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Comparison of *dnaA* Nucleotide Sequences of *Escherichia coli*, *Salmonella typhimurium*, and *Serratia marcescens*

OLE SKOVGAARD† AND FLEMMING G. HANSEN*

Department of Microbiology, Technical University of Denmark, DK-2800 Lyngby, Denmark

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The *dnaA* genes of *Salmonella typhimurium* and *Serratia marcescens*, which complemented the temperature-sensitive *dnaA46* mutation of *Escherichia coli*, were cloned and sequenced. They were very homologous to the *dnaA* gene of *E. coli*. The 63 N-terminal amino acids and the 333 C-terminal amino acids of the corresponding DnaA proteins were identical. The region in between, corresponding to 71 amino acids in *E. coli*, exhibited a number of changes. This variable region coincided with a nonhomologous region found in the comparison of *E. coli dnaA* and *Bacillus subtilis* "*dnaA*" genes. The regions upstream of the genes were also homologous. The ribosome-binding area, one of the promoters, the DnaA protein-binding site, and many GATC sites (*Dam* methyltransferase-recognition sequence) were conserved in these three enteric bacteria.

The DnaA protein is an essential factor in initiation of DNA replication at *oriC* in *Escherichia coli* (34). The DnaA protein affects the frequency of initiation. In *dnaA*(Ts) mutants, in which DnaA protein activity is low, the initiation frequency per cell mass is decreased (10, 15, 19, 32). In a cold-sensitive revertant of the *dnaA46* mutant, the initiation frequency is increased (18); this increase also occurs when the DnaA protein is overproduced (e.g., with a *dnaA* gene controlled by a λ p_L promoter) (3). The function of the DnaA protein is still not known, although it binds to DNA at specific sites (DnaA boxes) in the *oriC* region (12). The DnaA protein also binds to the DnaA box in the *dnaA* promoter region (4, 12). The DnaA protein probably interacts with several other proteins: the β subunit of the RNA polymerase (1), the DnaB protein (11), the DnaZ protein (37), and the *groE* gene products (9, 17). Thus, the DnaA protein is possibly involved in the assembly of the initiation complex.

The *dnaA* gene of *E. coli* has been sequenced. It encodes the DnaA protein with a M_r of 52,500 (13, 29). The *dnaA* gene is expressed from two promoters, *dnaA1p* and *dnaA2p* (14), and the expression is regulated by the DnaA protein itself. This autoregulation depends on the DnaA box located between the two promoters (2, 4, 18a).

The DNA sequence encompassing the origin of replication of *Bacillus subtilis* has been determined (24). It was found to contain a gene ("*dnaA*") coding for a protein homologous to the *E. coli* DnaA protein (28).

To extend the comparative study of this essential initiation factor, we cloned and sequenced the *dnaA* genes of two enteric bacteria, *Salmonella typhimurium* and *Serratia marcescens*. We compared the nucleotide sequences and the amino acid sequences (deduced from the nucleotide sequences) with those of the *E. coli dnaA* gene and DnaA protein, and we found that the three genes were very homologous. This study complements the study of Zyskind and co-workers (39), who have compared origins of replication from different enteric bacteria and found them to be very homologous.

* Corresponding author.
† Present address: Institute I, Life Sciences and Chemistry, University of Roskilde, DK-4000 Roskilde, Denmark.

MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* MT102 is an *hsdR* derivative of MC1000 (6) constructed by M. Trier-Hansen; *E. coli* FH1220 *trp-3 his-4 thi-1 dnaA46 pyrB*::Tn*5* and CM2517 *metB46 trp-3 his-4 thi-1 dnaA46 recA1* were used for complementation tests (both strains are derived from CM734) (16). Plasmid pFHC539 (35) was used as a hybridization probe. pGV455 is a sequencing vector analogous to those described in reference 33. Strain TA98 (23) was used to isolate *S. typhimurium* chromosomal DNA, and strain W225 (38) was used to isolate *S. marcescens* chromosomal DNA. Cultures were grown in NY medium (36).

DNA technology. Preparation of plasmid DNA, use of restriction enzymes (from New England BioLabs, Inc., or Amersham Corp.), ligation, and transformation were according to Maniatis et al. (20).

Southern DNA-DNA hybridization experiments (31) were carried out to determine the restriction map of the *dnaA* regions of *S. marcescens* and *S. typhimurium*. Chromosomal DNA was cut with different restriction enzymes, separated by agarose gel electrophoresis, denatured, transferred to filters (GeneScreen or GeneScreen Plus; New England Nuclear Corp.), and hybridized with nick-translated (kit from Amersham) pFHC539 DNA (pFHC539 carries the intact *dnaA* gene of *E. coli*). Hybridization and washings were carried out at 60°C.

Restriction fragments enriched for the *dnaA* gene of *S. marcescens* were purified from a digest of total chromosomal DNA as follows. Fragments of 4 to 6 kilobase pairs were electrophoresed 10 cm into an agarose gel and then collected by further electrophoresis onto DEAE membranes (NA45; Schleicher & Schuell, Inc.) which were repeatedly inserted into the same slit cut in front of the fragments. This process allowed us to pool chromosomal DNA fragments in well-defined size classes. The DNA fragments were eluted from the DEAE membrane with buffer (1.5 M NaCl, 7 mM Tris hydrochloride [pH 8.0], 1 mM EDTA, 0.1 mg of tRNA per ml) at 70°C for 30 min and recovered by ethanol precipitation. Fragment pools which were enriched for the *dnaA* gene were identified by dot-blot analysis (samples denatured at 95°C were spotted onto GeneScreen-Plus membrane) with nick-translated pFHC539 DNA as the hybridization probe.

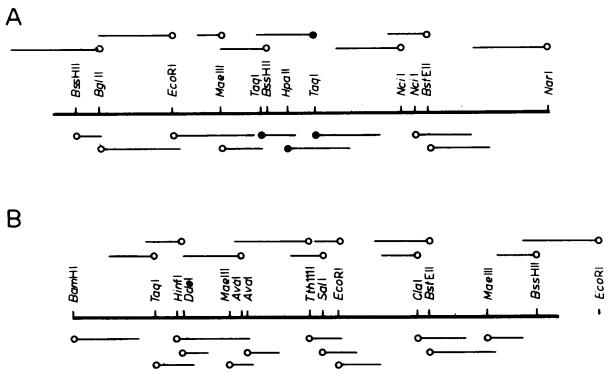


FIG. 1. Sequencing strategies for the *S. typhimurium* (A) and *S. marcescens* (B) *dnaA* genes. Only restriction sites used in the nucleotide sequencing are indicated. DNA fragments were end labeled by using Klenow polymerase and the appropriate ^{32}P-labeled deoxynucleoside triphosphate. Symbols: ○, fragments labeled directly; ●, fragments cloned in the sequencing vector pGV455 and then sequenced.

Nucleotide sequencing was according to Maxam and Gilbert (22). Plasmids pOSC277 (*S. marcescens*) and pOSC292 (*S. typhimurium*) were used as sources of DNA. The sequencing strategy is shown in Fig. 1. Part of the *S. typhimurium* sequence was obtained with fragments subcloned in the *Cla*I site of pGV455 (33).

RESULTS AND DISCUSSION

Restriction mapping and cloning of the dnaA regions of *S. typhimurium* and *S. marcescens*. Plasmid pFHC539 carrying the wild-type *E. coli dnaA* gene (35) was used to probe for homologous sequences in *S. typhimurium* and *S. marcescens*. We obtained hybridization signals from both bacteria, and we could construct restriction maps of the regions homologous to the *E. coli dnaA* gene (Fig. 2).

We cloned the *S. marcescens dnaA* gene as follows. The 4.7-kilobase-pair *Bam*HI fragment from *S. marcescens* (Fig. 2), which hybridized to pFHC539, was purified approximately 50 times (see Materials and Methods) and cloned into pBR322. A library of 100 clones was screened for the presence of the 1,000-base-pair (bp) *Eco*RI fragment characteristic of the *dnaA* region (Fig. 2). The plasmid DNA from one of these clones was subsequently found to hybridize to the *E. coli dnaA* region in a Southern experiment with nick-translated λ *tna-dnaA* phage DNA (16) as a probe. This plasmid (pOSC269) contained two different *Bam*HI fragments of 4.7 kilobase pairs. We subcloned the 1,950-bp *Bam*HI-*Eco*RV fragment (Fig. 2) and obtained plasmid

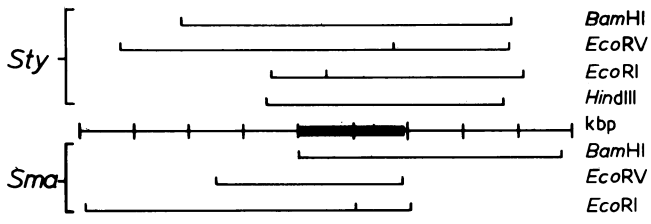


FIG. 2. Restriction enzyme map of the *dnaA* regions of *S. typhimurium* (*Sty*) and *S. marcescens* (*Sma*) derived from the hybridization experiments. ▬, Extent of *E. coli* DNA carried on plasmid pFHC539.

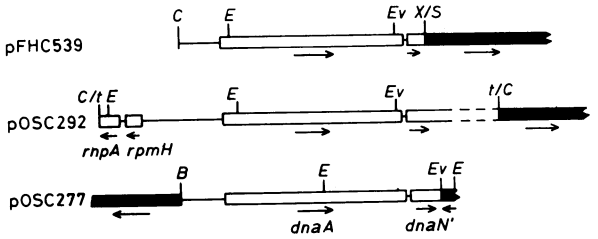


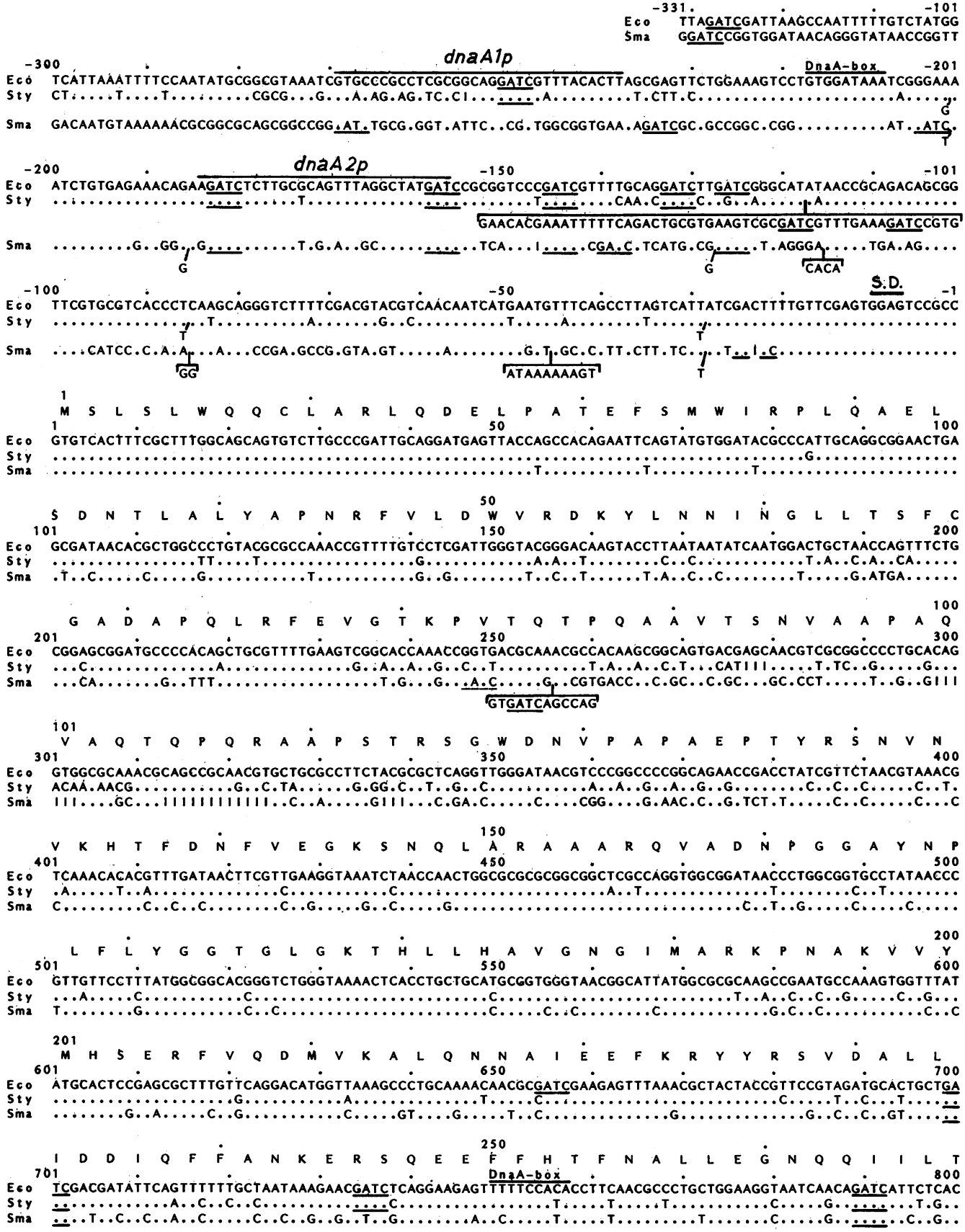
FIG. 3. Structure of plasmids carrying *dnaA* genes from *E. coli* (pFHC539 [35]), *S. typhimurium* (pOSC292), and *S. marcescens* (pOSC277). The three plasmids are pBR322 derivatives, with the chromosomal DNA inserted into the *tet* gene. Only the chromosomal part and the *tet* gene of pBR322 are shown. ▭, Chromosomal genes; ▬, *tet* gene. Arrows, orientation of the genes. Restriction enzyme sites: *C*, *Cla*I; *E*, *Eco*RI; *Ev*, *Eco*RV; *S*, *Sal*I; *X*, *Xho*I; *t*, *Taq*I.

pOSC277 (Fig. 3). The *dnaA*(Ts) strains (e.g., FH1220 [*dnaA46*] and CM2517 [*dnaA46 recA1*]) were transformed with plasmid pOSC277 at permissive temperature (30°C), and all transformants were found to form colonies also at 42°C. This complementation of dnaA(Ts) strains indicated that pOSC277 probably carried the intact *dnaA* gene.

The *Salmonella dnaA* gene complements *E. coli dnaA*(Ts) strains (21). Therefore, we used a library of clones containing partially *Taq*I-restricted *S. typhimurium* DNA inserted in pBR322 to transform a *dnaA46* strain (FH1220). One clone (pOSC292) was obtained at 42°C (approximately 10,000 transformants were obtained at 37°C), and restriction analysis demonstrated that it contained the 1,300-bp *Eco*RI-*Eco*RV and 1,000-bp *Eco*RI fragments in accordance with the restriction map of the *S. typhimurium dnaA* region (Fig. 2). This clone exhibited slow growth, which could be ascribed to the presence of the *rpmH* gene (data not shown). *E. coli* clones containing the *rpmH* and *dnaA* genes also exhibit slow growth (unpublished observation). The structure of plasmid pOSC292 is shown in Fig. 3.

Plasmids pOSC277 and pOSC292 were examined in maxicell experiments (30). In agreement with the complementation data, we found that the *dnaA* genes from *S. typhimurium* and *S. marcescens* were expressed efficiently in *E. coli* (data not shown).

Comparison of the *dnaA* gene sequences. The DNA sequences of the *dnaA* genes (and part of the *dnaN* genes) from the three enteric bacteria are shown in Fig. 4. The divergence of the *dnaA* genes was 12.2% between *E. coli* and *S. typhimurium*, 16.0% between *E. coli* and *S. marcescens*, and 17.1% between *S. typhimurium* and *S. marcescens* (deletions and insertions are not counted in this calculation). This comparison demonstrated that the *dnaA* genes from the three enteric bacteria were very homologous. Most of the nucleotide differences were situated in the third base position of the codon. Therefore, the relationships among the three bacteria become more striking when the amino acid sequences of the proteins are compared. The 63 N-terminal amino acids and the 333 C-terminal amino acids were identical (except for a shift from proline to alanine in *S. marcescens* at amino acid residue 194). Figure 5 shows the remaining 71 amino acids aligned for the three species. There were 15 amino acids which were different in *E. coli* and *S. typhimurium*, and the *S. typhimurium* protein was one amino acid shorter. The *S. marcescens* DnaA protein exhibited 22 amino acid differences in this region and had an insert of four and deletions of seven amino acids as compared with the *E.*


-331 -101
Eco TTAGATCGATTAAGCCAATTTTTGTCTATGG
Sma GGATCCGGTGGATAACAGGGTATAACCGGTT
dnaA1p
DnaA-box
-300 -201
Eco TCATTAAATTTTCCAATATGCGGCGTAAATCGTGCCCGCCTCGCGGCAGGATCGTTTACACTTAGCGAGTTCTGGAAAGTCCTGTGGATAAATCGGGAAA
Sty CT....T....T.....CGCG...G...A.AG.AG.TC.CI.....A.....T.CTT.C.....A....
Sma GACAATGTAAAAAACGCGGCGCAGCGGCCGG.AT.TGCG.GGT.ATTC..CG.TGGCGGTGAA.AGATCGC.GCCGGC.CGG.....AT..ATC.
dnaA2p
-200 -150 -101
Eco ATCTGTGAGAAACAGAAGATCTCTTGCGCAGTTTAGGCTATGATCCGCGGTCCCGATCGTTTTGCAGGATCTTGATCGGGCATATAACCGCAGACAGCGG
Sty .....T.....T.....T.....CAA.C....C..G..A.....A.....
GAACACGAAATTTTTCAGACTGCGTGAAGTCGCGATCGTTTGAAAGATCCGTG
Sma .....G..GG..G.....T.G.A..GC.....TCA...I....CGA.C.TCATG.CG.....T.AGGGA.....TGA.AG....
CACA
S.D.
-100 -50 -1
Eco TTCGTGCGTCACCCTCAAGCAGGGTCTTTTCGACGTACGTCAACAATCATGAATGTTTCAGCCTTAGTCATTATCGACTTTTGTTCGAGTGGAGTCCGCC
Sty .....T.....A.....G..C.....T.....A.....T.....
Sma ....CATCC.C.A.A...A...CCGA.GCCG.GTA.GT.....A.....G.T.GC.C.TT.CTT.TC.....T..I.C.....
GG
ATAAAAAAGT
1
M S L S L W Q Q C L A R L Q D E L P A T E F S M W I R P L Q A E L
1 50 100
Eco GTGTCACTTTCGCTTTGGCAGCAGTGTCTTGCCCGATTGCAGGATGAGTTACCAGCCACAGAATTCAGTATGTGGATACGCCCATTGCAGGCGGAACTGA
Sty .....G.....
Sma .....T.....T.....T.....
50
S D N T L A L Y A P N R F V L D W V R D K Y L N N I N G L L T S F C
101 150 200
Eco GCGATAACACGCTGGCCCTGTACGCGCCAAACCGTTTTGTCCTCGATTGGGTACGGGACAAGTACCTTAATAATATCAATGGACTGCTAACCAGTTTCTG
Sty .....TT....T.....G.....A.A..T.....C..C.....T.A..C.A..CA.....
Sma .T..C.....C.....G.....T.....G..G.....T..C..T.....T.A..C..C.....T.....G.ATGA.....
100
G A D A P Q L R F E V G T K P V T Q T P Q A A V T S N V A A P A Q
201 250 300
Eco CGGAGCGGATGCCCCACAGCTGCGTTTTGAAGTCGGCACCAAACCGGTGACGCAAACGCCACAAGCGGCAGTGACGAGCAACGTCGCGGCCCCTGCACAG
Sty ...C.....A.....G..A..A..G..C..T.....T.A..A..C.T...CATIII.....T.TC..G.....G...
Sma ...CA.....G..TTT.....T.G...G...A.C.....G..CGTGACC..C.GC..C.GC...GC.CCT.....T..G..GIII
GTGATCAGCCAG
101
V A Q T Q P Q R A A P S T R S G W D N V P A P A E P T Y R S N V N
301 350 400
Eco GTGGCGCAAACGCAGCCGCAACGTGCTGCGCCTTCTACGCGCTCAGGTTGGGATAACGTCCCGGCCCCGGCAGAACCGACCTATCGTTCTAACGTAAACG
Sty ACAA.AACG.....G..C.TA.....G.GG.C..T..G..C.....A..A..G..A..G..G.....C..C..C.....C..T.
Sma III....GC...IIIIIIIIIII..C..A.....GIII...C.GA.C.....C....CGG....G.AAC.C..G.TCT.T.....C..C.....C...C
150
V K H T F D N F V E G K S N Q L A R A A A R Q V A D N P G G A Y N P
401 450 500
Eco TCAAACACACGTTTGATAACTTCGTTGAAGGTAAATCTAACCAACTGGCGCGCGCGGCGGCTCGCCAGGTGGCGGATAACCCTGGCGGTGCCTATAACCC
Sty .A.....T..A.....C.....C.....A..T.....T.....C..T.....
Sma C.....C..C..C.....C..G.....G..C.....G.....C..T..G.....C.....C.....
200
L F L Y G G T G L G K T H L L H A V G N G I M A R K P N A K V V Y
501 550 600
Eco GTTGTTCCTTTATGGCGGCACGGGTCTGGGTAAAACTCACCTGCTGCATGCGGTGGGTAACGGCATTATGGCGCGCAAGCCGAATGCCAAAGTGGTTTAT
Sty ...A.....C.....C.....C.....T..A..C..C..G.....C..G...
Sma T.....G.....C..C.....C.....C..C.....C.....G.C..C.....C..C
201
M H S E R F V Q D M V K A L Q N N A I E E F K R Y Y R S V D A L L
601 650 700
Eco ATGCACTCCGAGCGCTTTGTTCAGGACATGGTTAAAGCCCTGCAAAACAACGCGATCGAAGAGTTTAAACGCTACTACCGTTCCGTAGATGCACTGCTGA
Sty .....G.....A.....T.....C.....C.....T..C...T.....
Sma .....G..A.....C..G.....C.....GT....G.....T..C.....G.....G..C..C..GT.....
250
I D D I Q F F A N K E R S Q E E F F H T F N A L L E G N Q Q I I L T
701 DnaA-box 800
Eco TCGACGATATTCAGTTTTTTGCTAATAAAGAACGATCTCAGGAAGAGTTTTTCCACACCTTCAACGCCCTGCTGGAAGGTAATCAACAGATCATTCTCAC
Sty .....A..C..C..C.....C.....T.....T.....T.....C.....G.....T.G..
Sma .....T..C..C..A..C.....C..C..G..G..T..G.....A..C.....T.....T.....T.....C.....G.....C..G..

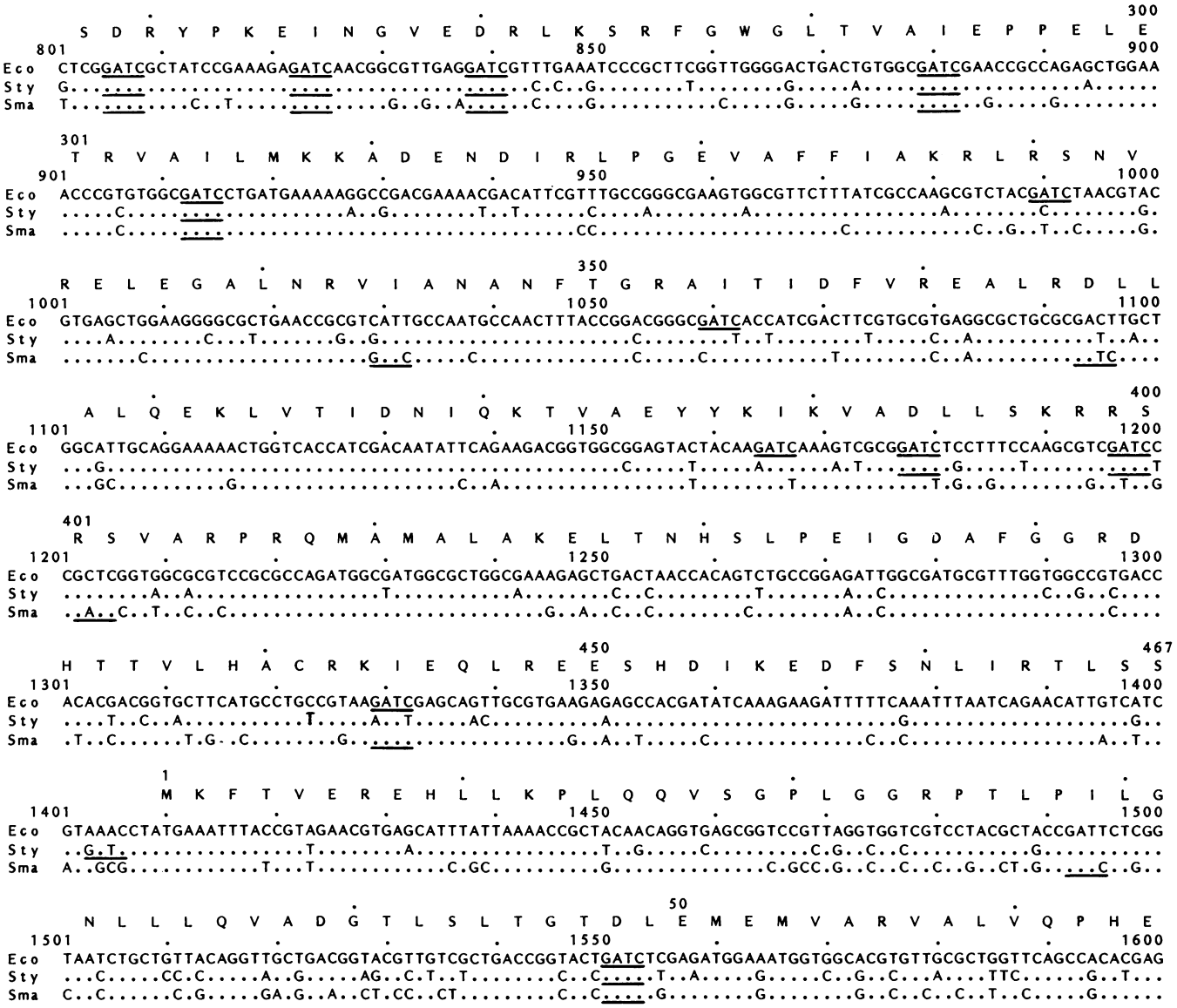


FIG. 4. Comparison of the nucleotide sequences of the dnaA genes and flanking sequences of *E. coli* (Eco), *S. typhimurium* (Sty), and *S. marcescens* (Sma). The *S. typhimurium* and *S. marcescens* sequences are aligned with the *E. coli* sequence, which is numbered relative to the first base in the *dnaA* start codon. ·, Conserved base; | , deleted base; inserted bases are indicated below the respective sequences. The *dnaA* promoters, ribosome-binding sites (S.D. [Shine-Dalgarno sequence; 30a]), and DnaA boxes are shown above the *E. coli* sequence. The amino acid sequences of the *E. coli* DnaA protein and of the first part of the DnaN protein are also indicated above the sequences. GATC sequences (Dam methylation sites) are underlined.

coli DnaA protein. This variable region of the DnaA protein from the three enteric bacteria coincides precisely with the region in the *B. subtilis* "*dnaA*" gene product which has no homology to the *E. coli* DnaA protein (28).

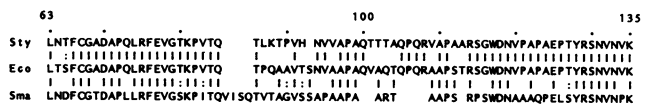


FIG. 5. Comparison of the amino acid sequences in the variable regions of *E. coli* (Eco), *S. typhimurium* (Sty), and *S. marcescens* (Sma). |, Identical amino acid residues; :, conservative changes of amino acid residues.

We suggest that the identity of the N-terminal and C-terminal domains of the DnaA protein indicates a constraint on the evolution of the gene, which may be due to many inherent functions of the DnaA protein, e.g., binding to the DnaA boxes, possible formation of oligomers, interaction with RNA polymerase, or interaction with other proteins.

S. typhimurium and *S. marcescens* showed the same pattern at the *dnaA-dnaN* transition as that seen in *E. coli* (29), i.e., the *dnaA* stop codon followed by four nucleotides and then the *dnaN* start codon (Fig. 4). We were able to compare the 64 N-terminal amino acids of the three *dnaN* gene products, and we found that this replication factor was also conserved; *S. typhimurium* showed a divergence of two amino acids, and *S. marcescens* showed a divergence of seven amino acids when compared with *E. coli*.

FIG. 6. Comparison of nucleotide sequences in the promoter regions of *E. coli* (Eco) and *S. marcescens* (Sma). |, Identical bases. Coordinates are the same as those described in the legend to Fig. 4.

A comparison of gene products in the three bacteria used in this study has been carried out in a number of other studies. The TrpG protein (194 amino acids) has 8 amino acid changes from *E. coli* to *S. typhimurium* and 35 amino acid changes from *E. coli* to *S. marcescens* (26). The TrpA protein (268 amino acids) has 40 changes from *E. coli* to *S. typhimurium* (27). The TrpB protein (397 amino acids) has 14 changes from *E. coli* to *S. typhimurium* (8). The Lpp protein (78 amino acids) has 6 changes from *E. coli* to *S. marcescens* (25). In all these cases, the amino acid differences are more evenly distributed in the proteins than was observed for the DnaA protein. Also, a comparison at the nucleotide level indicates that fewer constraints on these genes have been present during evolution.

The catabolite activator protein amino acid sequences from *E. coli* and *S. typhimurium* have also been compared. Only one amino acid divergence was found (7). The catabolite activator protein is also a protein with several functions, i.e., subunit-subunit interaction, binding of cyclic AMP, and binding to the specific DNA sequences. We believe that the very high conservation of the catabolite activator protein and of the two domains of the DnaA protein demonstrates that the more different molecular interactions are carried out by a protein, the higher the evolutionary contraint will be.

Comparison of the *dnaA* promoter regions. A comparison of the regions upstream of the three *dnaA* genes is shown in Fig. 4. It can be seen that the *dnaA*-proximal promoter, *dnaA2p*, was almost identical in the three species. Also, the DnaA box TTATCCACA was conserved. Sites for *Dam* methylation (GATC sequences) are underlined in Fig. 4. All three species exhibited a high number of *Dam* methylation sites in the *dnaA* promoter region, as well as in a region of the structural gene. This latter region in the *E. coli dnaA* gene contained a DnaA box-like sequence which had degenerated in the two other organisms. Methylation of a GATC sequence in the *dnaA2p* promoter region, which was conserved in the three species, is essential for initiation of transcription from this promoter in *E. coli* (5, 18a). Thus, the main regulatory region of the *dnaA* gene in *E. coli* (2, 4) was highly conserved.

The region upstream of the DnaA box in *S. typhimurium* also showed homology with the *dnaA1p* promoter region of *E. coli*. The −10 sequences (TACACT) were identical, whereas the −35 regions differed. For *S. marcescens*, no homology with the corresponding region in *E. coli* could be observed. A closer look (Fig. 6) revealed that the sequence upstream of the DnaA box in *S. marcescens* showed significant homology with the *dnaA2p* region. We conclude from this comparison that the *dnaA2p* promoter area has been duplicated in *S. marcescens*. However, several changes have occurred in the duplicated area, making it difficult to recognize a −10 sequence, and a mutation in the DnaA box has also occurred. The nucleotide sequencing has not been extended beyond the *Bam*HI site in *S. marcescens*. Therefore, we cannot exclude the possibility that a promoter analogous to *dnaA1p* is present upstream of the *Bam*HI site.

The region between the *dnaA2p* promoter and the *dnaA* gene is conserved in *S. typhimurium*, except for a large insertion of 53 bp in a region with otherwise good homology. *S. marcescens* showed less homology in this region. However, the 22 bp upstream of the start of the structural gene, which contain the ribosome binding site, were identical, indicating that *dnaA* gene expression at the translational level (translation initiation frequency) might be very similar in the three organisms.

We thus conclude that the regulation of expression of the *dnaA* gene in the three bacterial species probably is identical.

ACKNOWLEDGMENTS

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