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A small RNA acts as an antisilencer of the H-NS-silenced *rcsA* gene of *Escherichia coli*

(DsrA/*lon*/transcription initiation/capsule synthesis)

DARREN SLEDJESKI AND SUSAN GOTTESMAN*

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Building 37, Room 2E18, 37 Convent Drive, MSC 4255, Bethesda, MD 20892-4255

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ABSTRACT **The regulation of capsular polysaccharide synthesis in *Escherichia coli* K-12 depends on the level of an unstable positive regulator, RcsA. The amount of RcsA protein is limited both by its rapid degradation by Lon, an ATP-dependent protease, and by its low level of synthesis. We have found that the low level of expression from the *rcsA* promoter is due to transcriptional silencing by the histone-like protein H-NS; this silencing is sensitive to both sequence and context in a region upstream of the −35 region of the promoter. A small (85-nt) RNA, DsrA, when overproduced, activates transcription of *rcsA*::*lacZ* fusions by counteracting H-NS silencing. DsrA RNA does not show any extended homology with the *rcsA* promoter or other sequenced regions of *E. coli*. Since the stimulation of *rcsA* transcription by this small RNA does not depend on any sequences from within the *rcsA* transcript, DsrA acts, either directly or indirectly, on *rcsA* transcription initiation.**

Capsular polysaccharide synthesis in *Escherichia coli* K-12 depends on at least two positive regulators of transcription, RcsA and RcsB. In wild-type cells the level of expression of the *cps* genes, necessary for capsule synthesis, is low, and thus very little capsule is made. This is partly because the amount of RcsA that accumulates in the cell is also very low (1). Mutation of the Lon ATP-dependent protease extends the half-life of the very unstable RcsA protein, increasing its accumulation, which in turn increases capsule synthesis (for a review of capsule regulation, see ref. 2). It is likely that a regulatory mechanism exists for increasing RcsA synthesis and thereby increasing capsule synthesis.

In this paper we have investigated the regulation of transcription of *rcsA*. We show that *rcsA* is silenced by H-NS, a histone-like protein that silences the expression of a variety of bacterial genes and affects recombination and transposition (3–9), in a manner reminiscent of eukaryotic silencing (10, 11). We describe a small RNA, DsrA, a regulator of transcription that allows the otherwise silenced *rcsA* promoter to be expressed at high levels.†

MATERIALS AND METHODS

Bacterial Strains. All strains used in this paper were derivatives of *E. coli* K-12 strain SG20250 (12), a derivative of MC4100, unless otherwise indicated.

Transductions with P1vir were done as described by Miller (13). The *osmZ205* mutation was obtained from Higgins *et al.* (14); it has been renamed *hns-205* (15).

The *dsrA1*::*cat* insertion from pdsrACat1 was transferred onto the chromosome by means of the *rcsA*$^+$ *int*$^-$ bacteriophage SY6 as previously described (12).

mutD mutagenesis of pDDS106B1 (*dsrA*$^+$) was carried out as described by Silhavy *et al.* (16).

The *proU*::*lacZ* fusion (17) was moved from JM517 by P1 transduction into SG20250. The *papA*::*lacZ* fusion plasmid (18), derived from pBR322, was used to transform SG20250.

β-Galactosidase Assays. β-Galactosidase activity encoded by the various *lacZ* fusions was assayed as described by Miller (13). The pBluescript II vector had a 2- to 4-fold negative effect on the DDS1301 and DDS1303 *rcsA*::*lacZ* fusions. This negative effect was fully overcome by insertion of *dsrA* into the vector.

Construction of *rcsA*::*lacZ* Fusions. The *rcsA*::*lacZ* fusions were constructed by cloning PCR-generated fragments of the *rcsA* promoter from pDDS52 into the multicloning site of the *lacZ* transcriptional fusion vector pRS415 and were subsequently crossed into λRS45 (19). PCR amplification was performed by using the GeneAmp kit as recommended by the manufacturer (Perkin–Elmer/Cetus). Oligonucleotides were either synthesized on an Applied Biosystems 380B DNA synthesizer or purchased from BioServe Biotechnologies (Laurel, MD). The forward primers used for amplification of the *rcsA* promoter fragments were designed to have *Eco*RI restriction sites at the 5′ end. The *rcsA* +1 reverse primer was designed with a *Bam*HI site at the 5′ end. The G-91 to A (G-91A) mutation was created inadvertently by using a primer with this mutated sequence rather than the wild-type sequence. After amplification, DNA fragments were digested with *Eco*RI and *Hin*cII (for the +138 fusions) or *Eco*RI and *Bam*HI (for the +1 fusion), purified, and ligated into the multicloning site of pRS415. Orientation and fusion end points of the recombinant plasmids were checked by restriction enzyme digestion and DNA sequencing.

Subcloning of *dsrA* and *dsrB*. An insertion of a kanamycin-resistance transposon (from λNK1316; ref. 20) into the plasmid pVS107 [*dsrA*$^+$ *dsrB*$^+$; pBR322 carrying an *Eco*RV–*Bam*HI fragment from pATC400 (21)] was isolated that introduced a *Bam*HI restriction enzyme site 79 nucleotides downstream of the *rcsA* coding sequence. A 1.6-kb *Bam*HI fragment carrying *dsrA*$^+$ *dsrB*$^+$ was subcloned from this plasmid (pDDS106) into the multicloning site of pUC18 (pDDS106B1) or pBluescript II SK(+) (Stratagene; pDDS53). To allow more stable selection of the plasmid, a chloramphenicol (Cat) cassette flanked by *Bam*HI restriction sites was isolated from a *Sma* I digest of pCat19 (22) and inserted into a blunted *Bsm* I restriction site located between *dsrA* and *dsrB* of pDDS53 (Fig. 4), creating pDDS80. *dsrA* and *dsrB* were subcloned from pDDS80 into the *Bam*HI restriction site of pACYC184 (23), creating pDDS105 and pDDS95, respectively. The same cassette was inserted into the *Bsu*361 site of pDDS53 to create pdsrCat1. pDDS164 was created by ligating a 256-bp PCR fragment containing *dsrA* into the *Bam*HI site of pACYC184.

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*To whom reprint requests should be addressed.
†The *dsrA* and *rscA* sequences have been deposited in the GenBank data base (accession nos. U17136 and U17137, respectively).

Isolation of RNA. RNA was extracted from exponentially growing cells by using the hot phenol method II of Hinton (24).

Primer Extension. Primer extension analysis was performed by the extension of the 5′-^{32}P-end-labeled oligonucleotide CCATAATAATCGTTGACATGGCATACCCTC (rcsA9), GACCCTGAGGGGGTCGGGATG (rcsA24), or ACGA-CGCGGACCGCCATCC (rcsA25) following the method of Sambrook *et al.* (25) and using Moloney murine leukemia virus reverse transcriptase (United States Biochemical). Oligonucleotides were labeled by using [γ-^{32}P]ATP (10 mCi/ml in aqueous solution; Amersham; 1 Ci = 37 GBq) and T4 polynucleotide kinase (New England Biolabs) as described (25).

Ribonuclease Protection. The Ambion (Austin, TX) RPA II kit was used for ribonuclease protection, following the supplier's protocol. Antisense RNA probes were made by using the Ambion MAXIscript T7 and T3 *in vitro* transcription kit. The DNA templates for the *in vitro* transcription reactions were pDDS53 linearized with *Xba* I (sense riboprobe) or *Eco*RI (antisense riboprobe).

RNA Capping. RNA was 5′-capped and analyzed as described previously (26, 27) by using [α-^{32}P]GTP (3000 Ci/mmol; Amersham) and vaccinia virus guanylyltransferase (Bethesda Research Laboratories).

DNA Sequencing and Analysis. DNA sequencing was done by the dideoxynucleotide chain-termination method (28), using the Sequenase 2.0 sequencing kit (United States Biochemical). The University of Wisconsin Genetics Computer Group package was used for sequence analysis (29). Data base searching was performed with BLAST (30) through the BLAST network service at the National Center for Biotechnology Information.

Gel Electrophoresis and Western Blotting of H-NS. Total cellular extracts of *E. coli* were electrophoretically separated on SDS/15% polyacrylamide gels (31) and transferred (32) to a sheet of nitrocellulose. To verify equal protein loading in each lane the nitrocellulose was stained with Ponceau S (Sigma), following the manufacturer's protocol. The nitrocellulose was probed with rabbit anti-H-NS polyclonal antisera (a gift from E. Bremer, Marburg, Germany). The antibody–antigen complex was visualized with horseradish peroxidase-conjugated goat antibody to rabbit immunoglobulin (Calbiochem) and the Renaissance chemiluminescence reagent kit (DuPont/NEN), following the supplier's protocol.

RESULTS

***rcsA* Transcription Is Silenced by H-NS.** The *rcsA* transcription start site was determined by using primer extension (Fig. 1) and ribonuclease protection (data not shown) assays from both chromosomally encoded and plasmid-encoded *rcsA*; the single-copy RNA could be detected only in long exposures (not shown). The start point of transcription was 132 nucleotides upstream of the translation start codon for RcsA (Figs. 1 and 2). The 5′ end of the *rcsA* transcript was labeled with [γ-^{32}P]GTP by using guanylyltransferase, yielding a single major product, confirming that the 5′ end of the *rcsA* mRNA was not a ribonuclease processing site (data not shown).

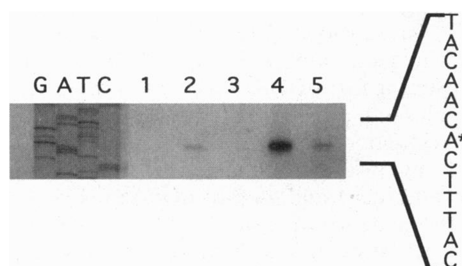

FIG. 1. Primer extension analysis of *rcsA* mRNA. Samples (5 μg) of RNA from SG20250 (lane 1), SG20250 carrying pDDS80 (multicopy *dsr*) (lane 2), SG20250 (lane 3), SG20250 carrying pATC352 [multicopy *rcsA* (21)] (lane 4), or SG20250 *hns-205* (lane 5) were used with primer rcsA9 for primer extension. An asterisk indicates the major extension product. The sequence is for the coding strand, which is complementary to the DNA sequence shown. In longer exposures a band of the size indicated by the asterisk is seen in lanes 1 and 3.

A series of *rcsA*::*lac* transcriptional fusions carrying various amounts of DNA upstream of the transcription start point (called +1 throughout this paper) and extending either just to the +1 position or just beyond the translation start point (+138) were assayed for expression of β-galactosidase (Table 1). Those fusions containing more than 400 bases upstream of +1 (DDS1301, DDS1308, and DDS1303) and a mutant derivative of a fusion containing 97 bases upstream of +1 (DDS1304) expressed β-galactosidase at relatively low levels. Fusions containing either 59 or 97 bases upstream of +1 (DDS1318, DDS1309, and DDS1310), on the other hand, expressed significantly higher levels of β-galactosidase. The transcription start points for the plasmid versions of DDS1304 (low level of transcription) and DDS1309 (high level of transcription) were confirmed by primer extension to be identical to that for the intact *rcsA* operon (data not shown).

hns mutant hosts are mucoid (3, 34). One possible mechanism for increasing capsule synthesis would be increased synthesis of RcsA. Isogenic *hns*$^+$ and *hns-205* derivatives of the *rcsA*::*lacZ* fusion strains were assayed for β-galactosidase expression (Table 1). In all cases in which the basal activity of the *rcsA*::*lacZ* fusion was low, the activity of the fusion in an *hns*$^-$ host was more than 10-fold higher than that in the *hns*$^+$ host (Table 1, lines 1–4). Experiments with another *hns* allele, *hns-651* (3) gave similar results (data not shown). Therefore, H-NS appeared to be necessary for maintaining the low basal level of *rcsA* expression. H-NS silencing seems to be dependent on sequences upstream of −59 of the *rcsA* promoter and apparently can be provided either by specific sequences (such as that in the DDS1304 mutant promoter) or by a different context (the −433 or −738 fusion with the wild-type promoter sequence). For fusions which expressed high levels of β-galactosidase in an *hns*$^+$ host, the *hns* mutant had no effect (DDS1318 or DDS1310) or had a modest effect (DDS1309), further supporting the idea that the low basal level is due to H-NS silencing.

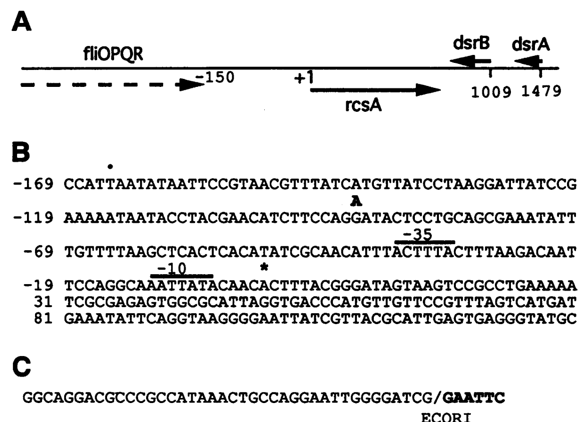

FIG. 2. (*A*) Structure of the *rcsA* region. *dsrA* and *dsrB* are transcribed in a direction opposite to *rcsA*. (*B*) Sequence of the *rcsA* promoter. An asterisk indicates the transcription start site (+1 position) of *rcsA* as determined in Fig. 1. The bold **A** indicates the position of the G-to-A mutation in the DDS1304 *rcsA*::*lacZ* fusion. The putative −10 and −35 regions of the *rcsA* promoter are overlined. The filled circle indicates the stop codon of *fliR*, the gene immediately upstream of *rcsA*. The sequence for *fliR* and part of this region was also determined by Matsumura and coworkers (33). (*C*) Vector sequence upstream of the *rcsA*::*lacZ* fusions. This sequence is from pRS415 (19).

Table 1. Expression of *rcsA–lacZ* fusions

Strain	*rcsA* region present	β-Galactosidase, units		
		WT	*hns-205*	p*dsr*$^+$
DDS1301	−738 to +138	170	2200	2200
DDS1308	−738 to +1	475	5600	2600
DDS1303	−433 to +138	240	3300	2600
DDS1304	−97 to +138, G-91A	220	2600	1600
DDS1318	−97 to +138	2800	2800	2700
DDS1309	−59 to +138	2700	8500	4500
DDS1310	−59 to +1	1900	1700	2000

All fusions are present as single-copy lysogens at attλ. Lysogens were isolated in SG20250 to give the parental strain listed. WT, wild type. Transformation with pDDS80 (p*dsr*$^+$) and transduction with *hns-205* were into these lysogens. Cells were grown with shaking at 32°C in LB medium supplemented with chloramphenicol for the p*dsr*$^+$-containing cells. Total β-galactosidase units were plotted against the optical density of the culture at 600 nm. The slope of the curve between OD values of 0.3 and 1.0 was used as the specific activity of the fusions. Slopes had an $r^2 > 0.95$. Specific activities varied less than 10% between experiments.

Primer extension analysis showed that the *hns* mutation influences the amount but not the start point of *rcsA* transcription (Fig. 1, lane 5).

***rcsA* Transcription Is Positively Regulated by *dsr*, a Region Downstream from *rcsA*.** Multicopy plasmids carrying a region closely linked to *rcsA* increase capsule synthesis if there is a functional *rcsA* gene in the chromosome (12, 21). Preliminary experiments localized the necessary element to a region beyond the end of the *rcsA* gene (the downstream region, *dsr*).

These results suggested that *dsr* might increase RcsA synthesis, leading to increased *cps* transcription. To test this model, the effect of the *dsr* region on *rcsA*::*lacZ* transcriptional fusions was determined (Table 1). In every case in which the basal level of transcription was low, the presence of the *dsr*$^+$ plasmid, pDDS80, increased the basal level expression of β-galactosidase 6- to 13-fold (Table 1, lines 1–4). Both the low basal level and the increase in *rcsA*::*lacZ* expression in the presence of multicopy *dsr* plasmid occurred in the absence of any sequences internal to the *rcsA* message (Table 1, DDS1308). An increase in the amount of *rcsA* mRNA was also detected by primer extension analysis in cells carrying the *dsr* plasmid (Fig. 1, lane 2).

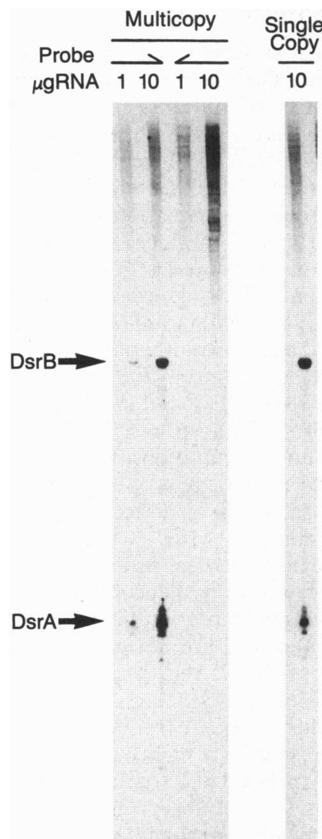

FIG. 3. Ribonuclease protection mapping of *dsr*. Either 1 or 10 μg of RNA from SG20250 carrying pDDS53 (multicopy) or 10 μg of RNA from SG20250 without a plasmid (single copy) was hybridized to labeled probes and treated with ribonuclease. The multicopy and single-copy samples shown were electrophoresed on the same gel and are identical exposures. Rightward arrow indicates a probe (sense probe) transcribed in the same direction as *rcsA* transcription. The leftward arrow indicates antisense probe, transcribed in the opposite direction to *rcsA* transcription. The single-copy sample was probed with the sense probe. The sizes of the *dsrA* and *dsrB* transcripts were estimated by comparison to a DNA sequencing ladder and are approximately 85 and 170 nt in size, respectively.

The 1.6-kb downstream region carried by the multicopy *dsr*$^+$ plasmid pDDS80 encoded two major transcripts, as determined by ribonuclease protection (Fig. 3) and primer extension experiments (data not shown). The smaller transcript, DsrA, was approximately 85 nt long and was transcribed toward the *rcsA* gene (Figs. 2 and 4). *dsrA* does not contain any extended open reading frames. A probable transcription termination stem-loop was found at the expected position of the 3′ end (see Fig. 4). The second transcript (DsrB) was approximately 170 nt long and was also transcribed toward *rcsA* (Fig. 2).

The 5′ ends of both the *dsrA* and *dsrB* transcripts could be labeled with [γ-^{32}P]GTP by using guanylyltransferase, demonstrating independent transcription start sites for the two RNAs (data not shown). The *dsrA* and *dsrB* transcripts were also detected by both primer extension and ribonuclease protection assays in wild-type cells (SG20250) containing no plasmids and were the same size and had the same transcription start sites as from cells containing *dsrA* and *dsrB* in multiple copies (Fig. 3).

***dsrA* Transcription Is Necessary for Antisilencing.** Subclones carrying fragments of pDDS80 in pACYC184 were isolated to map the region responsible for activation of *rcsA* transcription (Table 2). pDDS105, a subclone containing only *dsrA*, was sufficient to increase *rcsA* transcription; a subclone containing only *dsrB* (pDDS95) had unaltered *rcsA*::*lacZ* expression.

Insertion of a chloramphenicol-resistance cassette at the 3′ end of the plasmid-borne *dsrA* abolished the induction of *rcsA* by *dsr* (Table 2, pdsrCat1), as well as the accumulation of the 85-nt DsrA transcript (data not shown). When this *dsrA1*::*cat* insertion was recombined into the chromosomal copy of *dsrA*, no DsrA transcript was detected (data not shown). This chromosomal insertion mutation did not have any noticeable effect on cell growth, capsule production, or basal *rcsA* transcription.

Two point mutations in the *dsrA* plasmid which decreased or abolished the stimulation of *rcsA*::*lacZ* expression were isolated. One mutation, *dsrA7*, changes the conserved T in the −10 region of *dsrA* to C (Fig. 4). The mutation decreased the *in vivo* (data not shown) and *in vitro* (D. Jin, personal communication) transcription of *dsrA* below detectable levels, consistent with a promoter down mutation. *dsrA12* was an A to C transversion in the stem-loop of the terminator at the end of *dsrA* (Fig. 4). The terminator mutation led to the loss of the 85-nt DsrA transcript and the appearance of an equal or greater amount of a larger transcript, >600 nt in length. Ribonuclease protection experiments indicated that this larger

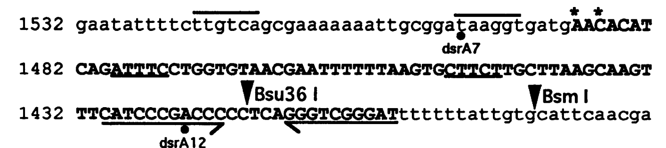

FIG. 4. Sequence of *dsrA*. The transcribed sequence for *dsrA* is capitalized and shown in boldface print. The overlined regions are the putative −10 and −35 regions of *dsrA*. The asterisks indicate the transcriptional start sites of *dsrA*. The arrows represent a potential rho-independent terminator of *dsrA*. The loops of two potential stem-loop structures of the DsrA RNA are underlined. Filled circles indicate the position of *dsrA* point mutations. Arrowheads represent restriction enzyme sites used for construction of some plasmids and RNA probes. All numbering is in relation to the *rcsA* transcription start site (+1) (see Fig. 2*A*).

Table 2. Analysis of *dsr* region

Plasmid	Description	β-Galactosidase, units
pACYC184	Vector	170
pDDS105	*dsrA*$^+$ alone	2000
PDDS95	*dsrB*$^+$ alone	190
pUC18	Vector	150
pDDS106B1	*dsrA*$^+$ *dsrB*$^+$	1700
pdsrCat1	*dsrA*::*cat dsrB*$^+$	110
pdsrA7	*dsrA7* (T-10C) *dsrB*$^+$	140
pdsrA12	*dsrA12* (terminator) *dsrB*$^+$	380

Plasmids were introduced into DDS1304 (−97, +138, G-91A). Cells were grown in LB medium and assayed as described in the legend for Table 1.

transcript has approximately the same 5′ end as the 85-nt DsrA transcript, with a 3′ end that extends into vector sequence (data not shown), consistent with loss of termination at the normal site. A plasmid carrying *dsrA7* did not increase *rcsA*::*lacZ* expression above the level seen with the parental vector (Table 2). Plasmids carrying *dsrA12* induced *rcsA*::*lacZ* expression only 2.5-fold (Table 2). Therefore, reducing *dsrA* transcription and elongating the transcript both interfere severely with antisilencing activity. This is most consistent with a role for the RNA itself in antisilencing.

Further support for a role for the RNA is provided by a comparison of the DsrA sequence and predicted secondary structure with the predicted product of the analogous gene from *Klebsiella pneumoniae* (Fig. 5). The *K. pneumoniae rcsB* gene is located in the same position and orientation relative to the *K. pneumoniae rcsA* gene as *dsrA* is relative to *E. coli rcsA* (37) and functions to stimulate *E. coli rcsA*::*lacZ* expression to an extent similar to that seen with *dsrA* plasmids (data not shown). The nucleotide sequence of *dsrA* (from the putative −35 through the terminator) shares 84% identity with the *K. pneumoniae rcsB* gene. No open reading frames are conserved between *dsrA* and *K. pneumoniae rcsB*, while a potential RNA secondary structure is conserved (Fig. 5). The sequence of the *dsrB* region, including an encoded open reading frame of 62 amino acids, is also conserved between *K. pneumoniae* and *E. coli* (47).

DsrA Interferes with H-NS Activity. In an *hns*$^-$ host, multicopy *dsr*$^+$ decreased rather than further increasing the specific activity of the DDS1304 *rcsA*::*lacZ* fusion (−97, G-91A, +138) from 2600 units to 1300 units and the DDS1309 *rcsA*::*lacZ* fusion (−59 to +138) from 8500 to 5000 units. This decrease may reflect poorer growth of these strains. In an *hns*$^-$ *dsrA*$^-$ double mutant, *rcsA*::*lacZ* expression was as high as that seen with the *hns*$^-$ single mutant. These results suggest that DsrA affects H-NS synthesis or activity rather than vice versa.

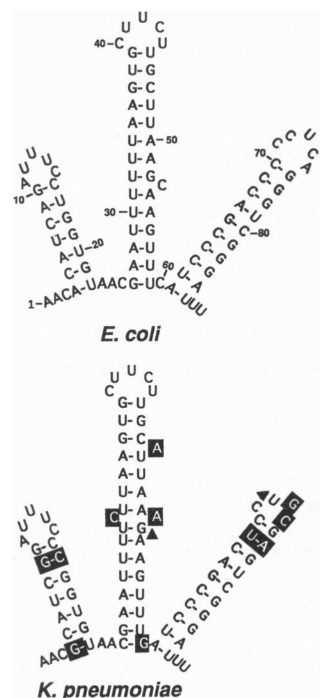

FIG. 5. Conservation of an RNA secondary structure between *E. coli dsrA* and *K. pneumoniae rcsB*. RNA secondary structure was analyzed by using the programs MULFOLD (35) and LOOPDLOOP (36). White letters on a dark background indicate the positions at which the *K. pneumoniae* sequence differs from the *E. coli dsrA* sequence. A filled triangle indicates a single nucleotide deletion in the *K. pneumoniae* sequence relative to the *E. coli* sequence. The 3′ ends are approximate. The *E. coli* DsrA RNA structure has a predicted energy of formation of −31.7 kcal/mol at 32°C (1 kcal = 4.18 kJ).

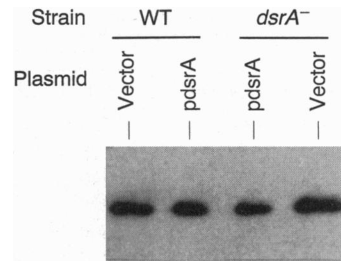

FIG. 6. SDS/PAGE Western blot analysis of H-NS accumulation. One microliter of a cell lysate from wild-type (WT; SG20250) or *dsrA*$^-$ [DDS3201, SG20250 Δ(*rcsA dsrA*)27 (12)] cells containing vector (pACYC184) or pdsrA (pDDS164) was analyzed.

The effect of overproduced DsrA on H-NS-silenced promoters is not limited to the *rcsA* promoter. The effects of either the *dsrA* plasmid or an *hns* mutant were compared for *lacZ* fusions to two promoters that are negatively regulated by H-NS, *proU* and *papA* (4, 17, 18). In both cases, the effects of the *dsrA* plasmid and of *hns* mutations were similar. The activity of the *proU*::*lacZ* fusion increased from 110 units to 4600 in the presence of the *dsrA* plasmid pDDS105 and to 4700 units in an *hns*$^-$ strain. The activity of the *papA*::*lacZ* fusion increased from 26 units to 400 units in the presence of pDDS105 and to 400 units in an *hns*$^-$ strain.

Overexpression of DsrA RNA had little or no effect on H-NS levels in *dsrA*$^+$ or *dsrA*$^-$ hosts (Fig. 6), suggesting that DsrA does not act by decreasing H-NS accumulation.

DISCUSSION

We have described DsrA, a small RNA that acts as an antisilencer for *rcsA* transcription. DsrA appears to define a new role for a small RNA as an activator of transcription. *dsrA* encodes an 85-nt RNA, contains no conserved open reading frames, has a conserved and stable RNA secondary structure, and loses activity when mutated to abolish either transcription initiation or the normal transcription termination site. The start point of transcription for the *rcsA* message is identical with and without DsrA stimulation; therefore DsrA stimulates the normal promoter rather than allowing use of a new promoter. A similar increase in transcription initiation occurs, in the complete absence of DsrA, under two other conditions: deletion of the region upstream of −59 from the *rcsA* promoter or introduction into the host of a mutation in the abundant histone-like protein H-NS. In hosts carrying either the truncated *rcsA* promoter or an *hns* mutation, DsrA overproduction had no further effect on *rcsA* transcription. These results suggest that, rather than acting as a direct activator, DsrA overcomes the silencing action of H-NS.

Requirements for H-NS Silencing. The *rcsA* promoter, like others in which H-NS is implicated as a silencer (38, 39), appears to be capable of high levels of transcription initiation, although the sequences at −10 and −35 are not particularly close to consensus. What is surprising is that a single base mutation in the −97 to +138 fusion (DDS1318 vs. DDS1304 in Table 1) is sufficient to turn this promoter from one which is insensitive to H-NS and expressed at a high level to one which is sensitive to H-NS and expressed at a low basal level. Since the same low H-NS-sensitive level is seen for fusions extending further upstream than −97, it would seem that,

rather than a specific recognition sequence for H-NS, these changes have disrupted or restored some aspect of DNA structure which allows H-NS to bind and/or act. H-NS is reported to bind preferentially to curved DNA (40–43). In Fig. 2 *B* and *C*, the sequences of the upstream region relevant to H-NS silencing are shown, as well as the vector sequences which are present upstream in the fusions. The wild-type upstream sequence above −97 is notably A+T rich compared with that present in the fusion, and a second A+T-rich stretch is present from −64 to −78. The G to A change at −91 in the fusion may help restore the structure (curved DNA?) normally provided by these two A+T-rich stretches.

Antisilencing by DsrA. For several promoters, a positive activator protein or other DNA-binding protein can act to overcome the silencing by H-NS and allow high levels of transcription initiation (44–46). The activator that overcomes silencing of the *rcsA* promoter appears to be, rather than a protein, the small DsrA RNA. DsrA action on *rcsA* cannot be on the *rcsA* message, since no sequences from within the *rcsA* transcript are necessary for the DsrA effect. DsrA shows no extended homology to the *rcsA* transcript, the *rcsA* promoter, or any other sequenced region of *E. coli*, including *hns* itself. While we cannot rule out distant message targets which have not yet been sequenced or have only limited homology with DsrA, the close linkage of *dsrA* to *rcsA*, in both *E. coli* and *K. pneumoniae*, supports a role for DsrA as a specific part of the capsule regulatory network.

How might DsrA act? Our results rule out effects on H-NS synthesis as the primary mode of DsrA action and support a general interference with the ability of H-NS to silence. DsrA might prevent H-NS binding to promoters or interact with the promoter (as the other antisilencers seem to do) to interfere with the "silencing activity" of H-NS. Such promoter-specific interference might take the form of preventing H-NS from forming a chromatin-like silenced structure.

Many other RNAs with roles in transcriptional regulation like DsrA may exist; the difficulty is detecting them by the usual genetic means. Short RNAs represent small targets for mutagenesis and are not easily recognized in DNA sequences, since the absence of an open reading frame tends to obscure their importance. We would not be surprised to see other examples of such small regulatory RNAs acting at unexpected points in regulatory cascades demonstrated in the near future.

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1. Stout, V., Torres-Cabassa, A., Maurizi, M. R., Gutnick, D. & Gottesman, S. (1991) *J. Bacteriol.* **173**, 1738–1747.
2. Gottesman, S. & Stout, V. (1991) *Mol. Microbiol.* **5**, 1599–1606.
3. Falconi, M., McGovern, V., Gualerzi, C., Hillyard, D. & Higgins, N. P. (1991) *New Biol.* **3**, 615–625.
4. Göransson, M., Sonden, B., Nilsson, P., Dagberg, B., Forsman, K., Emanuelsson, K. & Uhlin, B. E. (1990) *Nature (London)* **344**, 682–685.
5. Kawula, T. H. & Orndorff, P. E. (1991) *J. Bacteriol.* **173**, 4116–4123.
6. Shi, X., Waasdorp, B. C. & Bennett, G. N. (1993) *J. Bacteriol.* **175**, 1182–1186.
7. May, G., Dersch, P., Haardt, M., Middendorf, A. & Bremer, E. (1990) *Mol. Gen. Genet.* **224**, 81–90.
8. Levinthal, M., Lejeune, P. & Danchin, A. (1994) *Mol. Gen. Genet.* **242**, 736–743.
9. Bertin, P., Terao, E., Lee, E. H., Lejeune, P., Colson, C., Danchin, A. & Collatz, E. (1994) *J. Bacteriol.* **176**, 5537–5540.
10. Winston, F. & Carlson, M. (1992) *Trends Genet.* **8**, 387–391.
11. Laurenson, P. & Rine, J. (1992) *Microbiol. Rev.* **56**, 543–560.
12. Brill, J. A., Quinlan-Walshe, C. & Gottesman, S. (1988) *J. Bacteriol.* **170**, 2599–2611.
13. Miller, J. H. (1972) *Experiments in Bacterial Genetics* (Cold Spring Harbor Lab. Press, Plainview, NY).
14. Higgins, C. F., Dorman, C. J., Stirling, D. A., Waddell, L., Booth, L., May, I. R. & Bremer, E. (1988) *Cell* **52**, 569–584.
15. Dersch, P., Schmidt, K. & Bremer, E. (1993) *Mol. Microbiol.* **8**, 875–889.
16. Silhavy, T. J., Berman, M. L. & Enquist, L. W. (1984) *Experiments with Gene Fusions* (Cold Spring Harbor Lab. Press, Plainview, NY).
17. Mellies, J., Brems, R. & Villarejo, M. (1994) *J. Bacteriol.* **176**, 3638–3645.
18. Göransson, M., Forsman, K. & Uhlin, B. E. (1989) *Genes Dev.* **3**, 123–130.
19. Simons, R. W., Houman, F. & Kleckner, N. (1987) *Gene* **53**, 85–96.
20. Kleckner, N., Bender, J. & Gottesman, S. (1991) *Methods Enzymol.* **204**, 139–180.
21. Torres-Cabassa, A. S. & Gottesman, S. (1987) *J. Bacteriol.* **169**, 981–989.
22. Fuqua, W. C. (1992) *BioTechniques* **12**, 223–225.
23. Chang, A. C. Y. & Cohen, S. N. (1978) *J. Bacteriol.* **134**, 1141–1156.
24. Hinton, D. M. (1989) *J. Biol. Chem.* **264**, 14432–14439.
25. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
26. Moss, B. (1981) in *Gene Amplification and Analysis*, eds. Chirikjian, J. G. & Papas, T. S. (Elsevier/North-Holland, Amsterdam), 2nd Ed., pp. 253–266.
27. Adams, C. W., Forrest, M. E., Cohen, S. N. & Beatty, J. T. (1989) *J. Bacteriol.* **171**, 473–482.
28. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
29. Devereux, J., Haeberli, P. & Smithies, O. (1984) *Nucleic Acids Res.* **12**, 387–395.
30. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. (1990) *J. Mol. Biol.* **215**, 403–410.
31. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
32. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
33. Malakooti, J., Ely, B. & Matsumura, P. (1994) *J. Bacteriol.* **176**, 189–197.
34. Yasuzawa, K., Hayashi, N., Goshima, N., Kohno, K., Imamoto, F. & Kano, Y. (1992) *Gene* **122**, 9–15.
35. Gilbert, D. G. (1990) MULFOLD, A Mac Application, Port of Michael Zuker's MFOLD for Estimating RNA Secondary Structure (Biocomputing Office, Indiana Univ., Bloomington).
36. Gilbert, D. G. (1992) LOOPDLOOP, A Program for Visualizing RNA Secondary Structure (Biocomputing Office, Indiana Univ., Bloomington).
37. Allen, P., Hart, C. A. & Saunders, J. R. (1987) *J. Gen. Microbiol.* **133**, 331–340.
38. Reynolds, A. E., Felton, J. & Wright, A. (1981) *Nature (London)* **293**, 625–629.
39. Overdier, D. G. & Csonka, L. N. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 3140–3144.
40. Owen-Hughes, T. A., Pavitt, G. D., Santos, D. S., Sidebotham, J. M., Hulton, C. S. J., Hinton, J. C. D. & Higgins, C. F. (1992) *Cell* **71**, 255–265.
41. Tupper, A. E., Owen-Hughes, T. A., Ussery, D. W., Santos, D. S., Ferguson, D. J. P., Sidebotham, J. M., Hinton, J. C. D. & Higgins, C. F. (1994) *EMBO J.* **13**, 258–268.
42. Yamada, H., Muramatsu, S. & Mizuno, T. (1990) *J. Biochem. (Tokyo)* **108**, 420–425.
43. Zuber, F., Kotlarz, D., Rimsky, S. & Buc, H. (1994) *Mol. Microbiol.* **12**, 231–240.
44. Forsman, K., Sondén, B., Göransson, M. & Uhlin, B. E. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9880–9884.
45. Jordi, B. J. A. M., Dagberg, B., de Haan, L. A. M., Hamers, A. M., van der Zeijst, B. A. M., Gaastra, W. & Uhlin, B. E. (1992) *EMBO J.* **11**, 2627–2632.
46. Olsén, A., Arnqvist, A., Hammar, M., Sukupolvi, S. & Normark, S. (1993) *Mol. Microbiol.* **7**, 523–536.
47. Mengistu, Y. (1993) Thesis (Univ. of Liverpool, United Kingdom).