JOURNAL OF BACTERIOLOGY, Aug. 1987, p. 3850–3852
0021-9193/87/083850-03$02.00/0
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Vol. 169, No. 8

Spot 42 RNA of *Escherichia coli* Is Not an mRNA

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Received 20 February 1987/Accepted 4 May 1987

Spot 42 RNA of *Escherichia coli*, a 109-nucleotide RNA that influences the level of DNA polymerase I, has an AUG triplet preceded by a purine-rich potential ribosome-binding site and is followed by a short (14-triplet) potential open reading frame. Although the RNA bound to ribosomes, it did so inefficiently and nonproductively. When fused to *lacZ* sequences, spot RNA did not support the synthesis of β-galactosidase. Also, the biological effects of spot 42 RNA were not altered by mutation of the tyrosine UAU codon to the chain termination UAG. We conclude that the effects of spot 42 RNA are mediated by the RNA itself and not by a spot 42 RNA-encoded peptide.

The *spf* gene of *Escherichia coli*, which is located very close to the *polA* gene (6), encodes a small RNA called spot 42 RNA (5, 10). The exact function of this 109-nucleotide RNA is unclear, since deletion of the *spf* gene does not appear to affect normal cell growth (3). However, elevation of the level of the spot 42 RNA above the normal 100 to 200 copies per cell (11) results in an increased ability of cells to withstand treatment by agents that cause DNA damage, such as UV light or methyl methanesulfonate, whereas the absence of spot 42 RNA results in an increased sensitivity to such agents (D. A. Polayes, P. W. Rice, and J. E. Dahlberg, submitted for publication). Cells that overproduce spot 42 RNA, as a result of having multiple copies of the *spf* gene in a plasmid, grow slowly and adjust poorly to changes in the composition of media (9).

It is unknown whether spot 42 RNA functions as an mRNA or whether it participates directly (or as a cofactor) in reactions that elicit the associated phenotypes. Spot 42 RNA resembles a short mRNA in that it contains a polypurine sequence followed by AUG, 14 triplets, and UGA (10). The RNA contains two purine-rich potential ribosome-binding sites which are 4 and 11 nucleotides upstream of the AUG. Neither distance is considered optimal (12), but the RNA is bound to ribosomes as they are isolated from cells (11). In the experiments described here, we show that spot 42 RNA does not function efficiently as an mRNA and that the effects of the *spf* gene are likely to be due to interaction of spot 42 RNA with one or more cell components.

As was the case in vivo, spot 42 RNA bound to purified 70S ribosomes in vitro when assayed by either filter binding (Table 1) or sucrose gradient centrifugation (data not shown). Although this binding was factor dependent and subunit specific, it was inefficient and incomplete. A 250-fold molar excess of 70S ribosomes was required for maximal binding, and even then only half of the RNA was bound. These results contrast with the binding of the gene VIII mRNA of bacteriophage M13. Thus, spot 42 RNA can bind to ribosomes but it does so inefficiently.

The ability of the spot 42 RNA ribosome-binding site to function productively in the synthesis of protein was tested by fusing part of the *E. coli* β-galactosidase gene, *lacZ*, to the *spf* gene. Cells containing multiple copies of these chimeric *spf-lacZ* genes produced very little β-galactosidase (Table 2); the levels of enzyme activities in such cells were even lower than the level in uninduced cells containing a single copy of the wild-type *lacZ* gene (strain JED2118). Moreover, the level of β-galactosidase was not significantly affected by the presence of termination codons in, or the absence of an initiation codon from, the RNA. As might be expected from these results, the number of spot 42 RNA triplets upstream of the *lacZ* sequences also had no effect on expression. These results argue strongly that spot 42 RNA cannot normally be translated.

It was still possible that the ribosome-binding site of spot 42 RNA could be used to make a small peptide but not a large protein. For example, inefficient initiation would leave most of a large mRNA unprotected and subject to nuclease attack (13), but on a small RNA like spot 42 RNA, an initiating ribosome could protect most or all of the RNA. We investigated the possible translation of spot 42 RNA by using an indirect assay based on the fact that unusually high levels of the RNA cause cells to grow slowly on poor carbon sources such as succinate. This effect is illustrated in Fig. 1, in which the optical densities of cultures are displayed as cells were forced to adapt from glucose to succinate as a

TABLE 1. Binding of spot 42 RNA to ribosomes[a]

Ribosomes[b] (fmol added)	Spot 42 RNA bound[c] (fmol)	Gene VIII RNA bound[c] (fmol)
70S (100)	0.35	0.55
70S (500)	0.94	1.2
70S (1,000)	1.0	2.0
70S, washed (500)	0.06	0.08
30S (500)	0.7	0.96
50S[d] (500)	0.24	0.28

[a] 2.0 fmol of spot 42 RNA or gene VIII RNA (of phage M13) was mixed with 100 to 1,000 fmol of *E. coli* ribosomes, as indicated, in 50-μl reaction mixtures containing IB (100 mM Tris [pH 7.6], 50 mM NH_4Cl, and 5 mM magnesium acetate). After incubation at 25°C for 10 min, the mixture was passed through a 25-mm Millipore HAWP filter, the filter was washed three times with 5 ml of IB, and radioactivity was determined by scintillation counting. Background (no ribosomes) was subtracted from all results.

[b] Ribosomes were prepared as described by Held et al. (4).

[c] Spot 42 RNA and gene VIII RNA, synthesized in vitro by using purified *E. coli* RNA polymerase and linearized DNA templates, were gel purified before assay. The numbers of moles of RNA added or bound were calculated from the specific activities of the nucleoside triphosphates, assuming chain lengths of 109 and 360 nucleotides for spot 42 and gene VIII RNAs, respectively. Approximately 25% of these nucleotides were rGTP, the labeled nucleotide.

[d] About 15% of the optical density of this preparation of 50S ribosomes was due to particles that migrated at 30S on sucrose gradient centrifugation.

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TABLE 2. Structure and activity of *spf-lacZ* fusions

Strain[a]	Gene or plasmid[b]	Fusion sequence[c]	β-Galactosidase[d] (U)
	spf	GAGGTAAGATGTTCTATCTTTCAGACCTTTTACTTCACGTAATCGGATTTGGCTGA	
CAG1574	pΦ(*spf-7–lacZ*)*1*	GAGGTAAGATGTTCTATCTTTCAGACCTTTTACTTCACG.CAGATCCGAATT....CGGAT	5.8
	pΦ(*spf-7–lacZ*)*2*	GAGGTAAGATGTTCTATCTTTCAGACCTTTTACTTCACG.CAGATCCGAATTAATTCGGAT	1.9
	pΦ(*spf-8–lacZ*)	GAGGTAAGATGTTCTAG ....GGGGAT	4.5
	pΦ(*spf-9–lacZ*)	GAGGTAAGATG ....GGGGAT	3.0
	pΦ(*spf-10–lacZ*)	GAGGTAAG ....GGGGAT	15.7
	pMC1403	....GGGGAT	1.5
JED2118			
Without IPTG		AGGAAACAGCTATGACCATGATTACG ....GAT	21.4
With IPTG		AGGAAACAGCTATGACCATGATTACG ....GAT	1,697.0

[a] Strain CAG1574 [*araD* Δ(*ara-leu*) Δ*lac galU galK hsdR hsdM rpsL recA*) was from C. Gross (University of Wisconsin, Madison), and strain JED2118 was constructed by P1 *vir* (MG1655) transduction of M7042 to make it *lac*⁺; strains MG1655 and M7042 (*lacZ rpsL*) were from C. Gross. IPTG, Isopropyl-β-D-thiogalactopyranoside.

[b] Plasmid pΦ(*spf-7–lacZ*)*1* was constructed by cloning the 6.2-kilobase-pair *Bam*Hi-*Sal*I fragment which contains the *lacZYA* genes from pMC1403 (1) into the *Bgl*II and *Sal*I sites of pΦ*spf-13* (P. W. Rice, Ph.D. thesis, University of Wisconsin, Madison, 1986), and pΦ(*spf-7–lacZ*)*2* was constructed from pΦ(*spf-7–lacZ*)*1* by linearizing at the unique *Eco*RI site at the *spf-lac* fusion, filling in the ends by using the Klenow fragment of DNA Polymerase I (Boehringer Mannheim Biochemicals) and dATP and dTTP (Pharmacia Fine Chemicals), and religating. pΦ(*spf-8–lacZ*) was constructed by digesting pJD5544 (Polayes et al., submitted) with *Alu*I, gel purifying (2) the 156-base-pair *Alu*I fragment containing the 5′ flanking region of *spf* up to the TAG codon, and ligating this fragment into the *Sma*I site of pMC1403. pΦ(*spf-9–lacZ*) and pΦ(*spf-10–lacZ*) were generated by digesting pΦ(*spf-8–lacZ*) with *Mae*I and mung bean nuclease, recutting with *Eco*RI, and gel purifying a 158-base-pair fragment. This fragment was ligated into the *Eco*RI-*Sma*I sites of pMC1403.

[c] Sequences of the plasmids were determined by using the double-stranded plasmid DNA dideoxy method (14). For reference, the sequence of the putative coding region of *spf* is shown; the ribosome-binding site is indicated by dashes, and initiation and termination codons are underlined, as is codon 6 of the fused β-galactosidase gene. The asterisks above the top line indicate nucleotides that were mutated at position 27 (by oligonucleotide mutagenesis) (15) and position 36 (spontaneous) to make mutants G27 and A36. Periods indicate that the nucleotide shown in the top line is absent.

[d] β-Galactosidase assays were performed as described by Miller (7) and were normalized to a constant number of cells.

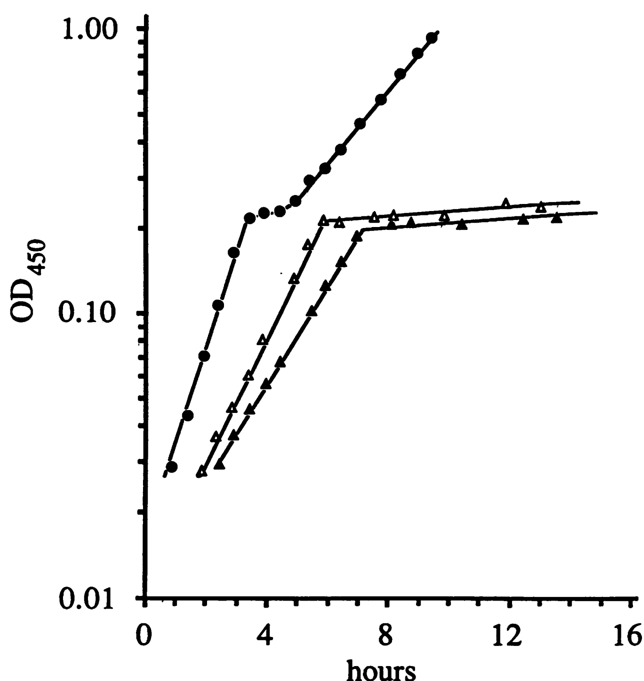

FIG. 1. Effect of multiple copies of *spf* on the ability of cells to adapt to a shift-down in carbon source. Cells of strain CAG1097 (M7042 *recA srl*::Tn*10*) (from C. Gross) were transformed with pBR322 DNA (●), pJD423 *spf*+ (pBR322 containing the *spf* gene with 103 nucleotides of 5′ flanking sequences) (▲), or pJD423/G27 (pJD423 with a T→G mutation at position 27) (△) and grown under ampicillin selection in MOPS (morpholinepropanesulfonic acid) minimal medium (8) supplemented with 0.01% glucose and 1% succinate at 37°C. Aeration was by shaking. At an optical density at 450 nm (OD_{450}) of approximately 0.2, the glucose was exhausted and cells were forced to adapt to growth on succinate.

carbon source. Cells containing the plasmid pBR322 were able to adjust, but cells with the *spf* gene cloned into pBR322 could not do so.

Mutation of a T to G at position +27 in the gene (G27) changes the third triplet from UAU to UAG (tyrosine to chain termination). This change, while destroying any ability of spot 42 RNA to be translated in these nonsuppressing cells, had no effect on the ability of the cells to grow in succinate (Fig. 1). In experiments to be published elsewhere (Polayes et al., submitted) we also showed that the G27 chain termination mutation did not alter the spot 42 RNA-associated resistance of cells to methyl methanesulfonate. Thus, if spot 42 RNA is an mRNA, the resulting peptide does not mediate known functions of the *spf* gene.

Although the G27 mutation had no detectable effect on the ability of cells to grow in succinate, it did result in slightly faster growth in glucose. Another point mutation, replacing C with A at position 36 (A36) (which would change aspartic acid to glutamic acid in a peptide), also increased the growth rate slightly in glucose but not in succinate (data not shown). Both of these point mutations could well alter the effects of the *spf* gene by altering the way spot 42 RNA folds.

We conclude that spot 42 RNA is not an mRNA, under normal circumstances. We cannot, of course, rule out the possibility that under special conditions which have not yet been tested, the RNA could have mRNA activity.

Because spot 42 RNA is not an mRNA, it is likely to influence cell physiology by binding to one or more components of cells. Such interaction could either alter or enhance preexisting activities. For example, spot 42 might associate with enzymes responsible for the synthesis or activity of components that participate in the repair of some types of DNA lesions.

We thank E. Lund, K. Loughney, and M. Davis for useful discussions and comments, C. A. Gross for bacterial strains, and L. Kahan for purified ribosomes.

P.W.R. was supported in part by Public Health Service training grant GM07215 from the National Institutes of Health, and D.A.P. was supported in part by a grant from the Damon Runyon-Walter Winchell Cancer Fund (DRG-765). This work was supported by National Science Foundation grant PCM 8309618 to J.E.D. and E. Lund.

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