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Possible presence and role of the promoter sequence for eukaryotic RNA polymerase III in bacteria

Sachiko Matsutani

Abstract The bacterial repetitive sequence IS*1*, is a translocatable DNA segment. The internal region of IS*1* acts as a *cis*-element to stimulate RNA synthesis from the upstream promoter. The product of the bacterial *artA* gene works with this *cis*-element to stimulate transcription. Eukaryotic genes for small RNAs and short interspersed repetitive elements (SINEs) have internal promoters, transcribed by RNA polymerase III (RNAP III). RNAP III requires the multi-subunit protein factor TFIIIC in transcription initiation. TFIIIC contains the B-block binding subunit which recognizes the internal promoter. Here, I report that the eukaryotic RNAP III promoter-like sequence was found in the *cis*-element of bacterial IS*1*. Mutations in the *cis*-element which affect transcription were present in the RNAP III promoter-like sequence. The RNAP III promoter sequence of *Alu*, which is a human SINE, was cloned into *Escherichia coli*, and was shown to stimulate bacterial transcription like the *cis*-element of IS*1*. Furthermore, the primary structures of ArtA protein and B-block binding subunits were compared. The amino acid sequence of ArtA appeared to be similar to the N- and C-terminal regions conserved in many B-block binding subunits. Prokaryotes and eukaryotes have been thought to have inherent transcription machineries. The results shown here, however, suggest a new aspect of the evolution of the RNAP III transcription machinery.
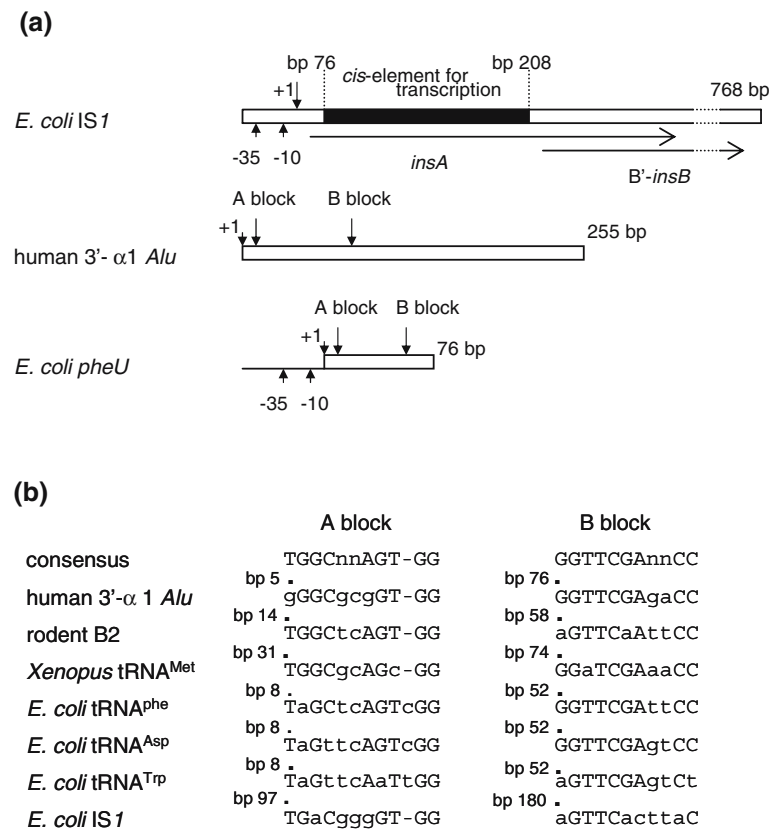
Introduction

IS*1* is a DNA segment of 768 bp which transposes to numerous sites on bacterial plasmids and chromosomes (Galas and Chandler 1989). IS*1* contains two genes encoding transposition related-proteins (Matsutani 1994), and the expression of these genes is driven by the promoter within the left end of IS*1* (Fig. 1; Machida et al. 1984). The internal region of IS*1* (bp positions 76–208 in IS*1*) acts as a *cis*-element to stimulate RNA synthesis from the IS*1* promoter and from exogenous promoters located upstream of the *cis*-element (Fig. 1; Matsutani 2005). The product of the bacterial *artA* gene works with the internal region of IS*1* and stimulates transcription (Matsutani 2005). Two-hybrid systems had been constructed in *E. coli*, and it is suggested that the ArtA protein participates in transcription initiation, and associates with the RNA polymerase α subunit (Matsutani 2006b). ArtA (or the protein it interacts with) possibly binds to the IS*1* internal region, and helps to tether RNA polymerase near the promoter.

Eukaryotic RNA polymerase III (RNAP III) synthesizes a variety of small RNAs like tRNAs, 5S rRNA, and several viral RNAs (Geiduschek and Tocchini-Valentini 1988). Short interspersed repetitive elements (SINEs) are also transcribed by RNAP III (Weiner et al. 1986). The promoters for RNAP III are located inside of the genes themselves, and split into two regions (Geiduschek and Tocchini-Valentini 1988). The anterior and posterior promoter regions,

S. Matsutani (✉)
Division of Microbiology, National Institute of Health Sciences, Tokyo 158-8501, Japan
e-mail: sachiko@nihs.go.jp

(a)

(b)

	A block	B block
consensus	TGGCnnAGT-GG	GGTTCGAnnCC
human 3'-α 1 *Alu*	bp 5 . gGGCgcgGT-GG	bp 76 . GGTTCGAgaCC
rodent B2	bp 14 . TGGCtcAGT-GG	bp 58 . aGTTCaAttCC
Xenopus tRNA^{Met}	bp 31 . TGGCgcAGc-GG	bp 74 . GGaTCGAaaCC
E. coli tRNA^{phe}	bp 8 . TaGCtcAGTcGG	bp 52 . GGTTCGAttCC
E. coli tRNA^{Asp}	bp 8 . TaGttcAGTcGG	bp 52 . GGTTCGAgtCC
E. coli tRNA^{Trp}	bp 8 . TaGttcAaTtGG	bp 52 . aGTTCGAgtCt
E. coli IS*1*	bp 97 . TGaCgggGT-GG	bp 180 . aGTTCacttaC

Fig. 1 Comparison of the eukaryotic genes transcribed by RNAP III with *E. coli* tRNA genes and IS*1*. (**a**) Organizations of human *Alu*, *E. coli* IS*1*, and *E. coli pheU* gene for tRNA^{phe}. *Alu* has an internal promoter consisting of A- and B-blocks. The B-block binding subunit of TFIIIC recognizes the promoter to initiate transcription with RNAP III. IS*1* and *pheU* have bacterial promoters upstream of the genes. The internal region of IS*1* and the product of the *E. coli artA* gene work together to stimulate bacterial transcription. Bacterial tRNA genes have RNAP III promoter sequences inside them. (**b**) Nucleotide sequences of RNAP III promoters in SINEs and tRNA genes. Nucleotides identical to those in the consensus sequence (Galli et al. 1981) are shown in capitals. There is a RNAP III promoter-like sequence in the *cis*-element region of *E. coli* IS*1*

called the A- and B-blocks, respectively, contain highly conserved sequences (Fig. 1; Galli et al. 1981). Transcription with RNAP III requires the multisubunit transcription factor TFIIIC, which plays an important role in transcription initiation (Lassar et al. 1983). The B-block binding subunit in TFIIIC recognizes the RNAP III promoter in the transcription of tRNAs and several viral RNAs (Willis 1993). Although B-block binding subunits are diverse in eukaryotes, there are four domains with conserved sequence similarities (Matsutani 2004). Interestingly, there are also the RNAP III promoter sequences in the bacterial tRNA genes (Fig. 1). These genes are transcribed from the upstream promoters by bacterial RNA polymerase (Fig. 1a), but it is shown that eukaryotic RNAP III can transcribe some of them in vitro (Folk et al. 1982).

In this paper, I show that the internal region of IS*1* that stimulates transcription contains the RNAP III promoter-like sequence. The RNAP III promoter sequence of a human *Alu* is cloned into *E. coli*, and demonstrated to function as a *cis*-element to stimulate bacterial transcription as well as the IS*1* internal region. Furthermore, the primary structure of bacterial ArtA protein is compared with those of the B-block binding subunits of TFIIICs.

Materials and methods

Bacterial strain, plasmid, and culture media

The bacterial strain used was the *E. coli* K12 derivative JM109 (Yanisch-Perron et al. 1985). The plasmid used was pSAM295 (Matsutani 2005). The culture media used were L-rich broth and supplemented 1 x A medium (Matsutani 1994). Media were supplemented with ampicillin (100 μg/ml).

DNA preparation and plasmid construction

Construction of the plasmid carrying the 5′ part of *Alu* was as follows: two oligonucleotides which contain the sequence of each strand of 3′-α1 *Alu* (bp positions 1–87 of the GenBank accession M13479; Perez-Stable et al. 1984) between the sequence of 5′-CTCACGGATCC-3′ and the sequence of 5′-GGATCCATGCC-3′, were chemically synthesized using an Applied Biosystems DNA synthesizer model 392, and purified on a 15% polyacrylamide/urea gel. These two oligonucleotides in equal molar amounts (160 pmol) were mixed, and heated at 100°C for 3 min in TE buffer in a total volume of 80 μl. The mixture was then cooled gradually to the room temperature, and additionally incubated on ice for 1 h. The annealed DNA sample was digested with *Bam*HI, and cloned into pSAM295 digested with *Bam*HI, to generate pSAM351. Nucleotide sequencing was carried out to orientate the cloned fragment and confirm that its sequence was the designed one. The constructions of pSAM354 and pSAM355 were described in Results.

To construct pSAM502, pSAM351 was digested partially with *Eco*RI and completely with *Xho*I, treated with the Klenow enzyme, and self-ligated. This DNA sample was introduced into JM109 cells. Plasmid DNAs were prepared from the transformants, and analyzed by digesting with appropriate restriction enzymes to select pSAM502.

Assay of β-galactosidase activity

E. coli JM109 cells having pSAM plasmids were grown and assayed for β-galactosidase as described by Matsutani (2005).

RNA analysis

Overnight cultures of *E. coli* JM109 cells having pSAM plasmids were diluted with fresh L-rich broth, and grown to an OD_{600} of about 0.3 at 37°C. IPTG was then added to the cultures at a final concentration of 2 mM, and incubation was continued for 2 h. Total RNAs were extracted, and 4 μg of the samples were electrophoresed in a 1.2% agarose gel in the presence of formaldehyde (Matsutani 2005). Blotting onto a nylon membrane was carried out by capillary action. The membrane was probed at 60 °C with the 0.5 kb fragment of the 5′ end of *lacZ* (bp positions 81–586 of the GenBank accession ECLACZ) which was labeled using the Random Primer Fluorescein Labeling Kit with Antifluorescein-HRP (NEN) (Matsutani 2005). The film was scanned, and hybridization signals were

quantified using Scion Image. To examine the stability of mRNAs, refampicin was added at a final concentration of 400 μg/ml to the cultures which had been incubated for 2 h with 2 mM of IPTG. Samples of the cultures just before the addition of refampicin and after 8-min incubation with refampicin, were used for Northern blot hybridization with the *lacZ* probe.
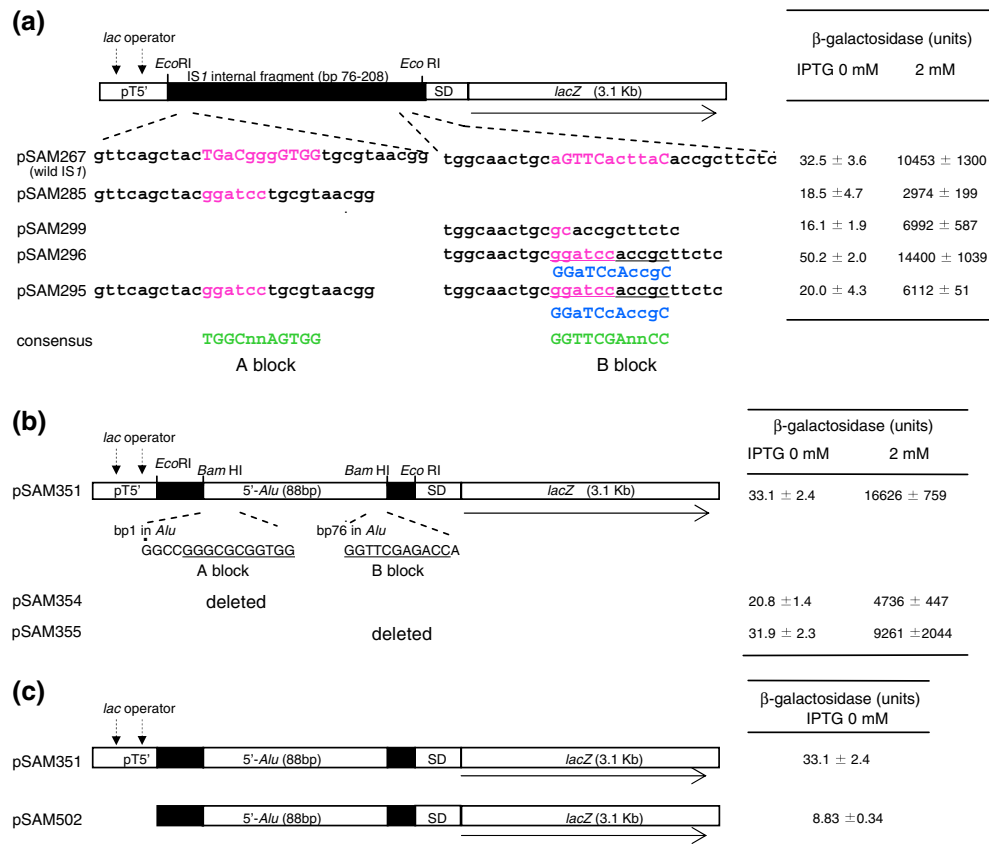
In silico analysis

To compare the primary structure of bacterial ArtA protein with those of eukaryotic B-block binding subunits, the Blast 2 sequences program (Altschul et al. 1997) in the NCBI website was used (http://www.ncbi.nlm.nih.gov/BLAST/). The Clustal W program (Thompson et al. 1994) in the EMBL-EBI website (http://www.ebi.ac.uk/clustalw/) was used to align multiple amino acid sequences. The Blast 2 sequences and Clustal W programs were performed by default.

Results

Presence of the RNAP III promoter-like sequence in the IS*1* internal region and its role in transcription

The promoters of the genes transcribed by RNAP III are located inside of the genes themselves, and split into two regions called the A- and B-blocks (Introduction). When considering how the internal region of IS*1* stimulates transcription, I found that the sequences of bp positions 97–107 of IS*1* are similar to the A-block sequence of human *Alu*, reported by Perez-Stable et al. (1984) (bp correspondences, eight out of eleven; Fig. 1b). This IS*1* sequence was also similar to the consensus sequence of the A-block (Fig. 1b). Moreover, the sequence of bp positions 180–190 of IS*1* was similar to the B-block sequence of rodent B2, reported by Krayev et al. (1982) (bp correspondences, nine out of eleven; Fig. 1b). The B2 sequence is one of the major families of SINEs in rodents (Krayev et al. 1982). In the previous study (Matsutani 2005), pSAM267, pSAM285, and pSAM299 were constructed, which are derivatives of pQE70 and contain *lacZ*, *Y*, *A* (Fig. 2a). In pSAM267, the IS*1* fragment of bp positions 76–208, has been inserted into the unique *Eco*RI site between the *E. coli* phage T5 promoter/two *lac* operators region (pT5′), and *lacZ* with translation initiation signals (Fig. 2a). pSAM285 and pSAM299 are identical to pSAM267, but pSAM285 carries a substitution of GGATCC from TGACGGGGTGGTGCG at bp positions 97–111 in IS*1*, and pSAM299 carries a substitution of GC from

(a)

β-galactosidase (units)

	IPTG 0 mM	2 mM

lac operator
IS*1* internal fragment (bp 76-208)   *Eco* RI

pT5'   [IS1 segment]   SD   *lacZ* (3.1 Kb)

		IPTG 0 mM	2 mM
pSAM267 (wild IS*1*)	gttcagctacTGaCgggGTGGtgcgtaacgg tggcaactgcaGTTCacttaCaccgcttctc	32.5 ± 3.6	10453 ± 1300
pSAM285	gttcagctacggatcctgcgtaacgg	18.5 ± 4.7	2974 ± 199
pSAM299	tggcaactgcgcaccgcttctc	16.1 ± 1.9	6992 ± 587
pSAM296	tggcaactgcggatccaccgcttctc GGaTCcAccgC	50.2 ± 2.0	14400 ± 1039
pSAM295	gttcagctacggatcctgcgtaacgg tggcaactgcggatccaccgcttctc GGaTCcAccgC	20.0 ± 4.3	6112 ± 51
consensus	TGGCnnAGTGG GGTTCGAnnCC		
	A block B block		

(b)

β-galactosidase (units)

	IPTG 0 mM	2 mM

lac operator   *Eco*RI   *Bam* HI   *Bam* HI *Eco* RI

pSAM351   pT5'   5'-*Alu* (88bp)   SD   *lacZ* (3.1 Kb)

bp1 in *Alu*   bp76 in *Alu*
GGCCGGGCGCGGTGG   GGTTCGAGACCA
A block   B block

		IPTG 0 mM	2 mM
pSAM351		33.1 ± 2.4	16626 ± 759
pSAM354	deleted	20.8 ± 1.4	4736 ± 447
pSAM355	deleted	31.9 ± 2.3	9261 ± 2044

(c)

β-galactosidase (units)
IPTG 0 mM

lac operator

		IPTG 0 mM
pSAM351	pT5' 5'-*Alu* (88bp) SD *lacZ* (3.1 Kb)	33.1 ± 2.4
pSAM502	5'-*Alu* (88bp) SD *lacZ* (3.1 Kb)	8.83 ± 0.34

Fig. 2 Bacterial gene expression in the presence of the RNAP III promoter sequence. Structures of DNA constructs and β-galactosidase activities specified by them, are shown. A filled box represents the IS*1* segment. *lacZ* from bp position 23, a promoter region (pT5'), and a translation initiation signal (SD) are also shown. (**a**) Effect of the presence of the RNAP III promoter-like sequence in the IS*1* internal region on *lacZ* expression. Data are from Matsutani (2005). Four plasmids just below pSAM267 are identical to pSAM267, but contain the sequence substitutions indicated in the figure. (**b**) Effect of the presence of the 5′ part of *Alu* on *lacZ* expression. pSAM351 is identical to pSAM295 (Fig. 2a), but contains the substitution of the 5′ part of *Alu* from the IS*1* internal region. pSAM354 and pSAM355 are identical to pSAM351, but have lost the A- and B-block sequences, respectively. (**c**) Effect of the deletion of the pT5′ region on *lacZ* expression. pSAM502 is identical to pSAM351, but has lost the pT5′ region

TTCACTTAC at bp positions 182–190 in IS*1*, respectively. It was already reported that in the absence and presence of IPTG, β-galactosidase activities specified by pSAM285 and pSAM299 were lower than those specified by pSAM267 (Fig. 2a; Matsutani 2005). The IS*1* internal regions in pSAM267, pSAM285, and pSAM299 are transcribed by pT5′, but not translated. Moreover, previous Northern blot analysis showed that the amount of *lac* mRNAs synthesized by pSAM285 is less than that of *lac* mRNAs synthesized by pSAM267 (Matsutani 2005). These results suggest that the RNAP III promoter-like sequence in IS*1* has a function to stimulate transcription.

pSAM296 is identical to pSAM267, except that pSAM296 carries a substitution of GGATCC from TTCACTTAC at bp positions 182–190 of IS*1* (Fig. 2a; Matsutani 2005). This substitution generates the stretch of GGATCCACCGC in which ACCGC is

from bp positions 191–195 in IS*1*. Compared with the B-block-like sequence in wild-type IS*1*, this stretch was more similar to the consensus B-block sequence (bp correspondences in pSAM296, eight out of eleven; bp correspondences in pSAM267, 7 out of 11; Fig. 2a). The seventh residue in the posterior sequence of pSAM267 is C (Fig. 2a), and in the case of pSAM296, the seventh residue is A. It should be noted that the seventh residue of the B-block is A in almost all internal promoters of eukaryotic tRNA genes (Dieci et al. 2002). In the absence and presence of IPTG, β-galactosidase activities specified by pSAM296 are always higher than those specified by pSAM267 and pSAM299 (Fig. 2a; Matsutani 2005). This result also suggests that the RNAP III promoter sequence acts as a *cis*-element to stimulate transcription in bacteria. pSAM295 is identical to pSAM296, except that pSAM295 carries a substitution of GGATCC from

TGACGGGGTGGTGCG at bp positions 97–111 in IS*1* (Matsutani 2005). β-Galactosidase activities specified by pSAM295 are lower than those specified by pSAM296, as well as β-galactosidase activities specified by pSAM285 are lower than those specified by pSAM267 (Fig. 2a; Matsutani 2005). In addition, in the presence of IPTG, β-galactosidase activity specified by pSAM295 is higher than that specified by pSAM285, as well as β-galactosidase activity specified by pSAM296 is higher than that specified by pSAM267 (Fig. 2a; Matsutani 2005). These results also suggest that bacterial transcription is stimulated by the RNAP III promoter-like sequence in IS*1*.

The RNAP III promoter sequence of human *Alu* also stimulates transcription in *E. coli*

The *Alu* sequence located on the 3′ side to the human α1-globin gene is called 3′-α1 *Alu*, and shown that the two intragenic regions of bp positions 4–37 and 70–82 are needed for its transcription (Fig. 1; Perez-Stable et al. 1984). Its anterior region contains an A-block sequence, and its posterior region overlaps with a B-block sequence (Fig. 1b; Perez-Stable et al. 1984). To examine whether the RNAP III promoter of 3′-α1 *Alu* stimulates the bacterial transcription, pSAM351, pSAM354, and pSAM355 were constructed (Fig. 2b). pSAM351 is identical to pSAM295 except that the *Bam*HI fragment containing the IS*1* segment was replaced by the 3′-α1 *Alu* fragment of bp positions 1–88. pSAM354 and PSAM355 are identical to pSAM351, but have lost the *Alu* regions of bp positions 1–15 and 76–87 containing the A- and B-blocks, respectively. As shown in pSAM351 and pSAM354 in Fig. 2b, deletion of the A block resulted in decreased β-galactosidase activities (1.6-fold and 3.5-fold lower activities in the absence and presence of IPTG, respectively). Deletion of the B-block also resulted in decreased activities (pSAM351 and pSAM355 in Fig. 2b). All of the results obtained with the 5′ part of *Alu* suggest the transcription activation function of the RNAP III promoter sequence in bacteria. Although decreases in B-block deletion were smaller than in A-block deletion, this is possibly because pSAM355 still has a B-block-like sequence derived from pSAM295 (see the section above; Fig. 2a).

pSAM502 is identical to pSAM351, but has lost its pT5′ region (Materials and methods). β-Galactosidase activity specified by pSAM502 was 8.83 units, and about 4-fold lower than that specified by pSAM351 (Fig. 2c). This result shows that A- and B-block sequences do not function as a promoter in bacteria, which is clearly different from eukaryotes.

Effect of the presence of the RNAP III promoter sequence on mRNA production in bacteria

To confirm that the RNAP III promoter sequence stimulates transcription in bacteria, *lac* mRNAs from strains carrying pSAM351, pSAM354, and pSAM355 were analyzed by Northern blot hybridization with the *lacZ* probe. mRNAs were prepared from cells grown in the presence of 2 mM of IPTG (Materials and methods). As shown in Fig. 3, all of the strains had similar band patterns, and in some, the bands of the intact *lacZ* mRNAs (3.1 kb) were superimposed on smears of significant backgrounds of mRNA. These corresponded to the patterns of previous Northern blot analyses which were carried out to confirm that the internal region of IS*1* stimulates gene expression at the transcriptional level (Matsutani 2005). The patterns in Fig. 3 were different in signal intensity, and mRNAs were present in the following order of decreasing abundance: pSAM351, pSAM355, and pSAM354. This was confirmed by scanning the film and quantifying all the hybridization signals: the ratios of the total hybridization signals of pSAM354, and of pSAM355 to that of pSAM351, were 0.20 and 0.52, respectively (Fig. 3). β-Galactosidase activities specified by these plasmids also gave the same order of decreasing
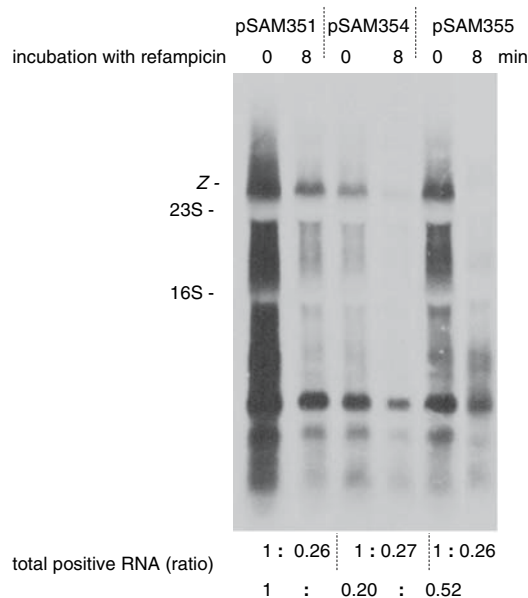

Fig. 3 Northern blot analysis of *lac* mRNAs in strains carrying pSAM351, pSAM354, and pSAM355. The positions of *lacZ* mRNA (3.1 kb), 23S rRNA (2904 bases), and 16S rRNA (1541 bases) are indicated. All hybridization signals were quantified, and the ratios of total signals of pSAM354 and pSAM355 to that of pSAM351 are shown. The decreasing ratio of signals when incubated with refampicin was also shown in each plasmid

abundance (Fig. 2b), showing that the decrease in the amount of *lac* mRNAs resulted in decreased β-galactosidase activity. The stability of *lac* mRNAs in strains carrying pSAM351, pSAM354 and pSAM355 was also examined. Transcription initiation was blocked with rifampicin, and degradation of *lac* mRNA was followed using Northern blot hybridization with the *lacZ* probe (Materials and methods). The decrease in *lac* mRNA was uniform in the three strains (Fig. 3): the ratios of total hybridization signals of pSAM351, pSAM354 and pSAM355 after 8-min incubation with refampicin to those just before adding refampicin were 0.26, 0.27, and 0.26, respectively.

Are there structural similarities between ArtA protein and B-block binding subunits of TFIIICs?

The *artA* gene is present in the *E. coli* F factor, and is suggested to express the ArtA protein (Wu and Ippen-Ihler 1989). Recently, it has been genetically shown that the product of the *artA* gene works with the internal region of IS*1* and stimulates transcription (Matsutani 2005). As shown in Figs. 2a and 4a, the *lacZ* construct in pSAM370 is identical to pSAM267, but has lost the IS*1* internal region (Matsutani 2005). The *lacZ* constructs in pSAM365 and pSAM372 are identical to pSAM299 and pSAM296, respectively, but
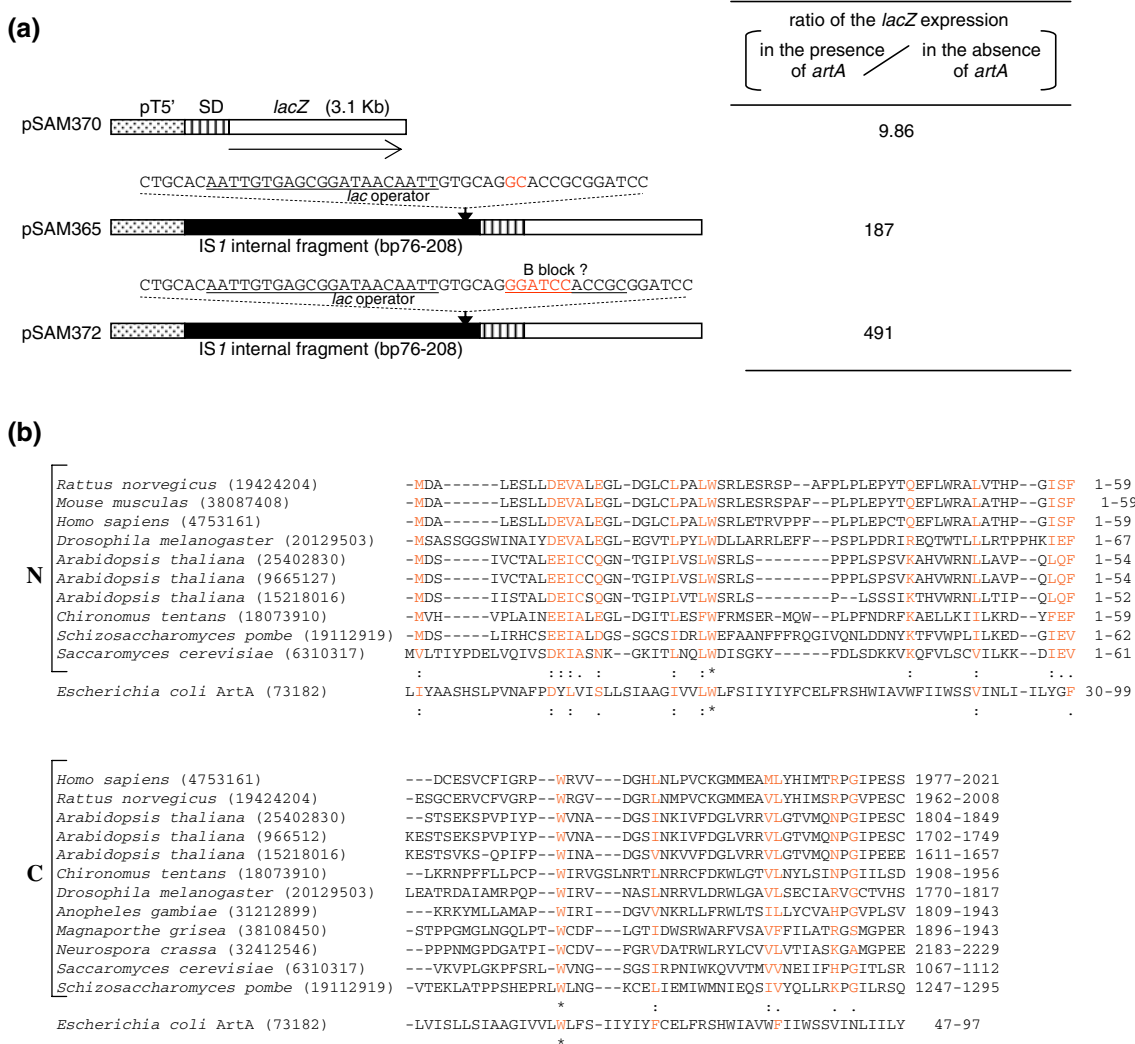
Fig. 4 Possible relation between the product of the bacterial *artA* gene and the B-block binding subunits of eukaryotic TFIIICs. (**a**) Effect of the presence of the RNAP III promoter-like sequence in IS*1* and the *artA* gene on *lacZ* expression. β-Galactosidase activities were measured in the absence of IPTG. Data are from Matsutani (2005). (**b**) Alignments of the amino acid sequences of the N- and C-terminal domains of the B-block binding subunits obtained using the Clustal W program. These sequences were aligned also with the amino acid sequence of bacterial ArtA protein using Clustal W. With or without the ArtA sequence, there were no differences in the alignments of the eukaryotic sequences

both have an insertion of the *lac* operator sequence near the mutated sites in the IS*1* internal regions (Figs. 2a, 4a; Matsutani 2005). β-Galactosidase activities specified by the three plasmids were measured, and the activity ratios in the presence of *artA* to those in the absence of *artA* have already been shown (Fig. 4a; Matsutani 2005). Comparison of these ratios demonstrates that the product of the *artA* gene works with the IS*1* internal region to stimulate transcription (Matsutani 2005). It is also suggested that in *E. coli*, there is a protein which binds to the IS*1* internal region (see Fig. 4 in Matsutani 2005). ArtA protein may not bind directly to the internal region of IS*1* (Introduction; Matsutani 2006b). However, it seemed that in the case of pSAM372, binding of the LacI repressor to the operator competed with the interaction between ArtA protein and B-block-like sequence in the IS*1* internal region. This is because *lac* operator sequences are close to the mutated sequences of the IS*1* internal regions in pSAM365 and pSAM372: pSAM365 has lost a B-block-like sequence in the IS*1* internal region; and pSAM372 has a sequence more similar to the B-block consensus compared with wild-type IS*1* (Figs. 2a, 4a). Therefore, I decided to compare the primary structure of ArtA protein with those of the B-block binding subunits of eukaryotic TFIIICs.

B-block binding subunits are diverse in eukaryotes, but there are four domains with conserved sequence similarities (Introduction; Matsutani 2004): there are three domains in the N-terminal one-third region, and one (named the domain N) is located in the N-terminal end region; and the fourth domain (named the domain C) is present near the C-terminal end. The amino acid sequences of each of these four domains had been aligned using the Clustal W program (Matsutani 2004). To examine the similarities between the primary structure of ArtA protein and those of B-block binding subunits, first, using the Blast 2 sequences program, ArtA protein was compared with many B-block binding subunits. However, I could not find significant similarities between them (data not shown). Next, I tried to align the sequences of each of the four domains in the B-block binding subunits with the ArtA sequence by Clustal W. Alignments of sequences of the two internal domains with the ArtA protein did not show similarities between the eukaryotic and prokaryotic sequences (data not shown). On the other hand, each of the alignments of domain N and C sequences with the ArtA sequence showed some sequence similarities (Fig. 4b): several amino acid residues were conserved in all of the sequences of B-block binding subunits and ArtA protein. The portions of eukaryotic

sequences aligned with the ArtA sequence were identical to the alignments previously obtained without the ArtA sequence. In other words, even with the ArtA sequence the alignments of domains N and C of the B-block binding subunits did not change. The results obtained here suggest that the ArtA protein is similar to the N- and C- terminal end regions of B-block binding subunits at the amino acid sequence level.

Discussion

In this paper, it was demonstrated that the eukaryotic RNAP III promoter-like sequence is present in the internal region of bacterial IS*1*, and can stimulate transcription from the bacterial promoter located upstream. The RNAP III promoter sequence of human *Alu* was cloned into *E. coli*, and was suggested to have the function of transcription stimulation like the IS*1* internal region. Furthermore, the primary structure of ArtA protein, which works with the IS*1* internal region, was compared with those of B-block binding subunits of TFIIICs, and some sequence similarities were suggested to be present.

It has been thought that prokaryotes and eukaryotes have inherent RNA polymerases and promoters (Fassler and Gussin 1996), and thus, the transcription machinery has evolved with the hosts. However, the results described here appear to be somewhat inconsistent with this notion. It is very intriguing to imagine another form of the evolution of transcription machinery. As described in the Introduction, eukaryotic tRNA and 5S rRNA genes, and SINEs have internal promoters, and are transcribed by RNAP III. SINEs are retrotransposons which transpose through RNA intermediates, and are suggested to be derived from tRNAs and 5S rRNAs (Matsutani 2006a). In this study it was suggested that bacterial IS*1* contains an RNAP III promoter-like sequence in the *cis*-element region which stimulates bacterial transcription. Although IS*1* is a DNA element and SINEs are retrotransposons, both can move on genomes.

ArtA protein consists of 104 amino acids, and is described to associate possibly with another protein(s) in transcription stimulation in *E. coli* (Matsutani 2005; 2006b). The molecular masses of eukaryotic B-block binding subunits are much higher than that of the ArtA protein. In addition, ArtA protein is bioinformatically predicted to contain two transmembrane domains (Matsutani 2005), which may suggest the indirect binding of ArtA protein to the B-block-like sequence in the IS*1* internal region. Nevertheless, it is interesting

that the small ArtA protein seemed to have sequence similarities to small domains conserved in large B-block binding subunits, which are highly diverse in eukaryotes.

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