JOURNAL OF BACTERIOLOGY, Mar. 1988, p. 1333–1338
0021-9193/88/031333-06$02.00/0
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Vol. 170, No. 3

Interaction of the *Bacillus subtilis* DnaA-Like Protein with the *Escherichia coli* DnaA Protein

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Received 14 July 1987/Accepted 23 November 1987

Plasmids carrying the intact *Bacillus subtilis dnaA*-like gene and two reciprocal hybrids between the *B. subtilis* and *Escherichia coli dnaA* genes were constructed. None of the plasmids could transform wild-type *E. coli* cells unless the cells contained surplus *E. coli* DnaA protein ($DnaA_{Ec}$). A *dnaA*(Ts) strain integratively suppressed by the plasmid R1 origin could be transformed by plasmids carrying either the *B. subtilis* gene ($dnaA_{Bs}$) or a hybrid gene containing the amino terminus of the *E. coli* gene and the carboxyl terminus of the *B. subtilis* gene ($dnaA_{Ec/Bs}$). In cells with surplus *E. coli* DnaA protein, expression of the *E. coli dnaA* gene was derepressed by the *B. subtilis* DnaA protein and by the hybrid $DnaA_{Ec/Bs}$ protein, whereas it was strongly repressed by the reciprocal hybrid protein $DnaA_{Bs/Ec}$. The plasmids carrying the different *dnaA* genes probably all interfere with initiation of chromosome replication in *E. coli* by decreasing the *E. coli* DnaA protein concentration to a limiting level. The $DnaA_{Bs}$ and the $DnaA_{Ec/Bs}$ proteins effect this decrease possibly by forming inactive oligomeric proteins, while the $DnaA_{Bs/Ec}$ protein may decrease $dnaA_{Ec}$ gene expression.

The *Escherichia coli dnaA* gene encodes an essential factor involved in initiation of chromosome replication, the DnaA protein. The *dnaA* gene was identified by the isolation of temperature-sensitive mutants and subsequent genetic mapping and physiological characterization (1, 3, 6, 14, 30). The *dnaA* gene is located on the chromosome 42 kilobases counterclockwise from *oriC* (27). It has been sequenced and found to encode a basic protein with a molecular mass of 52.5 kilodaltons (11, 21). The *dnaA* gene is autoregulated by the DnaA protein (2, 5, 15). As a repressor, it binds to a 9-base-pair (bp) sequence (5, 10), the DnaA box TTATCCACA, which is located in the *dnaA* promoter region (12). This 9-bp sequence is present at four sites within *oriC*, and the *oriC* DnaA boxes are also DnaA protein-binding sites (10). The DnaA boxes in *oriC* are essential for initiation of replication (22).

The region of the *Bacillus subtilis* chromosome where DNA replication is initiated was found to contain an open reading frame specifying a polypeptide showing a high degree of homology with the DnaA protein of *E. coli* (18). This *B. subtilis dnaA*-like gene is surrounded by the *B. subtilis* equivalents of the *E. coli gyrB*, *recF*, *dnaN*, *rpmH*, and *rnpA* genes. Thus, the gene orders in this region of the chromosome in these different organisms are very similar, except that *oriC* of *B. subtilis* is probably located very close to the *dnaA*-like gene (20). A comparison between the *B. subtilis* and *E. coli* DnaA proteins shows three domains with different degrees of homology (20). The role of the *B. subtilis dnaA*-like gene in chromosome replication is not known, since no mutations in the gene have been reported.

There are nomenclature problems when we discuss the *dnaA* gene of *B. subtilis*. The latest genetic linkage map of *B. subtilis* contains two *dnaA* genes, which, according to their map positions, are different (24) from the *dnaA*-like gene mentioned above. Therefore, we have used $dnaA_{Bs}$ and $dnaA_{Ec}$ for the *dnaA* genes of *B. subtilis* and *E. coli*, respectively, and $DnaA_{Bs}$ and $DnaA_{Ec}$ for the corresponding proteins. To describe a hybrid *dnaA* gene carrying *B. subtilis* sequences at the 5′ end and *E. coli* sequences at the 3′ end of the coding sequence, we have used $dnaA_{Bs/Ec}$, and for the sequences arranged in the reverse order, we have used $dnaA_{Ec/Bs}$. Similarly, we have used $DnaA_{Bs/Ec}$ and $DnaA_{Ec/Bs}$, respectively, for the corresponding hybrid proteins.

Earlier, we had tried unsuccessfully to construct a plasmid with the intact $dnaA_{Bs}$ of *B. subtilis* in *E. coli*. The failure of this construction suggested that the $dnaA_{Bs}$ gene product is toxic to the *E. coli* cell, possibly by interfering with some of the normal molecular interactions of the $DnaA_{Ec}$ protein. This assumption, as shown below, turned out to be correct. In this study, we describe the construction of plasmids containing the intact $dnaA_{Bs}$ gene. These plasmids could be maintained only in *E. coli* strains carrying a helper plasmid from which the $DnaA_{Ec}$ protein was overproduced or in *dnaA*(Ts) strains integratively suppressed by plasmid mini-R1. We also describe the construction of fusions of the *dnaA* genes of the two species which takes advantage of a common restriction site.

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MATERIALS AND METHODS

Bacterial strains and plasmids. Bacterial strains are listed and described in Table 1. Plasmid pBR322 (4) was used as the vector in the construction of recombinant plasmids.

DNA technology. Preparation and restriction enzyme analysis of plasmid DNA, treatment with alkaline phosphatase and DNA polymerase I (Klenow fragment), ligation, and transformation were done essentially as described by Maniatis et al. (16). The restriction enzyme and other enzymes were obtained from New England BioLabs, Inc., Boehringer Mannheim Biochemicals, and Amersham Corp.

Construction of plasmids carrying the $dnaA_{Bs}$ gene and hybrids between the *B. subtilis* and *E. coli dnaA* genes. A plasmid with the $dnaA_{Bs}$ gene was assembled as follows. First, plasmid pLA1526 (Fig. 1) was constructed by ligating

TABLE 1. *E. coli* strains and plasmids

Strain	Plasmid	Genotype	Source, reference, or construction
MC1000			7
FH1501	pSM1001	MC1000 Tc^r Ap^r	19
FH1502	pSM2002	MC1000 Ap^r Km^r	19
FH871[a]	pFHC871	MC1000 Cm^r	2
RB210		MC1000 (λRB1-*dnaA′* *′lacZ*)	5
FH1357	pFHC539	RB210 Ap^r	28
FH1358	pFHC871	RB210 Cm^r	This study
TC1926		RB210 *dnaA46*	—[b]
TC1962		RB210 *int*(mini-R1-*kan*)	—[c]
TC1963		TC1926 *int*(mini-R1-*kan*)	—[c]
FH1551	pFHC871	TC1963	This study

[a] Plasmid pFHC871 is a pACYC184 (8) derivative carrying a *ClaI-XhoI* fragment, containing the *dnaA* gene and promoter region, inserted into the *ClaI* and *SalI* sites of pACYC184.

[b] —Constructed in two steps: (i) P1(TC540 *ilv*::Tn*5*) × RB210, select for kanamycin resistance, and screen for Ilv^-; (ii) P1(CM734 *dnaA46*) (12) × RB210 *ilv*::Tn*5*, select for Ilv^+, and screen for temperature and kanamycin sensitivity.

[c] —Constructed by P1[CM1793 *int*(mini-R1-*kan*)] (27) × RB210 or TC1926 and select for kanamycin resistance.

the 1,302-bp *Eco*RV-*Bgl*II fragment, which carries the last 525 bp of the $dnaA_{Bs}$ gene (from pSM1001), with pBR322, digested with *Eco*RV and *Bam*HI by using the resulting DNA to transform strain MC1000. Second, plasmid pLA1540, which carries the intact $dnaA_{Bs}$ gene (Fig. 1), was constructed by inserting the 1,225-bp *Eco*RV fragment (from pSM2002), which spans the promoter region and the first part of the structural gene, into pLA1526. The isolated 1,225-bp *Eco*RV fragment was ligated to *Eco*RV-digested and alkaline phosphatase-treated pLA1526 and used to transform strain TC1963, which carries a mini-R1 replicon integrated in the chromosome. The integratively suppressed strain was chosen to circumvent a negative interference with the normal *E. coli* initiation control.

The *dnaA* genes of *B. subtilis* and *E. coli* have a common *Sph*I restriction site (Fig. 1). We constructed hybrid genes by using this *Sph*I restriction site. The plasmid pLA1511 (Fig. 1), which carries the $dnaA_{Bs/Ec}$ hybrid gene, was constructed as follows. Plasmid pSM2002 was digested with *Bam*HI and *Sph*I, ligated to pFHC539 (Fig. 1) DNA digested with *Bgl*II and *Sph*I, and used to transform strain FH1358, which produces $DnaA_{Ec}$ protein in excess because of the presence of plasmid pFHC871 (Fig. 1). The plasmid pLA1535, carrying the reciprocal fusion gene $dnaA_{Ec/Bs}$ (Fig. 1), was constructed in two steps, as follows. Plasmid pLA1527 was constructed by replacing the *Sph*I-*Eco*RV fragment of pFHC539 with a 331-bp *Sph*I-*Eco*RV segment from plasmid pSM2002 (Fig. 1). Plasmid pLA1535 was then constructed by inserting a 1,521-bp *Sal*I-*Bgl*II fragment (from pSM1001) into pLA1527. Plasmid pSM1001 was digested with *Bgl*II; the ends were filled with DNA polymerase I (Klenow fragment) and digested with *Sal*I. The isolated 1,521-bp fragment was ligated to pLA1527, digested with *Sal*I and *Eco*RV, and used to transform the integratively suppressed strain TC1963.

Determination of β-galactosidase activity. Bacterial cultures were kept in steady-state growth at 37°C in NY medium (29) with appropriate antibiotics (ampicillin [100 μg/ml] and chloramphenicol [150 μg/ml]) by repeated fivefold dilutions into fresh prewarmed medium. The optical density at 600 nm of the cultures never exceeded 0.5. When the cultures were diluted (repeated five times or more), samples were taken, toluenized, and used to assay β-galactosidase activity according to the method of Miller (17).

Determination of β-lactamase activity. β-Lactamase activity was determined in toluenized samples by a modified nitrocefin assay (9). The assays were started by the addition of extract (1 to 20 μl of toluenized cell suspension) to a 0.75-ml assay mixture (100 mM potassium phosphate buffer [pH 7.4], 50 μg of nitrocefin per ml) at room temperature (25°C); the assays were stopped by the addition of 0.25 ml of 8 M guanidinium chloride. A_{482} was determined, and the specific activity was calculated as [(A_{482} of the assay mixture)/(A_{600} of the culture)] × milliliters of sample × minutes of incubation. This modification of the nitrocefin assay reduces the cost, and assays can be carried out just as

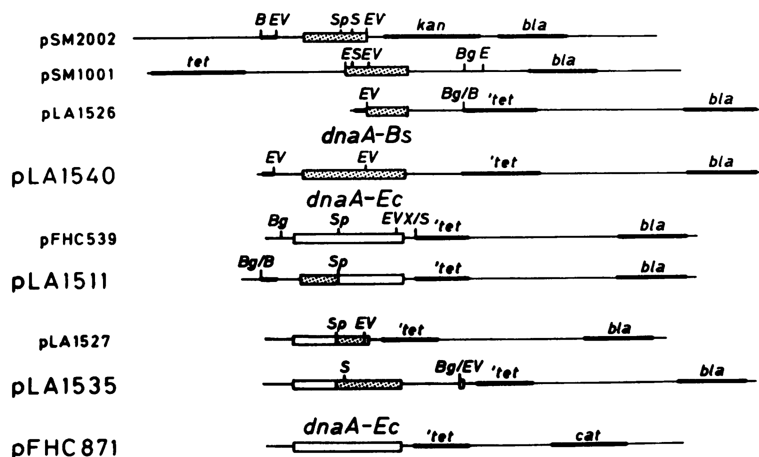

FIG. 1. Construction and structure of plasmids carrying the *dnaA* genes of *B. subtilis*, *E. coli*, and hybrids between the genes of the two species. Relevant restriction enzyme sites are indicated above the plasmids: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; EV, *Eco*RV; S, *Sal*I; Sp, *Sph*I. pFHC871 is a p15 replicon (8). The other plasmids are pBR322 replicons. The positions of the resistance genes are indicated with a thick line. All plasmids are aligned after their *dnaA* genes (or parts of them), which are transcribed from left to right. *B. subtilis* (▨) and *E. coli* (▭) structural *dnaA* gene regions ($dnaA_{Bs}$ and $dnaA_{Ec}$) are indicated. The plasmids used in the construction are indicated with small plasmid names. The large plasmid names indicate those used later in the study.

the standard β-galactosidase assays are. We found that the β-lactamase activity was proportional to the extract concentration and that the increase in absorption was linear in time. The limits for the assay are set by the absorption, not exceeding 0.8 in 5 min; in that case, less extract should be used. The absorption in the stopped samples is stable for at least 24 h.

Determination of plasmid copy number. Plasmid copy number was determined as described by Stüber and Bujard (25) or by measuring β-lactamase activity (see above; 26).

Determination of plasmid stability. Bacterial cultures were kept in exponential growth for four generations in NY medium with the appropriate antibiotics and then diluted 16-fold into unselective medium and kept in log-phase growth by repeated 16-fold dilutions. At the time that dilution samples were taken, they were (i) toluenized for determination of β-lactamase activity or (ii) diluted to give 300 to 500 colonies when spread on nonselective plates and replicated onto selective plates for determination of the fraction of plasmid-free cells.

RESULTS

Transformation analysis of plasmids carrying the intact *B. subtilis dnaA*-like gene or hybrids between *B. subtilis* and *E. coli dnaA* genes. Plasmids carrying the intact $dnaA_{Bs}$ gene and the hybrid *B. subtilis/E. coli dnaA* genes were constructed (Fig. 1). During the construction (see Materials and Methods for details), we found some indication that such plasmids might inhibit growth of *E. coli* cells. To test these findings, we used the plasmids to transform different strains, as shown in Table 2. Plasmid pLA1540, carrying the intact $dnaA_{Bs}$ gene, transformed neither strain RB210 ($dnaA^+$) nor TC1926 (RB210 *dnaA46*). As expected, it did transform strain TC1963, the mini-R1 integratively suppressed *dnaA46* derivative of RB210 which was used in the plasmid construction. However, these transformants, obtained at 37°C, at which temperature the *dnaA46* product is partially inactivated, were cold sensitive, i.e., they formed minute colonies at 30°C, at which temperature the DnaA(Ts) protein is more active. Corroborating this finding was the inability of pLA1540 ($dnaA_{Bs}$) to transform strain TC1962 [RB210 $dnaA_{Ec}^+$ *int*(mini-R1)].

Plasmid pLA1535 ($dnaA_{Ec/Bs}$) behaved in a manner similar to that described above, except that it gave a low percentage of transformants of the wild-type strain RB210. Plasmid DNAs from 20 independent RB210 transformants were analyzed. All of these contained exclusively dimeric plasmid molecules. Retransformation of strain RB210 with these dimeric molecules gave normal transformation frequencies. We conclude that plasmid pLA1535 ($dnaA_{Ec/Bs}$) affects normal *E. coli* cells in a way similar to that of pLA1540 ($dnaA_{Ec}$). The ability of RB210 to harbor plasmid pLA1535 ($dnaA_{Ec/Bs}$) as a dimer indicates that a lower level of fusion protein is produced from dimers than from monomers.

With normal frequencies, plasmid pLA1535 ($dnaA_{Ec/Bs}$) and pLA1540 ($dnaA_{Bs}$) transformed the strains (FH1358 and FH1551) containing elevated $DnaA_{Ec}$ protein levels due to the presence of plasmid pFHC871.

Transformation of strains RB210 and TC1963 with a mixture of the $dnaA_{Bs/Ec}$ plasmid, pLA1511, and the $dnaA_{Ec}$ plasmid, pFHC871, gave rise to transformants, almost all of which contained both plasmids. Rare transformants resistant only to ampicillin were found to contain altered forms of plasmid pLA1511 ($dnaA_{Bs/Ec}$). Some of these plasmids had large deletions, while others, though identical in size to pLA1511 ($dnaA_{Bs/Ec}$), apparently had acquired mutations allowing them to efficiently transform strain RB210.

Repression of DnaA protein and β-galactosidase synthesis in strains carrying various *dnaA* genes on high-copy plasmids. The RB210 strain (and its derivatives), used in the transformation experiments described above, is an MC1000 strain lysogenic for a lambda bacteriophage with the normal $dnaA_{Ec}$ regulatory region and the first part of the $dnaA_{Ec}$ gene fused in frame to the *lacZ* gene. Thus, the effects of the different DnaA proteins on the expression of the *dnaA* gene can be determined by measuring the amount of β-galactosidase (5). We measured $dnaA_{Ec}$ gene expression, as well as the relative copy numbers of the pLA plasmids and pFHC871 in the strains constructed in the transformations described above (Table 3).

The 2.4-fold derepression observed in strain TC1963 was caused by the presence of the *dnaA46* allele. Plasmid pLA1540 (p$dnaA_{Bs}$) and pLA1535 (p$dnaA_{Ec/Bs}$) decreased *dnaA''lacZ* expression in strain TC1963 by 30 to 40%, indicating that the $DnaA_{Bs}$ and $DnaA_{Ec/Bs}$ proteins repressed *dnaA* gene expression in *E. coli* slightly, possibly by binding to the DnaA box.

In wild-type cells (RB210) or cells of the integratively suppressed *dna46* strain (TC1963), the presence of a plasmid carrying the $dnaA_{Ec}$ gene (pFHC871) decreased *dnaA''lacZ* expression to approximately 75% of the expression observed in strain RB210 without the plasmid. However, if the cells contained, in addition, either a plasmid with the $dnaA_{Bs}$ gene (pLA1540) or a plasmid with the $dnaA_{Ec/Bs}$ gene (pLA1535), expression became derepressed. We have determined the relative copy numbers of plasmids in the different strains (Table 3). The degree of derepression correlated with the

TABLE 2. Transformation of *E. coli* strains with plasmids carrying the complete $dnaA_{Bs}$ gene or fusions of $dnaA_{Bs}$ and $dnaA_{Ec}$ genes

Strain	Genotype (chromosome/plasmid)	Transformation with plasmid[a]:			
		pBR322	pLA1540 ($dnaA_{Bs}$)	pLA1535 ($dnaA_{Ec/Bs}$)	pLA1511 ($dnaA_{Bs/Ec}$ [pFHC871])[b]
RB210	$dnaA_{Ec}$	+	−	2%[c]	2%[d]
TC1962	$dnaA_{Ec}$ *int*(mini-R1)	+	−	NT	NT
TC1963	*dnaA46 int*(mini-R1)	+	+	+	2%[d]
FH1358	$dnaA_{Ec}$/p$dnaA_{Ec}$	+	+	+	+
FH1551	*dnaA46 int*(mini-R1)/p$dnaA_{Ec}$	+	+	+	+

[a] +, Normal transformation frequency compared with the transformation obtained with pBR322; −, <0.05% transformants (no transformants); NT, not tested.
[b] pLA1511 can only exist with pFHC871 ($dnaA_{Ec}$; see footnote *d*).
[c] Low transformation frequency. All transformants carry the plasmid in a dimeric form, which gives a normal transformation frequency on retransformation.
[d] Low transformation frequency. Cotransformants constitute 95% and carry both plasmids. The remaining 5% are Ap^r transformants that are probably mutant derivatives of pLA1511, since after this first passage through the cell, they transform with a normal frequency.

TABLE 3. Expression of the *dnaA* gene in *E. coli* strains carrying the complete $dnaA_{Bs}$ gene or fusions of $dnaA_{Bs}$ and $dnaA_{Ec}$ genes[a]

Strain	*dnaA* allele on: Chromosome	*dnaA* allele on: Plasmid	No additional plasmid: β-gal	No additional plasmid: cp_{871}	pLA1540 ($dnaA_{Bs}$): β-gal	pLA1540: cp_{1540}	pLA1540: cp_{871}	pLA1535 ($dnaA_{Ec/Bs}$): β-gal	pLA1535: cp_{1535}	pLA1535: cp_{871}	pLA1511 ($dnaA_{Bs/Ec}$): β-gal	pLA1511: cp_{1511}	pLA1511: cp_{871}
RB210	$dnaA_{Ec}$		1										
TC1963	*dnaA46*		2.4		1.6	1		1.5	1				
FH1358	$dnaA_{Ec}$	p$dnaA_{Ec}$	0.7	0.8	1.4	0.75	0.65	0.9	0.75	0.75	0.2	0.6	0.8
FH1551	*dnaA46*	p$dnaA_{Ec}$	0.8	0.8	0.9	0.55	0.55	1.1	0.7	0.55	0.2	0.6	0.8

[a] *dnaA* gene expression is measured as β-galactosidase (β-gal) activity in strains carrying a *dnaA' 'lacZ* fusion on a λ phage integrated in the λ*att* site on the *E. coli* chromosome (5). The experimental values are normalized to the value of strain RB210. The relative value of β-galactosidase activity has been reproduced in three independent experiments. The relative copy numbers for the pLA plasmids (cp_{1540}, cp_{1535}, and cp_{1511}) were determined both as β-lactamase activity and from scanning of agarose gels; the relative copy number of pFHC871 (cp_{871}) was determined from scanning of agarose gels. The copy numbers of pLA1540 and pLA1535 in strain TC1963 are similar to the copy number of pBR322 in strain RB210.

ratio between plasmid copy numbers, i.e., the degree of derepression was greater when the plasmid with the $dnaA_{Bs}$ (or $dnaA_{Ec/Bs}$) gene was in excess of the plasmid with the $dnaA_{Ec}$ gene. We have also determined single-cell resistance to ampicillin (26) in cells carrying the pBR322-derived plasmids. The data from this analysis correlated nicely with the copy numbers given in Table 3. This method also gives a rough indication of the distribution of plasmid molecules among individual cells. We found that all cells in the population plated well up to a certain concentration of ampicillin and that the plating efficiency then decreased rapidly at higher concentrations. This result indicates that plasmid molecules are fairly evenly distributed among individual cells (data not shown). In the experiments shown in Table 3, we also analyzed the cultures for plasmid-free cells and found less than 1%, except for pLA1540 ($dnaA_{Bs}$) in strain FH1551 (TC1963/pFHC871), in which 10% of the cells were ampicillin sensitive.

The p$dnaA_{Bs/Ec}$ plasmid (pLA1511) behaved in a different way with respect to $dnaA_{Ec}$ gene regulation. When this plasmid was present in cells with the p$dnaA_{Ec}$ plasmid (pFHC871), it drastically reduced *dnaA''lacZ* expression (Table 3). This repression is close to the maximal repression that can be exerted at the $dnaA_{Ec}$ promoter (2, 5). The level of $DnaA_{Ec}$ protein in these cells was probably nearly normal, since the $dnaA_{Ec}$ gene is present in several copies.

Stability of plasmids in derivatives of strain RB210. It soon became clear that plasmids carrying the $dnaA_{Bs}$ or the hybrid genes were readily lost even when a selective pressure was kept on the cultures, as in the experiments described above. Figure 2A demonstrates the stability of plasmid pLA1540 (p$dnaA_{Bs}$) in various strains. The plasmid was stably maintained in the integratively suppressed strain TC1963, but it was lost in strains which carry plasmid pFHC871 (p$dnaA_{Ec}$). The higher loss rate in strain TC1963/pFHC871 was probably due to the lower copy number (Table 3). The pACYC184-derived p$dnaA_{Ec}$ plasmid showed no significant loss, although its starting copy number was the same (TC1963) or lower (RB210) than that of the pBR322-derived p$dnaA_{Bs}$ plasmid (Table 3). The plasmid with the $dnaA_{Bs/Ec}$ gene was also extremely unstable (Fig. 2B); in this case, the p$dnaA_{Ec}$ plasmid was also stably maintained. It is difficult to express the stability as absolute loss rates, since growth was inhibited by the plasmids with $dnaA_{Bs}$ or hybrid genes, giving rise to the biphasic segregation curves. However, that the loss rate is significant (1 to 5% per generation) was seen directly on the replicas from unselective to selective plates. Many of the colonies on the selective plates exhibited shapes (e.g., half colonies) indicating that the plasmids failed to segregate in the first divisions after cells were spread on the unselective plate (data not shown).

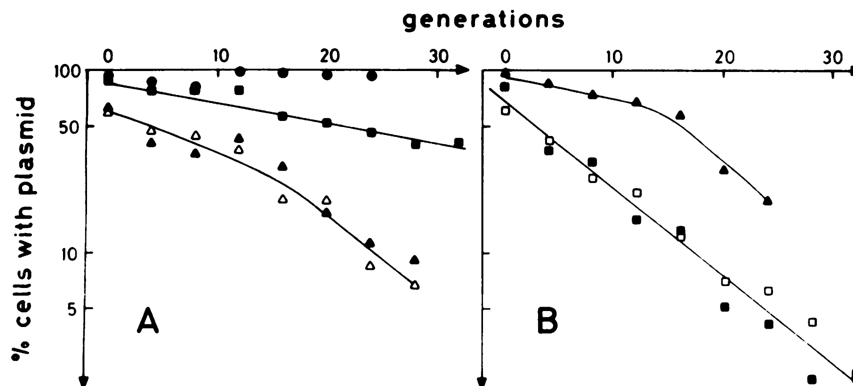

FIG. 2. Segregational instability of plasmids with the $dnaA_{Bs}$ gene (pLA1540) (A) or the $dnaA_{Bs/Ec}$ gene (pLA1511) (B). Plasmid instability was tested in different strains. Symbols: ○, TC1963 [*dna46 int*(mini-R1-*kan*)]; △, FH1358 (RB210/pFHC871); □, FH1551 (TC1963/pFHC871). Samples were taken with four-generation intervals. The fraction of plasmid-free cells was determined (closed symbols), or β-lactamase related activity was determined (open symbols) and related to the percentage of plasmid-free cells.

DISCUSSION

The plasmid carrying the *B. subtilis dnaA*-like gene ($dnaA_{Bs}$) constructed in this work transformed *E. coli* only if the strain carried a high-copy-number helper plasmid containing the $dnaA_{Ec}$ gene or if it was a *dnaA*(Ts) strain integratively suppressed by a mini-R1 replicon. The plasmid did not transform a normal strain, nor did it transform a *dnaA*(Ts) strain. These transformation data suggest that the $DnaA_{Bs}$ protein is inactive in the initiation of replication from *oriC* and interferes with the activity of the $DnaA_{Ec}$ protein at *oriC* either (i) by binding to the DnaA boxes in the origin but not having the right structure to interact with other essential components in the initiation reaction or (ii) by interacting with the $DnaA_{Ec}$ protein and preventing it from binding to *oriC*.

Expression of the $dnaA_{Ec}$ gene is repressed in a strain which carries a plasmid with the $dnaA_{Ec}$ gene and thus has a high $DnaA_{Ec}$ protein concentration, and it is derepressed in a *dnaA46*(Ts) strain (2, 5). In the integratively suppressed *dna46*(Ts) strain, the presence of the plasmid with the $dnaA_{Bs}$ gene led to a slight repression of $dnaA_{Ec}$ gene expression, indicating that the $DnaA_{Bs}$ protein is rather inefficient in binding to the DnaA box in the $dnaA_{Ec}$ promoter region. In the strains which contained the $dnaA_{Ec}$ plasmid, the introduction of the $dnaA_{Bs}$ plasmid led to derepression of the $dnaA_{Ec}$ expression. This derepression was not caused by the DnaA boxes in the $dnaA_{Bs}$ promoter region. A plasmid carrying this region did not affect $dnaA_{Ec}$ gene expression (data not shown). To explain the derepression, we propose that the DnaA proteins of the two species can form mixed oligomers which show little activity in binding to the DnaA box in the $dnaA_{Ec}$ promoter and to the boxes in *oriC*. From this interpretation of the data, we infer that the $DnaA_{Ec}$ and $DnaA_{Bs}$ proteins themselves form oligomers. Alternatively, one might imagine that the DnaA proteins act as monomers and that the $DnaA_{Bs}$ protein has a higher affinity for the DnaA box but is less efficient in repressing the $dnaA_{Ec}$ promoter than the $DnaA_{Ec}$ protein is. Intuitively, we believe that if this was the case, the $DnaA_{Bs}$ protein would also have a higher affinity for the DnaA boxes in *oriC* and thus effectively hinder initiation of replication no matter how much $DnaA_{Ec}$ protein was present. Therefore, we favor the first hypothesis.

Thus, in the cells carrying both *dnaA* genes on high-copy-number plasmids, in which the total amount of $DnaA_{Ec}$ protein is large, the amount of $DnaA_{Ec}$ oligomers, although smaller than in normal cells, as shown by the derepression of the promoter, is sufficient to sustain replication from *oriC*. In a wild-type cell carrying only the chromosomal copy of the $dnaA_{Ec}$ gene, we assume that the introduction of the $dnaA_{Bs}$ plasmid causes the concentration of $DnaA_{Ec}$ oligomers to drop below a threshold necessary for *oriC* function.

The $dnaA_{Bs}$ plasmid could also be introduced into a *dnaA*(Ts) strain integratively suppressed by the mini-R1 plasmid at 37°C, at which temperature the DnaA(Ts) protein was partially inactive but the transformants were cold sensitive. The plasmid could not be introduced into a $dnaA^+$ derivative of this strain. With the assumption that the mini-R1 origin requires *E. coli* DnaA protein for efficient initiation of replication, as indicated by in vitro experiments (23), these results suggest that the DnaA(Ts) protein is less efficient in forming mixed oligomers with the $DnaA_{Bs}$ protein, especially at high temperatures, and that the concentration of $DnaA_{Ec}$ protein thus is sufficient for mini-R1 replication. The inability to transform the $dnaA_{Bs}$ plasmid into the nonsuppressed *dnaA*(Ts) strain shows that it is insufficient for initiation from *oriC*.

The plasmid carrying a $dnaA_{Ec/Bs}$ gene, in which the first third originates from *E. coli* and the last two thirds from *B. subtilis*, behaves very similarly to the plasmid with the intact $dnaA_{Bs}$ gene. However, the inhibitory effect is not as strong. This difference could be caused by different levels of expression of the two genes.

The plasmid carrying the reciprocal *dnaA* fusion gene, $dnaA_{Bs/Ec}$, which contains the promoter region, the first third of the $dnaA_{Bs}$ gene and the last two thirds of the $dnaA_{Ec}$ gene, also interfered with the normal control of $dnaA_{Ec}$ gene expression. Only when *E. coli* was helped by the plasmid carrying the $dnaA_{Ec}$ gene could transformants with the $dnaA_{Bs/Ec}$ hybrid be obtained. The presence of the two plasmids in the cells led to a very strong repression of *dnaA' 'lacZ* expression, suggesting that the hybrid protein is very efficient in binding to the DnaA box in the $dnaA_{Ec}$ promoter. Therefore, the lack of transformants in a wild-type strain probably reflects a low expression of the chromosomal copy of the $dnaA_{Ec}$ gene and thereby a $DnaA_{Ec}$ protein concentration which becomes limiting for the initiation of chromosome replication. The lack of transformants in the mini-R1 integratively suppressed strain could be due either to a $DnaA_{Ec}$ concentration too low to support R1 replication or to interference with mini-R1 replication by binding of the $DnaA_{Bs/Ec}$ hybrid protein to the DnaA box in the R1 origin.

Both the $dnaA_{Bs}$ and $dnaA_{Bs/Ec}$ plasmids showed segregational instability. This result is probably due to the presence of the $dnaA_{Bs}$ promoter region which contains a number of DnaA boxes (20), since the coresident $dnaA_{Ec}$ plasmid was stable. The difference might also be due to the presence of a DnaA box in the pBR322 origin, which the p15 origin lacks. We interpret the instability as a clumping of plasmid molecules due to the binding of different DnaA protein forms to the DnaA boxes.

The construction of an intact *B. subtilis* $dnaA_{Bs}$ gene and hybrid *dnaA* genes will allow us to construct plasmids in which these genes are expressed from inducible promoters. We plan to use such plasmids to study the physiology of *E. coli* cells in which the initiation of replication and the regulation of the $dnaA_{Ec}$ gene is changed by foreign DnaA protein interference.

With respect to the initiation of replication in *B. subtilis*, construction of a plasmid with the intact $dnaA_{Bs}$ gene provides new possibilities for construction of *B. subtilis* minichromosomes. It could also be used for isolating mutations in the $dnaA_{Bs}$ gene.

ACKNOWLEDGMENTS

We are grateful to Lise Sørensen and Søs Koefoed for expert technical assistance and Kaspar von Meyenburg, Walter Messer, and Andrew Wright for discussions and help with the manuscript.

The work was supported by grants from the NOVO Foundation, Chr. Hansen Laboratory, the Danish Technical Research Council, and the Danish Natural Science Research Council.

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