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Small Cytoplasmic RNA of *Bacillus subtilis*: Functional Relationship with Human Signal Recognition Particle 7S RNA and *Escherichia coli* 4.5S RNA

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Small cytoplasmic RNA (scRNA; 271 nucleotides) is an abundant and stable RNA of the gram-positive bacterium *Bacillus subtilis*. To investigate the function of scRNA in *B. subtilis* cells, we developed a strain that is dependent on isopropyl-β-D-thiogalactopyranoside for scRNA synthesis by fusing the chromosomal *scr* locus with the *spac-1* promoter by homologous recombination. Depletion of the inducer leads to a loss of scRNA synthesis, defects in protein synthesis and production of α-amylase and β-lactamase, and eventual cell death. The loss of the scRNA gene in *B. subtilis* can be complemented by the introduction of human signal recognition particle 7S RNA, which is considered to be involved in protein transport, or *Escherichia coli* 4.5S RNA. These results provide further evidence for a functional relationship between *B. subtilis* scRNA, human signal recognition particle 7S RNA, and *E. coli* 4.5S RNA.

The protein export process is considered to be complex and to require the function of a number of different secretory mechanisms. Several proteins, such as SecA, SecY, and others, have been well characterized for *Escherichia coli* (24) and partially characterized for *Bacillus subtilis* (14, 21, 28). In higher eukaryotes, the signal recognition particle (SRP) is identified as one of the secretory proteins. SRP is a soluble ribonucleoprotein that is thought to be essential for targeting nascent secretory proteins to the endoplasmic reticulum membrane (10, 33). Experiments in vitro have shown that, when the signal peptide emerges from the ribosome, SRP can bind to it and maintain an export-competent conformation of presecretory proteins (23) consisting of one RNA molecule of 300 nucleotides (7SL RNA, referred to here as SRP 7S RNA) and six polypeptides of 6, 14, 19, 54, 68, and 72 kDa (31, 32). SRP 7S-like RNAs have been identified in a wide range of eukaryotes, including humans (30), plants (6), *Yarrowia lypolytica* (18), *Schizosaccharomyces pombe* (3), and a wide variety of archaebacterial species (11). It has been proposed that the structure of SRP 7S-like RNAs is divisible into four domains (domains I to IV) based on the predicted secondary structure of the human SRP 7S RNA (Fig. 1). Phylogenetic studies revealed that the primary sequences and secondary structures of domain IV are highly conserved among those organisms (19).

The small cytoplasmic RNA (scRNA; 271 nucleotides) of *B. subtilis* was first identified as the counterpart of *E. coli* 4.5S RNA (27). It is transcribed as a 354-nucleotide primary transcript and then processed to 271-nucleotide RNAs at both the 5′ and 3′ ends (25). As illustrated in Fig. 1, the left portion of *B. subtilis* scRNA is similar in size and secondary structure to the left portion of human SRP 7S RNA, whereas the nucleotides and secondary structure of the right portion of the scRNA are homologous to those of *E. coli* 4.5S RNA. The 4.5S RNA of *E. coli* is composed of 114 nucleotides and is metabolically stable in *E. coli* cells. The 4.5S RNA function is considered to be essential for cell viability (5). The loss of this RNA causes disruption of translation (1) and induction of the heat shock response and lambda prophage (2). Although recent studies showed that pre-β-lactamase accumulates in *E. coli* in which the 4.5S RNA is depleted, direct involvement of 4.5S RNA in protein secretion has yet to be determined (17, 20).

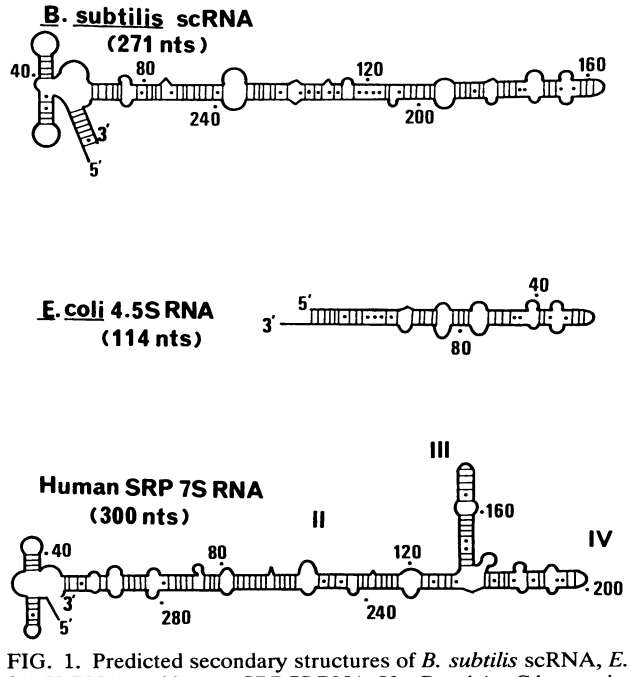

FIG. 1. Predicted secondary structures of *B. subtilis* scRNA, *E. coli* 4.5S RNA, and human SRP 7S RNA. U · G and A · G base pairs are indicated (●). Domains I to IV of human SRP 7S RNA (14) are also indicated. The line drawings were generated with the RNA structure-editing computer program DNASIS written by Takara Shuzo (Kyoto, Japan).

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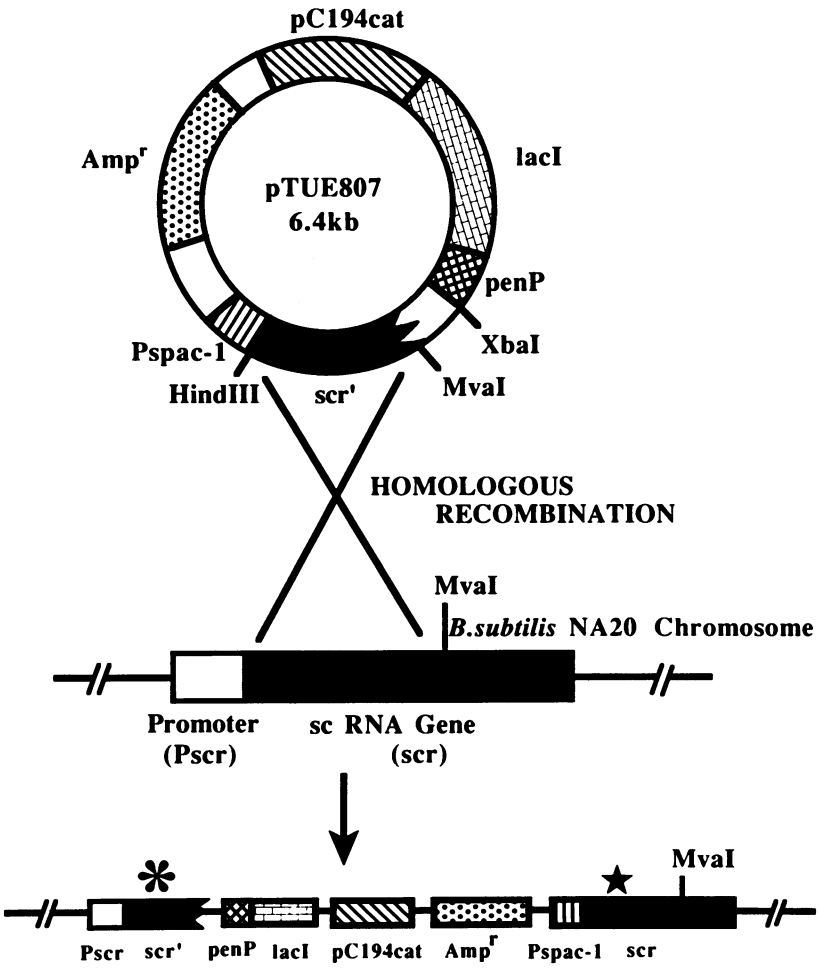

FIG. 2. Construction of an IPTG-dependent *B. subtilis* SC200NA strain in which the expression of *scr* is dependent on IPTG. *E. coli* plasmid pTUE807 was used to transform *B. subtilis* NA20 to Cm^r in the presence of 3 mM IPTG to give strain SC200NA. *lacI* is expressed by the promoter of *B. licheniformis* penicillinase. The approximate location of the *MvaI* site is indicated. Integration of pTUE807 into the *scr* locus results in a truncated copy of *scr* (scr') (*) under the control of the authentic *scr* promoter (Pscr) and an intact copy of *scr* (★) under the control of *spac-1* promoter (Pspac-1).

The sequence of domain IV of *B. subtilis* scRNA is highly homologous to those of *E. coli* 4.5S RNA (more than 90% identity) and human SRP 7S RNA (46% identity). Furthermore, *B. subtilis* scRNA can compensate for the loss of 4.5S RNA in *E. coli* (26). Therefore, scRNA may perform important functions for protein synthesis and transport in *B. subtilis* cells.

In this study, to understand the function of scRNA in *B. subtilis*, we constructed *B. subtilis* SC200NA, in which the scRNA gene (*scr*) is regulated by the isopropyl-β-D-thiogalactopyranoside (IPTG)-inducible promoter P*spac-1*, to control the level of scRNA. The strain is inviable and defective in protein synthesis. The production of α-amylase and β-lactamase is decreased in the absence of IPTG, and this leads to morphological changes. We also report that these characteristic features caused by the depletion of scRNA can be functionally compensated for by the expression of heterologous SRP 7S-like RNAs.

MATERIALS AND METHODS

Bacterial strains. *B. subtilis* NA20 (*purB6 trpB3 metB5*) and *E. coli* JM109 [Δ(*lac-proAB*) *thi recA1 endA1 gyrA96 hsdR17 supE44 relA1* F′ (*traD36 proAB*$^+$ *lacI*^{q} *lacZΔM15*)] were used as the host cells. *E. coli* K-12 was used as the DNA source for obtaining the 4.5S RNA gene by the polymerase chain reaction (22). *B. subtilis* SC200NA (*purB6 trpB3 metB5 scr*:: pTUE807) was constructed from *B. subtilis* NA20 as described below. The strains were cultured in the absence or presence of 5 μg of chloramphenicol per ml or 50 μg of ampicillin per ml. *B. subtilis* SC200NA was maintained and cultured in L broth containing 5 μg of chloramphenicol per ml and 3 mM IPTG.

Plasmids. The expression vector pDH88 (9), which contains the pBR322 replication origin, the *spac-1* promoter, the *lacI* gene expressed from the *penP* promoter, and the pC194 *cat* gene, was provided by D. J. Henner. It replicates in *E. coli* and can integrate into the *B. subtilis* chromosome when it contains a DNA fragment homologous to part of the

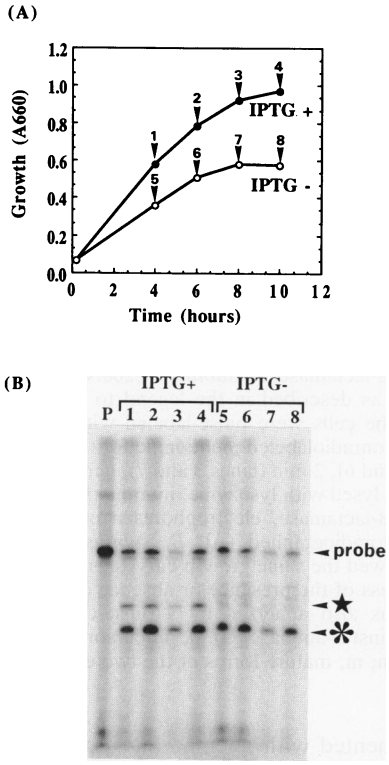

FIG. 3. Cell growth (A) and expression of the *scr'* and *scr* genes (B) of *B. subtilis* SC200NA in L broth in the presence (IPTG+) and absence (IPTG−) of IPTG. At the times indicated (points labeled 1 to 8) in panel A, cells were withdrawn for the RNase protection assay (B). RNA samples (10 μg each) extracted from the cells were analyzed by the RNase protection assay. Lanes: P, ^{32}P-labeled RNA probe; 1 through 4, RNA extracted from the cells after 4 through 10 h, respectively, of culture in the presence of 3 mM IPTG; 5 through 8, RNA extracted from the cells after 4 through 10 h, respectively, of culture in the absence of IPTG. ★, RNA transcribed from intact copy of the scRNA gene (*scr*); *, RNA transcribed from the truncated copy of the scRNA gene (*scr'*) (Fig. 2).

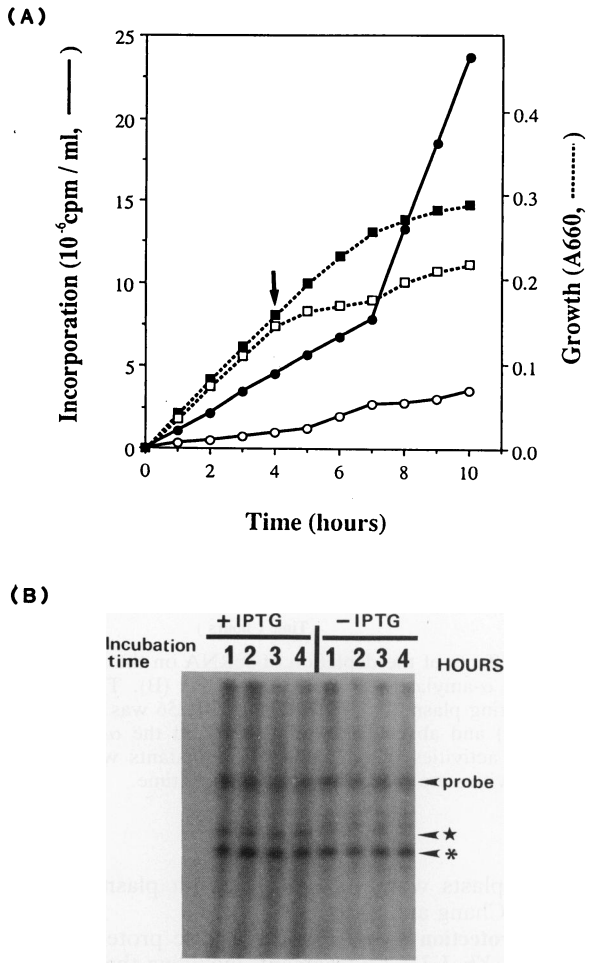

FIG. 4. Effects of the depletion of scRNA on protein synthesis. (A) *B. subtilis* SC200NA harboring pTUB256 was cultured in S7 medium in the presence or absence of IPTG. Samples were taken from the cultures at the times indicated, and the ability to incorporate [^{35}S]methionine into trichloroacetic acid-insoluble fractions was determined by using a 5-min incubation after 6 μCi of [^{35}S]methionine was added per 300 μl of the culture medium. After the incubation, an equal volume of 20% of trichloroacetic acid was added. Samples were heated at 90°C for 15 min, acid-insoluble materials were collected by filtration, and radioactivity was measured with a liquid scintillation counter. Symbols: ■ and □, A_{660} of cells grown with and without IPTG, respectively; ● and ○, counts per minute in the acid-insoluble fractions with and without IPTG, respectively; ↓, time at which samples were withdrawn for the pulse-chase experiments in Fig. 6. (B) The cells described above were withdrawn after 1, 2, 3, and 4 h of cultivation for the RNase protection assay after inoculation. Symbols: ★, RNA transcribed from intact copy of the scRNA gene (*scr*); *, RNA transcribed from the truncated copy of the scRNA gene (*scr'*).

chromosome. To put the *scr* gene in the chromosome under control of the *spac-1* promoter, an *E. coli* plasmid, pTUE807, was constructed from pDH88. Primers (18-mers 5′ ATATAC TTAAGCTTGCAT 3′, and 5′ CTGCGGCACATGAGAGGT 3′) and the polymerase chain reaction were used to synthesize a 251-bp DNA fragment containing 239 bp of the 5′ portion of the *scr* gene (22). Concurrently, a *Hin*dIII restriction site was introduced into the position immediately upstream of the transcription initiation site. The resultant DNA fragment was introduced between the *Hin*dIII and *Hin*cII sites of pUC19. A *Hin*dIII-*Xba*I 248-bp fragment was then inserted between the *Hin*dIII and *Xba*I sites of pDH88, in which those sites are located downstream of the *spac-1* promoter. The constructed plasmid was designated pTUE807 (Fig. 2).

An *E. coli-B. subtilis* shuttle vector, pTUBE809, which contains a 98-bp synthetic protein A promoter in the *Bam*HI-*Hin*dIII sites, was constructed from pHY300PLK (Takara Shuzo Co. Ltd., Kyoto, Japan). The promoter was expressed constitutively without the addition of IPTG. Plasmids pTUBE811, containing the gene for *E. coli* 4.5S RNA, and pTUBE810, containing the gene for human SRP 7S RNA, were derivatives of pTUBE809. The gene for 4.5S RNA was synthesized by the polymerase chain reaction, and that for human SRP 7S RNA was obtained from plasmid p7L30.1, which was provided by K. Sakamoto. The two genes were inserted downstream of the synthetic protein A promoter. *B. subtilis* plasmids pTUB101 and pTUB256 can direct the synthesis of the α-amylase of *B. subtilis* N7 (35) and pBR322 β-lactamase (16) with the aid of the *B. subtilis amyE* promoter and signal peptide, respectively.

Transformation of *B. subtilis*. The method used for DNA-mediated transformations with competent cultures of *B. subtilis* was slightly modified from that of Wilson and Bott

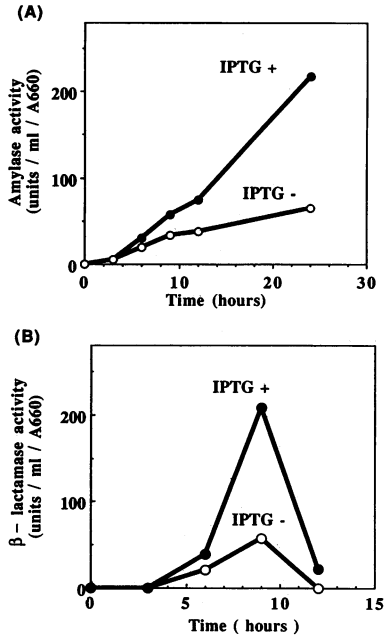

FIG. 5. Effects of the depletion of scRNA on the production of extracellular α-amylase (A) and β-lactamase (B). The SC200NA strain harboring plasmid pTUB101 or pTUB256 was grown in the presence (●) and absence (○) of IPTG, and the α-amylase and β-lactamase activities in the culture supernatants were assayed. Specific activities are plotted as a function of time.

(34). Protoplasts were transformed with plasmids by the method of Chang and Cohen (7).

RNase protection assay. For the RNase protection assay, the *Hin*dIII-*Xba*I 316-bp fragment, including the 307-bp mature scRNA, was synthesized by the polymerase chain reaction and inserted between the *Xba*I-*Hin*dIII sites of Bluescript II KS+ (Stratagene, La Jolla, Calif.) under the control of a bacteriophage T7 promoter. The T7 RNA polymerase and [α-^{32}P]UTP were used to synthesize the labeled probe, which is complementary to the region of mature scRNA.

B. subtilis SC200NA cells were grown in L broth in the presence or absence of 3 mM IPTG. At various times during incubation, total RNA was extracted with diethylpyrocarbonate by the method of Summers (29). Samples (10 μg) of each total RNA preparation and 12,000 cpm of the ^{32}P-labeled probe were hybridized at 45°C overnight. Then the mixture was digested with RNase A (0.5 U/ml) and RNase T_1 (100 U/ml) and precipitated by the addition of 2.5 volumes of ethanol. To determine the amount of scRNA, the samples were denatured by heating at 90°C for 3 min, applied to a DNA sequencing gel (6% polyacrylamide) containing 6 M urea, and autoradiographed.

Assay of extracellular enzymes. Amylase activity was measured at 40°C by a modification of the method of Fuwa (8) with 0.5% soluble starch as the substrate. The activity of β-lactamase was determined spectrophotometrically with nitrocefin (BBL Microbiology Systems, Cockeysville, Md.) by the method of O'Callaghan et al. (15).

Labeling, immunoprecipitation, and electrophoresis of α-amylase and β-lactamase. Signal peptide cleavage of α-amylase and β-lactamase was observed in pulse-chase experiments with *B. subtilis* cells carrying plasmid pTUB256 or pTUB101. Cells were first grown at 37°C in medium S7

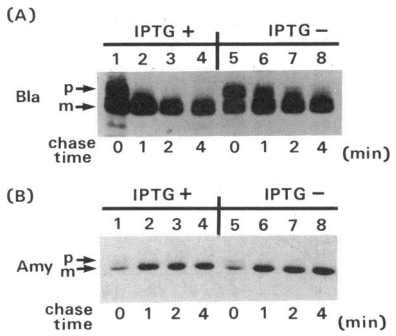

FIG. 6. Effect of the depletion of scRNA on the processing of α-amylase and β-lactamase. *B. subtilis* SC200NA harboring pTUB256 was cultured as described in the legend to Fig. 4. At 4.0 h after inoculation, the cells were pulse-labeled with [^{35}S]methionine and chased with nonradiolabeled methionine for 0 min (lanes 1 and 5), 1 min (lanes 2 and 6), 2 min (lanes 3 and 7), and 4 min (lanes 4 and 8). Samples were lysed with lysozyme, immunoprecipitated with antiserum against β-lactamase, electrophoresed on SDS-polyacrylamide gels, and autoradiographed (A). *B. subtilis* SC200NA harboring pTUB101 showed the same growth curves as those of the pTUB256 strain regardless of the presence or absence of IPTG. Processing of α-amylase was also analyzed by a pulse-chase experiment with antiserum against α-amylase (B). p, precursor of α-amylase or β-lactamase protein; m, mature forms of the two extracellular enzymes.

(13) supplemented with adenine (0.4 μg/ml), starch (0.1%), and 18 amino acids (20 μg/ml), excluding methionine and cysteine. IPTG (3 mM) was added as required. When cultures reached a reading of 200 to 300 in a Klett colorimeter, the cells were pulse-labeled with 20 μCi of [^{35}S]methionine (Amersham International Plc.) per ml for 30 s and then chased with 200 μg of unlabeled L-methionine per ml. The labeled cells were incubated in a buffer (50 mM Tris [pH 7.5]–10 mM EDTA–1 mM phenylmethylsulfonyl fluoride) containing lysozyme (10 μg/ml) at 37°C for 10 min and then immunoprecipitated with antiserum against β-lactamase or α-amylase. The precipitates were then separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Protein bands on the autoradiographs were quantified densitometrically with a Molecular Dynamics 300XE scanning imager.

Observation of cell morphology. To examine the effects of the depletion of scRNA on the morphology of *B. subtilis* SC200NA, cells cultured at 37°C for 8 h in the presence or absence of IPTG were subjected to cell wall staining (12). The samples were observed under a Nikon S-Ke light microscope at a magnification of 1,000.

RESULTS

Function of scRNA is essential for cell growth. To analyze whether scRNA is required for *B. subtilis* cell growth, we constructed *B. subtilis* SC200NA, in which the expression of the *scr* gene in the chromosome is dependent on IPTG (Fig. 2). Plasmid pTUE807 was transferred into *B. subtilis* NA20 by DNA-mediated transformation with competent cell cultures. The arrangement of the genes around the *scr* gene in the chromosome of *B. subtilis* SC200NA was determined by DNA-DNA hybridization with ^{32}P-labeled DNA fragments of pTUE807 as a probe. There were no chloramphenicol-resistant transformants that could grow in the absence of IPTG. Among the chloramphenicol-resistant transformants, *B. subtilis* SC200NA, which can grow only in the presence of IPTG, was selected for further experiments.

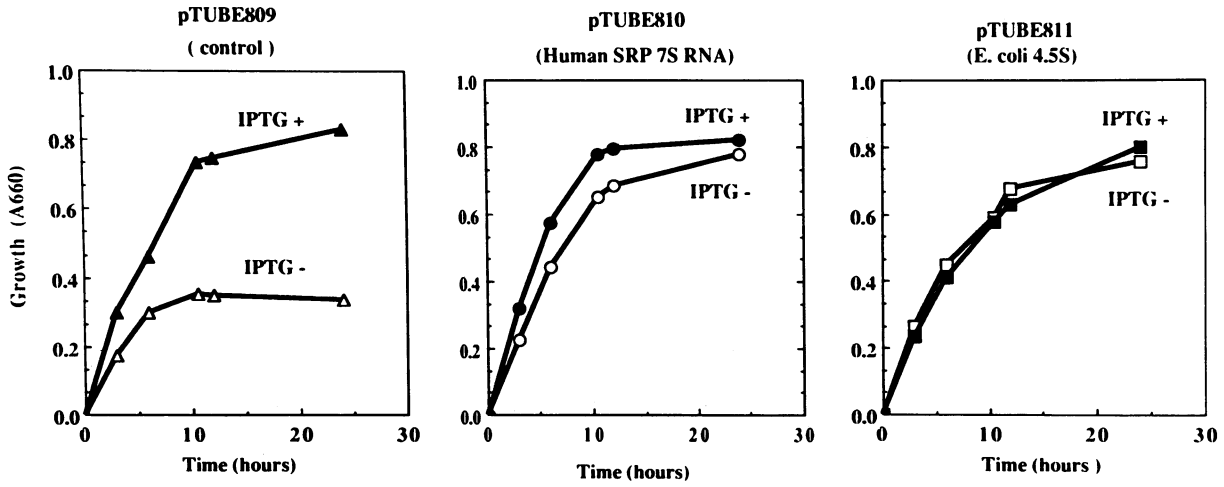

FIG. 7. Complementation of cell growth of *B. subtilis* strains in the depletion of scRNA by human SRP 7S RNA and *E. coli* 4.5S RNA. *B. subtilis* SC200NA strains harboring pTUBE809 (A), pTUBE810 (B), or pTUBE811 (C) were cultured in L broth at 37°C in the presence (▲, ●, ■) or absence (△, ○, □) of 3 mM IPTG.

To determine the effects of IPTG on cell growth, strain SC200NA was inoculated into L broth containing various concentrations of IPTG. The cells thrived in the presence of 1 to 3 mM IPTG. However, at IPTG concentrations of 0 to 0.5 mM or above 3.0 mM, growth was not observed or was inhibited, respectively. Figure 3A shows the growth curves of the cells in the presence and absence of 3 mM IPTG. In the absence of IPTG, the growth rate decreased immediately after incubation and the culture reached maximum density at approximately 6 h. To confirm that the complete scRNA was actually expressed in the constructed *scr* gene by the addition of IPTG, total RNAs were extracted from cells in the growth phase at the times indicated in Fig. 3A, and the transcripts were analyzed by the RNase protection assay. The complete scRNA was observed only in the presence of IPTG and almost disappeared after only a few doublings in the absence of IPTG (Fig. 3B, lane 5). On the other hand, the truncated scRNA was expressed throughout experiments in both the presence and absence of IPTG. As judged by densitometric analysis, the abundance of the complete molecule is approximately 20% of that of the truncated scRNA.

Effects of the depletion of scRNA on protein synthesis. The effects of IPTG withdrawal on total protein synthesis were evaluated by the incorporation of [^{35}S]methionine into the trichloroacetic acid-insoluble fractions. The rate of methionine incorporation into the trichloroacetic acid-insoluble fraction from pTUB256-transformant cells was constant during the exponential stage of growth and increased dramatically after 8 h of cultivation in the presence of IPTG (Fig. 4). In contrast, in the absence of IPTG, the rate of methionine incorporation stayed at a similar level during and after the exponential stage. The incorporation rate was only approximately 15% of that for cells in the presence of IPTG. This result is consistent with cell protein synthesis being strongly inhibited by the depletion of scRNA.

Effects of the depletion of scRNA on the extracellular protein synthesis and translocation. To examine the roles of scRNA in protein synthesis, including the translocation of extracellular enzymes, plasmids pTUB101 and pTUB256 (which direct the synthesis of extracellular α-amylase and β-lactamase, respectively) were introduced into *B. subtilis* SC200NA by protoplast transformation. The α-amylase activity in the medium of a pTUB101 transformant was increased to 200 U/ml per unit of A_{660} after 20 h of cultivation in the presence of IPTG. In contrast, in the absence of IPTG, the production of α-amylase was inhibited and the rate of synthesis was reduced to approximately 20% of the cells grown with IPTG after 20 h of cultivation (Fig. 5A). A similar inhibitory effect was observed on the production of β-lactamase in the pTUB256 transformant. The secretion of β-lactamase into the medium was prevented by the removal of IPTG (Fig. 5B).

To analyze whether scRNA is directly involved in the translocation of extracellular enzymes, *B. subtilis* strains containing pTUB101 or pTUB256 were pulse-chased with [^{35}S]methionine after incubation at 37°C for 4 h in the presence and absence of IPTG (arrow in Fig. 4). The enzymes were precipitated with antiserum against β-lactamase or α-amylase and then analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 6). Pre-β-lactamase accumulated in the cells grown without IPTG (Fig. 6A). Densitometric scanning of the autoradiogram showed that about 30% of β-lactamase precursor remained after a 1-min chase (Fig. 6A, lane 6). By this time, the scRNA in the cells was completely depleted, although growth inhibition was not yet observed (Fig. 4). No pre-β-lactamase was observed in cells in which scRNA synthesis had been induced (Fig. 6A, lanes 1 to 4). In contrast, translocation defects of extracellular α-amylase in *B. subtilis* (pTUB101) cultured in the absence of IPTG were not observed (Fig. 6B).

Human SRP 7S RNA and *E. coli* 4.5S RNA can functionally compensate for loss of scRNA in *B. subtilis*. Phylogenetic studies have suggested that *B. subtilis* scRNA is a structural homolog, and therefore a possible functional homolog, of heterologous SRP 7S-like RNAs. Therefore, we examined whether the scRNA can be replaced by other SRP 7S-like RNAs in *B. subtilis*. We introduced *B. subtilis-E. coli* shuttle plasmids pTUBE810 or pTUBE811 into *B. subtilis* SC200NA by protoplast transformation. In pTUBE811 and pTUBE810, the genes for *E. coli* 4.5S RNA and human SRP 7S RNA, in which their own promoters were omitted, were put under the control of a synthetic protein A promoter. Since this promoter was functional in *B. subtilis* throughout the culture period, *E. coli* 4.5S RNA and human SRP 7S RNA can be synthesized in both the presence and absence of

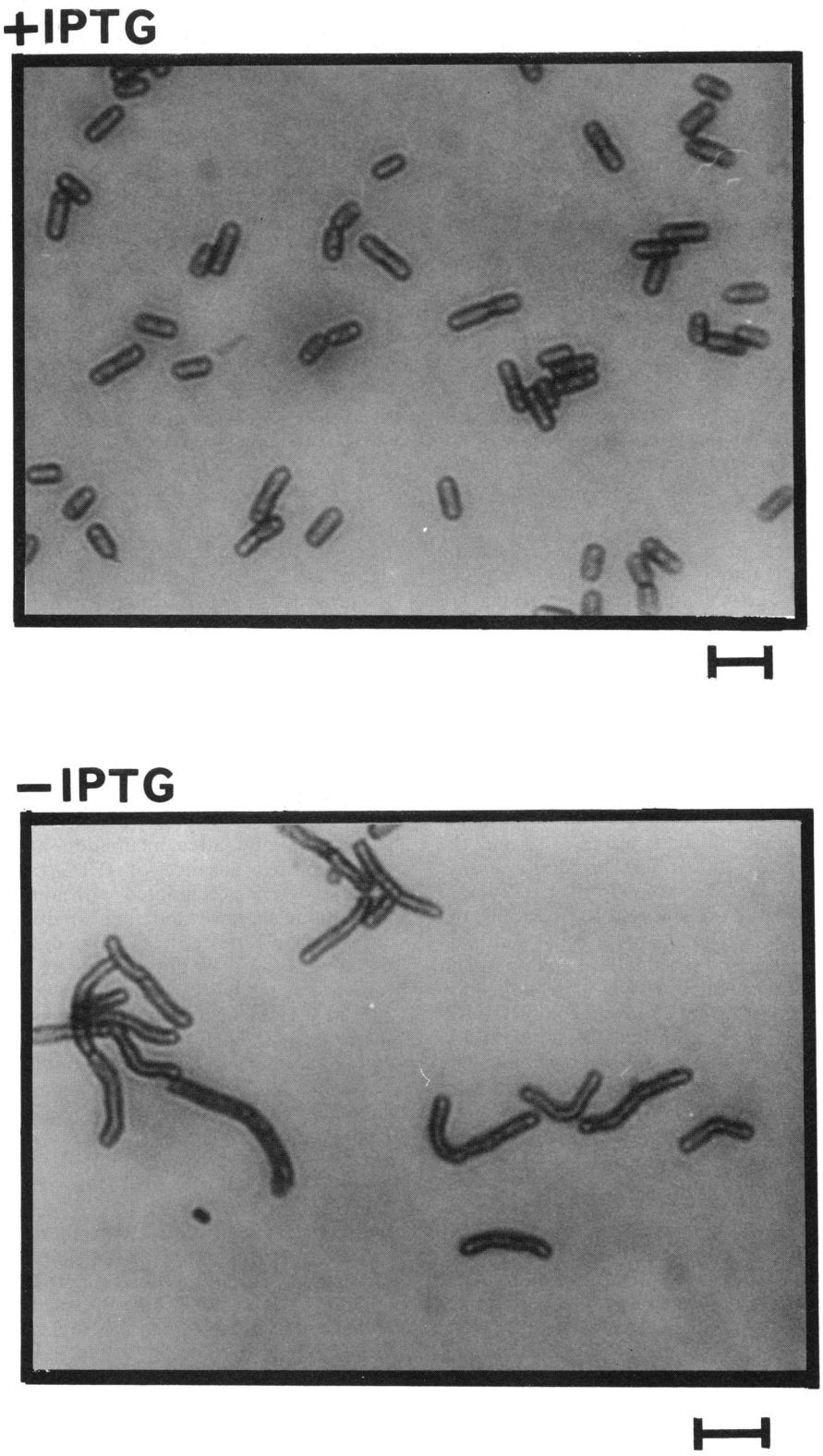

FIG. 8. Photographs of *B. subtilis* SC200NA cells. An exponential culture of SC200NA growing in the presence of 3 mM IPTG (A) and in the absence of IPTG (B) were subjected to cell wall staining and observed microscopically. Bars, 2 μm.

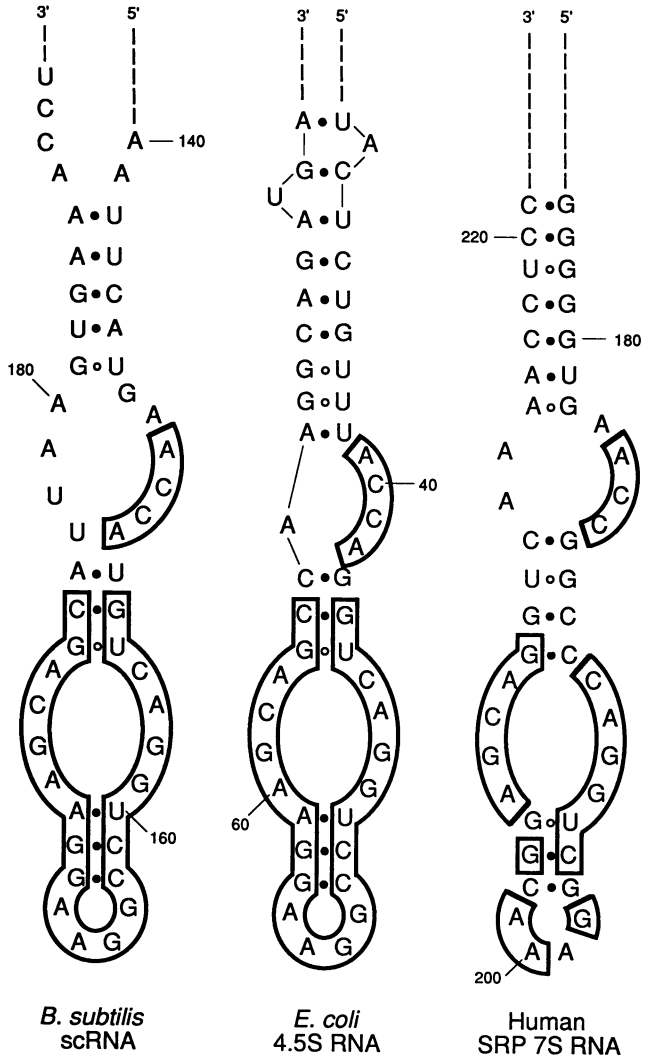

FIG. 9. Conservation of the domain IV motif of human SRP 7S RNA in *B. subtilis* scRNA and *E. coli* 4.5S RNA. The conserved bases among the three species are boxed. Base-paired nucleotides (●) and unpaired bases (○) are indicated. The RNA molecules are numbered from the 5′ end.

IPTG in *B. subtilis* SC200NA cells. The *B. subtilis* transformants can only survive when the *E. coli* 4.5S RNA and human SRP 7S RNA functionally replace scRNA. Removal of IPTG resulted in the growth arrest and cell death of the pTUBE809 transformant (Fig. 7A). In contrast, the growth rates of the pTUBE810 and pTUBE811 transformants in the absence of IPTG recovered to rates like those of the transformants and the parental *B. subtilis* SC200NA in the presence of IPTG. However, the growth of the pTUBE810 transformant was slightly inhibited (Fig. 7B and C). In the RNase protection assay the two foreign genes were actually expressed in the *B. subtilis* transformants. Furthermore, extracellular α-amylase and β-lactamase production was recovered by the pTUBE810 and pTUBE811 transformants in the absence of IPTG (data not shown). These results indicate that the *E. coli* 4.5S RNA and human SRP 7S RNA can compensate for the loss of scRNA in *B. subtilis* cells.

Depletion of scRNA leads to aberrant morphology of *B. subtilis* cells. To examine whether the depletion of scRNA affects the morphology of the bacterium, *B. subtilis* SC200NA cells growing in the presence and absence of IPTG were fixed and subjected to cell wall staining 8 h after inoculation. The depletion of scRNA led to significant effects on the shape of the bacterium (Fig. 8). Morphologically, cells grown in the absence of IPTG became filamentous and twisted and varied from 2 to 10 μm in length, whereas those grown in IPTG exhibited the characteristic rod shape of *B. subtilis* and were indistinguishable from the parental *B. subtilis* NA20 cells with respect to length and cell morphology.

DISCUSSION

Our data show that removal of the scRNA from *B. subtilis* results in cessation of cell growth and defects in protein synthesis and the production of α-amylase and β-lactamase. In *B. subtilis* SC200NA cells grown in the absence of IPTG, the RNase protection assay determined that scRNA very rarely reached the full size. Therefore, the defective growth and protein synthesis may be due to the direct effects of the scRNA depletion. Those defects were overcome by introducing plasmids that can direct the synthesis of human SRP 7S RNA or *E. coli* 4.5S RNA. Brown (4) and Struck et al. (27) reported that genes for SRP 7S-like RNAs from eukaryotes and archaebacteria can replace a defect in the gene for *E. coli* 4.5S RNA and that *B. subtilis* scRNA also complements the essential functions of the 4.5S RNA in vivo. These and our data suggest that these evolutionally divergent RNA molecules share common biological roles in many organisms and that they are interchangeable. When we compared the nucleotide sequences and structures of *B. subtilis* scRNA, *E. coli* 4.5S RNA, and human SRP 7S RNA, many similarities were observed in their domain IV regions, although the *E. coli* 4.5S RNA is composed of 114 nucleotides, which is approximately half of that of *B. subtilis* scRNA. There was no significant homology in the other parts of the domain IV regions of *B. subtilis* scRNA and human 7S RNA.

The sequence of the domain IV region of *B. subtilis* scRNA matched 26 of the 34 nucleotides in the same region of *E. coli* 4.5S RNA and 18 of the 34 nucleotides in the domain IV of human SRP 7S RNA (Fig. 9). Therefore, it is likely that the domain IV regions of the three RNAs are highly conserved with regard to primary and secondary structures and that these regions perform key functions for cell viability. It would be interesting to analyze whether the domain IV region is responsible for cell viability and, if so, what functions other domains of *B. subtilis* scRNA have.

Human SRP 7S RNA is a component of SRP that has an essential role in protein translocation. It is predicted that the *B. subtilis* scRNA is capable of forming a secondary structure which is strikingly similar to human SRP 7S RNA (Fig. 1). Therefore, we would like to ask whether scRNA has similar functions to the 7S RNA of SRP in protein translocation. Two independent groups have recently demonstrated that the depletion of 4.5S RNA in *E. coli* cells results in the accumulation of pre-β-lactamase, and they have proposed that the *E. coli* 4.5S/P48 ribonucleoprotein complex has a role in the primary protein export pathway in *E. coli* (17, 20). In the present study, we also observed the accumulation of pre-β-lactamase in *B. subtilis* cells with depleted scRNA.

On the other hand, we did not find any effects on the translocation of α-amylase; i.e., no pre-α-amylase accumulated in *B. subtilis* cells in the absence of IPTG. Decreased protein synthesis was observed even at earlier times during cell cultivation (Fig. 4), whereas the decreased production of α-amylase and β-lactamase was only significant after 6 h of

cultivation after the removal of IPTG (Fig. 5). Therefore, it is likely that cell protein synthesis was primarily interfered with by the depletion of scRNA and that after this the production of α-amylase and β-lactamase were decreased. Furthermore, the production of the two enzymes was recovered by the presence of *E. coli* 4.5S RNA and human 7SLRNA in the absence of IPTG. Hence, these results suggest that the scRNA is required for the maintenance of the translation or transcription activity in *B. subtilis* cells rather than for protein secretion.

The findings presented here provide further evidence for a close functional relationship between mammalian SRP 7SRNA, *E. coli* 4.5S RNA, and *B. subtilis* scRNA. More detailed analysis is needed to elucidate the exact function of scRNA in *B. subtilis*.

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