The tRNA function of SsrA contributes to controlling repression of bacteriophage Mu prophage

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The small regulatory RNA SsrA has both tRNA and mRNA activities. It charges alanine and interacts with stalled ribosomes, allowing for translation to resume on the SsrA mRNA moiety. Hence, unfinished peptides carry a short amino acid tag, which serves as a signal for degradation by energy-dependent proteases. In SsrA-defective *Escherichia coli* strains, thermoinducible mutants of the transposable bacteriophage Mu (Mu*cts*) are no longer induced at high temperature. Here we show that truncated forms of the key regulator of Mu lysogeny, the repressor Repc, accumulate in the absence of SsrA. These forms resemble C-terminally truncated dominant Mu repressor mutants previously isolated from Mu*cts*, which are no longer thermoinducible and bind operator DNA with a high affinity even at high temperature. Using various *ssrA* alleles, we demonstrate the importance of SsrA charging on the ribosome for controlling Mu prophage repression. Our results thus substantiate the previous observation that trans-translation is not the only function of the SsrA. The alternative function of SsrA appears to influence the stability of Mu lysogens by controlling the translation of the C-terminal domain of the repressor protein, which modulates the affinity of the protein for DNA and its susceptibility to proteolytic degradation.

Mu repressor | tmRNA | truncated proteins | lysis–lysogeny switch

Bacteriophage Mu is a temperate phage and a transposon, which infects *Escherichia coli* K-12 and many other enterobacteria (for review see refs. 1 and 2). In lysogenic hosts, repression of the Mu prophage results from the synthesis of a repressor protein, Repc (196 aa; calculated molecular mass, 22 kDa), which binds to a 184-bp operator region and is composed of nine repressor-binding motifs grouped into three complex operators sites (O1, O2, and O3), and two promoters, pE and pCM. The early promoter pE, overlapping O2, drives early lytic transcription through O3, while pCM, which is in O3, drives divergent repressor transcription through O2 and O1 (Fig. 1*A*; for a review see ref. 3).

As with other temperate phages, once established as a stable prophage, Mu undergoes spontaneous induction at low frequency. No known chemical or physical treatment provokes massive induction, which can be achieved only by using either one of two types of prophages expressing the mutant repressors Mu*vir* or Mu*cts*. Mu*vir* repressors carry a frameshift mutation that alters the C terminus end of Repc, making the protein hypersensitive to degradation by the host ATP-dependent protease ClpXP. This property is transmitted to the more stable wild-type (WT) and *cts* repressors, enabling Mu*vir* phages to induce the resident prophage on superinfection of a WT Muc^+ or Mu*cts* lysogen (4–9). Four point mutations in the N-terminal part of Repc (*cts45*:S18L; *cts71*:M28I; *cts25*:D43G; and *cts62*:R47Q) (Fig. 1*B*) render the protein thermosensitive for operator binding, and Mu*cts* lysogens thus become inducible at 42°C (10–12). The Mu prophage is also derepressed in stationary phase (called "S" derepression in this paper and refs. 13 and 14).

Mutations that truncate the C terminus of the *cts* repressors (amber mutations at one of the three CAG codons, Q179, Q187, and Q190) (Fig. 1*B*) compensate the heat-sensitivity of the thermosensitive proteins and confer to the double-mutant prophages a heat-stable dominant phenotype called Sts (for survival of temperature shifts or suppressor of thermosensitivity; ref. 12). Temperature resistance of the truncated proteins correlates with a more stable binding to Mu operator DNA *in vitro* (12). The C terminus of Repc thus "regulates" the two physical parameters of the protein involved in prophage induction: the affinity toward operator DNA and the susceptibility to proteolytic degradation. Reduced occupancy of the operators, whether because of a change in affinity or the degradation of the repressor, favors transcription from pE and thus expression of the early lytic genes. Although these properties had only manifested themselves in mutant proteins, here we describe a host factor, SsrA, that directly influences the amino acid sequence at the C terminus of the Mu repressor.

SsrA (also called 10Sa and tmRNA), the product of the *E. coli ssrA* gene, is a small (≈350 nt), stable RNA (15) present in most bacterial genomes as well as in the plastid genome (for references see: http//www.bioinf.au.dk/tmRDB). In *E. coli*, SsrA bears properties of both an alanine-tRNA and an mRNA (16–18). By a mechanism called trans-translation (19, 20), SsrA tags incomplete proteins expressed from broken or cleaved mRNA lacking in-frame stop codons (16, 21). The 11-aa-long C-terminal peptide tag AANDENYALAA, in conjunction with the ribosome-associated protein SspB (22), targets the tagged polypeptides to degradation by ClpXP or other energy-dependent proteases (ClpAP, FtsH/HflB, and Tsp, see refs. 16, 23, and 24).

SsrA-deficient *E. coli* strains are viable, but exhibit diverse phenotypes, including slow growth, especially at high temperature (25), increased expression of the Alp protease (26), inability to support growth of λimmP22 hybrid phages (27), and enhanced activity of several repressor proteins (28). SsrA acts in combination with another protein, SmpB, which is required for stable association of the RNA with the ribosome (29). Even though multiple effects and targets of SsrA have been documented (30), its connection to the overall physiology of the cell remains to be elucidated.

In this report we show that bacteriophage Mu exploits a peculiar feature of SsrA, its capacity to modulate the progression of translating ribosomes, to control the probability of prophage induction. The temperature induction of Mu*cts62* is blocked when the host bacterium carries a null mutation in either the *ssrA* or the *smpB* gene (T. A. Baker, personal communication; ref. 29). We investigated Mu repression in strains expressing various forms of SsrA to distinguish between two of its known functions, the charging of alanine and loading of the RNA onto ribosomes

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Abbreviations: WT, wild type; Cm, chloramphenicol.

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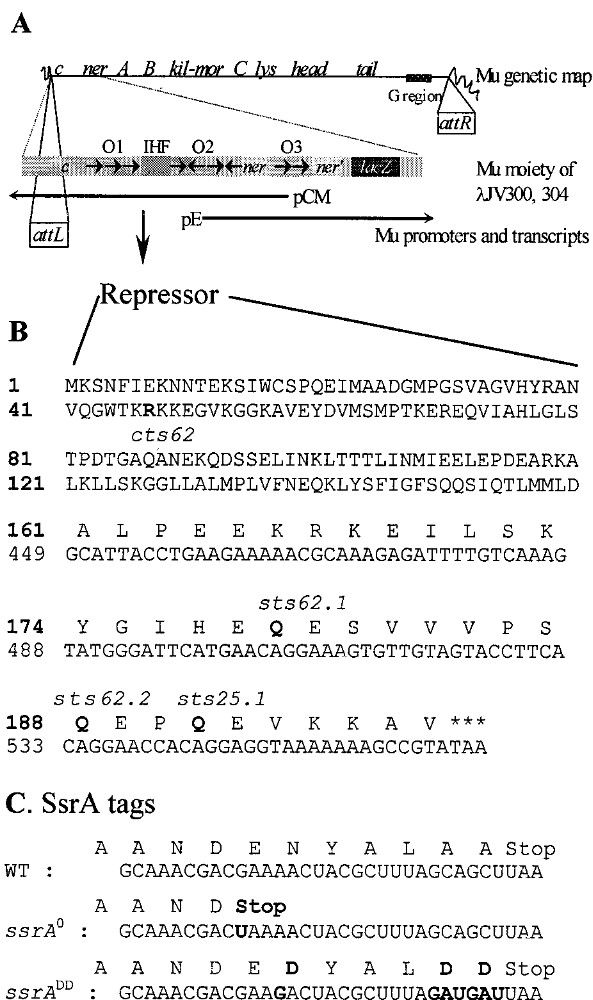

Fig. 1. (*A*) The genetic map of Mu and the indicator system of Mu repression–derepression. Some important landmarks on the Mu genome are shown on top of an enlarged view of the left end present in the λJV300 and pJV300 indicator constructs (see text for more details). Arrows indicate the transcripts initiating at pE (early lytic promoter) and pCM (lysogenic promoter). Light gray boxes show the O1–O3 operators with repressor-binding sequences represented by arrows indicating their relative orientations. By binding at the indicated positions, the host IHF and the Mu Ner proteins regulate expression from pE and pCM. *attR* and *attL*, Mu transposase-binding sites at Mu ends; *ner*′, truncated Mu *ner* gene. (*B*) Nucleotide and amino acid sequence of wild-type (WT) and mutant Mu repressor genes and proteins. The positions of the different mutations discussed in the text are shown in bold. The *sts* amber mutations are C-to-T transitions at the three last CAG codons (bold Q residues) in the *c* gene. The *cts62* mutation is a G-to-A transition (CGA to CAA) in codon 47. (*C*) SsrA tags. Nucleotide and amino acid sequences of the coding part of the WT and various mutant RNAs.

(31), and the tagging of the target protein toward proteolytic degradation (16). We assayed for whole-phage production in various Mu lysogens with these different *ssrA* alleles. We used an indicator system in which the Mu lytic promoter drives β-galactosidase expression to follow the influence of the bacterial host physiology on repression of the Mu prophage. Western blotting with a polyclonal antibody raised against purified Mu Repc allowed the analysis of the protein in strains carrying the various *ssrA* alleles. Our results explain the properties of previously characterized Mu*sts* repressor mutants and outline the molecular basis of one SsrA-mediated process that modulates the repression of the Mu prophage independent of its role in targeting proteins for degradation.

Table 1. Strains, plasmids, and phages

Strain	Characteristics, vector, and insert	Ref.
Bacteria		
MC4100	*araD139*, Δ(*lacIPOZYA*, *argF*)U169, *fla*, *relA*, *rpsL*	32
MC4100*ssrA*::Cm	Same as above but *ssrA*::Cm	This work
K8619	*galK*, *rpsL*, *ssrA*::Cm	33
K8664	K8619 (λ::*ssrA*$^+$) (at λ*att*), Amp^R	33
K8666	K8619 (λ::*ssrA*^{DD}) (at λ*att*), Amp^R	33
K8637	K8619 (λ::*ssrA*^{0}) (at λ*att*), Amp^R	33
K8650	K8619 (λ::*ssrA*^{UG}) (at λ*att*), Amp^R	This work
C600	*thr*, *leu*, *tonA*, *lacY*, *thi*, *supE*	34
Bacteriophages		
λRS45		35
λJV300	λRS45 with left end of Mu, *c*$^+$ pE::*lacZ*	11
λJV304	λRS45 with left end of Mu, *cts62* pE::*lacZ*	11
λJV313	λRS45 with left end of Mu, *cts62* *sts62.1* pE::*lacZ*	12
Muc$^+$		36
Mu*cts62*pAp1		37
Mu*cts4*		38
Plasmids		
pRS551	pBR322	35
pJV300	pRS551; *attL-Hae*dIII Mu *c*$^+$	11
pJV304	pRS551; *attL-Hae*III Mu*cts62*	11
pJV313	pRS551; *attL-Hae*III Mu*cts62*, *sts62-1*	12
pJV314	pRS551; *attL-Hae*III Mu *cts62*, *sts62-2*	12
pJV318	pRS551; *attL-Hae*III Mu *cts25*, *sts25-1*	12
pRS415	pBR322	35
pJW28	pRS415; *ssrA*$^+$	33
pJW34	pRS415; *ssrA*^{UG}	33
pJW29	pRS415; *ssrA*^{0}	J. Withey
pJW30	pRS415; *ssrA*^{DD}	J. Withey

Amp^R, ampicillin-resistant. J. Withey is at Univ. of Michigan, Ann Arbor.

Materials and Methods

Strains. Bacterial strains, phages, and plasmids are listed in Table 1. MupAp1 phages contain about 1 kb of Tn*3* DNA conferring ampicillin resistance, which substitutes for Mu DNA in the Mu G region (37).

Media. Bacteria were grown in LB (39) and titrated on LA plates (LB supplemented with 1.2% Difco agar). Ampicillin (50 μg/ml), streptomycin (50 μg/ml), chloramphenicol (Cm; 30 μg/ml), and kanamycin (50 μg/ml) were included when appropriate. Phage lysates were diluted in Tris/NaCl buffer (40) (but omitting tryptophan) and titrated on lawns of sensitive bacteria (0.1 ml of an overnight culture in LB poured with 2.5 ml of 0.7% LA agar on LA plates).

General Procedures. Lysates of thermoinducible Mu phages were prepared by thermal induction of a lysogen, and lysates of Mu*c*$^+$ were grown by infecting C600 on LA plates at 37°C, as described (41).

Mu lysogens were isolated by spotting phage suspensions at about 10^8 phage per ml on a lawn of bacteria on LA plates. After overnight incubation at 30°C, bacteria from the center of the lysis area were streaked for isolated colonies on LA plates at the same temperature and colonies were spotted on lawns of C600 to test for phage production at 42°C. When ampicillin resistance selection was possible where Mu*cts62*pAp1 was the prophage, lysogens were selected by streaking on LA plates supplemented with 50 μg/ml ampicillin.

Analysis of repressor sizes by Western blotting was performed

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Table 2. Influence of *ssrA* alleles on Muc+ and Mu*cts62* phage production

Strain	Mu phage	*ssrA* genotype	No. of phage produced			
			Overnight culture, 30°C	Exponential growth		
				30°C	40°C, 1 h	42°C, 1 h
K8619	Muc+	*ssrA*::Cm	<10^2	≈10^2	ND	<10^4
K8619	Mu*cts62*pAp1	*ssrA*::Cm[a]	<10^2	≈10^2	$4{\cdot}10^5$	$2.4{\cdot}10^9$
K8664	Muc+	*ssrA*+	$7{\cdot}10^4$	$4.6{\cdot}10^5$	ND	$8.3{\cdot}10^6$
K8664	Mu*cts62*pAp1	*ssrA*+	≈10^2	10^4	$4{\cdot}10^9$	$2.5{\cdot}10^9$
K8666	Muc+	*ssrA*DD	10^3	$1.2{\cdot}10^3$	ND	$3{\cdot}10^7$
K8666	Mu*cts62*pAp1	*ssrA*DD	≥10^4	$3.5{\cdot}10^5$	$3{\cdot}10^8$	10^9
K8637	Muc+	*ssrA*0	$6{\cdot}10^2$	$5{\cdot}10^3$	ND	$3.5{\cdot}10^7$
K8637	Mu*cts62*pAp1	*ssrA*0	≈10^2	$4{\cdot}10^3$	$4{\cdot}10^9$	$5{\cdot}10^9$
K8619	Muc+	pJW28 (*ssrA*+)	ND	$3{\cdot}10^4$	$4{\cdot}10^5$	ND
K8619	Muc+	pJW34 (*ssrA*UG)	ND	≈10^2	<10^2	ND
K8619	Mu*cts62*	pJW28 (*ssrA*+)	$5{\cdot}10^3$	10^3	$5{\cdot}10^9$	$4{\cdot}10^9$
K8619	Mu*cts62*	pJW34 (*ssrA*UG)	<10^2	<10^2	$2{\cdot}10^5$	$3{\cdot}10^9$

ND, not determined.

as described (4), except that detection of the Mu repressor was performed with Amersham Pharmacia's enhanced chemiluminescence Western blotting detection agents and x-ray film. Time course of β-galactosidase expression was measured as described (13).

DNA Manipulations. Enzymes were purchased from Life Technologies and used as recommended by the manufacturers. Plasmid DNA was extracted as described (42). The analysis of restriction fragments on 0.8% agarose gels in TAE buffer (40 mM Tris base/20 mM acetic acid/1 mM EDTA, pH 8.0) was essentially as described (43). Transformation of appropriate bacterial strains with plasmid DNA was as described (43). DNA sequencing was performed by the dideoxynucleotide chain-termination method (44) on an Amersham Pharmacia automated laser fluorescence sequencer.

Results

SsrA Charging with Alanine Decreases Mu Repression. A vast number of cellular proteins are tagged by SsrA (30) and subsequently degraded by ClpXP. Mu repressor is also a target of this protease (7, 9), and SsrA is essential for the thermal induction of Mu*cts62* (29). To elucidate the mechanism by which SsrA affects the physiology of Mu, and in particular the key regulator of the lysis–lysogeny decision, Repc, we have studied the effect of different *ssrA* alleles on prophage induction and on the physical properties of the repressor. We have tested two different conditions that are known to increase phage production: (*i*) entry into stationary phase, and (*ii*) elevated temperature. The temperature treatment is a particularly sensitive measure with a sharp transition around 41°C. As shown in Table 2, compared with SsrA+ lysogens, *E. coli* strains carrying a null *ssrA* allele and lysogenic for Muc+ produced very little phage, both at 30°C and at 42°C. When the Mu prophage carried the *cts62* mutation, phage production at 30°C was also lower in the *ssrA* host and, although in our hands thermal induction was normal at 42°C (lysis curves not shown), it was blocked at 40°C. No lysis occurred and phage production, which was the same as in the SsrA+ control strain at 42°C, decreased almost 1,000-fold at 40°C. As expected for measurements around the transition point, lysis was variable at 41°C (data not shown). Considering the experiments below 41°C (the transition point for thermal induction), the lack of SsrA thus decreased phage production on average by two orders of magnitude.

To distinguish between different functions of SsrA (charging of alanine and loading on the ribosome, trans-translation and tagging) involved in the regulation of Mu lysogeny, we used three *ssrA* alleles: *ssrA*^{DD}, *ssrA*^{0}, and *ssrA*^{UG} (Fig. 1*C*). The first mutant SsrA adds a tag that differs from the WT tag by three amino acid residues and thereby reduces dramatically the efficiency of targeting toward proteolytic degradation (16, 23, 24). The second mutant adds a truncated three amino acid tag, which also precludes recognition by the protease; the third mutant is unable to charge Ala and hence does not interact with the ribosome (33). As shown in Table 2, the *ssrA*^{DD} and *ssrA*^{0} alleles complemented the *ssrA*::Cm null mutation. At high temperature, they restored phage yields equivalent to those observed in the isogenic SsrA+ strain, for both Muc+ and Mu*cts62*. At 30°C, they also increased phage production although to various degrees that differed for Muc+ and Mu*cts62*. The *ssrA*^{UG} allele did not complement. This result was verified by two equivalent plasmid constructs (see last two lines in Table 2). Thus, as in the case of λ-P22 hybrid phage growth (27, 33), the addition of a tag productive for proteolytic degradation is not required for efficient Mu phage production. However, charging of SsrA with alanine is essential. Further experiments will show which of the intervening steps (e.g., interaction of SsrA with the ribosome, switching from the mRNA to the SsrA template, translation of the new template) is essential.

S Derepression Is Blocked in the Absence of SsrA Charging with Alanine. Despite derepression of pE in stationary-phase cultures, phage production remains low (overnight culture column of Table 2), probably because later stages of the lytic cycle cannot proceed efficiently in these physiological conditions. We therefore used a previously described reporter system for the expression from the Mu early lytic promoter pE (the λJV300 series of prophages, ref. 11 and Fig. 1*A*) to assess the effect of SsrA on Mu repression in stationary-phase cultures and on solid media. This system consists of single-copy prophages derived from λRS45 (35) inserted at the λ*att* site in a Δ*pro–lac E. coli* strain. The prophages contain the complete Mu repressor gene *c*, its promoter pCM, and the early lytic promoter pE, in their natural configuration. Mu repressor is expressed from its natural pCM promoter and is, hence, subject to negative autoregulation. pE is fused to a *lac* operon, such that *lacZ* replaces the Mu early lytic genes, allowing for quantitative measurements of expression from pE.

MC4100(Δ*pro–lac*) lysogenic for λJV300 or λJV304, respectively, carry the WT or *cts62* allele of the Mu *c* gene (Fig. 1*A*). On MacConkey lactose plates, strain MC4100(λJV304) forms red colonies (Lac+) at 42°C (because of the thermosensitivity of

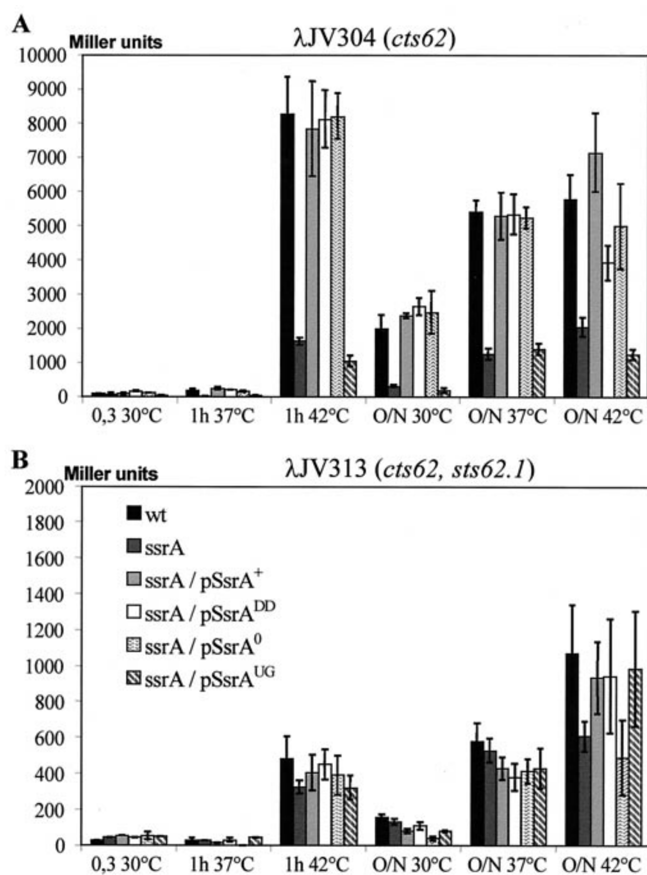

Fig. 2. β-Galactosidase measurements of Mu derepression in hosts carrying different *ssrA* mutations. Overnight cultures grown without aeration at 30°C were diluted 100-fold in fresh LB and grown with aeration at 30°C until OD_{600} = 0.3. β-Galactosidase activity was measured (0,3 30°C), and the remaining culture was divided in three parts. The first stayed at 30°C, the second was shifted to 37°C, and the third was shifted to 42°C. β-Galactosidase activity (Miller units) was measured after 1 h for cultures at 37°C and 42°C, and after overnight (O/N) incubation at 30°C, 37°C, and 42°C. The genotype of the Mu-reporter construct is indicated in each panel. The legend for the bars (shown in *B*) is the same in *A* and *B*.

Rep*cts62*), and after 48 h of incubation at 30°C (S derepression) (11, 13). The lysogens received the *ssrA*::Cm allele by bacteriophage P1 transduction and were transformed with plasmid pJW28, pJW30, pJW29, or pJW34, which express the WT, $ssrA^{DD}$, $ssrA^{0}$ and $ssrA^{UG}$, RNA, respectively. All strains were streaked on MacConkey lactose plates and incubated for up to 48 h at 30°C, and 24 h at 37°C and 42°C. Colonies of the λJV300 lysogens (c^+), although they did so more slowly than those with the λJV304 (*cts62*) prophage, turned red when carrying the WT, $ssrA^{DD}$, or $ssrA^{0}$ allele at 30°C and 37°C, but not when SsrA was absent or unable to load onto the ribosomes ($ssrA^{UG}$ allele). In another experiment, liquid cultures grown in LB at 30°C for various times were shifted to either 37°C or 42°C and sampled for β-galactosidase activity at various time intervals. None of the *ssrA* alleles tested affected the λJV300 (c^+) lysogens, which all produced barely detectable levels of the enzyme (data not shown). However, the λJV304 (*cts62*) lysogen in the *ssrA* null and $ssrA^{UG}$ mutant backgrounds was no longer activated in either stationary growth phase or at 37°C. At 42°C, pE-driven expression was reduced 4-fold. Here again the $ssrA^{0}$ and $ssrA^{DD}$ alleles complemented for pE derepression as efficiently as did the $ssrA^{+}$ allele, confirming that tagging for degradation was not the crucial function involved (Fig. 2*A*).

Consistent with the phage production data (Table 2), Muc^+ was also susceptible to S derepression. Except when they carried the $ssrA^{UG}$ allele, cells lysogenic for λJV300 turned red when grown as colonies on MacConkey lactose plates at 30°C (data not shown), although to a lesser degree than λJV304 lysogens. Most likely, this reflected the higher affinity of the WT repressor for the operators. Under physiological conditions that trigger S derepression, Mu repression was modulated by the ability of SsrA to charge alanine and hence to interact with the ribosome.

Truncated Forms of Mu Repressor Are Synthesized in the Absence of SsrA Charging with Alanine. Mu repressor seemed the most plausible target for the SsrA activity modulating Mu repression. We therefore analyzed Mu repressor by Western blotting in strains carrying the same *ssrA* alleles as above. As the protein could not be reliably detected in strains carrying a single copy of the Mu *c* gene, we used plasmid constructs of the pJV300 series (11). These pRS551 derivatives (based on the pBR322 replicon) carry the same Mu fragment as the λJV phages described above, providing autoregulated expression of various forms of Mu repressor from the pCM promoter. pJV304 (*cts62* repressor) or pJV300 (c^+ repressor) were introduced into strains K8619 (*ssrA*::Cm) and K8664 ($ssrA^+$). Fig. 3*A* shows that in the $SsrA^-$ strains, three new, truncated forms of repressor appeared. They had sizes similar to those of the three *sts* mutant proteins, which lack 7, 10, and 18 aa at their C terminus (Fig. 3*B*), respectively. Fig. 3*C* shows that, with the pJV304 plasmid, truncated forms could be seen in the K8650 ($ssrA^{UG}$) but not in the K8666 ($ssrA^{DD}$) or K8637 ($ssrA^{0}$) strains. The presence of truncated forms thus correlated with the absence of induction in the previous experiments. The shortest *sts* repressor (*sts62–1*) displayed a single form of the protein in an $SsrA^-$ strain (MC4100*ssrA*::Cm) (Fig. 3*D*), consistent with truncation occurring at the C terminus. Functional assays agree with this observation of the physical state of Repc. Indicator strains in which β-galactosidase expression was regulated by the truncated Mu repressor (*sts62–1*) were barely affected by any of the tested *ssrA* alleles (Fig. 2*B*). Moreover, Mu*cts4*, a thermoinducible mutant of Mu that carries an amino acid change in the central repressor domain and whose thermosensitivity is not suppressed by the *sts* mutations (J. E. Laachouch and A.T., unpublished results), was unaffected by the *ssrA*::Cm mutation (data not shown).

In summary, in the absence of charged SsrA, Repc proteins truncated at their C terminus were responsible for blocking spontaneous, temperature-induced, and S derepression of Mu. Consistent with this conclusion, none of the *ssrA* alleles further affected S or thermal derepression of a *cts,sts* indicator prophage (Fig. 2*B*).

Discussion

SsrA Connects Mu Repression to Host Physiology. The strong evolutionary conservation of SsrA (45) and the observation that the stability of a large number of proteins in *E. coli* is affected by SsrA (30) argue for an important role of this RNA for the physiology of the bacterium. Much of the regulation network that involves SsrA, and the relationship between SsrA activity and overall host physiology, remains to be elucidated. Our results emphasize a mechanism that is quite distinct from its effect on protein degradation, by which SsrA can control gene expression: SsrA adjusts the ratio of different forms, possessing different activities, of a repressor protein. In the absence of SsrA charging with alanine, truncated forms of Mu repressor accumulate in the lysogenic bacterium, preventing prophage induction. SsrA may thus allow phage Mu to sense its host physiology, in which case sensing would operate at the posttranslational level and through the phage lysogenic repressor.

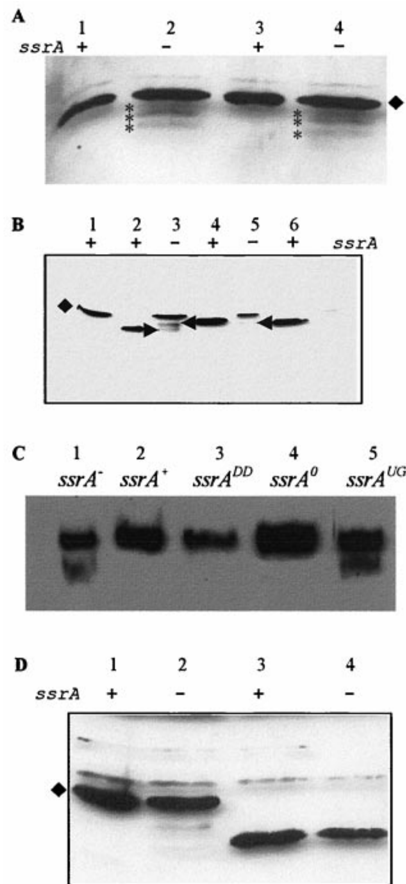

Fig. 3. Western blot analysis of Mu repressor in host with different *ssrA* mutations. (*A*) Three truncated forms of repressor appear in an *ssrA*⁻ strain. Lanes 1 and 3, MC4100; lanes 2 and 4, MC4100*ssrA*::Cm. The WT repressor (pJV300) is in lanes 1 and 2, the *cts62* (pJV304) is in lanes 3 and 4. (*B*) The truncated forms have size ranges similar to three previously characterized *sts* mutant proteins. Lane 1, MC4100/pJV304; lane 2, MC4100/pJV313 (*sts62-1*); lanes 3 and 5, MC4100*ssrA*::Cm/pJV304; lane 4, MC4100/pJV318 (*sts25-1*); lane 6, MC4100/pJV314 (*sts62-2*). (*C*) Complementation of the *ssrA*::Cm mutation by various *ssrA* alleles. Lane 1, K8619/pJV304 (*ssrA*::Cm); lane 2, K8664/pJV304 (*ssrA*$^{+}$); lane 3, K8666/pJV304 (*ssrA*^{DD}); lane 4, K8637/pJV304 (*ssrA*^{0}); lane 5, K8650(*ssrA*^{UG})/pJV304. (*D*) Truncation occurs at the repressor C-terminal end. Lane 1, MC4100/pJV304; lane 2, MC4100*ssrA*:: Cm/pJV304; lane 3, MC4100/pJV313 (Rep*cts62*, *sts62-1*); lane 4, MC4100*ssrA*:: Cm/pJV313. All these plasmids carry the *cts62* mutation. In *A*, *B*, and *D*, diamonds indicate full size repressor, asterisks and arrows indicate truncated forms.

Mutations in *ssrA* Confer the Same Phenotype as Truncated Forms of Mu-Repressor and Lead to Stable Repression of pE. The effect of SsrA on Mu repressor consists in preventing the accumulation of C-terminally shortened repressor proteins. Such truncated proteins, previously isolated as Sts repressor mutants, form exceptionally stable repression complexes (12). *E. coli* strains lysogenic for both Mu*cts62* and Mu*cts62*, *sts62-1* are not induced at 42°C, showing that the *sts* mutation is dominant (12). The appearance of equivalent truncated forms of Mu Repc in the absence of SsrA thus explains the partial block of derepression observed in the experiments presented here. By adjusting the ratio of full-length to truncated forms of the repressor, SsrA appears to influence the probability of Mu induction. Decreased availability of SsrA would lead to a diminished probability of lysis by Mu. The phage could use this mechanism to sense the overall state of the cell, more specifically the state of its translation machinery. SsrA is present at a higher concentration during stationary phase than in exponentially growing cells (31). The resulting increase in full-length repressor may be partly responsible for S derepression. Other phages also use SsrA for detecting host physiology: the λ*imm*P22 hybrid phages do not grow on SsrA⁻ strains (27, 28, 33). The *E. coli* LacI and LexA repressors, as well as the phage λ *c*I repressor (28, 30) are targets of SsrA, suggesting that SsrA could be part of a general sensing mechanism.

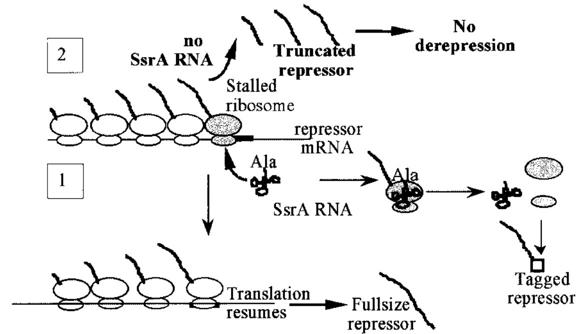

Fig. 4. Signal transduction for the control of Mu repression (adapted from ref. 33). Ribosomes translating Mu repressor mRNA arrest their progression at particular sequences (black rectangle). SsrA, if present (*1*), loads onto the first stalled ribosome, which resumes translation on the SsrA and produces a tagged peptide likely to be degraded by ClpXP. Trans-translation by the first ribosome clears the block for the following ribosomes, which produce full size repressor molecules. In the absence of SsrA (*2*), truncated repressor molecules are released from the ribosome. These truncated peptides possess a very high affinity for the operators and prevent derepression.

SsrA Is Only One Means by Which Mu Repressor Senses the Host Physiology. The host SsrA (this paper), the ClpXP host protease that degrades Mu repressor (9), the Lon protease, and the stationary phase-specific σ factor RpoS (13) are all influencing Mu repression in response to either temperature or stationary growth phase. These host factors are acting within complex host regulatory networks. ClpXP degrades SsrA-tagged peptides, Mu repressor, and RpoS in exponentially growing *E. coli* (see, for instance, ref. 46). The interplay between these different regulation networks finally elicits an appropriate response of Mu to very diverse physiological situations. SsrA appears to be an important component of this sensing mechanism. Nevertheless, many connections within this complex regulation scheme remain to be discovered.

How Are Truncated Forms of Mu Repressor Generated and Are They Tagged by SsrA? In addition to unraveling important aspects of the biology of Mu, our experiments raise new questions concerning the translation machinery of *E. coli* and the mode of action of SsrA. From the direct observation of Mu repressor forms in the WT, *ssrA*^{DD}, and *ssrA*^{0} strains we can exclude a role of tagging by SsrA for controlling Mu lysogeny. First, although we can readily separate repressor forms that differ by only three amino acids (the *sts* mutants), we never detected polypeptides with the size expected for tagged, truncated forms of Repc in *ssrA*^{DD}, *ssrA*^{0}, or ClpP-deficient hosts (data not shown). Second, we expected truncated forms of Repc with the three amino acids tag added by the *ssrA*^{0} RNA to retain the Sts phenotype, which was not the case.

We suppose that the ribosomes are prone to erroneous termination at three sites near the *sts* mutations in the *c* gene. Termination could involve translational bypassing (47, 48) to a stop codon, termination without a stop codon, blockage of the advancing ribosome by a cellular factor binding to the mRNA, or endoproteolytic cleavage of the nascent peptide on a stalled ribosome. Inspection of the termination sites revealed no runs of rare codons, nor any obvious secondary structure that could

explain an arrest of translation at these positions at the end of the Mu repressor coding sequence. All termination sites are at the sequence ACAGGA, but this motif is randomly distributed on the *E. coli* chromosome.

Abo *et al.* (30) recently reported that truncated LacI repressor forms appear in an SsrA$^-$ strain. They proposed a mechanism by which the binding of Lac repressor at the end of the *lacI* gene and DNA looping hinder RNA polymerase and, hence, ribosome progression. In Mu, the repressor binding sites in O1 are at the beginning of the *c* gene. Moreover, in SsrA$^-$ strains, truncated forms of the *cts62* repressor were present in similar amount at 42°C and 30°C (data not shown). Our results can therefore not be explained by an analogous model.

However, irrespective of the precise mechanism that produces the translation arrest, our observations argue for a mode of SsrA functioning similar to the one proposed by Withey and Friedman (33). In this model, once the first ribosome of a train of ribosomes succeeds in passing the termination point with the aid of SsrA, the arrest is abolished for subsequent ribosomes. SsrA would thus produce, at most, one tagged peptide (undetectable by using our methods) per repressor mRNA, whereas the following ribosomes would resume their progression to the normal stop codon (Fig. 4).

The selectable phenotype of temperature resistance on Mu*cts62* lysogens should provide an efficient screen for the identification of Mu and host components that affect translation of the repressor mRNA and modulate the influence of SsrA on Mu repressor. Some of them may turn out to be important factors for the general function of SsrA and for the progression of ribosomes along the RNA transcript.

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