RecA-Independent Pathways of Lambdoid Prophage Induction in *Escherichia coli*

DMITRY V. ROZANOV,[1] RICHARD D'ARI,[2] AND SERGEY P. SINEOKY[1]*

State Scientific Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow 113545, Russia,[1] and Institut Jacques Monod (Centre National de la Recherche Scientifique, Université Paris 7), 75251 Paris Cedex 05, France[2]

Two *Escherichia coli* genes, expressed from multicopy plasmids, are shown to cause partial induction of prophage λ in *recA* mutant lysogens. One is *rcsA*, which specifies a positive transcriptional regulator of the *cps* genes, which are involved in capsular polysaccharide synthesis. The other is *dsrA*, which specifies an 85-nucleotide RNA that relieves repression of the *rcsA* gene by histone-like protein H-NS. Genetic contexts known to increase Cps expression also cause RecA-independent λ induction: the *rcsC137* mutation, which causes constitutive Cps expression, and the *lon* and *rcsA3* mutations, which stabilize RcsA. Lambdoid phages 21, φ80, and 434 are also induced by RcsA and DsrA overexpression in *recA* lysogens. Excess λ cI repressor specifically blocks λ induction, suggesting that induction involves repressor inactivation rather than repressor bypass. RcsA-mediated induction requires RcsB, the known effector of the *cps* operon, whereas DsrA-mediated induction is RcsB independent in stationary phase, pointing to the existence of yet another RecA-independent pathway of prophage induction.

Temperate bacteriophages possess a dual mode of existence, able either to lyse sensitive cells or to form stable lysogens in which the phage genome is propagated as prophage by the bacterium, with phage lytic functions repressed by one or several phage repressors (28). For this reason, temperate phages have been attractive models for studying the mechanisms governing biological decisions: the choice between lysis and lysogeny after infection of a sensitive host and the choice of the established lysogen at each generation to continue propagating the prophage or to enter the lytic cycle.

The demonstration that, for certain lysogens, the vast majority of the population could be induced to enter the lytic cycle by UV irradiation was a major discovery, dispelling lingering doubts about the reality of the phenomenon of lysogeny (19, 20). Since that time, the combined efforts of many researchers over several decades have established the mechanism of lysogenic induction by UV irradiation and other DNA-damaging treatments. Under these conditions, the SOS response is induced; the RecA protein, activated by single-stranded DNA formed near DNA lesions, promotes autoproteolytic cleavage of certain prophage repressors as well as of the bacterial SOS repressor LexA (18, 28, 38, 39). The SOS response and SOS-inducible temperate phages have been described in a number of bacteria, including such distantly related species as *Escherichia coli* and *Bacillus subtilis*. In those cases tested, the SOS response is also the major pathway of spontaneous induction, which, under laboratory conditions at least, is severely reduced in *recA* mutant lysogens (28).

It has often been suggested that lysogenic induction via the SOS response is a selective mechanism permitting the phage to use its host's detection system for DNA damage in order to leave a severely damaged cell which is likely to die, like a rat leaving a sinking ship. If so, there may well be other conditions in which the phage, using other host detection mechanisms to sense imminent cell death, would similarly choose to enter the lytic pathway. Furthermore, many temperate phages are not SOS inducible yet still exhibit spontaneous induction, even in *recA* hosts. Well-known examples in *E. coli* are Mu and P2; host functions involved in their induction have not been identified.

For *E. coli*, a large number of stress responses have been described, including those permitting the cell to sense changes in temperature, osmolarity, pH, or nutrient availability and adjust its pattern of gene expression appropriately. In theory at least, it is possible that for each stress response there is a set of phages tuned in to the cell's detectors in such a way that, if cell survival seems threatened, the phage is induced.

In the present work we looked for *E. coli* genes, the amplification of which leads to RecA-independent induction of prophage λ. The selection used produced clones of the host genes *rcsA* and *dsrA*, which when overexpressed cause induction of λ and other lambdoid prophages in *recA* lysogens. Both genes code for positive regulators of capsular polysaccharide synthesis. Other genetic alterations leading to capsular polysaccharide overproduction also cause RecA-independent λ induction. From complementation studies, we conclude that DsrA participates in prophage induction via two different mechanisms. Results obtained for the induction of λcIind mutants suggest several possible mechanisms.

MATERIALS AND METHODS

Bacterial strains and bacteriophages. The bacterial strains used in this study, all derivatives of *E. coli* K-12, are listed in Table 1, together with the bacteriophages. Standard techniques were used for growth of bacteria and bacteriophages, phage crosses, generalized transduction with P1*vir*, and lysogenization (23, 30). All *recA* strains were checked for UV sensitivity and lack of growth of λ*bio*10 (or λ*imm*434 *bio*10) phage, as described elsewhere (42). The *rcsC137* and *lon* strains were mucoid on Luria-Bertani (LB) plates at 37°C and 30°C, respectively. The *rcsA3* mutant formed mucoid colonies at 30°C on M9 plates but not on LB plates.

* Corresponding author. Mailing address: State Scientific Research Institute of Genetics and Selection of Industrial Microorganisms, I Dorozhny Proezd 1, Moscow 113545, Russia. Phone: (7 095) 315 12 10. Fax: (7 095) 315 05 01. E-mail: vkpm@vnigen.msk.su.

TABLE 1. Bacterial strains and bacteriophages

Strain or phage	Relevant genotype or phenotype	Source or reference
Strains		
N99	galK2 galT22 rpsL IN(rrnD-rrnE)1	VKPM[a]
C600	supE44 hsdR thi-1 thr-1 leuB6 lacY1 tonA21	VKPM
LE392	supE44 supF58 hsdR514 galK2 galT22 metB1 trpR55 lacY	VKPM
XL1-blue	supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac F′[traD36 proAB⁺ lacI^q lacZΔM15 Tn10]	VKPM
MW1184	ara Δ(lac-proAB) rpsL thi (φ80 lacZΔM15) Δ(srlR-recA) 306::Tn10	VKPM
K802	supE44 hsdR2 galK2 galT22 metB1 lacY1 mcrA mcrB	VKPM
C600 Hfr	C600 + F′101(thr⁺ ara⁺ leu⁺)	VKPM
B3958	K802 recA9::Tn5	VKPM
B7218	LE392 Ap^r	VKPM
B7237	C600 hisG::Tn10	VKPM
B7349	B6905mal(λ) λ-resistant	VKPM
MT1	ilv his rpsL λN7N53 cI⁺ cro⁺::gal⁺	35
MT2	ilv his recA1 rpsL λN7N53 cI⁺ cro⁺::gal⁺	35
SG20322[b]	cps-11::lac-Mud1 lon-146::ΔTn10[c]	3
SG20645[d]	rcsA3 (RcsA*) zed-650::Tn10	3
SG20797[d]	Δlon-510 rcsB11::ΔTn10[c]	3
SG20803[d]	rcsC137 ompC::Tn5	3
SG20904[d]	Δlon-510 rcsA72::ΔTn10[c]	3
B6905	C600 Δ(srlR-recA)306::Tn10	C600 + P1(MW1184)
B7243	C600 rcsA3 (RcsA*) Δ(srlR-recA)306::Tn10	B7237 + P1(SG20645) + P1(MW1184)[e]
B7244	N99 Δ(srl-recA)306::Tn10	N99 + P1(MW1184)
B7246	N99 dsrA::ΔTn903[f]	N99 + pDR301[g]
B7252	C600 rcsA72::ΔTn10 recA9::Tn5[c]	C600 + P1(SG20904) + P1(B3958)
B7253	C600 rcsB11::ΔTn10 recA9::Tn5[c]	C600 + P1(SG20797) + P1(B3958)
B7254	C600 lon-146::ΔTn10 recA9::Tn5[c]	C600 + P1(SG20322) + P1(B3958)
B7307	C600 dsrA::ΔTn903 Δ(srlR-recA)306::Tn10[f]	C600 + P1(B7246) + P1(MW1184)
B7315	C600 rcsC137 ompC::Tn5 Δ(srlR-recA)306::Tn10	C600 + P1(SG20803) + P1(MW1184)
Bacteriophages		
λ⁺		VKPM
21		VKPM
φ80		VKPM
434		VKPM
Mucts62		VKPM
P1vir		VKPM
λbio10		VKPM
λplac5		VKPM
λimm⁴³⁴bio10		VKPM
λimm⁴³⁴plac5		VKPM
λGE112	b2 cI(Ind⁻)	8
λDY125	b2 cI(Ind⁻)	8
λEK117	b2 cI(Ind⁻)	8
λGR185	b2 cI(Ind⁻)	8
λGE112 plac5	cI(Ind⁻)	λGE112 + λimm⁴³⁴ plac5
λDY125 plac5	cI(Ind⁻)	λDY125 + λimm⁴³⁴ plac5
λEK117 plac5	cI(Ind⁻)	λEK117 + λimm⁴³⁴ plac5
λGR185 plac5	cI(Ind⁻)	λGR185 + λimm⁴³⁴ plac5
λprecA	λcI857 recA⁺	22
λ3B3	rcsA⁺ dsrA⁺ dsrB⁺	17

[a] VKPM, Russian National Collection of Industrial Microorganisms, GNIIGenetica, Moscow, Russia.
[b] Derived from MC4100, genotype F⁻ Δ(argF-lac)U169 araD139 rpsL150 deoC1 relA1 ptsF25 flbB5301 rbsR.
[c] ΔTn10 (Tn10Δ16Δ17) is defective Tn10 capable of transposition but deficient in transposase (40).
[d] Derived from MC4100 and contains cpsB10::lacMu-imm^λ.
[e] B7237 + P1(SG20645) + P1(MW1184) indicates that the strain was constructed by two successive P1 vir transductions in the order indicated.
[f] ΔTn903 contains a drug resistance marker from transposon Tn903 which encodes for an aminoglycoside 3′-phosphotransferase gene conferring kanamycin resistance (37).
[g] N99 + pDR301 indicates that the dsrA::ΔTn903 insertion of pDR301 was transferred to the chromosome by Hfr conjugation (see Materials and Methods).

TABLE 2. Plasmids

Plasmid	Genotype or phenotype	Source or reference
pUC19	bla^+	VKPM[a]
pGEM-7zf(−)	bla^+	Promega Corporation
pUC-4K	bla^+, Km^r	37
pCM4	bla^+, Cm^r	4
pEG5005	pBCO::Mud5005; bla^+, Km^r	14
pDR406	$rcsA^+$ $dsrA^+$ $dsrB^+$ derivative of pEG5005; Km^r	This work
pDR416	$rcsA^+$ $dsrA^+$ $dsrB^+$ derivative of pEG5005; Km^r	This work
pDR19	bla^+, Cm^r	pUC19 + pCM4
pDR500	$rcsA^+$ $dsrA^+$ $dsrB^+$ bla^+	pUC19 + pDR416
pDR501	$rcsA$::ΔTn903 $dsrA^+$ $dsrB^+$ bla^+, Km^r	pDR500 + pUC4K
pDR100	$rcsA^+$ $dsrA^+$ $dsrB^+$ bla^+	pUC19 + 3B3
pDR200	$rcsA^+$ bla^+	pUC19 + pDR100
pDR300	$dsrA^+$ $dsrB^+$ bla^+	pUC19 + pDR100
pDR201	$rcsA$::ΔTn903 bla^+, Km^r	pDR200 + pUC4K
pDR301	$dsrA$::ΔTn903 $dsrB^+$ bla^+, Km^r	pDR300 + pUC4K
pDR210	$rcsA^+$ bla::cat, Cm^r	pDR200 + pCM4
pDR310	$dsrA^+$ $dsrB^+$ bla::cat, Cm^r	pDR300 + pCM4
pDR211	$rcsA$::ΔTn903, bla::cat, Km^r Cm^r	pDR201 + pCM4
pDR311	$dsrA$::ΔTn903 $dsrB^+$ bla::cat, Km^r Cm^r	pDR301 + pCM4
pDR12	$dsrA^+$ $dsrB^+$ bla^+	pGEM-7zf(−) + pDR100
pDR1	cI^+, bla^+	pUC19 + λ$^+$

[a] VKPM, Russian National Collection of Industrial Microorganisms, GNIIGenetica, Moscow, Russia.

Media and chemicals. Permanent bacterial stocks were stored in 20% glycerol at −70°C; working stocks were maintained on LB agar at 4°C for up to 2 weeks. Except where otherwise stated, cells were grown in LB medium (23). M9 (23) and GT (24) plates were as described previously. Solid media were supplemented with 1.5% agar (Difco). Soft agar contained 0.2% MgSO$_4$ with 0.8% agar. Eosin-methylene blue (EMB) galactose plates (23) were used to screen for Gal$^+$ colonies. Plates containing 50 μg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) per ml and 50 μg of isopropyl-β-D-thiogalactopyranoside (IPTG) per ml were used to distinguish between Lac$^+$ and Lac$^−$ colonies. Transducing phages were grown in NZCYM medium, as described by Sambrook et al. (29). Bacterial strains carrying the mini-Mu element pEG5005 and helper phage Mucts62 were induced in LB medium with CaCl$_2$ (20 mg/ml) and MgSO$_4$ (10 mg/ml), as described by Groisman and Casadaban (14). The following antibiotics were added, when needed, at the indicated concentration: ampicillin (Ap), 50 μg/ml (to maintain plasmids) or 20 μg/ml (to measure infective centers); chloramphenicol (Cm), 20 μg/ml; kanamycin (Km), 10 μg/ml; streptomycin (Sm), 10 μg/ml; and tetracycline (Tc), 10 μg/ml. Dilutions of cells and phages were in 0.8% saline.

Plasmid construction. The plasmids used in this work are listed in Table 2. Isolation of plasmid and phage DNA and routine nucleic acid manipulations were as described previously (29). pUC19- and pGEM-7zf(−)-derived plasmids were constructed by restriction-enzyme digestion of the transducing phage and plasmid DNA followed by ligation with T4 DNA ligase. pDR406 and pDR416 were prepared in vivo after heat induction of strain C600, which carries both the mini-Mu element pEG5005 (Km^r) and a Mucts62 prophage, as described elsewhere (14). The resulting mixed phage lysate was used to transduce strain MT2 to Km^r on EMB galactose indicator plates containing Km to screen Gal$^+$ colonies.

The plasmid bearing $rcsA^+$ $dsrA^+$ $dsrB^+$ pDR500 was constructed by subcloning the isolated 2.0-kb PstI fragment of pDR416 in the unique PstI site of pUC19. A Lac$^−$ Ap^r mucoid transformant of XL1-blue was isolated, and plasmid DNA was extracted. pDR500 had the expected enzyme restriction pattern (Fig. 1A). pDR501 was constructed by insertion of ΔTn903 from pUC4K (37), cut with HincII, into the EcoRV site of pDR500.

To construct the plasmid bearing $rcsA^+$ $dsrA^+$ $dsrB^+$ pDR100, DNA preparations from phage λ3B3 from the Kohara collection (miniset 343) and from pUC19 were digested with EcoRI and PstI, mixed, and ligated. A Lac$^−$ Ap^r mucoid transformant of XL1-blue was isolated, and the plasmid was extracted. Restriction-enzyme and hybridization analysis confirmed the structure suggested by the genetics (Fig. 1B). Plasmids pDR200 ($rcsA^+$) and pDR300 ($dsrA^+$ $dsrB^+$) were constructed by subcloning in pUC19 isolated HincII and HincII-EcoRI fragments, respectively, from pDR100. It is known that E. coli K-12 strains become mucoid when they carry several copies of the $rcsA$ gene (36). pDR300 ($dsrA^+$ $dsrB^+$), like pDR200 ($rcsA^+$), increased capsular polysaccharide synthesis, and the transformants formed slime colonies, but the pDR300 ($dsrA^+$ $dsrB^+$)-mediated effect was less pronounced and depended on the strain. We used C600 to select mucoid transformants. pDR200 ($rcsA^+$) and pDR300 ($dsrA^+$ $dsrB^+$) had the expected 0.6-kb and 1.1-kb fragments, respectively. It should be noted that pDR300, in addition to the $dsrA$ gene, also carries the adjacent $dsrB$ gene. Both code for small RNAs, as described by Sledgjeski and Gottesman (31). The restriction map for the region containing $rcsA$, $dsrA$, and $dsrB$ is shown in Fig. 1B; it is similar to that published earlier (17, 31, 34). Plasmid $rcsA$ and $dsrA$ genes were disrupted in vitro by inserting the HincII fragment from pUC4K, containing ΔTn903, into pDR200 ($rcsA^+$) cut with EcoRV and pDR300 ($dsrA^+$ $dsrB^+$) cut with Bsu36I and blunted with Klenow polymerase. Nonmucoid Ap^r Km^r transformants of C600 were selected, and their plasmid DNA was extracted. The resulting plasmids, pDR201 ($rcsA$::ΔTn903) and pDR301 ($dsrA$::ΔTn903 $dsrB^+$), had the expected restriction-enzyme cleavage patterns (Fig. 1B).

To define the physical limits of the $dsrA^+$ and $dsrB^+$ regions that confer the phenotype observed, we cloned the isolated 1.1-kb HincII-EcoRI fragment of pDR100 in pGEM-7zf(−) digested with SmaI and EcoRI. The resulting plasmid, pDR12, was digested to completion with SphI or NsiI as demonstrated by electrophoresis. Then pDR12 was digested again with XbaI or HindIII, and DNA deletion with exonuclease III was carried out as described in the Erase-a-Base system from Promega Biotec. The endpoints of the deletions were determined by sizing the linearized DNAs on agarose gels.

To clone the λ cI gene, λ$^+$ DNA was digested with BglII and mixed with pUC19 digested with BamHI. Lac$^−$ Ap^r transformants of XL1-blue were checked for lambda immunity. Restriction analysis confirmed the genetic conclusion; plasmid pDR1 carries the fragment of λ DNA from 35,711 to 38,103 kb (5) (Fig. 2).

To measure infective centers it is necessary to prevent growth of lysogens. This is readily done by addition of ampicillin to the plates, provided the lysogens are Ap^s. We inactivated the plasmid bla gene in vitro as follows. First, we cloned the cat cassette from pCM4 (4) into the BamHI site of pUC19. From the resulting plasmid pDR19, the cassette was cut out by digestion with HincII and SmaI and inserted into the bla gene of pDR200 ($rcsA^+$) and pDR300 ($dsrA^+$ $dsrB^+$) cut with ScaI and into the bla gene of pDR300 ($dsrA^+$ $dsrB^+$), and pDR301 ($dsrA$::ΔTn903 $dsrB^+$) was partially digested with the same enzyme because of the presence of a second ScaI site outside of the bla gene. Restriction analysis of the selected plasmid confirmed insertion of the cat cassette in the bla gene.

Transfer of mutation from plasmid to chromosome. The insertion mutation constructed in plasmid pDR301 was transferred to the chromosome as described by Parker and Marinus (26). Plasmid pDR301 ($dsrA$::ΔTn903 $dsrB^+$) with a disrupted $dsrA$ gene was introduced into C600 Hfr, and the resulting strain was used as a donor in conjugation experiments with N99 as the recipient. Sm^r Km^r colonies were checked for sensitivity to ampicillin. P1 transduction confirmed that the $dsrA$::ΔTn903 allele was linked to $hisG$::Tn10 (3% cotransduction) as expected for insertions in the $rcsA$ $dsrA$ region.

Halo test and quantitative measurement of prophage induction. (i) Halo test. Lysogenic bacteria, grown overnight in LB medium at 30°C from single colonies on LB plates, were spotted on a lawn of the indicator strain LE392 in soft agar, and the plates were incubated overnight at 30°C. Herein, the term induction refers to appearance of a halo of lysis or of plaques within the spot.

FIG. 2. Structure of pDR1. pDR1 is a derivative of pUC19. The genetic and transcriptional map of the region surrounding the λ *cI* gene and a portion of the λ chromosome contained in pDR1 are indicated.
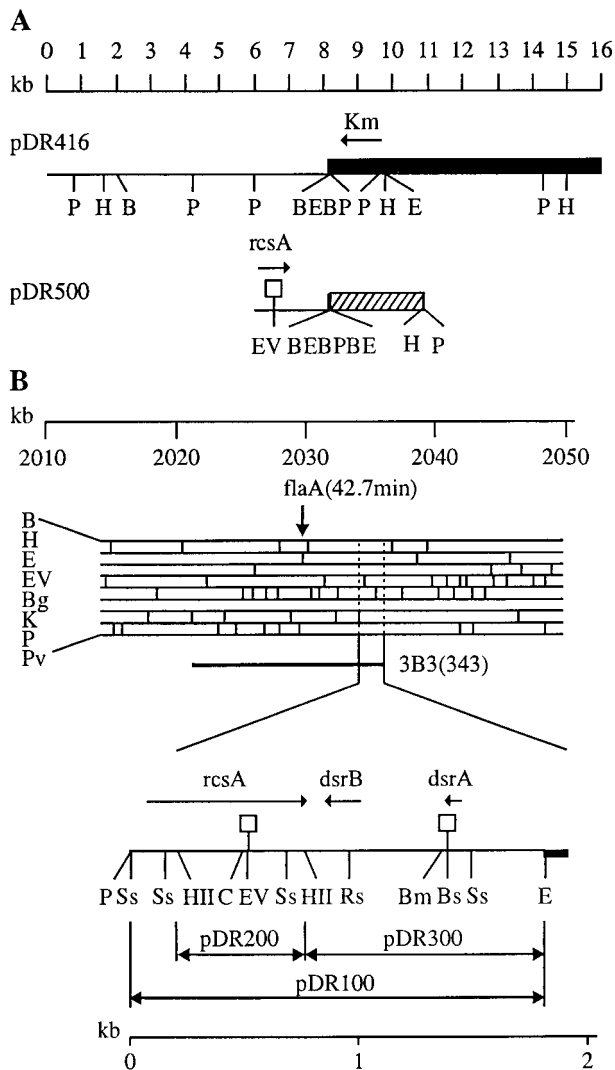
FIG. 1. Structure of plasmids used. (A) Plasmid pDR416 is a derivative of the mini-Mu element pEG5005 and plasmid pDR500 is a derivative of pUC19. The cloned chromosomal DNA is represented by a thin line, and mini-Mu material is represented by a black box. The position and direction of transcription of the *rcsA* gene were inferred from earlier data (34) and from restriction analysis. The open square indicates the position of the ΔTn*903* insertion which disrupts the *rcsA* gene in pDR500. The hatched box indicates the pUC19 DNA. (B) The physical structure of the chromosomal segment containing the *rcsA*, *dsrA*, and *dsrB* genes is shown together with the *E. coli* restriction map in the 42-min region (17). The position and direction of transcription of these genes were inferred from earlier data (31, 34) and from restriction analysis. The chromosomal insert in phage λ3B3 from the Kohara collection is indicated. The physical limits are indicated for the chromosomal DNA cloned in pDR100, pDR200, and pDR300, which are derivatives of pUC19. Open squares indicate the positions of the ΔTn*903* insertions which disrupted the *rcsA* and *dsrA* genes in pDR201 and pDR301, respectively. Abbreviations for restrictions enzymes are as follows: B, *Bam*HI; Bg, *Bgl*I; Bm, *Bsm*I; Bs, *Bsu*36I; C, *Cla*I; E, *Eco*RI; EV, *Eco*RV; H, *Hin*dIII; HII, *Hin*cII; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; Rs, *Rsr*II; Ss, *Ssp*II.

(ii) Quantification of induction efficiency. The efficiency of lysogenic induction was determined by calculating the ratio of infective centers to viable lysogens. Exponentially growing λ lysogens, washed to eliminate free phage, were mixed with indicator strain B7218 in soft agar and plated onto GT plates containing 20 μg of ampicillin/ml, as described by Moreau et al. (24). Plates were incubated overnight at 37°C. Ampicillin prevents uninduced bacteria from multiplying and releasing phage during overnight incubations but does not prevent induced lysogens from forming infective centers (25). This technique was used with Ap^s lysogens.
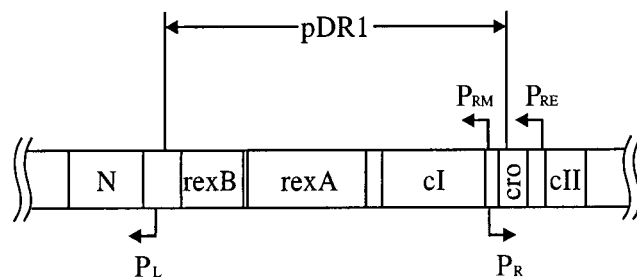
A second method of quantification of induction efficiency, measuring the concentration of free phage in the course of the induction experiment, was used. Overnight cultures from a single colony were prepared at 30°C in LB broth containing chloramphenicol when needed, 1 mM MgSO$_4$, and 0.2% glucose (reducing readsorption by repressing *lamB*, the structural gene of the λ receptor). The cultures, washed to eliminate free phage, were diluted 50- to 100-fold in the same medium and incubated at 30°C with aeration. At various times, samples were assayed for total viable cells and the optical density at 590 nm was measured. Several drops of chloroform were then added, and the free phages were mixed with LE392 indicator bacteria in soft agar and plated on LB plates. Plates were incubated overnight at 37°C.

RESULTS

Screening for clones of *E. coli* DNA that cause RecA-independent λ induction. To screen for genes which, when overexpressed, can induce λ prophage, we took advantage of the characteristics of the MT strains constructed by Toman et al. (35). These strains carry a defective λ prophage with the *N*, *cI*, and *cro* genes. The *gal* operon is fused to the *cro* gene (and the normal *gal* promoter is deleted). When cI repressor is expressed, the strains are Gal⁻. If cI repressor is removed, the *cro-gal* operon is transcribed. The Cro protein then represses the *cI* gene and prevents turning off of *cro-gal*, so the strain becomes locked in a Gal⁺ configuration. The strains thus guard a "memory" of a past induction event. We used strain MT2, which carries a *recA* mutation and normally displays a much lower frequency of Gal⁺ colonies, <10⁻⁶, than the frequency, 10⁻³ to 10⁻⁴, in the *recA*⁺ strain MT1.

A gene library of *E. coli* wild type was prepared in vivo by using the mini-Mu element pEG5005 (14). Strain MT2 was then transformed with the library, and the transformants were plated on EMB galactose plates to identify Gal⁺ clones. Among 3,000 transformants, 6 Gal⁺ clones were isolated. Plasmid DNA was extracted and hybridized with the λprecA phage DNA. Four plasmids carried the *recA* gene. The other two plasmids, pDR406 and pDR416, were reintroduced into MT2 and also into a B6905(λ) (Δ*recA*) lysogen. Both plasmids caused induction, as judged by the appearance of Gal⁺ clones in MT2 and by the formation of a halo of lysis when B6905(λ) (Δ*recA*) transformants were spotted on a sensitive lawn.

Excess RcsA or DsrA causes RecA-independent induction of λ. The two plasmids, pDR406 and pDR416, in addition to inducing λ in strains MT2 and B6905(λ) (Δ*recA*), conferred a mucoid phenotype. Furthermore, in MT2 the mucoid phenotype was restricted to the Gal⁺ colonies, presumably reflecting the requirement for GalE enzyme to synthesize UDP-Gal, a precursor of colanic acid (21). Since this made scoring of MT2
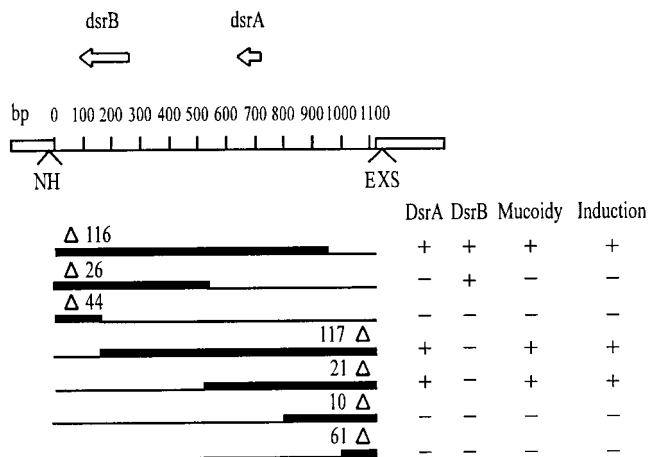
FIG. 3. Detailed map of deletions generated in pDR12. Deletions were generated by the Erase-a-Base system (see Materials and Methods). The extent of each deletion is shown by a thin line. Mucoidy and induction were scored in strain B6905(λ) (ΔrecA) transformed with the various plasmids. Abbreviations for restrictions enzymes are as follows: E, EcoRI; H, HindIII; N, NsiI; S, SphI; X, XbaI.
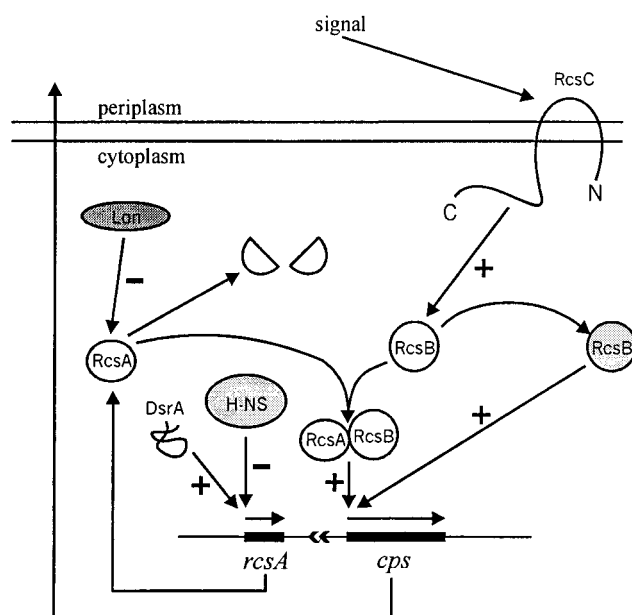

FIG. 4. Model of the regulation of cellular capsule synthesis in E. coli. Based in part on a figure presented by Stout and Gottesman (33).

difficult, we tested induction by monitoring the formation of a halo of lysis.

Mucoidy has been observed in cells overproducing RcsA (36). We therefore hybridized these plasmids with DNA from Kohara phage λ3B3, which carries the rcsA gene. Both plasmids carried the rcsA region. Furthermore, the restriction map of the cloned inserts accorded with that reported by Kohara et al. (17) for the region from 2,030 to 2,040 kb (Fig. 1). The 2.0-kb PstI fragment from pDR416 containing rcsA and dsrA was subcloned into pUC19. The resulting plasmid, pDR500, caused mucoidy and induced λ prophage in B6905(λ) (ΔrecA) cells. When the rcsA gene of pDR500 was interrupted by ΔTn903 (Fig. 1A; see Materials and Methods), the resulting plasmid, pDR501, caused mucoidy and induced λ. However, this plasmid carries the dsrA gene, overexpression of which can derepress chromosomal rcsA expression (reference 31; see below).

To determine conclusively the role of RcsA and DsrA in the induction observed, we recloned the rcsA and dsrA genes from Kohara phage λ3B3, making plasmids pDR200 (rcsA+) and pDR300 (dsrA+) (Fig. 1B). Both plasmids caused mucoidy and induced λ prophage in B6905(λ) (ΔrecA) cells. Inactivation of rcsA (plasmid pDR201; rcsA::ΔTn903) or of dsrA (plasmid pDR301; dsrA::ΔTn903) abolished both phenotypes. The dsrB gene, of unknown function, is present on the dsrA+ and dsrA::ΔTn903 plasmids but does not induce λ. Furthermore, it is not required for DsrA-mediated induction: reducing the size of the insert in plasmid pDR12 by the Erase-a-Base system showed that the dsrA gene alone was sufficient for RecA-independent λ induction (Fig. 3).

These data confirm that overproduction of RcsA or DsrA (which derepresses the chromosomal rcsA gene) causes induction of λ in a recA lysogen.

We verified that the induced phage was indeed λ and not some hitherto unknown prophage. First, the induced phage was unable to grow on a λ lysogen. Second, transformants of the B6905 (ΔrecA) nonlysogen with plasmids pDR200 (rcsA+) and pDR300 (dsrA+) produced no lysis zone on a lawn of the indicator strain.

Effect of RcsA or DsrA overproduction on other lambdoid phages. To determine whether the induction was strain or phage specific, we analyzed the effect of RcsA and DsrA overproduction on another recA mutant lysogenic for various lambdoid phages. Plasmids pDR200 (rcsA+) and pDR300 (dsrA+) were introduced into four lysogens of the ΔrecA strain B7244, and the ability of the resulting strains to produce halos on a sensitive lawn was observed. Lysogens having phages λ, 21, φ80, and 434 and both plasmids produced halos, but those without any plasmid did not. We conclude that overproduction of either RcsA or DsrA causes RecA-independent induction of all four of these phages and that induction is not strain specific.

Quantification of RecA-independent λ induction by RcsA or DsrA overproduction. RcsA is known to be a transcriptional activator of the cps genes, which are involved in the synthesis of colanic acid, the capsular polysaccharide of E. coli. RcsA is normally limiting for the transcription of these genes, and its overexpression results in a mucoid phenotype (10, 12, 13, 34). The closely linked dsrA gene codes for a small, 85-nucleotide RNA molecule which, when overexpressed, derepresses rcsA transcription (overcoming repression by H-NS) (31). In addition to RcsA, the Cps regulon includes the RcsB and RcsC regulators, which form a sensor-effector pair. In response to an unknown environmental signal, the membrane sensor RcsC is thought to phosphorylate RcsB, thereby increasing expression of the Cps regulon (10, 12, 33) (Fig. 4).

RcsA is highly unstable and is degraded by Lon protease; lon mutants consequently have high levels of RcsA and high Cps expression (34, 36). The dominant rcsA3 mutation makes a stabler RcsA* protein and thus also induces high level of Cps expression (3). Finally, the rpsC137 allele results in constitutive activation of RcsB and high level of Cps expression, even in rcsA mutants (10, 12, 33). The rcsB gene product is absolutely necessary for Cps induction in all cases.

To quantify the level of induction due to RcsA and DsrA overproduction, we assayed the number of lysogens able to

TABLE 3. Efficiency of induction of prophage λ^a

Plasmid and gene(s)	Concn (cells/ml) at t_1	Frequency of infective centers (λc^+) at t_1	Concn of phage (λc^+/ml) at:		
			t_1	t_2	t_3
C600*recA*(λ)					
No plasmid	1.4×10^8	1.1×10^{-7}	1.8×10^2	60	20
pDR211 (*rcsA*::ΔTn*903*)	1.4×10^8	2.0×10^{-7}	1.0×10^2	50	30
pDR311 (*dsrA*::ΔTn*903*)	1.0×10^8	2.7×10^{-7}	40	40	1.0×10^2
pDR210 (*rcsA*$^+$)	9.0×10^7	1.8×10^{-6}	2.0×10^3	9.0×10^2	4.0×10^2
pDK311 (*dsrA*$^+$)	9.0×10^7	7.8×10^{-6}	8.0×10^2	9.0×10^2	3.6×10^3
lon	1.0×10^8	1.3×10^{-6}	5.0×10^2	2.0×10^2	60
rcsA3	1.0×10^8	1.9×10^{-6}	1.5×10^3	6.0×10^2	1.0×10^2
rcsC137	1.1×10^8	1.4×10^{-5}	1.5×10^3	9.5×10^3	7.0×10^3
C600(λ)	1.0×10^8	9.0×10^{-5}	2.0×10^4	8.0×10^4	2.0×10^4

^{a} An overnight culture, washed to eliminate free phage, was diluted 50- to 100-fold in fresh LB broth and incubated at 30°C. The concentrations of cells at the start of the experiment ranged from 2×10^7 to 4×10^7. The frequency of infective centers in exponential phase and the free-phage concentration were measured at various times, as described in Materials and Methods. t_1 (2 to 3 h), t_2 (6 to 8 h), and t_3 (24 h) correspond to exponential-, postexponential-, and stationary-phase time points. All experiments were carried out at least three times; averages are given in the table. Deviations from the averages were insignificant.

form an infective center on a sensitive lawn. To limit the growth of uninduced lysogens, ampicillin was added to the plates and an ampicillin-resistant indicator was used. Ampicillin does not affect phage growth but kills uninduced cells after about one generation (25). This prevents the formation of tiny plaques due to late induction in microcolonies of lysogens. Since these assays require the use of Ap^s lysogens, we inactivated the *bla* gene in the plasmids carrying *rcsA*$^+$, *dsrA*$^+$ and their inactivated derivatives. The resulting plasmids—pDR210 (*rcsA*$^+$ *bla*::*cat*), pDR310 (*dsrA*$^+$ *bla*::*cat*), pDR211 (*rcsA*::ΔTn*903 bla*::*cat*) and pDR311 (*dsrA*::ΔTn*903 bla*::*cat*)—were introduced into the tester strains and analyzed for inducibility. All conditions leading to high level of Cps expression also caused RecA-independent induction of λ (Table 3). Our results do not contradict those of Gottesman et al. (11), who observed no influence of *lon* mutation on the frequency of λ prophage induction, because the level of RcsABC-mediated induction is several orders of magnitude lower than that of induction via the RecA pathway.

The concentration of free phage was determined at several time points during the induction experiment, to see whether the level depended on the phase of cell growth.

The event we are interested in is λ^+ prophage induction caused by RcsA or DsrA overproduction, but lytic phage development can also result when λcI mutants arise, contributing to the number of infective centers. To distinguish between induction and mutation, we periodically monitored the levels of free phage in growing cultures, scoring λc^+ and λc separately. λc^+ and λc phages can be recognized because they form turbid and clear plaques, respectively. Both *rcsA*$^+$ and *dsrA*$^+$ that were cloned on plasmids, like the *rcsC137*, *rcsA3*, or *lon* mutation, stimulated λ prophage induction (Table 3). The results confirm that the genes regulating the synthesis of capsular polysaccharide affect prophage stability. It is interesting that RcsA overproduction causes an increase in free phage in exponential phase whereas DsrA overproduction produces the maximum free-phage titer on entry to the stationary phase of growth.

The mucoid phenotype of strains carrying *rcsA*$^+$ and *dsrA*$^+$ plasmids could reduce phage readsorption, thereby apparently increasing the free-phage titer. Indeed, adsorption to these cells was slower (data not shown). However, similar free-phage curves were obtained with λ-resistant *recA*(λ) lysogens, in which readsorption was prevented (data not shown). Further-

more, the number of infective centers, which should not be significantly influenced by phage readsorption, showed a similar 10- to 100-fold increase in *recA* lysogens bearing *rcsA*$^+$ or *dsrA*$^+$ plasmids or carrying an *rcsC137*, *rcsA3*, or *lon* allele (Table 3). The growth rates of the various strains used differed little, although mucoid strains reached stationary phase at a lower concentration (data not shown). We thus conclude that *rcsA*$^+$ or *dsrA*$^+$ plasmids increase the free-phage titer not by changing cell vigor, phage burst size, or adsorption efficiency but rather from genuine prophage induction.

Neither pDR210 (*rcsA*$^+$) nor pDR310 (*dsrA*$^+$) significantly increased the concentration of λc in the culture medium, showing that the burst of λc mutants is independent of RcsA and DsrA. This once again implies that the observed increase in the concentration of λc^+ free phage reflects true induction of λ^+ prophage, since other factors would be expected to affect λc^+ and λc titers similarly.

Genetics of RcsA- or DsrA-mediated induction. To clarify the role of the *rcsA*, *dsrA*, and *rcsB* genes in RecA-independent λ induction, we carried out complementation tests in which lysogenic strains B7252(λ) (*rcsA recA*), B7307(λ) (*dsrA recA*), and B7253(λ) (*rcsB recA*) were tested for their ability to induce the prophage in the presence of *rcsA*- and *dsrA*-bearing plasmids (Table 4).

Induction mediated by RcsA, as seen from the frequency of infective centers and the free-phage concentration, does not require intact chromosomal *rcsA* and *dsrA* genes but is strictly dependent on an intact *rcsB* gene (Table 4). It is clear that the activation of *cps* transcription and prophage induction by RcsA have common regulatory steps.

Since DsrA overproduction stimulates RcsA synthesis, we expected DsrA induction to reflect the same genetic requirements. However, we found a 10- to 100-fold increase in the frequency of infective centers in both *rcsA recA* mutants and *rcsB recA* mutants compared to control cells (Table 4). This observation suggests that induction by DsrA is independent of the RcsABC system.

For DsrA-mediated induction, there was a difference between the exponential and stationary phases of growth, as seen from the free-phage concentration. In exponential phase, *rcsA* and *rcsB* mutations reduced the effect of pDR310 (*dsrA*$^+$) about 10-fold, compatible with the positive action of DsrA RNA on *rcsA* transcription. However, in stationary phase there was a marked increase in the titer of λc^+ free phage in *rcsA*

TABLE 4. Role of the *rcsA*, *dsrA*, and *rcsB* genes in λ induction[a]

Plasmid and gene(s)	Concn (cells/ml) at t_1	Frequency of infective centers (λc⁺) at t_1	Concn of phage (λc⁺/ml) at:		
			t_1	t_2	t_3
B7252(λ) (*rcsA recA*)					
No plasmid	2.5×10^8	2.9×10^{-7}	70	40	40
pDR211 (*rcsA*::ΔTn*903*)	1.7×10^8	1.4×10^{-7}	70	60	20
pDR311 (*dsrA*::ΔTn*903*)	1.3×10^8	8.0×10^{-8}	70	60	30
pDR210 (*rcsA⁺*)	1.3×10^8	1.5×10^{-6}	5.0×10^2	4.0×10^2	2.0×10^2
pDR311 (*dsrA⁺*)	9.0×10^7	1.2×10^{-5}	2.0×10^2	1.5×10^3	4.0×10^3
B7307(λ) (*dsrA recA*)					
No plasmid	1.0×10^8	1.1×10^{-7}	10	20	1.0×10^2
pDR211 (*rcsA*::ΔTn*903*)	9.0×10^7	3.0×10^{-7}	30	30	60
pDR311 (*dsrA*::ΔTn*903*)	9.0×10^7	4.7×10^{-7}	50	60	1.5×10^2
pDR210 (*rcsA⁺*)	1.2×10^8	9.1×10^{-7}	8.0×10^2	9.0×10^2	3.0×10^2
pDR311 (*dsrA⁺*)	1.1×10^8	8.9×10^{-6}	8.0×10^2	1.1×10^3	3.7×10^3
B7253(λ) (*rcsB recA*)					
No plasmid	2.5×10^8	2.3×10^{-7}	20	50	30
pDR211 (*rcsA*::ΔTn*903*)	1.0×10^8	1.0×10^{-7}	10	30	20
pDR311 (*dsrA*::ΔTn*903*)	9.0×10^7	1.6×10^{-7}	10	40	20
pDR210 (*rcsA⁺*)	1.2×10^8	1.9×10^{-7}	20	40	10
pDR311 (*dsrA⁺*)	1.4×10^8	5.9×10^{-6}	4.0×10^2	1.0×10^3	4.0×10^3

[a] Experimental methods and conditions were as described in footnote *a* of Table 3. Data presented are as described in footnote *a* of Table 3.

and *rcsB* mutants carrying a *dsrA⁺* plasmid. This observation is difficult to reconcile with the hypothesis that DsrA RNA causes prophage induction only through its positive regulation of *rcsA* transcription (antagonizing repression by H-NS). It suggests that in the late phase of cell growth, an increased level of DsrA RNA induces λ prophage via an RcsA-independent pathway.

The levels of λc in all experiments were similar (data not shown).

Effect of λ repressor overproduction on RcsA-mediated induction. If the RcsABC system provides a repressor bypass for the various phages, overproduction of repressor would not be expected to affect induction. If, on the other hand, some component of the RcsABC system (or a gene product induced by these activators) interacts directly with the various phage repressors, then repressor overproduction should reduce induction frequency. Using a multicopy plasmid pDR1 (*cI⁺*), we overproduced λ repressor in *recA rcsC137*(λ) and *recA lon* (λ) lysogens. Induction was completely abolished (data not shown). This suggests that RcsA-mediated induction involves repressor inactivation. The same plasmid in *recA lon* strains that are lysogenic for the heteroimmune phages 21, φ80, and 434 had no effect on induction. Similar specificity has been shown for SOS induction, where excess λ *cI*, although preventing λ induction, does not saturate the system since 434 repressor can still be inactivated (1).

Influence of *cIind* mutations on λ induction by RcsA and DsrA. To clarify the mechanism of prophage induction by RcsA and DsrA, we tested the inducibility of mutant λ*cIind* prophages, described by Gimble and Sauer (8). The repressors of all λ*cIind* mutants used—λGR185, λEK117, λDY125, and λGE112—have a defect in RecA-stimulated proteolysis, but all except λGE112 (mutated in the cleavage site) can carry out self cleavage, at various efficiencies, under alkaline condition in vitro (9). Since the λ*cIind* mutants all carried the *b2* deletion, covering the att site, we crossed them with λimm⁴³⁴ plac5 to obtain λ*cIind* plac5 att⁺ recombinants, which we tested for inducibility (Table 5).

As can be seen in Table 5, spontaneous induction of λDY125 plac5 in exponential phase increases two- to tenfold when the cells carry pDR210 (*rcsA⁺*) or pDR311 (*dsrA⁺*), as judged by the frequency of infective centers and the level of free phage. This observation shows that the action of RcsA and DsrA on the prophage is not inhibited by the *cIind* mutation. Similar results were obtained with λGR185 plac5 and λEK117 plac5 (data not shown). However, the action of RcsA was lowered in the case of prophage λGE112 plac5 (Table 5). As mentioned above, the repressor of this phage is mutated in the cleavage site and differs from the other *cIind* proteins in that it does not undergo self cleavage under alkaline condition in vitro (9). This result suggests that prophage induction in exponential phase is by repressor inactivation, possibly reflecting the action of an alternative RecA coprotease.

As shown in Table 5, on entry to stationary phase pDR311 (*dsrA⁺*) causes a similar, severalfold increase in the concentration of free phage with λplac5 and λ*cIind*. The effects of the plasmid were the same on all λ*cIind* phages. DsrA RNA thus acts on the prophage independently of *cIind* mutations in the repressor gene.

The amounts of λc mutants differed little among the strains tested (data not shown).

DISCUSSION

Prophage stability is coupled to cell physiology. Under conditions in which cell viability is jeopardized, the prophage may manage to survive by entering the lytic pathway. The paradigm for lysogenic induction—and in fact the only induction mechanism known at present to exist—is the SOS response and its positive regulator, the RecA protein. In response to DNA damage, RecA stimulates the autoproteolytic cleavage of certain prophage repressors (28).

Other stress responses of the bacterial cell, alongside the SOS response, may also induce certain prophages, allowing them to abandon a host in physiological difficulty. Under lab-

TABLE 5. Efficiency of induction of λcI*ind* prophages[a]

Plasmid and gene(s)	Concn (cells/ml) at t_1	Frequency of infective centers (λc$^+$)[b] at t_1	Concn of phage (λc$^+$/ml)[b] at:		
			t_1	t_2	t_3
C600Δ*recA*(λ plac5)					
No plasmid	2.3×10^8	9.0×10^{-9}	1.5×10^2	1.3×10^2	1.6×10^2
pDR211 (*rcsA*::ΔTn*903*)	2.4×10^8	3.3×10^{-8}	2.8×10^2	1.8×10^2	3.7×10^2
pDR311 (*dsrA*::ΔTn*903*)	1.4×10^8	1.5×10^{-8}	1.0×10^2	1.0×10^2	1.4×10^2
pDR210 (*rcsA*$^+$)	1.8×10^8	2.0×10^{-7}	1.7×10^3	9.2×10^2	4.3×10^2
pDR311 (*dsrA*$^+$)	1.4×10^8	1.6×10^{-6}	8.0×10^2	3.1×10^3	8.5×10^3
C600 (λDY125 plac5)					
No plasmid	3.6×10^8	4.0×10^{-8}	3.0×10^2	1.7×10^2	1.6×10^2
pDR211 (*rcsA*::ΔTn*903*)	2.8×10^8	3.6×10^{-8}	1.7×10^2	2.0×10^2	2.1×10^2
pDR311 (*dsrA*::ΔTn*903*)	3.3×10^8	1.8×10^{-8}	2.9×10^2	2.4×10^2	2.1×10^2
pDR210 (*rcsA*$^+$)	3.6×10^8	1.1×10^{-7}	4.3×10^3	2.2×10^3	6.2×10^2
pDR311 (*dsrA*$^+$)	2.7×10^8	2.5×10^{-7}	1.4×10^3	4.0×10^3	2.9×10^3
C600(λGE112 plac5)					
No plasmid	1.8×10^8	2.0×10^{-8}	90	50	50
pDR211 (*rcsA*::ΔTn*903*)	2.9×10^8	2.0×10^{-8}	50	30	40
pDR311 (*dsrA*::ΔTn*903*)	2.2×10^8	2.0×10^{-8}	50	30	30
pDR210 (*rcsA*$^+$)	2.0×10^8	4.0×10^{-8}	3.6×10^2	6.0×10^2	2.0×10^2
pDR311 (*dsrA*$^+$)	2.3×10^8	3.8×10^{-7}	1.2×10^3	9.4×10^2	7.9×10^3

[a] Experimental methods and conditions were as described in footnote *a* of Table 3. Data presented are as described in footnote *a* of Table 3.
[b] λc$^+$, turbid plaques of λcI*ind* phages.

oratory conditions, these putative systems of lysogenic induction are likely to be inefficient unless the right stress is applied. Here we present evidence that this is indeed the case for *E. coli*. In particular, we have demonstrated RecA-independent induction of λ and other lambdoid prophages by overproduction of RcsA, a transcriptional regulator of the *cps* operons involved in capsular polysaccharide synthesis (10, 12, 13, 34), and by overproduction of DsrA, an 85-nucleotide RNA able to antagonize H-NS repression (31). In both cases, the numbers of free phage and of infective centers in overproducing strains were increased 10- to 100-fold compared to those in control strains.

The synthesis of capsular polysaccharide, or colanic acid, is regulated by the two component system RcsC-RcsB, in which RcsC, a membrane protein, is similar to histidine kinase sensor proteins and RcsB, which is absolutely required for *cps* gene expression, is similar to DNA-binding effectors (33). RcsA, and probably RcsF as well (7), are additional transcriptional activators of the Cps regulon. The RcsA level is regulated by H-NS repression of *rcsA*, which is antagonized by DsrA RNA (31), and by degradation by protease Lon (34, 36).

We show here that the RcsABC-dependent system induces λ, 21, φ80, and 434, and its action may well extend to other lambdoid phages and possibly also to nonlambdoid, SOS-inducible phages. It is interesting that spontaneous induction of lambdoid phages is principally via the SOS response, as evinced by its sharp reduction in *recA* lysogens (cf. Table 3). The second mechanism seems to be via RcsABC, since induction is essentially abolished in *recA rcsB*(λ) lysogens (cf. Table 4).

At present we have no information as to the nature of the environmental signal transduced by the RcsABC system that stimulates capsular polysaccharide synthesis. Nevertheless, all strains with genetic backgrounds that resulted in increased *cps* gene expression also displayed RecA-independent prophage induction. These include strains overproducing RcsA or DsrA from multicopy plasmids, *lon* and *rcsA3* mutants in which RcsA

is stabilized (and therefore present at high levels), and the *cps* constitutive mutant *rcsC137*, thought to phosphorylate RcsB spontaneously. It is a curious fact that, although *lon* mutants have higher *cps* expression than the *rcsA3* mutant (3), the latter strain has a higher level of λ induction (Table 3). This suggests that prophage induction does not result from transcriptional activation of a *cps* operon.

Prophage induction by the RcsABC system, which takes place in exponential phase, depends on the concentration of repressor in the cells. One can speculate that this induction results either from a lowering of *c*I repressor synthesis from the promoter P_{RM} or from decreased repressor activity via a direct interaction between *c*I and some cell protein(s) whose synthesis induces the RcsABC system. The second hypothesis seems more likely since the mutation in phage λGE112, which blocks self cleavage of the repressor, lowers prophage induction via RcsA. The RcsABC system may regulate the expression of an alternative RecA coprotease. The known homology between RcsA and the LuxR family of transcriptional activators (34) suggests the activation of the gene of an alternative RecA coprotease. In addition, the complete dependence of RcsA action on the presence of an intact *rcsB* gene and the effect of the *rcsC137* mutation also argue in favor of this proposition.

Prophage induction in the presence of excess DsrA RNA in exponential phase depends on the presence of intact *rcsA* and *rcsB* genes. This indicates that the action of DsrA RNA on the prophage in exponentially growing cells is via *rcsA* transcription. However, on entry to stationary phase, as we have seen, induction by DsrA RNA no longer depends on the RcsABC system. This leads us to postulate the existence of a second induction pathway of λ prophage by DsrA RNA, independent of both RecA and the RcsABC system. Furthermore, this pathway is not affected by the presence of a *c*I*ind* mutation in the prophage repressor gene, even if the normal cleavage site is mutated. DsrA action on the prophage in this phase of

growth is probably at the transcriptional level, possibly creating a repressor bypass.

Retallack and Friedman (27) have reported that 10Sa RNA, the *ssrA* gene product, binds to λ *c*I repressor, competing with binding to the operator O_{R2} and reducing repression. It is not known whether an excess of this small RNA, like DsrA, can cause RecA-independent prophage induction. However, the recently established role of 10Sa RNA in tagging proteins produced from truncated mRNA molecules (16) suggests that gene regulation is not its primary function.

DsrA RNA was originally discovered because of its ability, when overproduced, to derepress *rcsA* expression (31). It was shown to relieve turning off by H-NS of several operons, including *rcsA* and *rpoS*, the structural gene of sigma S (31, 32). Derepression of *rcsA* is clearly involved in DsrA-mediated prophage induction in exponential-phase cells, since chromosomal *rcsA* and *rcsB* mutations lead to a significant drop in the induction level (Table 4). In stationary phase, however, excess DsrA causes efficient prophage induction even in the absence of RcsA and RcsB (Table 4). Stationary-phase cells normally have high levels of both H-NS (6) and RpoS (15). DsrA increases the amount of RpoS (32) and antagonizes H-NS action (31), which itself normally decreases the amount of RpoS (2, 41). Since both H-NS and RpoS regulate a number of operons, DsrA overexpression, with a larger-than-normal increase in RpoS and less-efficient action of H-NS, could radically alter the pattern of gene expression in stationary phase. How this configuration results in prophage induction remains to be shown.

Induction of λ prophage via RecA-assisted repressor cleavage was an important step forward in elucidating the molecular regulation of the SOS response. We have presented here two new, RecA-independent systems of prophage induction. One is observed in stationary phase in the presence of excess DsrA RNA and probably involves sigma S and the histone-like protein H-NS; its characterization will add to our knowledge of gene expression in stationary phase. The other induces prophages via RcsABC regulatory network; knowledge of this circuit should help reveal the environmental signal inducing capsular polysaccharide synthesis. It is likely that additional mechanisms of lysogenic induction exist. Our search, for example, would not have identified systems inoperative on λ or systems that require RecA in a non-SOS role. A complete catalogue of lysogenic induction mechanisms would reveal what aspects of host physiology viruses are sensitive to and would provide new tools for elucidating the molecular signals indicating cellular disorders.

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