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Identification and Characterization of Starvation-Regulated Genetic Loci in *Salmonella typhimurium* by Using Mu d-Directed *lacZ* Operon Fusions

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We used the technique of Mu d-directed *lac* operon fusion formation in an effort to identify loci in *Salmonella typhimurium* which are transcriptionally regulated by nutrient starvation conditions. We identified *lacZ* operon fusions in eight genetic loci, all of which exhibited increased transcription when starved for two or more of the following nutrients: nicotinate, phosphate, ammonium, glucose, and sulfate. The loci have been designated *stiA* to *stiH* for starvation-inducible loci. Mutations in two *sti* loci (*stiC* and *stiD*) significantly decreased cell viability during prolonged periods of nicotinate starvation. *stiA* and *stiD* are linked and map at 30 min. The *stiC*, *stiE*, *stiG*, and *stiH* loci mapped at approximately 77, 43, 88, and 56 min, respectively, on the *S. typhimurium* linkage map.

In nature, *Salmonella typhimurium* can encounter a variety of environmental conditions. Some of these conditions permit optimal or nearly optimal growth, whereas others can cause mild-to-severe metabolic physiological stress, creating feast-or-famine-like situations. It is reasonable to assume that physiological mechanisms have evolved to enable bacteria to survive periods of suboptimal growth conditions or physiological stress. Several different genetic systems which respond to various environmental stresses have been studied in *Escherichia coli*, including the heat shock regulon (16), the SOS regulon (27), and the phosphate starvation stimulon (28–30).

The phosphate starvation stimulon of *E. coli* appears to be analogous to the system described in this communication for *S. typhimurium*. Wanner and McSharry (30) identified several genes regulated under phosphate starvation conditions, which were designated *psi* for phosphate starvation inducible. The *psi* loci were classified into different genetic and physiological types on the basis of the extent of their regulation by known *pho* regulatory genes (*phoM*, *phoR*, and *phoB*) (29, 30).

Since glucose, ammonium, and phosphate are important for NAD biosynthesis and because NAD(P) participates in hundreds of enzymatic reactions (both anabolic and catabolic), it was reasoned that nicotinate (NA) starvation might result in numerous stress signals which, in turn, would trigger the induction of many genes associated with the maintenance or restoration of balanced growth. We report the identification of a system in *S. typhimurium* which appears to be analogous to the *psi* system of *E. coli* (30) but in which the initial selection was for NA starvation-inducible genetic loci. We identified and characterized, using Mu d-directed *lac* operon fusion construction (1, 2), eight loci which respond to starvation of two or more of the following: NA, phosphate, ammonium, glucose, and sulfate. We refer to the genes which make up this stimulon as *sti* for starvation inducible. The locus *stiA* was originally reported as *sinA* (6). The genetic designation has been changed to avoid potential confusion with a previously used mnemonic.

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MATERIALS AND METHODS

Bacterial strains, phage, and transductions. The strains used in this study were all derivatives of *S. typhimurium* LT-2 and are listed in Table 1. Transductions were performed by using a high-transducing derivative of *S. typhimurium* bacteriophage P22, P22 HT 105/1-*int* (3).

Culture media and chemicals. The following minimal basal media were used for starvation studies, depending on the growth condition being tested. These included minimal E medium (26) and modifications of minimal MOPS (morpholinepropanesulfonic acid) and minimal M9 media (15). The medium used for carbon source utilization studies was NCE medium (5). The complex media used were LB medium (5), NB medium, MacConkey agar medium, and MacConkey base medium. Antibiotics were present at the following amounts per milliliter: 30 μg of ampicillin, 10 μg of tetracycline, 50 μg of kanamycin, 1 mg of streptomycin. 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was used at a final concentration of 40 μg/ml.

Isolation of *sti-lac* fusion strains of *S. typhimurium*. An appropriate ampicillin-sensitive (Ap^s) strain was transduced with an HT lysate propagated on a strain lysogenic for either Mu d1 (1, 2) or Mu d1-8 (11) as described by Holley and Foster (9). Initial selection was for the transfer of Ap^r on LB medium containing ampicillin. Ap^r transductants were then replicated onto minimal E or MOPS medium supplemented with 0.4% glucose, 40 μg of X-Gal per ml, ampicillin, and either nonlimiting nicotinate (NA; 100 μM) or limiting NA (0.5 to 1.0 μM). Colonies which appeared to be a more intense blue on limiting NA medium than on nonlimiting NA medium were selected and purified for further testing of the starvation-inducible character. Derivatives which lacked P22 prophage were identified with the green medium of Chan et al. (3) and checked for sensitivity to P22 H5 phage.

Starvation conditions. To test the effects of NA, phos-

TABLE 1. Bacterial strains

Strain	Genotype[a]	Source or reference
JF235	Δ*nadA100*	
JF344	Δ*nadB103*	
JF420	Δ*nadA100 stiA1*::Mu dl(Ap^r *lac*)	6
JF428	Δ*nadA100 zde-6025*::Tn*10* (83% linked to *stiA*$^+$)	6
JF450	Δ*nadA100 stiB2*::Mu dl (Ap^r *lac*)	This work
JF519	Δ*nadA100 stiC4*::Mu dl (Ap^r *lac*)	This work
JF560	*nadA540*::Tn*10 leu-515*(Am) *su-19*	This work
JF595	*nadA540*::Tn*10 leu-515*(Am) *su-19 stiD5*::Mu dl-8	This work
JF704	Δ*nadA135 leu-515*(Am) *su-19 stiD5*::Mu dl-8	This work
JF715	Δ*nadB541*::Tn*10 supD10*	This work
JF746	Δ*nadA100 stiF11*::Mu dl	This work
JF759	Δ*nadA100 stiE9*::Mu dl (Ap^r *lac*)	This work
JF788	Δ*nadA100 zec-1914*::Tn*10* (90% linked to *stiE*$^+$)	This work
JF807	Δ*nadA100* Δ*stiA18*	This work
JF918	Δ*nadA100 zhg-1923*::Tn*10* (3% linked to *stiC*$^+$)	This work
JF1074	*nadB541*::Tn*10 supD10 stiG1060*::Mu dl-8 (Ap^r *lac*)	This work
JF1075	*nadB541*::Tn*10 supD10 stiH1061*::Mu dl-8 (Ap^r *lac*)	This work
JF1098	*pyrD95 zfg-1930*::Tn*10* (97% linked to *stiH*$^+$)	This work
JF1128	*stiG1060*::Mu dl-8 (Ap^r *lac*)	This work
JF1129	*stiH1061*::Mu dl-8 (Ap^r *lac*)	This work
JF1142	Δ*nadA100* Δ*stiB13*	This work
JF1145	Δ*nadA100* Δ*stiC15*	This work
JF1146	Δ*nadA100* Δ*stiE16*	This work
JF1148	Δ*nadA100 stiD5*::Mu dl-8 (Ap^r *lac*)	This work
JF1164	Δ*nadA100 stiG1060*::Mu dl-8 (Ap^r *lac*)	This work
JF1165	Δ*nadA100 stiH1061*::Mu dl-8 (Ap^r *lac*)	This work
JF1222	Δ*nadA100 stiA1*::Mu dJ	
SF26	*leu-am su-19*	D. Botstein
SF32	*pyrD2266*::Tn*10*	J. Roth
SF127	*pur884*::Tn*10*	J. Roth
SF129	*ilvA597*::Tn*10*	J. Roth
SF131	*thi550*::Tn*10*	J. Roth
SF142	*hisD9953*::Mu dl-8 (Ap^r *lac*)	J. Roth
FF20	*pyrC7 rpsL*/F′ ts*114 lac*$^+$ *zzf-21*::Tn*10* (orientation B)	J. Roth
FF21	*pyrC7 rpsL*/F′ ts*114 lac*$^+$ *zzf-22*::Tn*10* (orientation A)	J. Roth
SA464	*serA13 rfa-3058* (Hfr 117 cw)	K. Sanderson; 19
SA722	*serA15 pur-268* (Hfr 123 cw)	K. Sanderson; 19
MU25	Δ*nadB132 serA13 rfa-3058* (Hfr 117 cw)	This work
MU26	Δ*nadB132 serA13 pur-268* (Hfr 123 ccw)	This work
MU29	*serA15 pur-268 zec-1914*::Tn*10* (90% linked to *stiE*$^+$) (Hfr 117 ccw)	This work
MU37	*metP760 gal stiD5*::Mu dl-8 (Ap^r *lac*$^+$/F′ ts*114 lac*$^+$ *zzf-22*::Tn*10* (Hfr 25–34 cw)	This work
MU41	Δ*nadA100 zhg-1923*::Tn*10* (3% linked to *stiC*$^+$)/F′ ts*114 lac*$^+$ *zzf-21*::Tn*10* (Hfr 73–79 ccw)	This work
MU47	*stiG1060*::Mu dl-8 (Ap^r *lac*$^+$)/F′ ts*114 lac*$^+$ *zzf-22*::Tn*10* (Hfr 83–90 cw)	This work
MU48	*stiH1061*::Mu dl-8 (Ap^r *lac*$^+$)/F′ ts*114 lac*$^+$ *zzf-22*::Tn*10* (Hfr 52–60 ccw)	This work
MU52	Δ*nadA100 stiA1*::Mu dJ (Km^r *lac*$^+$)/F′ ts*114 lac zzf-22*::Tn*10* (Hfr 30–34 ccw)	This work

[a] cw, Clockwise; ccw, counterclockwise.

phate, ammonium, glucose, and sulfate starvation on *sti-lac* fusions, each fusion strain was grown overnight in 5 ml of nonlimiting medium. For NA, phosphate, glucose, and ammonium starvation, cells were grown overnight in minimal MOPS containing 0.4% glucose, 13.2 mM phosphate, 9.5 mM ammonium, 280 μM sulfate, and 100 μM NA (all nonlimiting concentrations). Before sulfate starvation, each strain was grown overnight in minimal M9 medium containing 0.4% glucose, 64 mM phosphate, 19 mM ammonium, 100 μM NA, and 280 μM sulfate (all nonlimiting concentrations). Subsequently, cultures were washed in 10 ml of MOPS buffer and suspended in 5 ml of the same.

Washed cells were then grown under nonlimiting and limiting (starvation) growth conditions. Cells were inoculated into nonlimiting MOPS or MOPS supplemented with either 0.5 μM NA for NA starvation or 0.132 mM phosphate for phosphate limitation. For sulfate starvation, cells were inoculated into nonlimiting M9 or M9 containing 2.8 μM sulfate (limiting) and 8 mM L-methionine. Methionine was added to decrease the severity of this starvation and permit some growth. Ammonium and glucose starvation followed growth under nonlimiting conditions (MOPS medium) to a cell density of 2×10^8 to 3×10^8 cells per ml. Cells were collected by centrifugation and suspended in MOPS containing either 0.08% glucose for glucose limitation or 0.6 mM ammonium plus 8 mM L-arginine for ammonium limitation (28). Pyrimidine, purine, amino acid, and thiamine starvation required uracil, adenine, isoleucine-valine-, or thiamine-requiring derivatives of *sti-lac* strains. Starvation for these nutrients was as described previously (6).

Growth in all cases was monitored as a function of the increase in optical density at 600 nm. β-Galactosidase activities were assayed as described by Miller (14).

Cell viability over prolonged starvation. The effects of *sti* mutations on long-term viability were determined by using appropriate parent strains and *sti* deletion or stabilized Mu d

insertion mutants. *sti* deletion mutations were constructed as Ts^+ Ap^s Lac^- strains by the method of Maloy and Roth (13). Stabilized Mu d1-8 insertions were constructed by transferring the Mu d1-8 insertion into a nonsuppressor background via P22 HT transduction.

Samples (2 ml) of overnight cultures (minimal E) were inoculated into 100 ml of limiting minimal E glucose containing 1 μM NA and grown at 30°C with aeration for up to 21 days. At designated time intervals, cell densities were recorded and 10-μl samples of each culture were removed and used to make serial 10-fold dilutions. Samples of appropriate dilutions were plated on LB agar and incubated overnight at 30°C. The number of colonies per plate was used to estimate viable counts for each culture. Data were then plotted as the log% of the maximum viability determined for each culture versus time (18).

Construction of Tn*10* insertions near *sti* loci. Tn*10* insertions near sti^+ loci were identified by crossing *sti*::Mu d fusion strains with an HT lysate propagated on a pool of cells containing random chromosomal Tn*10* insertions (5). Tc^r transductants were replicated to nonlimiting MOPS glucose plates containing 0.5 μM NA and tetracycline. Colonies which appeared white, indicating repair of a Mu d-*lac* insertion, were restreaked and purified for further study.

Mapping of various *sti* loci on the *S. typhimurium* genetic map. Three methods were used to map the *sti* loci on the *S. typhimurium* linkage map (20). The first involved transducing a known Hfr strain (19) to Tc^r with an HT lysate grown on a strain containing a Tn*10* insertion near a particular *sti* locus (see above). Subsequent Tc^r Hfr strains were purified and tested for the ability to repair known auxotrophic markers and to transfer Tc^r to a known F^- Sm^r recipient. Tc^r Hfr strains exhibiting a good ability to transfer desired markers were used to map the Tn*10* insertion, as well as to repair known auxotrophic mutations via interrupted mating experiments (19).

The second mapping method was that of Tn*10*-directed Hfr formation as described by Chumley et al. (4). For this, an F'ts *lac* Tn*10* plasmid was transferred via conjugation to a strain containing a Tn*10* insertion near a specific sti^+ locus. Temperature-resistant Lac^+ derivatives were isolated and used as Hfr donor strains. Alternatively, *lac*-directed Hfr formation was used to map some loci (13). This method is similar to the Tn*10*-directed Hfr formation method. However, instead of using the Tn*10* insertions present on the chromosome and plasmid as areas of homology, the *lac* sequences of a stabilized Mu d fusion (Mu d1-8 fusions in nonsuppressive backgrounds) and the *lac* region on the donor plasmid were used as areas of homology in the formation of subsequent ts^+ Tc^r Hfr strains.

RESULTS

Identification of starvation-inducible loci. NA-requiring strains (JF235, JF560, and JF715) were transduced with HT phage lysates propagated on either a Mu d1 or a Mu d1-8(Ap^r *lac*) lysogen of *S. typhimurium* as described in Materials and Methods. Initial screening was for the transfer of Ap^r on LB-ampicillin medium. Approximately 40,000 Ap^r colonies were screened for increased β-galactosidase production under NA starvation with X-Gal as an indicator of β-galactosidase activity. Colonies which appeared more intensely blue on minimal E glucose medium containing a limiting concentration of NA than on the same media containing a nonlimiting concentration of NA were selected for further study. All potential starvation-inducible (*sti*) operon fusions were then screened in vitro for NA starvation induction.

Characterization of *sti* loci based on expressions under various starvation conditions. Starvation-inducible fusion strains were subsequently characterized as to their expression under several starvation conditions including NA, phosphate, ammonium, sulfate, glucose, amino acid, purine, pyrimidine, and thiamine starvation. Table 2 presents the base β-galactosidase activities under nonlimiting conditions and the fold inductions obtained under NA, phosphate, ammonium, glucose, and sulfate limitation. Table 2 also presents the results of isoleucine-valine, adenine, uracil, and thiamine limitation of the appropriate derivative strains. The values represent the average activities calculated from at least three separate experiments run under similar conditions. The *stiB-lac* fusion (JF450) was induced under NA, phosphate, and glucose starvation to various degrees but was unaffected by ammonium or sulfate limitation. Induction ranged from approximately sevenfold under NA starvation to less than fourfold under glucose starvation. The *stiC-lac* (JF519) fusion was induced under NA, phosphate, ammonium, and glucose, but not sulfate, limitation to various degrees, ranging from 13-fold induction under phosphate and NA starvation to 3.3-fold induction under glucose starvation. The *stiD-lac* (JF595) fusion exhibited significant induction only under NA and phosphate starvation, showing consistent and reproducible induction ranging from 1.5- to 2-fold under both of these conditions. The *stiE-lac* (JF759) fusion was significantly induced under NA, phosphate, and ammonium limitation but not under glucose or sulfate starvation. Its induction ranged from approximately 2.6-fold

TABLE 2. Effects of nutrient limitations on starvation-inducible (*sti*) locus expression

sti-lac fusion	β-Galactosidase activity (nonlimiting)[a]	Fold increase in β-galactosidase activity after starvation for[b]:								
		NA	PO_4	Ammonium	Glucose	SO_4	Ile-Val	Uracil	Adenine	Thiamine
stiA	25	3.9	6.3	5.4	2.1	0.7	3.6	1.0	1.7	3.8
stiB	23	7.0	5.0	1.5	3.8	0.8	1.0	1.0	1.2	1.7
stiC	7.5	12.9	13.3	7.1	3.3	0.8	3.4	1.1	6.3	3.0
stiD	97	1.6	1.9	1.1	0.9	0.4	0.4	1.0	1.0	1.2
stiE	162	2.6	2.6	1.9	1.2	0.5	1.6	0.9	1.7	2.8
stiF	29	1.4	2.7	1.8	ND[c]	5.0	ND	ND	ND	ND
stiG	38	3.0	2.4	1.7	1.0	0.7	ND	ND	ND	ND
stiH	26	3.9	1.9	1.3	2.8	0.6	ND	ND	ND	ND

[a] Starvation conditions and β-galactosidase assays were as described in Materials and Methods. β-Galactosidase activity was measured in Miller units.
[b] *ilvA*::Tn*10* derivatives were starved for Ile-Val, *pyrD*::Tn*10* derivatives were starved for uracil, *purE*::Tn*10* derivatives were starved for adenine, and *thi*::Tn*10* derivatives were starved for thiamine.
[c] ND, Not determined.

under NA and phosphate starvation to about 1.9-fold under ammonium starvation. The *stiG-lac* (JF1074) fusion was consistently induced under NA, phosphate, and ammonium starvation but not under glucose or sulfate limitation. Induction ranged from 2.8-fold under NA limitation to just over 1.6-fold under ammonium starvation. The *stiH-lac* (JF1075) fusion showed consistent induction during NA, phosphate, and glucose starvation but did not show significant induction under either ammonium or sulfate limitation. Induction ranged from 3.9-fold under NA limitation to about 1.9-fold under phosphate starvation. None of these fusions were induced by sulfate limitation. One fusion strain (JF746) was identified initially as sulfate starvation inducible (fivefold). *stiF* was also expressed during phosphate (2.6-fold) and ammonium (1.8-fold) limitation (Table 2).

Several of the *sti-lac* fusion strains were further tested for the regulatory effects of purine (adenine), pyrimidine (uracil), amino acid (isoleucine and valine), and thiamine limitation. For Ile-Val starvation, an *ilvA*::Tn*10* insertion (SF129) was introduced via transduction into each of the *sti-lac* fusion strains to be tested so that each resulting strain required Ile-Val to grow. For adenine and uracil starvation, *purE*::Tn*10* (SF127) and *pyrD*::Tn*10* (SF32) insertions were similarly introduced into each of the *sti-lac* fusion strains. For thiamine starvation, a *thi*::Tn*10* insertion (SF131) was introduced.

The *stiB-lac* fusion was significantly induced only under thiamine limitation but not by any of the other secondary conditions tested, exhibiting a 1.7-fold induction, whereas the *stiD-lac* fusion was not significantly induced under any of the secondary starvation conditions tested. As noted for *stiA-lac* (6), both the *stiC-lac* and *stiE-lac* fusions were significantly induced under Ile-Val, adenine, and thiamine, but not uracil, starvation. The *stiC* induction ranged from 2.9-fold under thiamine limitation to greater than sixfold under adenine starvation. The *stiE* induction ranged from approximately 1.7-fold under both Ile-Val and adenine starvation to 2.8-fold under thiamine limitation.

Several NA starvation-inducible loci were found to be induced by NA limitation only. These genes were associated with NAD metabolism and were described earlier (10, 24).

Effect of carbon source shifts on *sti-lacZ* expression. Shifting cells from a preferential carbon source such as glucose to one that is more difficult to metabolize (i.e., an energy source downshift) could provide some insight into the mechanisms controlling some of these genes. Preliminary experiments on solid media suggested that *stiA*, *stiC*, *stiH*, and, to a lesser extent, *stiG* all respond to energy downshifts. These strains were subsequently examined in a more quantitative manner (Table 3). The data indicate that transcription of *stiA*, *stiC*, and *stiH* increased dramatically after shifts from glucose to acetate or succinate. As one might expect, all three of these loci also increased β-galactosidase activity in response to glucose starvation (Table 2). This correlation does not hold for all of the *sti* genes, however. For example, *stiB*, which does respond to glucose starvation, was not noticeably affected by energy downshift. Also, whereas *stiG* responded somewhat to energy downshift, it was unaffected by glucose starvation. Thus, whereas common signals probably occur after glucose starvation and carbon source downshift, there must be additional signals produced which are unique to each of these stresses. It is unlikely that catabolite or glucose repression is solely responsible for controlling the expression of these loci, since (i) these genes are regulated in minimal salts glucose medium, (ii) *relA* is involved in the responses of several *sti* loci, and (iii) the correlation between cAMP and classic glucose repression does not hold (data not shown). Point iii can be made by using *stiA* or *stiC*. Data on *cya*::Tn*10* derivatives of *stiA* or *stiC* suggest that cAMP is involved with maintaining repressed levels of expression (nonlimiting). However, growth on acetate and succinate when intracellular cAMP levels are high results not in decreased expression but in increased expression of both loci.

Effects of *sti* mutations on cell viability during prolonged NA starvation. Since the *sti* loci respond to a variety of starvation conditions, it seemed reasonable to assume that some of these genes might play a role in maintaining cell viability during prolonged starvation. *sti* deletion mutants or nonsuppressor strains containing *sti*::Mu dl-8 insertions (JF1142, JF1145, JF1146, and JF1148) and the parent strain (JF235) were inoculated into minimal E glucose containing 1 μM NA (limiting). Samples were removed periodically and treated as described in Materials and Methods. The results (Fig. 1) indicate that at least two of the *sti* loci participated in maintaining cell viability, at least over the 24-day period in which the experiment was run. Both the *stiD* and *stiC* mutations significantly affected viability over the period of the experiment (each starvation was conducted two or three times). The *stiD* mutation (JF1148) decreased viability as a function of the log% of the maximum viability achieved for each culture, down to approximately 0.29% as compared with 1.2% for the wild-type culture after a 24-day period. A *stiC* mutation caused an even more dramatic effect. After 24 days, the log% of the maximum viability of the *stiC* deletion (JF1145) culture was approximately 0.013%, compared with 1.2% for the wild-type cells. Thus, *stiC* and, to a lesser extent, *stiD* both appear to be involved with maintaining cell viability over prolonged starvation periods. The *stiC-lac* fusion mutation generated several additional phenotypic effects. Loss of *stiC* function caused the morphology of individual colonies to become wrinkled, resulting in an almost square appearance. This mutation also made the resulting strain very resistant to transduction by P22 HT phage as compared with its parent strain or a strain repaired for this insertion. Thus, the function of the *stiC* gene product appears to be pleiotropic in nature, affecting viability, colony morphology, and P22 sensitivity.

TABLE 3. Effect of carbon source on *sti-lac* expression

Strain (fusion genotype)	β-Galactosidase activity in[a]:			
	0.4% glucose	40 mM glycerol	0.4% acetate	0.6% succinate
JF420 (*stiA-lacZ*)	20.3	48.4	232.0	202.0
JF519 (*stiC-lacZ*)	4.9	11.6	88.0	84.8
JF1074 (*stiG-lacZ*)	54.6	60.0	84.9	53.7
JF1075 (*stiH-lacZ*)	23.2	87.5	188.0	278.5

[a] Cells were grown to logarithmic phase (optical density at 600 nm, 0.2) in NCE medium containing 0.4% glucose and centrifuged, and the pellet was suspended in an equal volume of NCE medium containing the carbon source indicated. Each culture was grown to an optical density of 0.6 to 0.7 before assay.

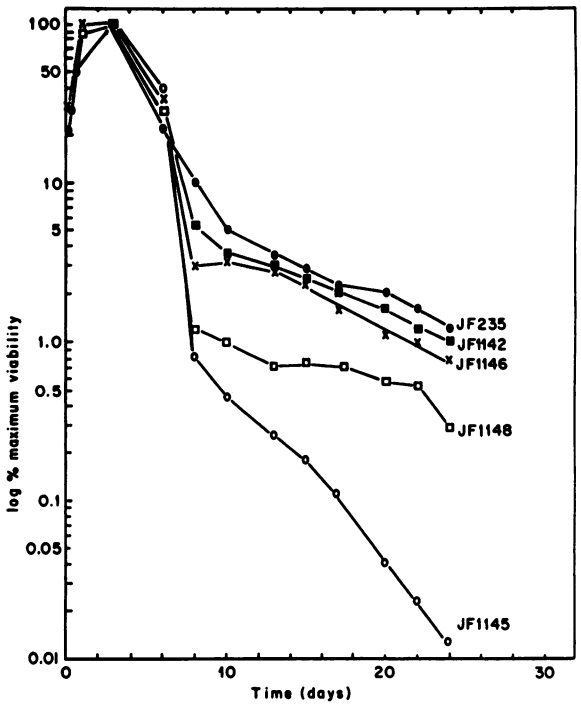

FIG. 1. Cell viability curves for wild-type and *stiB-stiE* mutant strains. Cultures were grown under limiting NA conditions as described in Materials and Methods. Cell viability was plotted as a function of the log% of the maximum viability achieved for each culture versus time in days. Symbols: ●, JF235 (wild type; parent strain); ■, JF1142 (Δ*stiB*); ○, JF1145 (Δ*stiC*); □, JF1148 (*stiD5*::Mu d1-8 *sup*$^+$); ×, JF1146 (Δ*stiE*).

Mapping of *sti* loci on the *S. typhimurium* linkage map. Several of the *sti* loci have been approximately mapped on the *S. typhimurium* linkage map (20). The *stiD*, *stiG*, and *stiH* genes were all mapped by using the technique of *lac*-directed Hfr formation (13), with JF991, JF1128, and JF1129 as recipient strains. One subsequent Hfr, MU37, was used to map *stiD* at approximately 30 min on the *S. typhimurium* linkage map (Fig. 2). Cotransduction experiments indicated that the *stiD5*::Mu d1-8 fusion is 80% cotransducible with a Tn*10* insertion (*zde-6025*::Tn*10*) located near *stiA*$^+$ (JF428; reference 6).

The Hfr strain MU47 was used to map the *stiG1060*::Mu d1-8 fusion in the region of 83 to 90 min on the linkage map, and MU48 was used to map the *stiH1061*::Mu d1-8 fusion in the region between 52 and 60 min on the *S. typhimurium* linkage map (Fig. 2). By this technique, both the *stiD-lac* and *stiH-lac* fusions were determined to be transcribed in the clockwise direction (Fig. 2). The *stiG-lac* fusion was found to be transcribed in the counterclockwise direction (Fig. 2). Construction of the *lac*-directed Hfr strain MU52 revealed that transcription of the *stiA* locus is clockwise.

The *stiC* locus was mapped by using the technique of Tn*10*-directed Hfr formation (4). For this, a Tn*10* strain (JF918) containing an insertion near *stiC*$^+$ was isolated and used to construct the Hfr strain MU41 as described in Materials and Methods. This Hfr strain was used to map the *stiC* locus in the region between 73 to 79 min on the *S. typhimurium* linkage map (Fig. 2).

The *stiE* locus was mapped by moving a Tn*10* insertion found to be 90% cotransducible with *stiE*$^+$ (JF788) to two known Hfr strains, SA722 and SA464 (19). Subsequent Tc^r Hfr strains exhibiting good ability to transfer both known auxotrophic markers and Tc^r to recipient strains were then used to map the Tn*10* insertion near *stiE*$^+$ via interrupted mating experiments as discussed in Materials and Methods. These experiments indicate that the location of the *stiE* locus is in the region between 40 and 45 min on the *S. typhimurium* linkage map (Fig. 2).

DISCUSSION

Unlike *Escherichia coli*, *S. typhimurium* is suited for long periods of nutrient deprivation such as those encountered in various environmental situations. This communication describes a systematic study of starvation-regulated gene expression in *S. typhimurium*. These studies with Mu d-*lacZ* operon fusion methodology, along with simultaneous studies using O'Farrell two-dimensional gel electrophoresis, help illustrate how *S. typhimurium* responds to various nutrient limitations (23).

Table 4 presents an overview of the physiological and genetic data. The *sti* loci were placed into five classes based on their response to the primary starvation conditions but, as is obvious after examination of the information in Table 4, there are overlapping controls between groups. For example, whereas the members of class I responded in an identical manner over all conditions, one member of class II responded to alternate carbon sources in a fashion more consistent with a class I gene. Table 4 also shows that several *sti* loci require *relA*$^+$ for full induction and *cya*$^+$ for full repression. The significance of this phenomenon is not apparent.

The *sti* genes and the *psi* loci (induced only under phosphate starvation; 7) may be analogous to the phosphate starvation-inducible (*psi*) stimulon of *E. coli* (17, 28–31). The phosphate starvation stimulon of *E. coli* was also shown to be affected by a variety of different starvation conditions, including phosphate, nitrogen (ammonium), and carbon (glucose) starvation. The loci were designated *psi* regardless of whether they were induced by other types of starvation, because their initial selection was based on phosphate starvation. We favor the *sti* designation since it reflects the general nature of the starvation response. It is preferable to

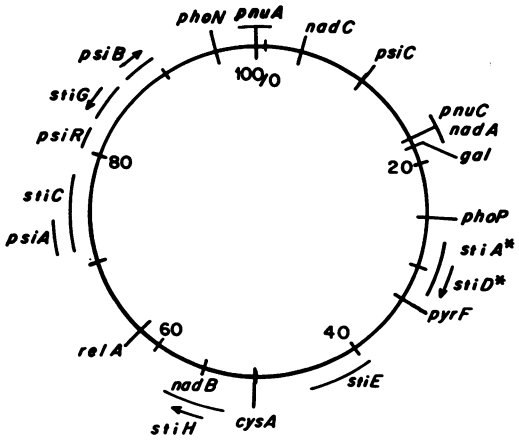

FIG. 2. Linkage map of *S. typhimurium*. Arcs represent the approximate map positions of the gene indicated. Arrows indicate the directions of transcription of the genes indicated. See the text for an explanation of the mapping procedures used for individual genes. An asterisk indicates that the map positions relative to adjacent markers is not known.

TABLE 4. Summary of physiological and genetic regulation of *sti* loci

Class and locus	Level of induction with the following[a]:												Effect on expression by[b]:	
	Primary condition					Secondary condition				Carbon source				
	NA	PO_4	Glucose	NH_4	SO_4	Ile-Val	Adenine	Uracil	Thiamine	Acetate	Glycerol	Succinate	*cya*	*relA*
I														
stiA	+	++	+	++	−	+	+	−	+	++++	+	++++	r	i
stiC	+++	+++	+	++	−	+	++	−	+	++++	+	++++	r	i
II														
stiB	++	++	+	−	−	−	−	−	+	−	ND[c]	−	r	i
stiH	+	+	+	−	−	ND	ND	ND	ND	++++	+++	++++	i	i
III														
stiE	+	+	−	+	−	+	+	−	+	−	−	−	r	o
stiG	+	+	−	+	−	ND	ND	ND	ND	−	−	−	ND	ND
IV *stiF*	−	+	−	+	++	ND	ND	ND	ND	−	ND	−	ND	ND
V *stiD*	+	+	−	−	−	−	−	−	−	−	ND	−	r	o

[a] +, Detectable induction; ++, moderate induction; +++, strong induction; ++++, very strong induction; −, no induction.
[b] r, Regulator involved in maintaining repressed levels during NA excess; i, regulator required for full induction during NA limitation; o, no effect.
[c] ND, Not done.

use designations such as *psi* when referring to genes which only respond to one condition.

Wanner (30) and Wanner and McSharry (31) have shown that several of the *E. coli psi* loci are under complete or partial control by the various *pho* regulatory loci (22, 25, 28). Schlesinger and Olsen (21) and Kier et al. (12) have reported that, when a plasmid-borne *phoA* (alkaline phosphatase) gene is placed into *S. typhimurium*, *phoA* is regulated by phosphate availability as in *E. coli*. This suggests that a type of phosphate regulation similar to that reported in *E. coli* may exist in *S. typhimurium*. However, no subsequent study characterizing such a system in this organism has been reported. Consequently, we are unable to evaluate the potential role that *pho* regulatory loci may have in controlling *sti* gene expression. The system we describe differs from the *E. coli* system dramatically as to the extent of induction for specific genes. Whereas the *S. typhimurium sti* loci are regulated 2- to 15-fold, the *psi* loci of *E. coli* are induced 10- to 200-fold. The difference could be that our initial selection involved NA starvation, which might uncover a set of genes which show only modest induction ratios. However, we have also performed experiments which initially screened for phosphate starvation-inducible genes (7). Several *S. typhimurium psi* genes were discovered, but none were affected by other forms of starvation. Studies on the synthesis of starvation-inducible proteins as detected by two-dimensional polyacrylamide gel electrophoresis have also been conducted in our laboratory (23). Evidence that the fusion studies can be correlated with the earlier protein studies was found in that the SIN-8 polypeptide was missing in all of the *stiA* mutants tested (data not shown).

Recent studies by Groat et al. (8) examined carbon starvation proteins in *E. coli* with findings similar to those obtained with *S. typhimurium* (23). As proposed by Groat et al. (8), the process of molecular realignment at the onset of starvation with both of these organisms may be analogous to what occurs during sporulation in bacilli. This is not surprising, since the physiological needs of starving organisms are much different from those of cells in balanced growth. It is also evident that not all *sti* products are essential for starvation survival, as noted for the *stiB* and *stiE* deletion mutants (Fig. 1).

The complexity of overlapping regulatory circuits which control the various *sti* loci is evident when the effects of different nutrient limitations on *sti* gene expression are noted. The results suggest that the promoter-operator regions of these genes respond to a variety of regulatory signals. What constitutes these regulatory signals remains unknown. The modest response of some of the fusions could reflect increased stability of specific messages during starvation rather than a direct effect on transcription. This would be an intriguing way for a cell to cope with starvation stress. The elucidation of specific starvation response mechanisms will be important, not only from an evolutionary standpoint but also for gaining insight into how other cells cope with starvation.

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