is generated that targets *oriC* and inhibits re-initiation after a single round of replication (FIG. 4a). Inactivation of *seqA* causes extra initiations at a moderate level that does not inhibit cell growth[33]. Subcellular localization studies suggest that SeqA molecules assemble on the nascent, hemimethylated DNA region around the replication fork[40–42].

Negative regulation of DnaA: transcriptional repression. *E. coli* has at least three systems that negatively regulate DnaA function to ensure that replication occurs only once per cell cycle (FIG. 4a; TABLE 1). The first of these is the repression of *dnaA* transcription immediately after replication initiation[43]. The *dnaA* gene is located near *oriC*, and immediately after their replication the multiple hemimethylated GATC sequences in the gene are bound by SeqA[1,33,44], which represses transcription of the gene[33,43] (FIG. 4a; TABLE 1). Placing *dnaA* near the terminus

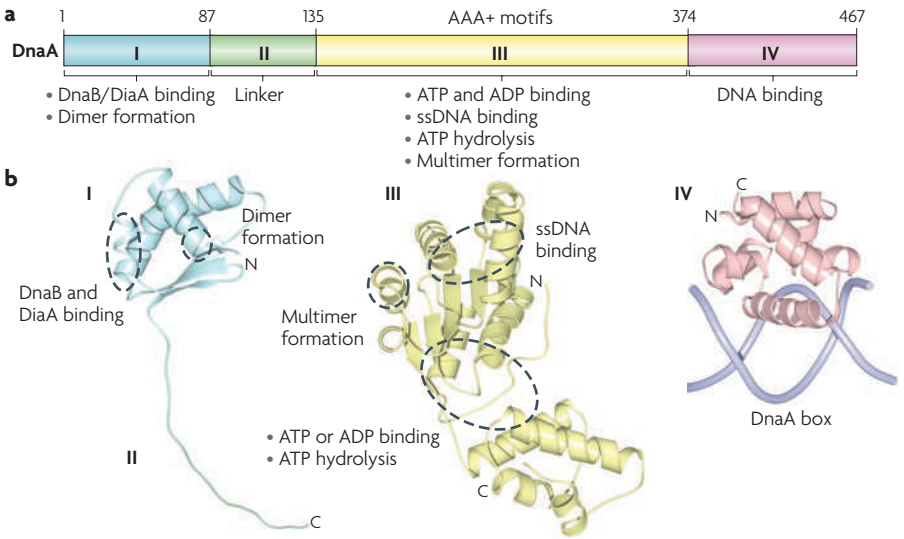

Figure 3 | **Tertiary structures of *Escherichia coli* DnaA domains. a** | The four functional domains of *Escherichia coli* DnaA are shown. **b** | Tertiary structures of *E. coli* DnaA domains I–IV. Several functional regions in each domain are indicated by dotted outlines. DnaA domains I–II and IV were obtained from the protein data bank (Protein Data Bank accession codes 2E0G and 1J1V, respectively). A structural model of *E. coli* DnaA domain III was obtained from the SWISS-MODEL WORKSPACE using the tertiary structure of *Thermotoga maritima* DnaA domain III (Protein Data Bank accession code 2Z4S). AAA+, ATPase associated with diverse cellular activities; C, carboxyl terminus; DiaA, DnaA initiator-associating protein; DnaB, replicative DNA helicase; N, amino terminus; ssDNA, single-stranded DNA.

(*ter*) region (which is replicated last) results in only a moderate level of extra initiations[45] and the total cellular DnaA concentration is not increased[45], consistent with the idea that the timely inhibition of *dnaA* transcription is crucial for repressing extra initiations. This system can reduce the rate of *de novo* ATP–DnaA synthesis immediately after replication initiation, which is important for initiation repression. As such, this system adds another layer to initiation regulation that is coupled with chromosomal replication. In addition, *dnaA* is subject to transcriptional autoregulation through the binding of DnaA to DnaA boxes in the promoter region, which prevents an over-abundance of DnaA and additional initiation events[46,47,48] (FIG. 4a; TABLE 1).

Negative regulation of DnaA: regulatory inactivation by Hda and the clamp. The second system of negative regulation of DnaA is the regulatory inactivation of DnaA (RIDA) system, which promotes ATP hydrolysis in a replication-coupled manner to yield initiation-inactive ADP–DnaA[9,14,49,50] (FIGS 4a;5). As ATP is 10-fold more abundant than ADP[51], most of the newly synthesized DnaA will bind ATP. Thus, the RIDA system is required for reducing the ATP–DnaA level after the initiation of replication.

RIDA is the predominant mechanism by which *E. coli* represses excessive initiations[52]. It requires a complex consisting of DnaA homologue protein (Hda), ADP and the DNA-loaded clamp[49,50]. Hda has a clamp-binding site and an AAA+ domain that interacts with DnaA. This interaction stimulates the hydrolysis of DnaA-bound ATP[50,53,54]; cells bearing altered DnaA that is inactive in ATP hydrolysis exhibit excessive initiation and growth inhibition, as do cells that encode mutant Hda[27,50,55,56]. Inhibition of *dnaA* transcription further supports RIDA by inhibiting *de novo* DnaA synthesis, as mentioned above[14,45]. Hda is activated by ADP binding, although how ADP functions remains to be elucidated[57].

The requirement of a DNA-loaded clamp for RIDA ensures that feedback is coupled to active replication (FIG. 5). Subcellular localization studies suggest that clamps co-localize with replication forks[58]. The principle of RIDA (that is, replication-coupled negative feedback) is widely conserved in bacterial and eukaryotic mechanisms of initiation regulation (see below).

Recently, a system for the regeneration of ATP–DnaA from ADP–DnaA was discovered in *E. coli*[59]. Two chromosomal DNA regions termed DnaA-reactivating sequence 1 (DARS1) and DARS2 bear three flanking DnaA boxes. ADP–DnaA oligomers are formed on these sites, promoting dissociation of ADP from DnaA and subsequent ATP binding *in vitro*, most likely through specific structural changes to the DnaA in the oligomers (FIG. 4a). DARS1 and DARS2 are required for the increase in ATP–DnaA levels and the timely initiation of chromosomal replication that are observed *in vivo* during the cell cycle. The cellular factors that regulate DARS activity are as yet unidentified, although there is evidence for the presence of DARS2-activating proteins[59]. These factors could couple activation of DnaA and replication initiation with the progress of the cell cycle (FIG. 1). Interestingly, the essential sequence elements in DARS regions are widely conserved in the genomes of bacterial species[59].

Negative regulation of DnaA: titration by the datA locus. Titration of DnaA to a specific site termed *datA* is the third way of regulating the initiation function of DnaA. Approximately 200–300 of the 500–2,000 DnaA molecules present in an *E. coli* cell can bind to a unique chromosomal locus known as *datA*, effectively preventing them from functioning at *oriC*[60–62] (FIG. 4a; TABLE 1). The *datA* locus (~1 kb) contains five DnaA boxes and a single binding site for IHF, a protein that stimulates the binding of DnaA molecules[61,63].

Deletion of *datA* results in extra initiations at a moderate level that does not inhibit growth of cells[62]. The *datA* locus resides near *oriC*, and this location is important for the repression of extra initiations in rapidly growing cells[62]. Titration of DnaA molecules by *datA* is enhanced by the nascent copies of the locus that are duplicated immediately after initiation at *oriC*. Thus, repression of extra initiations by *datA* in rapidly growing cells depends in part on chromosomal replication. In addition, the *datA*–DnaA complex could interact with the *oriC*–DnaA complex and inhibit initiation reactions[64]. This type of regulation is proposed for low-copy number plasmids and is termed the hand-cuffing model[65].

The regulatory method used by *datA* fits with the principle of the initiator titration model[66]. According to this model, most DnaA molecules bind to many non-*oriC* sites on the chromosome, thereby inhibiting initiation until the level of DnaA reaches a threshold. It also hypothesizes that when the chromosomal non-*oriC* sites are fully occupied with DnaA, newly synthesized DnaA can bind to *oriC*, forming active initiation complexes.

There remains some controversy about the localization of DnaA in the cell. A recent report by Ogawa and co-workers showed that foci of a yellow fluorescent protein (YFP) fusion to DnaA (DnaA–YFP) are present in the region of *oriC* that

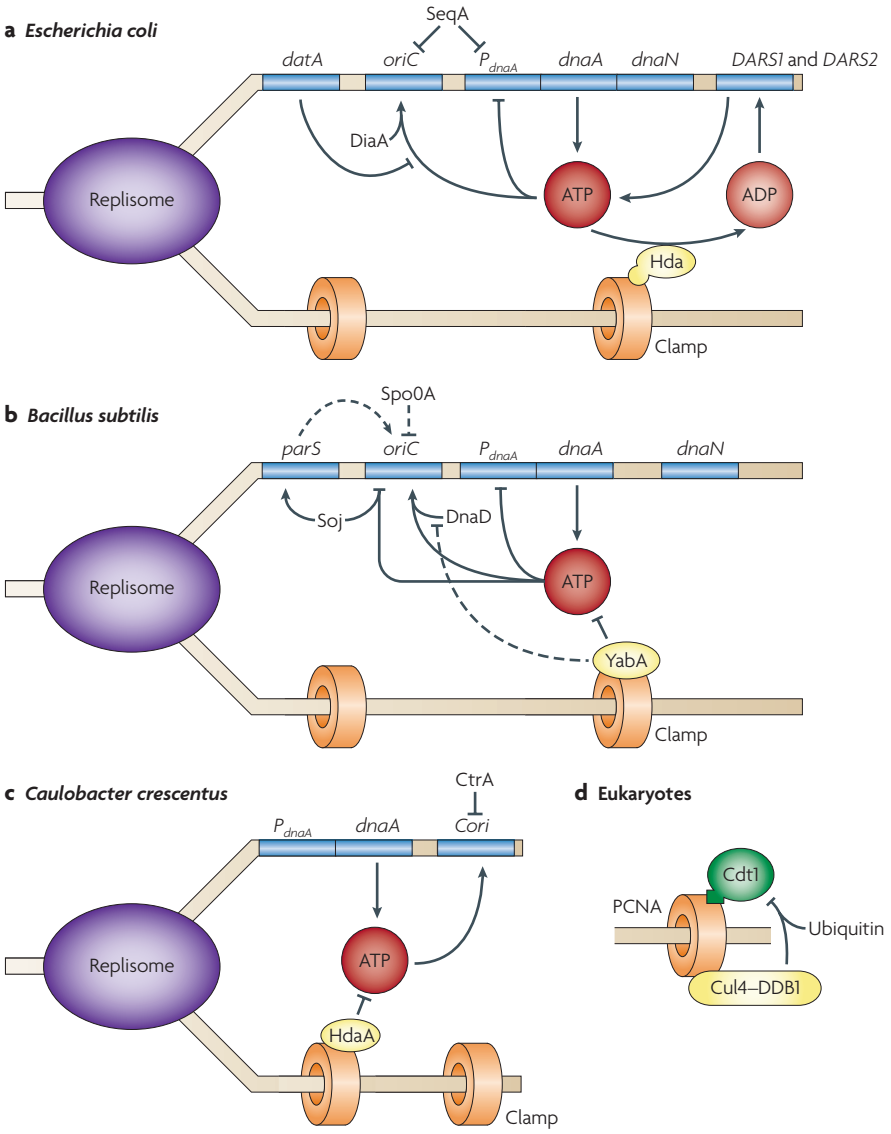

Figure 4 | **Regulatory systems for chromosomal replication initiation. a,b** | Mechanisms in *Escherichia coli* and *Bacillus subtilis*. The initiator protein in bacteria, DnaA, binds to the chromosomal replication origin (*oriC*) to trigger DNA replication. DnaA can be bound to ATP (dark red circles) or ADP (light red circles), but only activates initiation in its ATP-bound form. The DNA polymerase III subunit-β gene (*dnaN*) encodes the clamp and forms an operon with *dnaA*. Various methods of controlling DnaA–ATP concentrations and binding to *oriC* are shown, along with other mechanisms acting on *oriC* to prevent initiation of replication. **c** | Mechanisms in *Caulobacter crescentus*. Cell cycle transcriptional regulator (CtrA) binds to the *Caulobacter* replication origin (*Cori*) and inhibits initiation. Degradation of CtrA (not shown) in a timely manner allows replication only at the correct stage. DnaA–ATP also acts on *Cori* to initiate replication and may be inhibited by HdaA in a replication-coupled negative-feedback loop. **d** | Mechanisms in eukaryotes. Following initiation, the DNA-loaded clamp (proliferating-cell nuclear antigen (PCNA)) interacts with cullin 4 (Cul4)–DNA damage-binding protein 1 (DDB1) to cause ubiquitylation and degradation of Cdt1, the initiation protein. Regulation mechanism functioning specifically during sporulation or under starved conditions are omitted in this figure for simplicity. DARS, DnaA-reactivating sequence;DiaA, DnaA initiator-associating protein; Hda, DnaA homologue protein.

includes *datA*[63]. By contrast, Crooke and co-workers showed that a DnaA–GFP fusion protein forms a spiral filament along the longitudinal cell axis[67]. In both studies, DnaA was linked to a fluorescent protein in domain II and did not induce extra initiations. It is possible that excess DnaA might be pooled in both forms — *datA*-bound protein and cytosolic spiral multimers — and that the quantitative balance between the two pools might be affected by slight structural differences between the recombinant DnaA derivatives.

Recently, a *datA* mutant was shown to have a considerable level of extra initiations that occurred in a rifampicin-resistant manner, unlike normal initiation in wild-type cells[68]. Rifampicin inhibits transcription, which is required for initiation of replication but not DNA chain elongation in wild-type cells[69]. Extra initiations in *datA* mutant cells have been detected in most cases by flow cytometry analysis using cells that were incubated in the presence of rifampicin for run-out replication of the chromosome[62]. The effect of *datA* on repressing extra initiations in growing cells needs to be further elucidated.

Regulation of initiation in *B. subtilis*

Negative regulation of* oriC*: Spo0A and SirA. *B. subtilis* lacks *seqA* and *dam* orthologues[70] but contains several other proteins that regulate *oriC*, including Spo0A and sporulation inhibitor of replication (SirA). Spo0A is a global transcriptional regulator of various genes in stationary phase as well as a master regulator of sporulation-specific transcription[71]. When *B. subtilis* sporulates, initiation of chromosomal replication is repressed. *In vitro*, Spo0A binds to specific sites in *oriC* and inhibits duplex unwinding[71] (FIG. 4b; TABLE 1). *B. subtilis* phage ϕ29 also has Spo0A-binding sites, and replication of this phage is inhibited *in vivo* in a Spo0A-dependent manner. These results suggest that Spo0A represses chromosomal replication by binding directly to *oriC in vivo*[71].

SirA also represses initiation during sporulation[72,73]; in cells that lack *sirA*, extra initiations occur during sporulation. Spo0A induces SirA, although SirA can inhibit initiation independently of Spo0A when expressed from an isopropyl-β-D-thiogalactoside (IPTG)-inducible promoter[72]. Analysis using a DnaA–GFP fusion suggests that DnaA complexes, which are probably located on *oriC*, are dissociated by the induced SirA[73]. Direct binding of SirA to DnaA has been demonstrated with a two-hybrid method[73] (TABLE 1). These negative regulations are of the cascade type of repression of initiation, in that the expression and function of Spo0A and SirA are coupled with changes in cell proliferation or differentiation states (FIG. 1).

Negative regulation of DnaA: regulation by YabA and the clamp. Is the RIDA system conserved in *B. subtilis*? Interactome analysis has suggested that the protein YabA can bind both DnaA and the clamp[74]. Indeed, purified YabA forms homotetramers that bind DnaA and the clamp directly[75]. Inactivation of *yabA*, as well as

the introduction of mutations in *yabA* that render the encoded protein defective in binding to DnaA and the clamp, result in extra initiations at a level that is not inhibitory to cell growth[74–76]. These results indicate that YabA represses superfluous DnaA activity in a clamp-dependent manner, similarly to Hda of *E. coli* (FIG. 4b; TABLE 1). Therefore, despite the lack of substantial sequence similarity, YabA and Hda are functional homologues[74,75]. Consistent with this idea, YabA–YFP foci are formed in a clamp binding-dependent manner[75,77] and colocalize with foci of DNA polymerase III subunit γ/τ (DnaX) fused to cyan fluorescent protein (CFP) in cells[76,77], which suggests that the assembly of YabA molecules at or near the replication fork is coupled to replication. This regulatory mechanism is similar in principle to the RIDA mechanism in *E. coli*. YabA is conserved in Gram-positive bacterial species that have genomes with a low GC content[74,75].

What, then, is the role of YabA–DnaA binding in the repression of extra initiations? Unlike Hda, YabA does not contain AAA+ domains and forms a stable complex with DnaA[75]. Studies of subcellular localization in *B. subtilis* using DnaA–GFP or DnaA–YFP fusions have shown that DnaA specifically localizes at or near the replication fork during most of the replication period, in a manner that is dependent on the clamp and YabA[78]. DnaA foci colocalize with *oriC* only at the initiation of replication or when chromosomal replication is complete (that is, at the point at which the clamp is released from the DNA)[78]. Consistent with these observations, the clamp localizes at or near the replication fork when DNA is replicating and disperses during the inter-replication period of the cell cycle in *B. subtilis*[79], as was also seen for *E. coli*[58]. Taken together, these observations indicate that *B. subtilis* DnaA is titrated to the clamp–YabA complexes at or near the replication forks, thereby sequestering DnaA from *oriC*. Recently, DnaA–GFP in *B. subtilis* was shown to colocalize with *oriC* or its proximal region[73], although it remains possible that sufficient DnaA molecules are present on the replication forks to prevent additional initiations.

A secondary role for YabA has been proposed, namely the inhibition of DnaA–DnaD interactions[80] (FIG. 4b). DnaD is a subunit of a putative helicase loader complex. YabA-binding sites on DnaA are located on the surface of domain III, opposite the nucleotide-binding pocket, and overlap the DnaD-binding region[80].

Table 1 | **Negative regulatory systems involved in replication initiation**

Factors	Strategy
Escherichia coli	
Hda and clamp	Hydrolysis of DnaA-bound ATP by the ADP–Hda–DNA–clamp complex
datA	Binding to DnaA molecules, reducing the number available for initiation
SeqA	Direct binding to hemimethylated GATC sites in *oriC*, inhibiting binding by DnaA
	Binding to hemimethylated GATC sites in the *dnaA* promoter region
DnaA	DnaA box binding in the *dnaA* promoter region
Bacillus subtilis	
YabA and clamp	Binding to DnaA molecules, reducing the number available for initiation
Soj	Direct binding to DnaA complexed with *oriC*
SirA	Direct binding to DnaA and inhibition of its binding to *oriC* during sporulation
Spo0A	Inhibition of DnaA binding to *oriC* (*in vitro*)
DnaA	DnaA box binding in the *dnaA* promoter region
Caulobacter crescentus	
HdaA and clamp	Inactivation of DnaA in the replisome
ClpP	Degradation of DnaA
SpoT	Degradation of DnaA in response to carbon starvation
CtrA	Inhibition of DnaA binding to *oriC*

Clamp, DNA polymerase III subunit-β (DnaN); ClpP, Clp protease, proteolytic subunit; CtrA , cell cycle transcriptional regulator; Hda, DnaA homologue protein; *oriC*, chromosomal replication origin; SirA , sporulation inhibitor of replication.

Although *dnaA* is also transcriptionally autoregulated in *B. subtilis*[81] (FIG. 4b; TABLE 1), the cells are very sensitive to the copy number of DnaA boxes. Unlike *E. coli* cells, they cannot maintain a multicopy plasmid bearing DnaA boxes, owing to the negative effect that these boxes have on chromosomal replication initiation[82,83]. This supports the idea that titration of DnaA molecules to non-*oriC* sites is a more effective mechanism of inhibition of initiation in *B. subtilis* than it is in *E. coli*. The relative importance of this mechanism in the two organisms might be related to the different roles of YabA and Hda at the replication forks: YabA is important for DnaA titration, whereas Hda operates by DnaA–ATP hydrolysis (TABLE 1). To date, there has been no concrete evidence that only the ATP-bound form, and not the ADP-bound form, of *B. subtilis* DnaA is active in initiation.

Negative regulation of DnaA: Soj. The multifunctional protein Soj can bind directly to DnaA and regulate its activity[84] (FIG. 4b; TABLE 1) in addition to regulating sporulation and playing a part in partitioning the chromosomes. It is a Walker-type ATPase with cooperative and sequence-non-specific DNA-binding activity. The protein Spo0J can stimulate the ATPase activity of Soj, and together these proteins form a complex with the chromosomal *parS* locus, which resides near *oriC*, and promotes separation of *oriC* on sister chromosomes[85]. Soj and Spo0J are orthologues of ParA and ParB, respectively, which are two proteins required for the partitioning of sister molecules of low-copy number plasmids and which form a heterocomplex on the centromere-equivalent site of the plasmid[86].

In vivo analysis of *soj* mutants that are defective in ATP binding, ATP hydrolysis or cooperative DNA binding *in vitro* suggests that ATP-bound, DNA-free Soj, but not DNA-bound Soj, binds DnaA assembled on *oriC* in a Spo0J-independent manner, resulting in repression of extra initiations[84] (FIG. 4b; TABLE 1). It has been suggested that when initiation must occur Soj binds directly to the *oriC–parS* region of the DNA in a cooperative manner, allowing DnaA to function in initiation. At this step, Soj might alter higher-order chromosomal structure in the *oriC–parS* region so as to facilitate replication initiation[84,87].

Following initiation, Spo0J is proposed to interact with Soj, which would stimulate the ATPase activity and reduce the DNA-binding activity of Soj, thereby releasing it from the *oriC–parS* region[84]. In mutants with an ATPase-defective Soj protein and in *spo0J*-null mutants, over-initiation of chromosomal replication occurs, perhaps owing to the formation of a stable Soj–DNA complex at the *oriC–parS* region. This would induce checkpoint regulation, thereby indirectly inhibiting sporulation[84,88]. Soj might affect

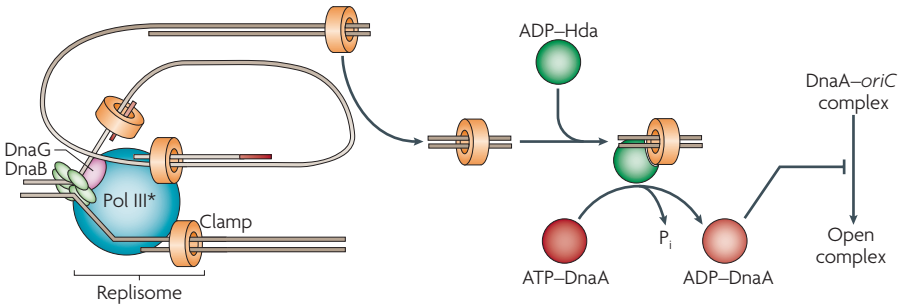

Figure 5 | **Replication initiation and regulatory inactivation of DnaA (RIDA) in *Escherichia coli*.** After formation of the replisome and completion of an Okazaki fragment, the clamp (subunit-β of the DNA polymerase III holoenzyme (DnaN)) remains on the DNA and binds ADP–DnaA homologue protein (Hda). The resultant ADP–Hda–clamp–DNA complex promotes DnaA–ATP hydrolysis, yielding ADP–DnaA, which is not active in promoting duplex unwinding at the chromosomal replication origin (*oriC*). DnaB, replicative DNA helicase; DnaG, DNA primase; P_i, inorganic phosphate; Pol III*, DNA polymerae III subassembly.

the conformation of DnaA bound to *oriC*, but the molecular mechanisms underlying Soj dynamics and the Soj–DnaA interaction remain to be elucidated.

Regulation of initiation in *C. crescentus*

Negative regulation of* oriC*: CtrA. *C. crescentus* is a Gram-negative alphaproteobacterium that it is evolutionarily distant from *E. coli*[70]. *C. crescentus* undergoes asymmetrical cell division, yielding a stalked cell and a swarmer cell; whereas the stalked cell replicates the chromosomal DNA and divides, the swarmer cell does not replicate the chromosome or divide until it converts to a stalked cell[89,90]. In swarmer cells, cell cycle transcriptional regulator (CtrA), a master regulator of cell cycle-dependent transcription, is bound to the cognate *Caulobacter* replication origin (*Cori*), thereby inhibiting initiation[89,90] (FIG. 4c; TABLE 1). CtrA is abundant in swarmer cells, and CtrA phosphorylation by CckA enhances CtrA binding to multiple sites in *Cori*[91]. On differentiation into stalked cells, the ClpXP protease degrades CtrA, allowing initiation to take place[92,93]. This CtrA-dependent regulation is considered to be a cascade type of regulation (FIG. 1).

Negative regulation of DnaA: HdaA and the clamp. Recently, it was found that *C. crescentus* also regulates DnaA. HdaA, the *C. crescentus* orthologue of *E. coli* Hda, is required to repress extra initiations in stalked cells and colocalizes with the clamp throughout DNA replication[94]. This suggests that HdaA inactivates DnaA in a replication-coupled negative-feedback manner (FIG. 4c; TABLE 1).

Unlike *E. coli* DnaA, *C. crescentus* DnaA is unstable and gradually degrades after initiation of replication[93]. When *C. crescentus* is starved of carbon, DnaA is degraded in a manner that depends on a stringent response control protein SpoT, a ppGpp synthetase[95] (TABLE 1).

Feedback regulation in eukaryotes

The principle of RIDA is conserved in eukaryotic replication regulation systems. The inactivation of initiation proteins by the clamp (that is, proliferating-cell nuclear antigen (PCNA) in eukaryotes) has been demonstrated in yeasts, *Caenorhabditis elegans*, *Xenopus laevis* and humans[96–100]. Eukaryotic Cdt1 and cell division cycle 6 (Cdc6) proteins bind to ORC, a counterpart of DnaA, at the origin region, permitting the minichromosome maintenance (MCM) helicase complex to load; this is followed by additional protein–protein interactions that occur in a CDK-dependent manner and, then, replication initiation[4,5,10,11]. After initiation, the DNA-loaded form of the clamp interacts with the E3 ubiquitin ligase cullin 4 (Cul4)–DNA damage-binding protein 1 (DDB1) complex and Cdt1, leading to ubiquitylation and degradation of Cdt1[96–100] (FIG. 4d).

In S phase of the cell cycle in *Drosophila melanogaster*, Cul4–Cdt2–clamp complexes promote degradation of E2F, a key transcription factor that activates important genes for S phase onset and DNA replication[101]. Thus, the clamp-mediated feedback inhibition of replication initiation is a highly conserved principle, despite the diversity in clamp-binding proteins across species.

Concluding remarks

Recent progress in the field of protein subcellular localization in bacteria has revealed important mechanisms of cell cycle regulation. In particular, the crucial role of the replication fork in repressing the function of the initiation protein is a common principle used in a wide range of bacteria and eukaryotes. Hda, YabA, HdaA and the Cul4–DDB1 complex are crucial regulators of the initiation protein (DnaA in bacteria and Cdt1 in eukaryotes) that cooperate with the clamp at replication forks. As for regulation of the origin DNA, SeqA-dependent inactivation is one of the replication-coupled systems used in *E. coli*. In *B. subtilis* and *C. crescentus* (which both lack Dam and SeqA), Spo0A, SirA and CtrA regulate the function of the replication origin according to the differentiation state or cell cycle stage of the cell. Moreover, the presence of multiple pathways for regulating initiation at different layers is also a common feature.

Many important questions remain unanswered to date, such as how Hda activity is regulated by ADP binding and how YabA activity is controlled during the cell cycle. It is still unclear how the functional mechanisms of Hda and YabA were differentiated during evolution. Hda is conserved in alphaproteobacteria, betaproteobacteria and gammaproteobacteria, whereas YabA is present in *Bacillus* spp. and related genera. In addition, the molecular mechanisms that operate in DNA–clamp–(ADP–Hda)–(ATP–DnaA) complexes and in DnaA functional regulation by Soj or HdaA are still unknown. It is also important to elucidate mechanisms that inhibit cell growth in response to over-initiation. Only fragments of roles for a possible cell division regulator and for stalled replication forks have been suggested to date[102,103].

Roles for novel positive regulators must also be further elucidated. The molecular mechanisms that operate in DARS-dependent DnaA–nucleotide exchange and the regulation of DARS function remain unclear. The core elements of DARS elements are conserved in sequence on the genomes of many bacterial species[59], but it is not known if these function as DARS elements *in vivo*. The regulation of DiaA dynamics in the initiation process also requires further study. DiaA is structurally conserved in gammaproteobacteria[17] and even in the epsilonproteobacterium *Helicobacter pylori*, in which the functional homologue is HobA[104–106].

Moreover, analysis of archaeal cells is important work for the future. How is the initiation of chromosomal replication regulated in archaeal cells? These cells have an initiation protein, origin recognition complex subunit 1 (Orc1)/Cdc6, and PCNA homologues but do not have Hda, YabA, Dam or SeqA homologues. Efforts to solve these and related questions would be invaluable.

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1. Messer, W. The bacterial replication initiator DnaA. DnaA and *oriC*, the bacterial mode to initiate DNA replication. *FEMS Microbiol. Rev.* **26**, 355–374 (2002).
2. Mott, M. L. & Berger, J. M. DNA replication initiation: mechanisms and regulation in bacteria. *Nature Rev. Microbiol.* **5**, 343–354 (2007).
3. Ozaki, S. & Katayama, T. DnaA structure, function, and dynamics in the initiation at the chromosomal origin. *Plasmid* **62**, 71–82 (2009).
4. Nishitani, H. & Lygerou, Z. Control of DNA replication licensing in a cell cycle. *Genes Cells* **7**, 523–534 (2002).
5. Bell, S. P. & Dutta, A. DNA replication in eukaryotic cells. *Annu. Rev. Biochem.* **71**, 333–374 (2002).
6. Kornberg, A. & Baker, T. A. *DNA Replication* 2nd edn (Freeman and Co., New York, 1992).
7. O'Donnell, M. Replisome architecture and dynamics in *Escherichia coli*. *J. Biol. Chem.* **281**, 10653–10656 (2006).
8. Georgescu, R. E. *et al.* Structure of a sliding clamp on DNA. *Cell* **132**, 43–54 (2008).
9. Katayama, T. Feedback controls restrain the initiation of *Escherichia coli* chromosomal replication. *Mol. Microbiol.* **41**, 9–17 (2001).
10. Zegerman, P. & Diffley, J. F. Phosphorylation of Sld2 and Sld3 by cyclin-dependent kinases promotes DNA replication in budding yeast. *Nature* **445**, 281–285 (2007).
11. Tanaka, S. *et al.* CDK-dependent phosphorylation of Sld2 and Sld3 initiates DNA replication in budding yeast. *Nature* **445**, 328–332 (2007).
12. Nguyen, V. Q., Co, C. & Li, J. J. Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. *Nature* **411**, 1068–1073 (2001).
13. Weinreich, M., Liang, C., Chen, H. H. & Stillman, B. Binding of cyclin-dependent kinases to ORC and Cdc6p regulates the chromosome replication cycle. *Proc. Natl Acad. Sci. USA* **98**, 11211–11217 (2001).
14. Kurokawa, K., Nishida, S., Emoto, A., Sekimizu, K. & Katayama, T. Replication cycle-coordinated change of the adenine nucleotide-bound forms of DnaA protein in *Escherichia coli*. *EMBO J.* **18**, 6642–6652 (1999).
15. Roth, A., Urmoneit, B. & Messer, W. Functions of histone-like proteins in the initiation of DNA replication at *oriC* of *Escherichia coli*. *Biochimie* **76**, 917–923 (1994).
16. Ishida, T. *et al.* DiaA, a novel DnaA-binding protein, ensures the timely initiation of *Escherichia coli* chromosome replication. *J. Biol. Chem.* **279**, 45546–45555 (2004).
17. Keyamura, K. *et al.* The interaction of DiaA and DnaA regulates the replication cycle in *E. coli* by directly promoting ATP DnaA-specific initiation complexes. *Genes Dev.* **21**, 2083–2099 (2007).
18. Erzberger, J. P., Mott, M. L. & Berger, J. M. Structural basis for ATP-dependent DnaA assembly and replication-origin remodeling. *Nature Struct. Mol. Biol.* **13**, 676–683 (2006).
19. Ozaki, S. *et al.* A common mechanism for the ATP-DnaA-dependent formation of open complexes at the replication origin. *J. Biol. Chem.* **283**, 8351–8362 (2008).
20. McGarry, K. C., Ryan, V. T., Grimwade, J. E. & Leonard, A. C. Two discriminatory binding sites in the *Escherichia coli* replication origin are required for DNA strand opening by initiator DnaA-ATP. *Proc. Natl Acad. Sci. USA* **101**, 2811–2816 (2004).
21. Kawakami, H., Keyamura, K. & Katayama, T. Formation of an ATP-DnaA-specific initiation complex requires DnaA Arginine 285, a conserved motif in the AAA+ protein family. *J. Biol. Chem.* **280**, 27420–27430 (2005).
22. Ryan, V. T., Grimwade, J. E., Nievera, C. J. & Leonard, A. C. IHF and HU stimulate assembly of pre-replication complexes at *Escherichia coli oriC* by two different mechanisms. *Mol. Microbiol.* **46**, 113–124 (2002).
23. Swinger, K. K. & Rice, P. A. IHF and HU: flexible architects of bent DNA. *Curr. Opin. Struct. Biol.* **14**, 28–35 (2004).
24. Keyamura, K., Abe, Y., Higashi, M., Ueda, T. & Katayama, T. DiaA dynamics are coupled with changes in initial origin complexes leading to helicase loading. *J. Biol. Chem.* **284**, 25038–25050 (2009).
25. Abe, Y. *et al.* Structure and function of DnaA N-terminal domains: specific sites and mechanisms in inter-DnaA interaction and in DnaB helicase loading on *oriC*. *J. Biol. Chem.* **282**, 17816–17827 (2007).
26. Felczak, M. M., Simmons, L. A. & Kaguni, J. M. An essential tryptophan of *Escherichia coli* DnaA protein functions in oligomerization at the *E. coli* replication origin. *J. Biol. Chem.* **280**, 24627–24633 (2005).
27. Nishida, S. *et al.* A nucleotide switch in the *Escherichia coli* DnaA protein initiates chromosomal replication: evidence from a mutant DnaA protein defective in regulatory ATP hydrolysis *in vitro* and *in vivo*. *J. Biol. Chem.* **277**, 14986–14995 (2002).
28. Felczak, M. M. & Kaguni, J. M. The box VII motif of *Escherichia coli* DnaA protein is required for DnaA oligomerization at the *E. coli* replication origin. *J. Biol. Chem.* **279**, 51156–51162 (2004).
29. Kawakami, H. *et al.* The exceptionally tight affinity of DnaA for ATP/ADP requires a unique aspartic acid residue in the AAA+ sensor 1 motif. *Mol. Microbiol.* **62**, 1310–1324 (2006).
30. Iyer, L. M., Leipe, D. D., Koonin, E. V. & Aravind, L. Evolutionary history and higher order classification of AAA+ ATPases. *J. Struct. Biol.* **146**, 11–31 (2004).
31. Erzberger, J. P., Pirruccello, M. M. & Berger, J. M. The structure of bacterial DnaA: implications for general mechanisms underlying DNA replication initiation. *EMBO J.* **21**, 4763–4773 (2002).
32. Fujikawa, N. *et al.* Structural basis of replication origin recognition by the DnaA protein. *Nucleic Acid Res.* **31**, 2077–2086 (2003).
33. Lu, M., Campbell, J. L., Boye, E. & Kleckner, N. SeqA: a negative modulator of replication initiation in *E. coli*. *Cell* **77**, 413–426 (1994).
34. Slater, S. *et al. E. coli* SeqA protein binds *oriC* in two different methyl-modulated reactions appropriate to its roles in DNA replication initiation and origin sequestration. *Cell* **82**, 927–936 (1995).
35. Fujikawa, N. *et al.* Structural and biochemical analyses of hemimethylated DNA binding by the SeqA protein. *Nucleic Acids Res.* **32**, 82–92 (2004).
36. Han, J. S., Kang, S., Kim, S. H., Ko, M. J. & Hwang, D. S. Binding of SeqA protein to hemi-methylated GATC sequences enhances their interaction and aggregation properties. *J. Biol. Chem.* **279**, 30236–30243 (2004).
37. Guarne, A. *et al.* Crystal structure of a SeqA-N filament: implications for DNA replication and chromosome organization. *EMBO J.* **24**, 1502–1511 (2005).
38. Bach, T., Krekling, M. A. & Skarstad, K. Excess SeqA prolongs sequestration of *oriC* and delays nucleoid segregation and cell division. *EMBO J.* **22**, 315–323 (2003).
39. Nievera, C., Torgue, J. J., Grimwade, J. E. & Leonard, A. C. SeqA blocking of DnaA-*oriC* interactions ensures staged assembly of the *E. coli* pre-RC. *Mol. Cell* **24**, 581–592 (2006).
40. Onogi, T., Niki, H., Yamazoe, M. & Hiraga, S. The assembly and migration of SeqA-Gfp fusion in living cells of *Escherichia coli*. *Mol. Microbiol.* **31**, 1775–1782 (1999).
41. Brendler, T., Sawitzke, J., Sergueev, K. & Austin, S. A case for sliding SeqA tracts at anchored replication forks during *Escherichia coli* chromosome replication and segregation. *EMBO J.* **19**, 6249–6258 (2000).
42. Molina, F. & Skarstad, K. Replication fork and SeqA focus distributions in *Escherichia coli* suggest a replication hyperstructure dependent on nucleotide metabolism. *Mol. Microbiol.* **52**, 1597–1612 (2004).
43. Bogan, J. A. & Helmstetter, C. E. DNA sequestration and transcription in the *oriC* region of *Escherichia coli*. *Mol. Microbiol.* **26**, 889–896 (1997).
44. Braun, R. E. & Wright, A. DNA methylation differentially enhances the expression of one of the two *E. coli dnaA* promoters *in vivo* and *in vitro*. *Mol. Gen. Genet.* **202**, 246–250 (1986).
45. Riber, L. & Løbner-Olesen, A. Coordinated replication and sequestration of *oriC* and *dnaA* are required for maintaining controlled once-per-cell-cycle initiation in *Escherichia coli*. *J. Bacteriol.* **187**, 5605–5613 (2005).
46. Messer, W. & Weigel, C. DnaA initiator—also a transcriptional factor. *Mol. Microbiol.* **24**, 1–6 (1997).
47. Braun, R. E., O'Day, K. & Wright, A. Autoregulation of the DNA replication gene *dnaA* in *E. coli* K-12. *Cell* **40**, 159–169 (1985).
48. Atlung, T., Løbner-Olesen, A. & Hansen, F. G. Overproduction of DnaA protein stimulates initiation of chromosome and minichromosome replication in *Escherichia coli*. *Mol. Gen. Genet.* **206**, 51–59 (1987).
49. Katayama, T., Kubota, T., Kurokawa, K., Crooke, E. & Sekimizu, K. The initiator function of DnaA protein is negatively regulated by the sliding clamp of the *E. coli* chromosomal replicase. *Cell* **94**, 61–71 (1998).
50. Kato, J. & Katayama, T. Hda, a novel DnaA-related protein, regulates the replication cycle in *Escherichia coli*. *EMBO J.* **20**, 4253–4262 (2001).
51. Bochner, B. R. & Ames, B. N. Complete analysis of cellular nucleotides by two-dimensional thin layer chromatography. *J. Biol. Chem.* **257**, 9759–9769 (1982).
52. Camara, J. E. *et al.* Hda inactivation of DnaA is the predominant mechanism preventing hyperinitiation of *Escherichia coli* DNA replication. *EMBO Rep.* **6**, 736–741 (2005).
53. Su'etsugu, M., Shimuta, T., Ishida, T., Kawakami, H. & Katayama, T. Protein associations in DnaA-ATP hydrolysis mediated by the Hda-replicase clamp complex. *J. Biol. Chem.* **280**, 6528–6536 (2005).
54. Xu, Q. *et al.* A structural basis for the regulatory inactivation of DnaA. *J. Mol. Biol.* **385**, 368–380 (2009).
55. Fujimitsu, K. *et al.* Modes of overinitiation, *dnaA* gene expression, and inhibition of cell division in a novel cold-sensitive *hda* mutant of *Escherichia coli*. *J. Bacteriol.* **190**, 5368–5381 (2008).
56. Felczak, M. M. & Kaguni, J. M. DnaAcos hyperinitiates by circumventing regulatory pathways that control the frequency of initiation in *Escherichia coli*. *Mol. Microbiol.* **72**, 1348–1363 (2009).
57. Su'etsugu, M., Nakamura, K., Keyamura, K., Kudo, Y. & Katayama, T. Hda monomerization by ADP binding promotes replicase clamp-mediated DnaA-ATP hydrolysis. *J. Biol. Chem.* **283**, 36118–36131 (2008).
58. Onogi, T., Ohsumi, K., Katayama, T. & Hiraga, S. Replication-dependent recruitment of the β-subunit of DNA polymerase III from cytosolic spaces to replication forks in *Escherichia coli*. *J. Bacteriol.* **184**, 867–870 (2002).
59. Fujimitsu, K., Senriuchi, T. & Katayama, T. Specific genomic sequences of *E. coli* promote replicational initiation by directly reactivating ADP-DnaA. *Genes Dev.* **23**, 1221–1233 (2009).
60. Sekimizu, K., Yung, B. Y. & Kornberg, A. The dnaA protein of *Escherichia coli*. Abundance, improved purification, and membrane binding. *J. Biol. Chem.* **263**, 7136–7140 (1988).
61. Kitagawa, R., Mitsuki, H., Okazaki, T. & Ogawa, T. A novel DnaA protein-binding site at 94.7 min on the *Escherichia coli* chromosome. *Mol. Microbiol.* **19**, 1137–1147 (1996).
62. Kitagawa, R., Ozaki, T., Moriya, S. & Ogawa, T. Negative control of replication initiation by a novel chromosomal locus exhibiting exceptional affinity for *Escherichia coli* DnaA protein. *Genes Dev.* **12**, 3032–3043 (1998).
63. Nozaki, S., Yamada, Y. & Ogawa, T. Initiator titration complex formed at *datA* with the aid of IHF regulates replication timing in *Escherichia coli*. *Genes Cells* **14**, 329–341 (2009).
64. Nozaki, S., Niki, H. & Ogawa, T. Replication initiator DnaA of *Escherichia coli* changes its assembly form on the replication origin during the cell cycle. *J. Bacteriol.* **191**, 4807–4814 (2009).
65. Paulsson, J. & Chattoraj, D. K. Origin inactivation in bacterial DNA replication control. *Mol. Microbiol.* **61**, 9–15 (2006).
66. Hansen, F. G., Christensen, B. B. & Atlung, T. The initiator titration model: computer simulation of chromosome and minichromosome control. *Res. Microbiol.* **142**, 161–167 (1991).
67. Boeneman, K. *et al. Escherichia coli* DnaA forms helical structures along the longitudinal cell axis distinct from MreB filaments. *Mol. Microbiol.* **72**, 645–657 (2009).
68. Morigen, Molina, F. & Skarstad, K. Deletion of the *datA* site does not affect once-per-cell-cycle timing but induces rifampin-resistant replication. *J. Bacteriol.* **187**, 3913–3920 (2005).
69. Boye, E. & Løbner-Olesen, A. Bacterial growth control studied by flow cytometry. *Res. Microbiol.* **142**, 131–135 (1991).
70. Hiraga, S., Ichinose, C., Onogi, T., Niki, H. & Yamazoe, M. Bidirectional migration of SeqA-bound hemimethylated DNA clusters and pairing of *oriC* copies in *Escherichia coli*. *Genes Cells* **5**, 327–341 (2000).
71. Castilla-Llorente, V., Munoz-Espin, D., Villar, L., Salas, M. & Meijer, W. J. Spo0A, the key transcriptional regulator for entrance into sporulation, is an inhibitor of DNA replication. *EMBO J.* **25**, 3890–3899 (2006).

72. Rahn-Lee, L., Gorbatyuk, B., Skovgaard, O. & Losick, R. The conserved sporulation protein YneE inhibits DNA replication in *Bacillus subtilis*. *J. Bacteriol.* **191**, 3736–3739 (2009).
73. Wagner, J. K., Marquis, K. A. & Rudner, D. Z. SirA enforces diploidy by inhibiting the replication initiator DnaA during spore formation in *Bacillus subtilis*. *Mol. Microbiol.* **73**, 963–974 (2009).
74. Noirot-Gros, M. F. *et al.* An expanded view of bacterial DNA replication. *Proc. Natl Acad. Sci. USA* **99**, 8342–8347 (2002).
75. Noirot-Gros, M. F. *et al.* Functional dissection of YabA, a negative regulator of DNA replication initiation in *Bacillus subtilis*. *Proc. Natl Acad. Sci. USA* **103**, 2368–2373 (2006).
76. Hayashi, M., Ogura, Y., Harry, E. J., Ogasawara, N. & Moriya, S. *Bacillus subtilis* YabA is involved in determining the timing and synchrony of replication initiation. *FEMS Microbiol. Lett.* **247**, 73–79 (2005).
77. Goranov, A. I., Breier, A. M., Merrikh, H. & Grossman, A. D. YabA of *Bacillus subtilis* controls DnaA-mediated replication initiation but not the transcriptional response to replication stress. *Mol. Microbiol.* **74**, 454–466 (2009).
78. Soufo, C. D. *et al.* Cell-cycle-dependent spatial sequestration of the DnaA replication initiator protein in *Bacillus subtilis*. *Dev. Cell* **15**, 935–941 (2008).
79. Meile, J. C., Wu, L. J., Ehrlich, S. D., Errington, J. & Noirot, P. Systematic localisation of proteins fused to the green fluorescent protein in *Bacillus subtilis*: identification of new proteins at the DNA replication factory. *Proteomics* **6**, 2135–2146 (2006).
80. Cho, E., Ogasawara, N. & Ishikawa, S. The functional analysis of YabA, which interacts with DnaA and regulates initiation of chromosome replication in *Bacillus subtils*. *Genes Genet. Syst.* **83**, 111–125 (2008).
81. Ogura, Y., Imai, Y., Ogasawara, N. & Moriya, S. Autoregulation of the *dnaA-dnaN* operon and effects of DnaA protein levels on replication initiation in *Bacillus subtilis*. *J. Bacteriol.* **183**, 3833–2841 (2001).
82. Moriya, S., Fukuoka, T., Ogasawara, N. & Yoshikawa, H. Regulation of initiation of the chromosomal replication by DnaA-boxes in the origin region of the *Bacillus subtilis* chromosome. *EMBO J.* **7**, 2911–2297 (1988).
83. Moriya, S., Atlung, T., Hansen, F. G., Yoshikawa, H. & Ogasawara, N. Cloning of an autonomously replicating sequence (*ars*) from the *Bacillus subtilis* chromosome. *Mol. Microbiol.* **6**, 309–315 (1992).
84. Murray, H. & Errington, J. Dynamic control of the DNA replication initiation protein DnaA by Soj/ParA. *Cell* **135**, 74–84 (2008).
85. Lee, P. S. & Grossman, A. D. The chromosome partitioning proteins Soj (ParA) and Spo0J (ParB) contribute to accurate chromosome partitioning, separation of replicated sister origins, and regulation of replication initiation in *Bacillus subtilis*. *Mol. Microbiol.* **60**, 853–869 (2006).
86. Ebersbach, G. & Gerdes, K. Plasmid segregation mechanisms. *Annu. Rev. Genet.* **39**, 453–479 (2005).
87. Ogura, Y., Ogasawara, N., Harry, E. J. & Moriya, S. Increasing the ratio of Soj to Spo0J promotes replication initiation in *Bacillus subtilis*. *J. Bacteriol.* **185**, 6316–6324 (2003).
88. Veening, J. W., Murray, H. & Errington, J. A mechanism for cell cycle regulation of sporulation initiation in *Bacillus subtilis*. Genes *Dev.* **23**, 1959–1570 (2009).
89. Quon, K. C., Yang, B., Domian, I. J., Shapiro, L. & Marczynski, G. T. Negative control of bacterial DNA replication by a cell cycle regulatory protein that binds at the chromosome origin. *Proc. Natl Acad. Sci. USA* **95**, 120–125 (1998).
90. Bastedo, D. P. & Marczynski, G. T. CtrA response regulator binding to the *Caulobacter* chromosome replication origin is required during nutrient and antibiotic stress as well as during cell cycle progression. *Mol. Microbiol.* **72**, 139–154 (2009).
91. Siam, R. & Marczynski, G. T. Cell cycle regulator phosphorylation stimulates two distinct modes of binding at a chromosome replication origin. *EMBO J.* **19**, 1138–1147 (2000).
92. McGrath, P. T., Iniesta, A. A., Ryan, K. R., Shapiro, L. & McAdams, H. H. A dynamically localized protease complex and a polar specificity factor control a cell cycle master regulator. *Cell* **124**, 535–547 (2006).
93. Gorbatyuk, B. & Marczynski, G. T. Regulated degradation of chromosome replication proteins DnaA and CtrA in *Caulobacter crescentus*. *Mol. Microbiol.* **55**, 1233–1245 (2005).
94. Collier, J. & Shapiro, L. Feedback control of DnaA-mediated replication initiation by replisome-associated HdaA protein in *Caulobacter*. *J. Bacteriol.* **191**, 5706–5716 (2009).
95. Lesley, J. A. & Shapiro, L. SpoT regulates DnaA stability and initiation of DNA replication in carbon-starved *Caulobacter crescentus*. *J. Bacteriol.* **190**, 6867–6880 (2008).
96. Arias, E. E. & Walter, J. C. PCNA functions as a molecular platform to trigger Cdt1 destruction and prevent re-replication. *Nature Cell Biol.* **8**, 84–90 (2006).
97. Nishitani, H. *et al.* Two E3 ubiquitin ligases, SCF-Skp2 and DDB1-Cul4, target human Cdt1 for proteolysis. *EMBO J.* **25**, 1126–1136 (2006).
98. Senga, T. *et al.* PCNA is a cofactor for Cdt1 degradation by CUL4/DDB1-mediated N-terminal ubiquitination. *J. Biol. Chem.* **281**, 6246–6252 (2006).
99. Ralph, E., Boye, E. & Kearsey, S. E. DNA damage induces Cdt1 proteolysis in fission yeast through a pathway dependent on Cdt2 and Ddb1. *EMBO Rep.* **7**, 1134–1139 (2006).
100. Kim, Y. & Kipreos, E. T. The *Caenorhabditis elegans* replication licensing factor CDT-1 is targeted for degradation by the CUL-4/DDB-1 complex. *Mol. Cell Biol.* **27**, 1394–1406 (2007).
101. Shibutani. *et al.* Intrinsic negative cell cycle regulation provided by PIP box- and $Cul4^{Cdt2}$-mediated destruction of E2f1 during S phase. *Dev. Cell* **15**, 890–900 (2008).
102. Katayama, T., Takata, M. & Sekimizu, K. CedA is a novel *Escherichia coli* protein that activates the cell division inhibited by chromosomal DNA overreplication. *Mol. Microbiol.* **26**, 687–697 (1997).
103. Simmons, L. A., Breier, A. M., Cozzarelli, N. R. & Kaguni, J. M. Hyperinitiation of DNA replication in *Escherichia coli* leads to replication fork collapse and inviability. *Mol. Microbiol.* **51**, 349–358 (2004).
104. Zawilak-Pawlik, A. *et al.* HobA – a novel protein involved in initiation of chromosomal replication in *Helicobacter pylori*. *Mol. Microbiol.* **65**, 979–994 (2007).
105. Natrajan, G., Hall, D. R., Thompson, A. C., Gutsche, I. & Terradot, L. Structural similarity between the DnaA-binding proteins HobA (HP1230) from *Helicobacter pylori* and DiaA from *Escherichia coli*. *Mol. Microbiol.* **65**, 995–1005 (2007).
106. Natrajan, G., Noirot-Gros, M. F., Zawilak-Pawlik, A., Kapp, U. & Terradot, L. The structure of a DnaA/HobA complex from *Helicobacter pylori* provides insight into regulation of DNA replication in bacteria. *Proc. Natl Acad. Sci. USA* **106**, 21115–21120 (2009).

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Competing interests statement
The authors declare no competing financial interests.

DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/genomeproj
Bacillus subtilis | *Caenorhabditis elegansCaulobacter crescentus* | *Drosophila melanogaster* | *Escherichia coli* | *Xenopus laevis*
Protein Data Bank: http://www.pdb.org/pdb/home/home.do
1J1V | 2E0G | 2Z4S
UniProtKB: http://www.uniprot.org
CtrA | DiaA | DnaA | DnaB | DnaC | DnaD | DnaG | DnaN | DnaX | Hda | SeqA | SirA | Soj | Spo0A | YabA

FURTHER INFORMATION
Author's homepage: http://210.233.60.66/~bunsei/BUNSEI-ENG.html
ALL LINKS ARE ACTIVE IN THE ONLINE PDF