Bacterial Gre factors associate with RNA polymerase (RNAP) and stimulate intrinsic cleavage of the nascent transcript at the active site of RNAP. Biochemical and genetic studies to date have shown that Escherichia coli Gre factors prevent transcriptional arrest during elongation and enhance transcription fidelity. Furthermore, Gre factors participate in the stimulation of promoter escape and the suppression of promoter-proximal pausing during the beginning of RNA synthesis in E. coli. Although Gre factors are conserved in general bacteria, limited functional studies have been performed in bacteria other than E. coli. In this investigation, ChAP-chip analysis (chromatin affinity precipitation coupled with DNA microarray) was conducted to visualize the distribution of Bacillus subtilis GreA on the chromosome and to determine the effects of GreA inactivation on core RNAP trafficking. Our data show that GreA is uniformly distributed in the transcribed region from the promoter to coding region with core RNAP, and its inactivation induces RNAP accumulation at many promoter or promoter-proximal regions. Based on these findings, we propose that GreA would constantly associate with core RNAP during transcriptional initiation and elongation and resolves its stalling at promoter or promoter-proximal regions, thus contributing to the even distribution of RNAP along the promoter and coding regions in B. subtilis cells.
in vivo function of GreA in Bacillus Subtilis

The transcription elongation factors NusA, NusB, and NusG are concentrated in specific regions of the nucleoid termed transcription foci, which represent major sites of rRNA synthesis in B. subtilis cells. In contrast, B. subtilis GreA localizes uniformly throughout the nucleoid, suggesting its constant association with RNAP synthesizing mRNA (5, 7).

Recent studies have explored the trafficking of core RNAP and the transcription factors, i.e., the sigma factor (E. coli σ70 and B. subtilis σA) and elongation factor NusA, on the chromosomes of E. coli and B. subtilis using ChiP-chip and CHAP-chip (chromatin affinity precipitation coupled with DNA microarray) methods (15, 22, 29a). The results suggest that the sigma factor in the initiation complex of RNAP is replaced by several bases to give one amino acid change (Asp44 to Ala) of GreA and the region upstream of greA was amplified by PCR from B. subtilis chromosomal DNA using the primer sets D44AgreA1F-D44greA1R and D44greA2F-Cm-D44AgreA2R, respectively. Primers D44AgreA1F and D44AgreA2R introduced substitutions of several bases to give one amino acid change (Asp44 to Ala) of GreA and the recognition site of the restriction enzyme, ApaLI, used for the confirmation of the substitution in greA gene. The D44greA2F-Cm primer contained a 22-bp sequence complementary to the PCR-CmR2 primer at the 5′ end. The region upstream of greA was amplified from B. subtilis 168 chromosomal DNA by using the primers D44greA3F and D44greA3R. The D44greA3R primer contained a 22-bp sequence complementary to the PCR-CmR2 primer at the 5′ end. The resulting four fragments were fused by PCR using the D44greA3F-D44greA3R primer set and integrated into the B. subtilis chromosome via homologous recombination through the 5′ and 3′ flanking regions.

A strain expressing C-terminal histidine-tagged RpoC (168ropCHis) was transformed with chromosomal DNA of YK03 to obtain YK04 (ropC-his greAg). The GreA-D44A strain [greA(D44A)] harboring a point mutation altering Asp44 of GreA to Ala (YK05) was constructed by using PCR (shown schematically in Fig. 1). The chloramphenicol-resistance gene with the terminator region was obtained from the pDLT3 plasmid (23) by using the rPCR-CmF2 and rPCR-CmR2 primer sets. The D44greA1F-D44greA2R and D44AgreA1F-D44greA1R primers were subsequently purified from antiserum by using peptide affinity column treatments with formaldehyde (1% final concentration) for 30 min at 57°C. Cells were washed with Tris-buffered saline buffer (pH 7.5) and stored at −80°C. Affinity purification of RNAP complexes was performed according to a previously described procedure for CHAP-chip experiments (17) with the following modifications. Dithiothreitol was removed from the UT buffer, Dynabead Talon (50 μl, Invitrogen) used instead of MagNeHis beads, and elution of complexes from Dynabeads was performed in twice with 400 μL of elution buffer. Recovered RNAP complexes were heated at 95°C for 30 min to remove cross-linking, and the appropriate amounts of proteins were separated by using a 5 to 20% SDS-PAGE gradient gel, followed by transfer to polyvinylidene difluoride membrane (GE Healthcare) via electroblotting at 100 V for 1.5 h (RpoC, SigA, and NusA) or 4 h (GreA). Western blotting was performed according to the instructions of the Amersham ECL Plus Western blotting detection system (GE Healthcare) using horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Bio-Rad). Mouse polyclonal anti-RpoB antibody was obtained from Neoclone, and rabbit polyclonal anti-σA antibody was kindly provided by Fujio Kawamura (24). Rabbit polyclonal anti-NusA and anti-GreA antibodies were prepared as described below.

Preparation of anti-GreA or NusA peptide antibody. Peptides corresponding to residues 21 to 37 (EAGDRSKISVRTDDP), 57 to 73 (RVFARKDVVDEVYDQRL), 168sigAHis, 168nusAHis, and YK02 (expressing His-tagged GreA) were obtained in 400 μL of Luria-Bertani (LB) medium containing chloramphenicol (0.5 μg/ml) under aerobic conditions at 37°C until cultures reached an optical density at 600 nm (OD600) of 0.4. Each culture was treated with formaldehyde (1% final concentration) for 30 min at 57°C. Cells were washed with Tris-buffered saline buffer (pH 7.5) and stored at −80°C. Affinity purification of RNAP complexes was performed according to a previously described procedure for CHAP-chip experiments (17) with the following modifications. Dithiothreitol was removed from the UT buffer, Dynabead Talon (50 μL, Invitrogen) used instead of MagNeHis beads, and elution of complexes from Dynabeads was performed in twice with 400 μL of elution buffer. Recovered RNAP complexes were heated at 95°C for 30 min to remove cross-linking, and the appropriate amounts of proteins were separated by using a 5 to 20% SDS-PAGE gradient gel, followed by transfer to polyvinylidene difluoride membrane (GE Healthcare) via electroblotting at 100 V for 1.5 h (RpoC, SigA, and NusA) or 4 h (GreA). Western blotting was performed according to the instructions of the Amersham ECL Plus Western blotting detection system (GE Healthcare) using horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG (Bio-Rad). Mouse polyclonal anti-RpoB antibody was obtained from Neoclone, and rabbit polyclonal anti-σA antibody was kindly provided by Fujio Kawamura (24). Rabbit polyclonal anti-NusA and anti-GreA antibodies were prepared as described below.

Preparation of anti-GreA or NusA peptide antibody. Peptides corresponding to residues 21 to 37 (EAGDRSKISVRTDDP), 57 to 73 (RVFARKDVVDEVYDQRL), 228 to 244 (EAGDRSKISVRTDDP), and integrated into the B. subtilis chromosome via homologous recombination.

Preparation of anti-GreA or NusA peptide antibody. Peptides corresponding to residues 21 to 37 (EAGDRSKISVRTDDP), 57 to 73 (RVFARKDVVDEVYDQRL), 228 to 244 (EAGDRSKISVRTDDP), and integrated into the B. subtilis chromosome via homologous recombination.

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OD$_{600}$ of 0.4. The procedure for ChAP fraction preparation was similar to that for pulldown purification of the RNAP complex, and the final volume of the elution fraction was 40 µl. Cross-linked whole-cell extract fractions before purification of RNAP in each experiment were used to prepare control DNA for ChAP-chip analysis. Protein-DNA cross-links were dissociated by heating overnight at 65°C. DNA was subsequently purified by using QiaQuick (Qiagen) and eluted with 50 µl of nuclease-free water (Ambion).

Random amplification and terminal labeling of DNA in whole-cell extracts or affinity-purified fractions and hybridization to the custom Affymetrix tiling chip were performed as described previously (17). The signal intensities of DNA isolated from the affinity purification and whole-cell extract fractions before purification (control DNA) were adjusted to confer a signal average of 500. The signal intensities of DNA in the affinity-purified fraction were divided by those of control DNA for quantitative estimation of the enrichment of DNA fragments by affinity purification (37). The binding signals represented by the enrichment values were visualized along the genome coordinate by using the In Silico Molecular Cloning Program, Array Edition (In Silico Biology, Japan). All experiments were performed in duplicate.

Analysis of the TR of RNAP. The $\sigma^\beta$ binding peaks were automatically detected (15), with the threshold value set as 2.0. We selected genes positioned immediately downstream of the $\sigma^\beta$ binding sites, removing those located divergently and sharing the same $\sigma^\beta$ binding sites. Consequently, we selected 268 genes with sufficient RNAP signal intensities (>0.95) and lengths (>150 bp) for traveling ratio (TR) calculation (15, 29a).

Transcriptome analysis. Total RNA was purified from wild-type, YK03, and YK05 strains cultured in 200 ml of LB medium at 37°C under aerobic conditions to an OD$_{600}$ of 0.4. Synthesis of cDNA, terminal labeling, and hybridization to the custom Affymetrix cDNA chip were performed as described previously (4). The signal intensities of perfectly matched probes (only) were used in this analysis and were adjusted to confer a signal average of 500. Data visualization was performed by the In Silico Molecular Cloning Program. The average signal intensities of probes in each gene were calculated, and 2,824 genes with average signal intensities of >100 in wild-type, $\Delta$greA, and GreA-D44A cells were used to search for genes that were up- or downregulated upon inactivation of GreA. Comparison of transcriptome between the wild type and each greA mutant was performed by four different combinations using duplicate data for each strain.

Array data. Raw data (CEL format) from ChAP-chip and transcriptome experiments have been deposited in ArrayExpress under accession numbers E-MEXP-3056 and E-MEXP-3055, respectively.

RESULTS

Distribution of GreA on the B. subtilis chromosome. To visualize the genome-wide association of GreA with RNAP, we created a strain expressing GreA tagged with 12 histidines at the C terminus under the control of the original promoter on
the chromosome. GreA-His-expressing cells were cultivated in LB medium under aerobic conditions and harvested at an OD_{600} of 0.4, followed by ChAP-chip analysis, as described earlier (15). In parallel, we performed ChAP-chip analysis of the core RNAP (β subunit), σ^A, and NusA, as well as transcription analysis using cells cultured under similar conditions. Typical distributions of protein-binding and transcription signals are shown in Fig. 2, and the complete data set is presented in Fig. S2 in the supplemental material. The core RNAP binding signals started from the transcription start site (5' end of contiguous transcription signals; gray line) and were evenly distributed along the transcribed region (Fig. 2A and B). The σ^A signals were observed symmetrically at the transcription start site (Fig. 2C), while the NusA signals started slightly downstream of the transcription start site and were distributed throughout the transcribed regions (Fig. 2D). These features are consistent with our previous findings (15). We observed that GreA signals were distributed along the transcribed regions (Fig. 2E), a finding similar to those for core RNAP and NusA. However, absolute signal intensities were lower and background signals were higher than binding signals of other proteins, probably because of indirect interactions of GreA with DNA and/or lower accessibility of His tag in the GreA-RNAP complex. In addition, we found no regions where GreA signals are observed without RNAP signals. Furthermore, we detected genome-wide positive correlation between the RNAP and GreA binding signals (r = 0.86, Fig. 3A) in the coding regions, similar to NusA binding signals (r = 0.94, Fig. 3B). These results suggest that GreA is constantly associated with the majority of core RNAP during transcription elongation in B. subtilis cells, which is consistent with the overlapping localization of RNAP-green fluorescent protein (GFP) and GreA-GFP fluorescence (7). The reduced correlation of signal intensities between GreA and RNAP in the low-signal-intensity region in Fig. 3A would be caused by the higher background signals of GreA.

**GreA is involved in the initiation and elongation of RNAP complexes.** Several biochemical and structural studies have established that sigma factor and NusA compete for the same binding surface of core RNAP (10, 41), while GreA associates with core RNAP at a different site (secondary channel). This finding suggests that, in addition to association with the elongation complex of RNAP, Gre factor also interacts with the initiation complex of RNAP. In support of this hypothesis, start sites of the GreA binding signals appeared to shift to transcription start sites, compared to those of NusA signals (Fig. 2 and see Fig. S2 in the supplemental material). To confirm GreA association with the RNAP initiation complex, we analyzed the composition of RNAP complexes with the pulldown assay using His-tagged RpoC, GreA, NusA, or σ^A as bait. Strains expressing 12×His-tagged GreA, NusA, σ^A, and RpoC were cultivated to an OD_{600} of 0.4, and cellular proteins were cross-linked with formaldehyde. Subsequently, cross-linked protein complexes were purified with nickel magnetic beads, and proteins included within the purified complexes were fractionated by SDS-PAGE after the removal of cross-linking by heat treatment. For precise comparison of the amounts of components within each complex, protein mixtures containing similar concentrations of RpoB (representing the amount of core RNAP) were subjected to SDS-PAGE (see Fig. S3 in the supplemental material), and RpoB, σ^A, NusA, and GreA were detected using specific antibodies (Fig. 4A). Upon purification of the RNAP complex using RpoC-His, we detected σ^A, NusA, and GreA (lane 4). However, the RNAP complex purified with σ^A-His (initiation complex) did not contain NusA (lane 3), while the complex purified using NusA-His (elongation complex) did not contain σ^A (lane 2), indicating mutually exclusive binding of σ^A and NusA to core RNAP. In contrast, GreA was detected in both complexes, albeit weakly, probably due to indirect interactions. Furthermore, both NusA and σ^A were detected in the RNAP complex purified by using GreA-His (lane 1), supporting the association of GreA with both initiation and elongation complexes of RNAP.

We further investigated the composition of RNAP complexes purified by using RpoC-His in rifampin-treated cells (Fig. 4B). Rifampin inhibits transition from the initiation to the elongation complex of RNAP that allows the progression of transcription (40), leading to the accumulation of the RNAP initiation complex only. As expected, NusA disappeared from the RNAP complex rapidly after the addition of rifampin to cell culture, while the σ^A level was not affected. However, GreA was detected in the RNAP complex after rifampin addition, supporting the association of GreA with both the initiation and the elongation complexes of RNAP.

**RNAP accumulates in the promoter-proximal regions of many genes in ΔgreA and GreA-D44A mutant cells.** In E. coli cells, Gre factors are involved in restarting paused complexes of RNAP during transcriptional elongation (36) and, additionally, directly stimulate transcription initiation for some promoters (34, 35). We investigated whether GreA is involved in the regulation of RNAP trafficking in the B. subtilis chromosome by comparing the core RNAP distribution patterns in wild-type and greA deletion (ΔgreA) mutant strains. Furthermore, we evaluated the effects of GreA inactivation using cells expressing mutant GreA protein defective in stimulating nucleolytic cleavage activity of RNAP. Two conserved acidic residues of GreA, Asp 41 and Glu 44, promote intrinsic transcript cleavage of RNAP in E. coli, and replacement of Asp 41 with Ala abolishes GreA activity (20). B. subtilis GreA displays 66 and 55% sequence similarities to E. coli GreA and GreB, respectively, and these important catalytic amino acids are conserved (see Fig. S4 in the supplemental material). Accordingly, we introduced a mutation altering Asp 44 (corresponding to Asp 41 in E. coli GreA) to Ala in B. subtilis greA to obtain the GreA-D44A strain.

Although no clear effects on distribution of elongating RNAP were observed, accumulation of core RNAP at the promoter or promoter-proximal regions was induced upon greA deletion and GreA-D44A mutation (see Fig. S5 in the supplemental material; see also Fig. 6). To further investigate this phenomenon, we compared the relative ratios of the core RNAP binding signal intensities of the probes in the promoter and coding regions (traveling ratio [TR]; Fig. 5A) (15, 29a) in the promoter region (see Table S2 in the supplemental material). Histograms of distribution of the TR values of these genes in wild-type (Fig. 5B), ΔgreA (Fig. 5C), and GreA-D44A (Fig. 5D) cells clearly indicate that GreA inactivation results in reduction of TR. Furthermore, scatter plots comparing TR values in wild-type,
FIG. 2. Distribution of binding signals of core RNAP (β’), σ^A, NusA, and GreA compared to transcription signals at the metK-asnB-ynA operon. The arrangement of genes (thick arrows) and predicted terminators (arrows with open circles) is schematically shown at the bottom. Transcript (A), core RNAP (β’) (B), σ^A (C), NusA (D), and GreA (E) binding signals for each probe are indicated by vertical bars at the appropriate genomic coordinates. The gray line represents the transcriptional start site. The results of two independent experiments are shown.
ΔgreA (Fig. 5E) and GreA-D44A strains (Fig. 5F) disclose decreased TR values in the majority of genes examined and not specific genes. Our findings suggest that GreA inactivation results in the stalling of RNAP at the promoter or promoter-proximal region, supporting its general involvement in stimulation of promoter escape or suppression of promoter-proximal pausing in *B. subtilis* cells.

Notably, the GreA-D44A mutation exerted a more significant effect on RNAP trafficking than GreA deletion. Thus, it appears that GreA activity in assisting nucleolytic cleavage activity of RNAP is essential to resolve stalling at the promoter or promoter-proximal regions. Furthermore, it is possible that the GreA-D44A protein retains the ability to bind RNAP, similar to the *E. coli* mutant protein, which interferes with the intrinsic nucleolytic activity of active RNAP (20), although no direct evidence to support this theory has been obtained.

**GreA contributes to resolving the stall of α3-RNAP.** Next, we attempted to characterize the stalled RNAP complexes in GreA-inactivated cells, focusing on genes for which core RNAP peaks appeared clearly at the promoter or promoter-proximal regions in ΔgreA and GreA-D44A cells. We selected genes whose TR values were reduced by more than 0.20, followed by visual inspection to strictly define core RNAP accumulation in *greA* mutants. As a result, 13 genes were identified

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**FIG. 3.** Genome-wide correlation between core RNAP and GreA or NusA binding signals. Log scatter plots of the average signal intensities of core RNAP (represented by β') and GreA (A) or NusA (B) for 1,066 genes positioned immediately downstream of the α3 binding peaks are presented. The correlation coefficient (r) is indicated in each panel.

**FIG. 4.** Pulldown assay of components of the RNAP complexes. (A) RNAP complexes purified using His-tagged GreA (lane 1), NusA (lane 2), SigA (lane 3), and RpoC (lane 4) as bait were separated using SDS-PAGE and detected with antibodies against RpoB, SigA, NusA, and GreA, as indicated at the left of each panel. Because a large amount of His-tagged bait protein was present in each sample, diluted sample was used to detect the band of bait proteins indicated by black circles (●). (B) Whole-cell lysates and RNAP complexes purified using RpoC-His from *B. subtilis* cells treated with rifampin for 0, 1, 2, or 3 min were separated by SDS-PAGE and probed with antibodies against RpoB, SigA, NusA, and GreA, as indicated at the left of each panel.
in ΔgreA cells (see Fig. S6 in the supplemental material, genes 1 to 13) and 34 genes in GreA-D44A cells (see Fig. S6 in the supplemental material, genes 2 to 35). Among these, 1 and 22 genes were reproducibly detected only in ΔgreA and GreA-D44A cells, respectively, and 12 genes reproducibly detected in both strains. We further examined whether these stalled complexes were σ^A-RNAP or NusA-RNAP by searching for genes in which σ^A and NusA peaks could be discriminated. We identified 10 genes (marked by asterisks in Fig. S6 in the supplemental material) in which σ^A and NusA peaks overlapped to a lesser extent. In most of these genes, accumulated RNAP peaks overlapped with σ^A peaks (Fig. 6), suggesting that the majority of RNAP peaks induced by GreA inactivation constitute the σ^A-RNAP complex.

The greA deletion and D44A substitution have little impact on the transcriptome. Finally, we investigated the impact of GreA inactivation on genome-wide transcriptional regulation in B. subtilis cells. Total RNA was prepared from wild-type,
ΔgreA, and GreA-D44A cells, cultivated in LB medium under aerobic conditions, and harvested at an OD₆₀₀ of 0.4, and transcriptome profiles were obtained by using the tiling chip used for the ChAP-chip experiments, as described earlier (4).

We selected 2,824 genes with average signal intensities of >100 in the coding regions in all three strains and generated scatter plots of their transcription signal intensities, as shown in Fig. 7.

Next, we searched for genes that are up- or downregulated by >2.8-fold (i.e., log₂ 1.5) in ΔgreA and GreA-D44A cells, compared to wild-type cells, with P values (Student t test) lower than 0.05. As a result, 28 upregulated and 35 downregulated genes were identified (see Table S3 in the supplemental material). Among the 28 upregulated genes, 17 genes were upregulated in both mutant strains, and 24 and 21 genes were upregulated in ΔgreA and GreA-D44A cells, respectively. Similarly, 15 genes were downregulated in both mutant strains, and 24 and 26 genes were downregulated in ΔgreA and GreA-D44A, respectively. Furthermore, we observed no correlation between changes in the transcription level and RNAP accumulation (Fig. 7). These results indicate that inactivation of GreA has a limited impact on the transcriptome, and these effects are not directly related to RNAP accumulation in the promoter or promoter-proximal regions.

FIG. 6. Induction of the RNAP binding signal peak overlapping with the SigA or NusA signal peak. Distribution of core RNAP binding signals (β' binding signals) in wild-type, ΔgreA, and GreA-D44A cells, together with those of σ⁵⁴ and NusA binding signals, in yfnI. The dotted line indicates the middle position of the σ⁵⁴ binding signals. The TR values of RNAP are indicated in each panel.

DISCUSSION

To our knowledge, this is the first report on genome-wide distribution analysis of the bacterial elongation factor, Gre. The cellular level of *B. subtilis* GreA is twice that of RNAP, and the majority of GreA associates with RNAP (7). We have shown here that GreA is evenly distributed from the promoter to coding regions and overlaps with RNAP engaged in transcription in *B. subtilis* (Fig. 2 and 3 and see Fig. S2 in the supplemental material). Gre factors were previously proposed to transiently associate with stalled RNAP (9). However, our data strongly suggest that GreA is not specifically recruited to stalled RNAP. In addition, pulldown assays of the components of RNAP complexes demonstrated that GreA associates with not only the elongation complex of RNAP (NusA-RNAP) but also the initiation complex (σ⁵⁴-RNAP) (Fig. 4). However,
although the copurification analysis suggests that His-tagged GreA minimally retains binding ability to core RNAP (Fig. 4A), it is possible that the His tag addition affects some GreA function and/or its binding affinity to RNAP, and this requires further investigation.

GreA inactivation had no clear effects on the distribution of elongating RNAPs but induced a genome-wide shift in TR values, a finding indicative of RNAP pausing at promoter or promoter-proximal regions (Fig. 5). Clear RNAP peaks were detected at the promoter or promoter-proximal regions of 35 genes in GreA-inactivated cells (Fig. 6 and see Fig. S6 in the supplemental material). Furthermore, the majority of the induced RNAP peaks colocalized with $\sigma^{\ast}$ peaks, suggesting the accumulation of $\sigma^{\ast}$-RNAP. In E. coli, Gre factors enhance promoter escape and suppress promoter-proximal pausing of $\sigma^{\ast}$-RNAP (11, 13, 21, 34, 35). Based on these findings, we propose that B. subtilis Gre factor plays a similar role during the initiation of RNA synthesis in many promoters or promoter proximal regions. Although the resolution of our ChAP-chip analysis did not permit discrimination of RNAP accumulation at promoters or promoter-proximal regions, we favor the possibility of accumulation at promoter-proximal pausing, since B. subtilis RNAP is known to form unstable open complexes and synthesize smaller amounts of abortive transcripts than E. coli RNAP (2, 40). Recently, it was reported that the pausing of RNAP in E. coli is induced by direct and sequence specific interactions of RNAP with promoter-like sequences (6, 29). However, we have not yet found any correlation between RNAP stalling and promoter-like sequences at the promoter-proximal regions in B. subtilis. Further in vitro analysis of the effects of GreA on transcription initiation by B. subtilis RNAP and bioinformatics studies on the signals inducing RNAP pausing at promoter-proximal sites are required to elucidate the molecular mechanism of RNAP accumulation in greA mutants.

The RNAP accumulation observed in $\Delta$greA cells was also detected in GreA-D44A cells, supporting the hypothesis that Asp44 of the B. subtilis GreA is essential to resolve the pausing of RNAP through stimulation of nucleolytic cleavage activity of RNAP. Interestingly, the effects of the GreA-D44A mutation on RNAP pausing were more extensive than those of the $\Delta$greA mutation. The E. coli Gre protein mutated at D41 (corresponding to D44 in B. subtilis GreA) inhibits elongation of transcription in vitro (20). Recently, overexpression of a yeast TFIIIS mutant harboring substitutions of two amino acids that stimulate intrinsic nucleolytic activity of RNAP was found to be lethal in yeast cells (30). Based on the collective results, we propose that the B. subtilis GreA-D44A protein retains the ability to bind RNAP, and this binding interferes with the intrinsic nucleolytic activity of RNAP, which remains active, even without stimulation by Gre. However, in contrast to the data obtained with yeast, growth defects were not observed upon expression of the GreA-D44A protein, suggesting that the pausing of RNAP during the initiation and elongation of transcription does not occur frequently in B. subtilis cells, and the problem of stalling may be resolved by ways other than cleavage of the extruded $\beta$' terminus of nascent RNA such as, for example, collapse of the association of RNAP with the DNA template.

In E. coli cells, GreA inactivation has direct and negative effects on the transcription initiation frequencies of a number of genes (34). However, we could not establish a direct impact of GreA inactivation on the transcriptome in B. subtilis cells under normal growth (LB medium and aerobic) conditions, even though RNAP trapping or pausing at promoters or promoter-proximal regions was induced in many genes in mutant strains. These observations suggest that the trapping or pausing frequency is lower in B. subtilis cells, compared to that in E. coli, probably due to differences in the biochemical properties of the two RNAP types. Initiation complexes of B. subtilis RNAP are efficiently converted to elongation complexes in vitro and in vivo, compared to E. coli RNAP (2, 15, 29a, 40). As in several other bacteria, GreA function may be essential for B. subtilis growth under stress conditions that induce frequent pausing of RNAP. However, the phenotypes of greA mutants under various conditions are yet to be established, and further studies are required to understand the biological importance of GreA in B. subtilis cells.

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