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Deletion of the *spf* (Spot 42 RNA) Gene of *Escherichia coli*

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To investigate the function of spot 42 RNA, a small RNA of *Escherichia coli*, we constructed a strain in which *spf*, the structural gene for this RNA, is deleted. We achieved this by using a Δ*att* phage λ carrying a DNA fragment spanning the *spf* region but with a precise deletion of *spf*. By integration of this phage at the *spf* locus and by its subsequent excision, we were able to cross the *spf* deletion onto the bacterial chromosome. The fact that such a deletion could be obtained indicated that *spf* is not an essential gene. We did not observe any major defect in Δ*spf* cells, although in one strain background the deletion caused a slight growth impairment.

Spot 42 RNA of *Escherichia coli* is a moderately abundant (100 to 200 copies per cell) RNA, 109 nucleotides long (14, 15). It is encoded by a single gene (*spf*) located immediately downstream from *polA* at 86.6 min on the *E. coli* genetic map (2, 7, 13). The function of the *spf* gene is unknown, although the abundance of the RNA suggested to us that the gene product may play an important, perhaps essential, role in vivo. The cellular distribution of spot 42 RNA gives little clue to its function; the RNA is found associated with membranes, with ribosomes, and with the nucleoid (15).

Currently we do not know whether the ultimate gene product of *spf* is RNA or protein. The RNA itself could be a structural or catalytic component of a ribonucleoprotein particle, like the M1 RNA component of RNase P (5). On the other hand, spot 42 RNA has sequence features suggesting that it may be an mRNA: a ribosome-binding site followed by an initiation codon and an open reading frame with the coding capacity for a 15-residue peptide (14). The potential translated product is rich in hydrophobic amino acids, particularly leucine, but also contains a total of four charged residues (including the N- and C-terminal amino acids). The latter give an alternating pattern of positively and negatively charged groups with adjacent pairs separated by approximately one turn of an α-helix. Consideration of the hydrophobic nature of the putative peptide gene product has led us and others to suggest that it may be inserted into membranes, perhaps serving a structural role or forming channels to allow the passage of certain ions or small molecules (7, 13). Sahagan and Dahlberg have shown that expression of *spf* is negatively regulated by cyclic AMP (15). This could be taken as further circumstantial evidence linking the *spf* gene product with membrane structure or function, since cyclic AMP is also a negative regulator of several membrane and envelope proteins (1, 10, 12) as well as of cell division (17).

The problem of assigning a function to a gene locus can be approached by first asking whether the gene of interest (which must be available as a cloned fragment) is essential for viability in *E. coli*. This can be done by determining whether the gene can be replaced on the chromosome by an in vitro-constructed deletion, using a strategy which we have described previously (8). If the target gene is not essential for survival, one will obtain a strain in which the gene has been deleted. This null mutation can then be used to test for functions of the gene.

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MATERIALS AND METHODS

Materials. Restriction endonucleases were obtained from New England Biolabs and Boehringer Mannheim Biochemicals. T4 DNA ligase was from Collaborative Research, Inc., and Boehringer Mannheim Biochemicals. Oligonucleotide linkers were from Collaborative Research, Inc., and New England Biolabs. Bacterial strains used in this work are listed in Table 1. Plasmids and general techniques relevant to this work have been described in detail previously (8). The plasmids were all derived from the vector pNG16, a deletion derivative of pBR322.

Construction of a genetically marked *spf* deletion. This construction is outlined in Fig. 1. pGH78 contains a 2.1-kilobase-pair (kb) *Sac*I-*Hin*dIII fragment carrying the end of the *polA* coding region and the *spf* gene, with a *Bam*HI linker inserted in the *Alu*I site 46 base pairs (bp) beyond the termination codon of *polA* (8). This *Bam*HI site defined the upstream endpoint for deletion of *spf*. The downstream endpoint was defined using a plasmid, ΔN3058, carrying an in vitro-constructed deletion of *polA* (6). This plasmid contains DNA from the *Sau*3A site at position 3058 on our published sequence (7), to the *Hin*dIII site at position 3822, with a *Bam*HI site at the position of the original *Sau*3A site. As a genetic marker we used the kanamycin resistance (Km^r) gene of Tn*903*; plasmid pGH54 contains this gene as a 950-bp *Bam*HI fragment inserted at the *Bam*HI site of pNG16 (8). The 800-bp *Bam*HI-*Eco*RI fragment from ΔN3058 and the 950-bp *Bam*HI fragment of pGH54 were purified and ligated to the large *Eco*RI-*Bam*HI fragment of pGH78, to give a structure in which the Km^r gene (transcribed in the same direction as *polA* and *spf*) replaces *spf*. The *Sal*I site in the vector DNA was replaced with an *Eco*RI linker and the resulting 3.2-kb *Eco*RI fragment was recovered by cloning into the *Eco*RI site of pNG16, to give pGH84. The Km^r Δ*spf* construction was then inserted into the *Eco*RI vector, λgt1-λB (4). The structure of the resulting phage, λGH84, was verified by Southern blot analysis, using pCJ1 as the probe. A control phage, λGH86, which carries the Km^r gene without deletion of *spf*, was constructed in an analogous way after insertion of the Km^r fragment into the *Bam*HI site of pGH78.

Isolation and analysis of lysogens. Lysogens of λGH84 in *E. coli* CM4722 were selected at 30°C on L-broth plates seeded with λ*ch*80del9 (11) and were subsequently checked for λ immunity and Km^r. Chromosomal DNA from candidate

TABLE 1. Bacterial strains used in this work

Strain	Genotype	Source
CM4722[a]	F$^+$ Δ(*gal-bio*) *thi-1 relA1 spoT1*	W. S. Kelley
CJ200	CM4722 *spf*$^+$ Km^r	This work
CJ205	CM4722 Δ*spf* Km^r	This work
S165	*his* Δ*galS165 rpsL*	Our strain collection
LE392	*lacY galK galT metB trpR supE supF hsdR*	Our strain collection
AW330	*lac gal ara xyl thr leu thi fhuA rpsL*	R. Macnab
20SOK$^-$	*galK galP*	R. Macnab
JC1552	*leuB trp hisG argG metB lacY gal malA xyl mtl rpsL fhuA tsx supE*	B. Bachmann (CGSC)[b]
AB2557	*purF dsdA aroC ilvD lac malA xyl mtl rpsL fhuA tsx supE*	B. Bachmann (CGSC)
KL188	*pyrD trp his thyA thi galK malA xyl mtl rpsL*	B. Bachmann (CGSC)

[a] CM4722 is an F$^+$ derivative of the HfrH strain KS302 (16).
[b] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

lysogens was analyzed by Southern blots to determine the structure at the *spf* locus.

Curing of lysogens. Lysogens were grown overnight at 30°C in L broth. Cured cells were selected by plating appropriate dilutions at 42°C on L-broth plates containing 1 mM EDTA. The proportion of survivors that retained Km^r was determined by replica plating. To obtain an independent estimate of the relative frequencies of the recombination events giving rise to Km^r and kanamycin-sensitive (Km^s) cells, a sample of each lysogen culture was induced to give a phage lysate. The composition of the lysate was determined by forming lysogens in strain LE392 and then determining the relative numbers of Km^r and Km^s lysogens. (For further details, see reference 8.)

Hybridization probes. Southern blots were probed either with nick-translated pCJ1, which carries a 5-kb *Hin*dIII fragment containing the *polA* and *spf* genes (9), or with spot 42 RNA labeled by in vitro transcription as described previously (7).

Other methods. Transduction of *spf*$^+$ Km^r from *E. coli* CJ200, and Δ*spf* Km^r from *E. coli* CJ205, was carried out using P1 *kc* and following standard procedures (11). The desired transductants were selected on plates containing 40 μg of kanamycin per ml.

Growth of CJ200 and CJ205 in liquid medium was compared using a competition experiment. Each strain was mixed with an equal number of cells of the parent strain, CM4722 (Km^s). After a period of growth, the mixture was plated on L broth, and the proportion of Km^r cells (CJ200 or CJ205) was determined.

The sensitivity of *spf*$^+$ and Δ*spf* strains to salt, detergents, and drugs was compared in two ways. In the first method, a heavy inoculum of each strain to be tested was streaked out within a sector of an L-broth plate. The substance to be tested was placed in a well in the center of the plate. After incubation, face-upwards, the position of the inhibition zone for isogenic Δ*spf* and *spf*$^+$ pairs was compared. Alternatively, the strains were plated in top agar and the substance to be tested was placed on a filter paper disk on the lawn of cells, as described by Benson and DeCloux (3). Similar results were obtained with both methods.

Swarming behavior of motile strains was examined on tryptone plates containing 0.38% agar.

RESULTS

Our method for determining whether a particular gene is essential for survival (Fig. 2) has been described in detail previously (8). Briefly, it involves the insertion of a Km^r marker between the sequences immediately flanking the gene of interest, using standard in vitro manipulations. The genetically marked deletion is cloned onto a phage λ vector that is Δ*att* and thermoinducible (*c*I857). At the permissive temperature, this phage can lysogenize by homologous recombination at the cloned bacterial sequences, giving a structure in which the integrated prophage is flanked by a wild-type and a deleted (Km^r) copy of the target gene. Spontaneous curing of such a lysogen will either regenerate the wild-type configuration or leave the Km^r-marked deletion on the chromosome, depending on the position of the recombinational crossover. Failure to obtain Km^r cells among the temperature-resistant cured survivors could indicate either that deletion of the target gene is lethal or that the necessary recombinational events did not take place at a high enough frequency. The latter possibility can be addressed by an independent estimate of the fraction of deletion-producing events, either by examining the other product

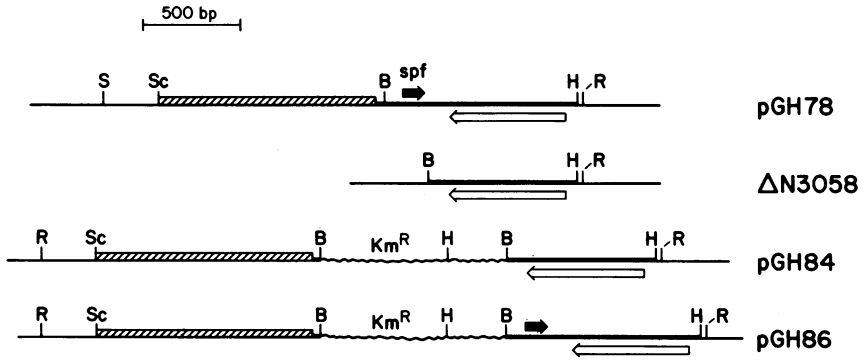

FIG. 1. Construction of plasmids used in this work. For details see the text. The thick line represents DNA derived from the *E. coli* chromosome. Within this region are indicated part of the *polA* coding sequence (hatched area), the *spf* gene (black arrow), and a functional open reading frame for a 22-kilodalton protein (open arrow) (7). The DNA fragment carrying the Km^r gene is represented by the wavy line. DNA derived from the plasmid vectors is drawn as a thin line and is not shown in its entirety. B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; S, *Sal*I; Sc, *Sac*I.

of the excision process, the phage produced on induction of the same lysogen, or by repeating the curing with an additional copy of the target gene in *trans* so as to remove any selective pressure against the deletion.

Phage and lysogen structure. The construction of λGH84, in which the *spf* gene has been replaced by Km^r while retaining the surrounding sequences, is described in the Materials and Methods section and in Fig. 1. The deletion endpoints were carefully chosen so as to remove completely the *spf*-transcribed region without encroaching on either of the extremely close neighboring genes (7). Lysogens of λGH84 in CM4722 were selected, and their chromosomal DNA was analyzed by Southern blots to identify those in which integration had taken place at the *spf* locus. Digestion with *Eco*RI and *Sal*I gives a pair of fragments (of sizes 8.2 and 8.3 kb, or 7.5 and 9.0 kb, depending on the position of the recombinational crossover) if integration has taken place at *spf* (Fig. 3). Integration elsewhere on the chromosome results in a 13.3-kb fragment diagnostic of an unrearranged *spf* locus and a 3.2-kb fragment derived from λGH84. We chose four independent lysogens, two of type A and two of type B, for the curing experiment.

Curing of lysogens shows that *spf* can be deleted. Cured cells were selected from overnight cultures of the chosen lysogens, and the proportion of Km^r colonies was determined (Table 2). In every case, a substantial proportion of Km^r, and therefore *spf*-deleted, cells was obtained. For each lysogen tested, the ratio of Km^r to Km^s cured cells was approximately reciprocal to the ratio of Km^r to Km^s transducing phage obtained on induction of that lysogen, indicating that there was no selective bias against *spf*-deleted cells. There was a consistent three- to fourfold bias in favor

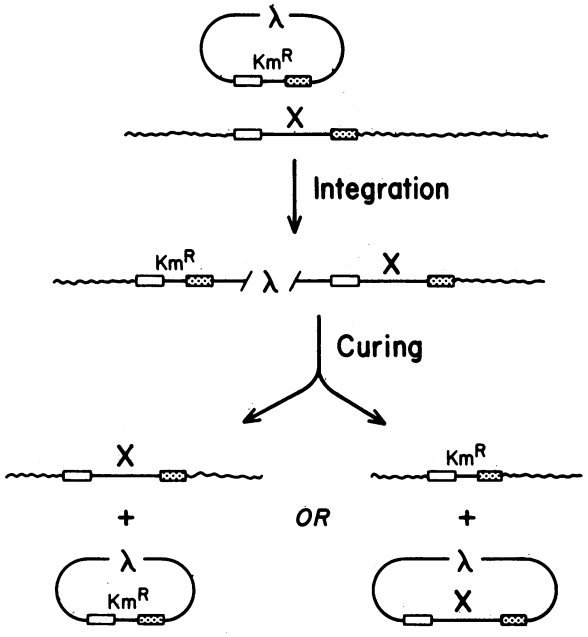

FIG. 2. Method for determining whether a gene can be deleted. Phage integration and excision are used to cross a deletion of the target gene (X) onto the bacterial chromosome. For details see the text. The regions of homology between the phage and the chromosome are boxed: ▭, upstream flanking DNA; ▨, downstream flanking DNA. Chromosomal DNA external to these regions of homology is shown as wavy lines. (Note that only one of the two possible integrated structures is shown.)

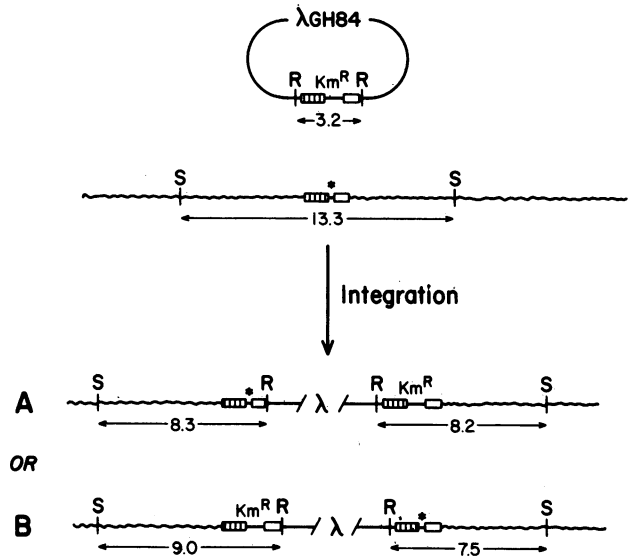

FIG. 3. Integration of λGH84 at the *spf* locus. Two alternative structures, designated A and B, can result from integration of λGH84 via homologous recombination at *spf*. Symbols: *, position of the *spf* gene; ▥, upstream flanking DNA carried on λGH84; ▭, downstream flanking DNA. Chromosomal DNA external to the region of homology with λGH84 is shown as a wavy line. The position of relevant *Eco*RI (R) and *Sal*I (S) sites are indicated, along with the sizes (in kilobases) of fragments that would hybridize to a pCJ1 probe.

of recombination in the upstream flanking region, so that lysogens of structure A gave more Km^r cells, and lysogens of structure B gave more Km^s cells (Table 2 and Fig. 3). This is probably due to the greater length of the upstream region of flanking homology (1,150 bp as opposed to 770 bp).

Southern blot analysis of chromosomal DNA from cured cells obtained in this way showed that Km^s cells had regained the 5-kb *Hin*dIII fragment diagnostic of an unrearranged *spf* locus (data not shown). Km^r cells, on the other hand, had two new *Hin*dIII fragments of 4.7 and 1.1 kb (Fig. 4B, lanes 1 and 2). Moreover, DNA from these cells did not hybridize to spot 42 RNA (Fig. 4B, lanes 5 and 6). This observation confirms the hybridization data of Rice and Dahlberg (13) which indicated that the wild-type *E. coli* chromosome contains a single locus homologous to spot 42 RNA.

The curing experiment provided us with cells lacking the *spf* gene, which could serve as a tool to examine the phenotype of an *spf* null mutation and thus probe the function of the *spf* gene. To be sure that any phenotype was attributable to the Δ*spf* mutation, we generated an isogenic *spf*$^+$ Km^r strain, using the control phage λGH86, in which Km^r has been inserted without removing *spf* (Fig. 1). The strains CJ200 (*spf*$^+$ Km^r) and CJ205 (Δ*spf* Km^r) are identical except for the presence in CJ200 of 230 bp spanning the *spf* gene (Fig. 4). Southern blot analysis of DNA from the *spf*$^+$ Km^r cells confirmed that the smaller *Hin*dIII fragment was 1.3 kb, compared with 1.1 kb in Δ*spf* cells (Fig. 4B, lanes 3 and 4), and that this fragment hybridized to spot 42 RNA (lanes 7 and 8).

Phenotype of Δ*spf* cells. We compared the growth of CJ205 (Δ*spf*) and CJ200 (*spf*$^+$) on various solid media: L broth or MacConkey agar, each supplemented with glucose, lactose, galactose, or maltose, and M9 minimal medium (11) supple-

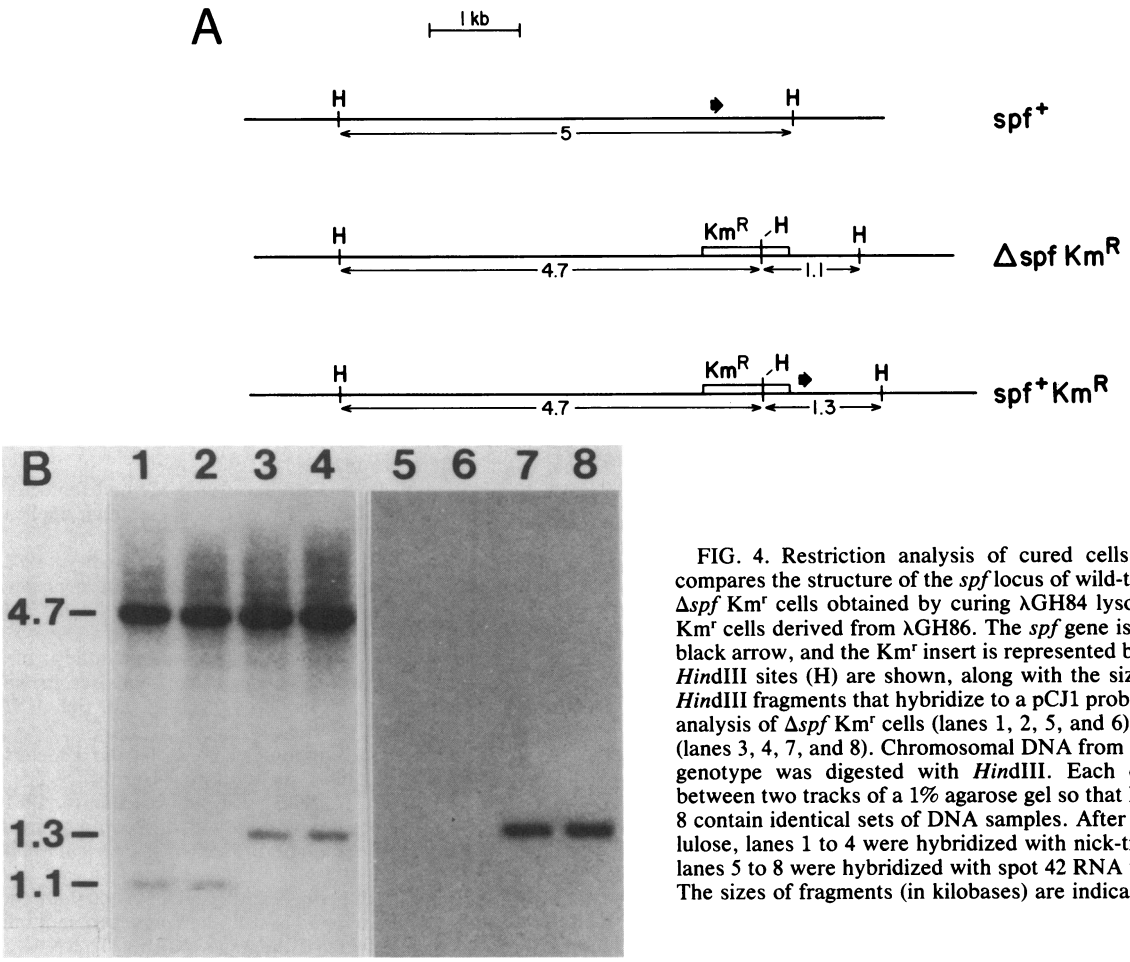

FIG. 4. Restriction analysis of cured cells. (A) The diagram compares the structure of the *spf* locus of wild-type cells (*spf*$^+$), the Δ*spf* Km^r cells obtained by curing λGH84 lysogens, and the *spf*$^+$ Km^r cells derived from λGH86. The *spf* gene is represented by the black arrow, and the Km^r insert is represented by the boxed region. *Hin*dIII sites (H) are shown, along with the sizes (in kilobases) of *Hin*dIII fragments that hybridize to a pCJ1 probe. (B) Southern blot analysis of Δ*spf* Km^r cells (lanes 1, 2, 5, and 6) and *spf*$^+$ Km^r cells (lanes 3, 4, 7, and 8). Chromosomal DNA from two isolates of each genotype was digested with *Hin*dIII. Each digest was divided between two tracks of a 1% agarose gel so that lanes 1 to 4 and 5 to 8 contain identical sets of DNA samples. After transfer to nitrocellulose, lanes 1 to 4 were hybridized with nick-translated pCJ1, and lanes 5 to 8 were hybridized with spot 42 RNA transcribed in vitro. The sizes of fragments (in kilobases) are indicated.

mented with glucose or glycerol. We found no difference in colony size or morphology at 37°C or 42°C. However, when the plates were incubated at 23°C, CJ205 (Δ*spf*) gave smaller colonies than CJ200. The difference was most noticeable on L-broth plates, and amounted to about a twofold difference in colony diameter after 2 days of growth. We believe that the slower growth at 23°C is attributable to the *spf* deletion and not to a secondary mutation, since we observed the same result with several independent isolates of the CJ205 genotype obtained both by curing and by transduction. However, the effect was dependent on the CM4722 background. No difference in growth at 23°C was seen when we compared Δ*spf* Km^r and *spf*$^+$ Km^r derivatives of the other strains listed in Table 1. Since CM4722 is the only strain of this group that is F$^+$, we transferred the F factor from CM4722 to the Δ*spf* derivative of strain S165. The resulting strain was indistinguishable from its F$^-$ parent on plates at 23°C, ruling out any effect due to the F factor in CM4722. Likewise, transduction of CJ205 and CJ200 to *gal*$^+$ *bio*$^+$ failed to eliminate the difference.

We also compared the growth of CJ205 and CJ200 in liquid culture (L broth). At 37°C, there was no detectable difference in growth rate. However, CJ205 grew slightly more slowly than CJ200 at both 23°C and 42°C. The difference was most easily demonstrated by competition between CJ205 or CJ200 and the parent strain, CM4722, in a mixed culture (see Materials and Methods section). While the ratio of CJ200 to CM4722 cells stayed approximately constant throughout 10 generations at either temperature, the proportion of CJ205 cells decreased (typically from 50% at the start of the experiment to 25% after 10 generations). We suspect that the growth difference in liquid medium at 42°C may also be peculiar to the CM4722 background, since no such effect was seen in the S165 strain background. Moreover, the effect in liquid medium at 42°C was not accompanied by an observed difference in plating efficiency or colony morphology at the same temperature.

We tested a number of properties diagnostic for an alteration in membrane structure or function. The Δ*spf* mutation did not affect the sensitivity of cells to NaCl, EDTA, sodium dodecyl sulfate, or various antibiotics (carbenicillin, tetracycline, nalidixic acid, and rifampin), at either 37°C or 23°C. (Increased sensitivity to such compounds is characteristic of

TABLE 2. Curing of λGH84 lysogens[a]

Lysogen[b]	No. of cured cells		No. of phage	
	Km^r	Km^s	Km^r	Km^s
A1	70	18	15	47
A2	67	20	14	28
B1	16	74	28	8
B2	21	59	37	13

[a] This procedure is described briefly in the text and in more detail in reference 8.

[b] The structures of these lysogens are shown in Fig. 2.

strains having altered membrane permeability; see reference 3.) Δ*spf* did not affect the motility or chemotaxis of the strains CM4722, AW330 and 20SOK$^-$. The *spf* deletion was compatible with the multiple auxotrophic markers in strains JC1552, AB2557, and KL188, indicating that the *spf* gene product is not essential for transport of the relevant metabolites.

DISCUSSION

Our ability to construct strains having a deletion of *spf* shows that this gene is not essential for cell viability under the growth conditions used. Moreover, the frequency at which such deletions were obtained suggests that there is little or no selective bias against Δ*spf* cells. This observation is borne out by our subsequent comparison of isogenic Δ*spf* and *spf*$^+$ pairs of strains. The *spf* null mutation results in a slight impairment of growth only under certain conditions and in only one strain background that we have tested. This may indicate that the *spf* gene product is involved in a process that is required only under certain circumstances, perhaps because alternative pathways exist. Our results show that, even though a gene may be efficiently expressed and responsive to the metabolic state of the cell (as is *spf*), one cannot assume that the gene is essential, and this must be determined directly.

Our preliminary experiments have failed to provide any support for the hypothesis that an *spf*-encoded peptide may be a membrane component. However, since processes such as transport frequently have multiple pathways, we cannot rule out this possibility. For the present, the observed Spf$^-$ phenotype should be useful as a tool with which to examine *spf* point mutations (derived in vitro or in vivo). We hope that further study of Δ*spf* mutants will shed light on the basis for the phenotype and provide additional clues to the role of the *spf* gene in vivo.

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LITERATURE CITED

1. **Aono, R., M. Yamasaki, and G. Tamura.** 1978. Changes in composition of envelope proteins in adenylate cyclase- or cyclic AMP receptor protein-deficient mutants of *Escherichia coli*. J. Bacteriol. **136**:812–814.
2. **Bachmann, B. J.** 1983. Linkage map of *Escherichia coli* K-12, edition 7. Microbiol. Rev. **47**:180–230.
3. **Benson, S. A., and A. DeCloux.** 1985. Isolation and characterization of outer membrane permeability mutants in *Escherichia coli* K-12. J. Bacteriol. **161**:361–367.
4. **Cameron, J. R., S. M. Panasenko, I. R. Lehman, and R. W. Davis.** 1975. *In vitro* construction of bacteriophage λ carrying segments of the *Escherichia coli* chromosome: selection of hybrids containing the gene for DNA ligase. Proc. Natl. Acad. Sci. USA **72**:3416–3420.
5. **Guerrier-Takada, C., K. Gardiner, T. Marsh, N. Pace, and S. Altman.** 1983. The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell **35**:849–857.
6. **Joyce, C. M., D. M. Fujii, H. S. Laks, C. M. Hughes, and N. D. F. Grindley.** 1985. Genetic mapping and DNA sequence analysis of mutations in the *polA* gene of *Escherichia coli*. J. Mol. Biol. **186**:283–293.
7. **Joyce, C. M., and N. D. F. Grindley.** 1982. Identification of two genes immediately downstream from the *polA* gene of *Escherichia coli*. J. Bacteriol. **152**:1211–1219.
8. **Joyce, C. M., and N. D. F. Grindley.** 1984. Method for determining whether a gene of *Escherichia coli* is essential: application to the *polA* gene. J. Bacteriol. **158**:636–643.
9. **Joyce, C. M., W. S. Kelley, and N. D. F. Grindley.** 1982. Nucleotide sequence of the *Escherichia coli polA* gene and primary structure of DNA polymerase I. J. Biol. Chem. **257**: 1958–1964.
10. **Mallick, U., and P. Herrlich.** 1979. Regulation of synthesis of a major outer membrane protein: cyclic AMP represses *Escherichia coli* protein III synthesis. Proc. Natl. Acad. Sci. USA **76**:5520–5523.
11. **Miller, J. H. (ed.).** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Movva, R. N., P. Green, K. Nakamura, and M. Inouye.** 1981. Interaction of cAMP receptor protein with the *ompA* gene, a gene for a major outer membrane protein of *Escherichia coli*. FEBS Lett. **128**:186–190.
13. **Rice, P. W., and J. E. Dahlberg.** 1982. A gene between *polA* and *glnA* retards growth of *Escherichia coli* when present in multiple copies: physiological effects of the gene for spot 42 RNA. J. Bacteriol. **152**:1196–1210.
14. **Sahagan, B. G., and J. E. Dahlberg.** 1979. A small unstable RNA molecule of *Escherichia coli*: spot 42 RNA. I. Nucleotide sequence analysis. J. Mol. Biol. **131**:573–592.
15. **Sahagan, B. G., and J. E. Dahlberg.** 1979. A small unstable RNA molecule of *Escherichia coli*: spot 42 RNA. II. Accumulation and distribution. J. Mol. Biol. **131**:593–605.
16. **Shimada, K., R. A. Weisberg, and M. E. Gottesman.** 1973. Prophage lambda at unusual chromosomal locations. II. Mutations induced by bacteriophage lambda in *Escherichia coli* K-12. J. Mol. Biol. **80**:297–314.
17. **Utsumi, R., H. Tanabe, Y. Nakamoto, M. Kawamukai, H. Sakai, M. Himeno, T. Komano, and Y. Hirota.** 1981. Inhibitory effect of adenosine 3′,5′-phosphate on cell division of *Escherichia coli* K-12 mutant derivatives. J. Bacteriol. **147**:1105–1109.