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Suppression of the Pleiotropic Effects of HisH and HisF Overproduction Identifies Four Novel Loci on the *Salmonella typhimurium* Chromosome: *osmH*, *sfiW*, *sfiX*, and *sfiY*

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Insertion mutations that suppress some or all the pleiotropic effects of HisH and HisF overproduction were obtained by using transposons Tn*10d*Tet and Tn*10d*Cam. All suppressor mutations proved to be recessive, indicating that their effects were caused by loss of function; thus, the suppressors identify genes that are necessary to trigger the pleiotropic response when HisH and HisF are overproduced. Genetic mapping of the suppressor mutations identifies four novel loci on the *Salmonella typhimurium* genetic map. Mutations in *osmH* (min 49) behave as general suppressors that abolish all manifestations of the pleiotropic response. Mutations in *sfiY* (min 83) suppress cell division inhibition and thermosensitivity but not osmosensitivity. Mutations that suppress only cell division inhibition define another locus, *sfiX* (min 44). A fourth novel locus, *sfiW* (min 19), is also involved in cell division inhibition. The phenotype of *sfiW* mutations is in turn pleiotropic: they suppress cell division inhibition, make *S. typhimurium* unable to grow in minimal media, and cause slow growth and abnormal colony and cell shape. The inability of *sfiW* mutants to grow in minimal medium cannot be relieved by any known nutritional requirement or by the use of carbon sources other than glucose. The hierarchy of suppressor phenotypes and the existence of epistatic effects among suppressor mutations suggest a pathway-like model for the His^c pleiotropic response.

Constitutive expression of the *Salmonella typhimurium* histidine operon causes growth inhibition at 42°C (24), wrinkled morphology of colonies grown in either 2% glucose or "green" plates (28, 42), and growth inhibition on plates containing three to five times the normal concentration of E salts (12, 37). The wrinkled-colony morphology is due to cell filamentation which is also observed in liquid cultures, especially if grown with a high concentration of glucose (25, 28, 42). This filamentation is *sulA* independent and unrelated to the SOS response (28).

The His^c pleiotropic response is caused by increased levels of proteins HisH and HisF (12, 25, 42). In the close relative *Escherichia coli*, a similar response is observed when HisH and HisF are overproduced (27). HisH and HisF catalyze the release of 5-amino-4-imidazole carboxamide riboside 5′-monophosphate (AICAR), which is also an intermediate in purine biosynthesis (43, 44, 53). Since AICAR is a potential precursor of the alarmone 5-amino-4-imidazole carboxamide riboside 5′-triphosphate (9, 46), several authors had proposed that increased synthesis of AICAR might be responsible for the pleiotropic effects of *his* overexpression (11, 22). However, AICAR does not seem to be involved in the pleiotropic response (25, 26).

We have addressed the identification of genes involved in the His^c pleiotropic response by the classical approach of studying intergenic (external) suppression (30). Mutations that suppress some or all the manifestations of the pleiotropic response identify four novel loci on the *S. typhimurium* chromosome: *osmH* (min 49), *sfiW* (min 19), *sfiX* (min 44), and *sfiY* (min 83). The hierarchy of suppressor phenotypes and the existence of epistatic effects in certain suppressor combinations suggest that the pleiotropic response of His^c mutants involves a complex network or pathway whose ultimate physiological significance remains unknown.

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MATERIALS AND METHODS

Bacterial strains, bacteriophages, and strain construction. The *S. typhimurium* strains used in this study, all derived from strain LT2, are listed in Table 1. Strain TR6753, carrying the attenuator deletion *hisO1242*, was used as the standard His^c strain (12). Strain *hisΔ3050*, obtained from J. R. Roth (Department of Biology, University of Utah, Salt Lake City), carries a complete deletion of the histidine operon. Transductional crosses using phage P22 HT 105/1 *int201* (44a, 49) were used for strain construction; the transducing phage will be henceforth referred as P22 HT. Phage sensitivity was tested by cross-streaking with the clear-plaque mutant P22 H5. To obtain phage-free isolates, transductants were purified by streaking on green indicator plates.

Plasmids and transposons. The episome F′128 *pro*$^+$ *lac*$^+$ *zzf-1836*::Tn*10d*Cam was described by Elliott and Roth (23). pNK972 (Amp^r) is a pBR333 derivative carrying the IS*10* transposase gene under the control of a *tac* promoter (41). pNK2880 contains the IS*10 tnpA*(*ats1 ats2*) gene expressed from a *tac* promoter; this plasmid contains a *lac*I^q allele and confers ampicillin resistance (35). The *ats1* and *ats2* mutations confer relaxed specificity to Tn*10* transposase (5). Tn*10d*Tet, Tn*10d*Cam, and Tn*10d*Kan are deletion derivatives of transposon Tn*10* that do not carry transposition functions (52). The *E. coli* episome F′128 *pro*$^+$ *lac*$^+$ *zzf-1831*::Tn*10d*Tet and the *S. typhimurium* episome F′152 *nad*$^+$ *zzf-1833*::Tn*10d*Kan were both obtained from J. R. Roth. Mud1-8(Amp Lac) (32) and MudI1734(Kan Lac) (13) are transposition-deficient Mu derivatives that generate operon fusions upon insertion. MudI1734(Kan Lac) (13) was renamed Mu*dJ* by Hughes and Roth (34).

Media, chemicals, and culture conditions. The E medium of Vogel and Bonner (51) supplemented with 0.2% glucose was used as minimal medium. In certain experiments, glucose was replaced by 0.2% fumarate. High-salt (3× E) plates contained three times the normal concentration of E salts (12). NCE medium is E medium without citrate. The rich medium was nutrient broth (NB; 8 g/liter; Difco) with added NaCl (5 g/liter). Solid media contained agar at a final concentration of 1.5%. Antibiotics were prepared and used as described by Maloy (38). For the selection of Tet^s derivatives of Tet^r strains, we used the medium of Bochner et al. (10) as modified by Maloy and Nunn (39). Green indicator plates were prepared as described by Chan et al. (14) except that methyl blue (Sigma) substituted for aniline blue. Nutritional supplements and auxanography pools were prepared as described by Davis et al. (19). Casamino Acids were used at concentrations of 0.2, 0.4, and 0.6%. Except when specifically indicated, cultures were incubated at 37°C. Liquid cultures were grown in a Gallenkamp orbital incubator at 160 rpm.

TABLE 1. *S. typhimurium* strains used

Strain	Genotype	Reference or source[a]
LT2	Wild type	Laboratory stock
PP1002	*cya-1091*::Tn*10 trpB223*	SGSC[b]
SA4247	*aroA554*::Tn*10*	SGSC
SMS409	*aspC409*::Tn*10*	SGSC
SV2056	*his*Δ*3050*/F′128 *lac*$^+$ *pro*$^+$ *zzf-1836*::Tn*10d*Cam	
SV2057	*his*Δ*3050*/pNK972	
SV2058	*hisO1242*/F′128 *lac*$^+$ *pro*$^+$ *zzf-1831*::Tn*10d*Tet	
SV2059	*hisO1242*/pNK2880	
SV2060	*hisO1242 osmH1*::Tn*10d*Cam	
SV2061	*hisO1242 osmH2*::Tn*10d*Cam	
SV2064	*hisO1242 osmH3*::Tn*10d*Tet	
SV2068	*hisO1242 sfiW1*::Tn*10d*Tet	
SV2069	*hisO1242 sfiX1*::Tn*10d*Tet	
SV2070	*hisO1242 sfiX2*::Tn*10d*Tet	
SV2071	*hisO1242 sfiY1*::Tn*10d*Tet	
SV2072	*hisO1242 sfiX3*::Tn*10d*Tet	
SV2073	*hisO1242 sfiW2*::Tn*10d*Tet	
SV2074	*hisO1242 sfiY2*::Tn*10d*Tet	
SV2082	*hisO1242 osmH4*::Tn*10d*Tet	
SV2090	*hisO*$^+$ *hisC9968*::MudJ	
SV2091	*hisO1242 hisC9968*::MudJ	
SV2092	*hisO*$^+$ *hisC9968*::MudJ *osmH1*::Tn*10d*Cam	
SV2093	*hisO1242 hisC9968*::MudJ *osmH1*::Tn*10d*Cam	
SV2129	*hisO1242 osmH3*::Tn*10d*Cam *sfiX2*::Tn*10d*Tet	
SV2130	*hisO1242 osmH3*::Tn*10d*Cam *sfiY1*::Tn*10d*Tet	
SV2131	*hisO1242 osmH3*::Tn*10d*Cam *sfiW1*::Tn*10d*Tet	
SV2132	*hisO1242 sfiW1*::Tn*10d*Tet *sfiX2*::Tn*10d*Kan	
SV2133	*hisO1242 sfiW1*::Tn*10d*Tet *sfiY1*::Tn*10d*Kan	
SV2134	*hisO1242 sfiX2*::Tn*10d*Kan *sfiY1*::Tn*10d*Tet	
SV2135	*hisO1242 osmH3*::Tn*10d*Cam *sfiW1*::Tn*10d*Tet *sfiY1*::Tn*10d*Kan	
SV2136	*hisO1242 osmH3*::Tn*10d*Cam *sfiW1*::Tn*10d*Tet *sfiX2*::Tn*10d*Kan	
SV2137	*hisO*$^+$ *hisIE9969*::Mud1-8	
SV2138	*hisO1242 hisIE9969*::Mud1-8	
SV2139	*hisO*$^+$ *hisIE9969*::Mud1-8 *osmH1*::Tn*10d*Tet	
SV2140	*hisO1242 hisIE9969*::Mud1-8 *osmH1*::Tn*10d*Tet	
SV2150	*hisO1242* DUP [*hisH9962* ∗MudP∗ *cysA1586*][c]	
SV2155	*hisO1242* DUP [*purE2154* ∗MudP∗ *purB1879*][c]	
TR6753	*hisO1242*	12
TT2242	*aroD5 hisW1824 purF145 rpsL metG319 zee-78*::Tn*10*	SGSC
TT7294	*zea-1032*::Tn*10*	SGSC
TT10423	*proAB47*/F′128 *pro*$^+$ *lac*$^+$ *zzf-1836*::Tn*10d*Tet	J. Roth
TT10425	*nadA56*/F′152 *nad*$^+$ *zzf-1833*::Tn*10d*Kan	J. Roth
TT10604	*proAB47*/F′128 *pro*$^+$ *lac*$^+$ *zzf-1836*::Tn*10d*Cam	J. Roth
TT13736	*eut-153*::MudJ	SGSC
TT15222	*leuA414* r$^-$ m$^+$ *fels2 thr-458*::MudQ	N. Benson
TT15225	*leuA414* r$^-$ m$^+$ *fels2 pyrA2413*::MudP	N. Benson
TT15228	*leuA414* r$^-$ m$^+$ *fels2 nadC220*::MudP	N. Benson
TT15233	*leuA414* r$^-$ m$^+$ *fels2 purE2155*::MudP	N. Benson
TT15234	*leuA414* r$^-$ m$^+$ *fels2 purE2155*::MudQ	N. Benson
TT15251	*leuA414* r$^-$ m$^+$ *fels2 hisH9950*::MudP	N. Benson
TT15252	*leuA414* r$^-$ m$^+$ *fels2 hisH9950*::MudP	N. Benson
TT15252	*leuA414* r$^-$ m$^+$ *fels2 aroC566*::MudP	N. Benson
TT15253	*leuA414* r$^-$ m$^+$ *fels2 cysA1586*::MudQ	N. Benson
TT15259	*leuA414* r$^-$ m$^+$ *fels2 nadB226*::MudP	N. Benson
TT15260	*leuA414* r$^-$ m$^+$ *fels2 nadB226*::MudQ	N. Benson
TT15262	*leuA414* r$^-$ m$^+$ *fels2 cysHIJ1574*::MudQ	N. Benson
TT15269	*leuA414* r$^-$ m$^+$ *fels2 ilvA2648*::MudP	N. Benson
TT15274	*leuA414* r$^-$ m$^+$ *fels2 purD1874*::MudP	N. Benson
TT17163	*leuA414* r$^-$ m$^+$ *fels2 hisH9962*::MudP	N. Benson

[a] Omitted for strains first described in this study.
[b] SGSC, Salmonella Genetic Stock Centre, University of Calgary, Calgary, Alberta, Canada.
[c] Nomenclature for duplications (DUP) follows the rules described in references 15 and 33.

Mutagenesis with Tn*10d*Cam and isolation of salt-resistant derivatives of a His^c strain. We used a variant of the nonhomologous transduction procedure described by Elliott and Roth (23). A lysate grown on strain SV2056 was used to transduce strain SV2057, selecting Cam^r Amp^r transductants. Transducing mixtures were made on NB plates and preincubated for 4 to 6 h at 37°C before replica plating to NB supplemented with chloramphenicol and ampicillin. Use of a recipient carrying the deletion *his*Δ*3050* prevented the insertion of the mini-Tn*10* in the histidine operon. Cam^r Amp^r transductants were replica plated more than three times to NB-chloramphenicol plates containing EGTA (10 mM); the latter was added to prevent reinfection (thus allowing the isolation of phage-free

TABLE 2. Loci involved in the pleiotropic effects of HisH and HisF overexpression

Locus	Map location (min)	Characteristics of mutants	Original alleles fully characterized	Other allele versions available[a]
osmH	49	Suppression of osmosensitivity, thermosensitivity, and cell division inhibition	*osmH1*::Tn*10d*Cam	Tet^r
			osmH2::Tn*10d*Cam	Tet^r
			osmH3::Tn*10d*Tet	Kan^r, Cam^r
			osmH4::Tn*10d*Tet	None
sfiW	19	Suppression of cell division inhibition; inability to grow in minimal medium	*sfiW1*::Tn*10d*Tet	Kan^r
			sfiW2::Tn*10d*Tet	Kan^r
sfiX	44	Suppression of cell division inhibition only	*sfiX1*::Tn*10d*Tet	Kan^r
			sfiX2::Tn*10d*Tet	Kan^r
			sfiX3::Tn*10d*Tet	Kan^r
sfiY	83	Suppression of cell division inhibition and thermosensitivity	*sfiY1*::Tn*10d*Tet	Kan^r
			sfiY2::Tn*10d*Tet	Kan^r

[a] Not included in the strain list.

derivatives). Pools of 1,000 to 2,000 Cam^r colonies were made and lysed with phage P22 HT. The pools were then used to transduce strain TR6753; Cam^r transductants were selected on 3× E plates. The total number of Cam^r isolates pooled was around 60,000. To guarantee independence, only one Cam^r, salt-resistant isolate per pool was further studied.

Mutagenesis with Tn*10d*Tet. A lysate grown on strain SV2058 was used to transduce strain SV2059; the latter contains plasmid pNK2880 (35) to permit *trans* complementation of the defective Tn*10d*Tet element by ATS transposase. Transducing mixtures were directly made on NB plates and preincubated for 4 h at 37°C. The cultures were then replica plated to NB plates supplemented with tetracycline and to appropriate media to detect suppression of the mutation *hisO1242*: 3× E plates to select salt-resistant derivatives, E plates incubated at 42°C to select thermoresistant isolates, and green plates to detect the presence of nonfilamenting (smooth) colonies. The total number of Tet^r isolates scored (all of independent origin) was around 110,000. Candidates were made phage free on green plates and used to transduce TR6753. This second transduction allowed the isolation of plasmid-free Tet^r derivatives.

Transposon substitutions. Lysates grown on strains TT10604, TT10423, and TT10425 were used for transposon substitutions. These strains carry F-prime plasmids containing Tn*10d*Cam, Tn*10d*Tet, and Tn*10d*Kan (Table 1). The lysates were irradiated with UV light, using a 15-W Sylvania lamp at a distance of 30 cm during 30 s; irradiation of the phage suspensions can be expected to increase recombination in the recipients (45). Transductions selecting the incoming marker (Tet^r, Cam^r, or Kan^r) were carried out. Transductants were then scored for loss of the resident marker (Tet^r or Cam^r). These procedures allowed us to obtain allele variants tagged with different antibiotic resistance genes. Strains carrying allele variants are not included in Table 1; the allele variants available are listed in Table 2.

Rapid mapping with Mud-P22 prophages. We followed the procedure described by Benson and Goldman (6), using 67 "locked-in" Mud-P22 prophages. In addition to the 54-lysogen collection described in reference 6, we used 13 additional Mud-P22 lysogens (strains TT15222, TT15225, TT15228, TT15233, TT15234, TT15251, TT15252, TT15253, TT15259, TT15260, TT15262, TT15274, and TT17163, all obtained from Nick Benson, Department of Biology, University of Utah). These additional lysogens (but not the original collection described in reference 6) are included in Table 1.

Cotransductional mapping. Upon transduction with phage P22 HT, transductants were replica plated to suitable plates to score transduction of unselected markers. Compatible marker combinations were made possible by using different versions (Tet^r, Kan^r, or Cam^r) of the alleles to be mapped. Cotransduction frequencies are averages of more than four independent crosses, scoring at least 300 transductants from each cross. The relative order of markers was determined by three-factor crosses. Cotransduction frequencies below 1% were not taken into account (38). Table 1 contains the original strains carrying markers cotransducible with the loci studied (TT7294, TT15253, SA4247, SMS409, TT2242, PP1002, and TT15269) but not their derivatives constructed by adding one of the alleles to be mapped; such constructions were required to perform certain three-factor crosses. Strains used in unsuccessful cotransductional mapping attempts are also omitted in Table 1.

Complementation analysis. Complementation was achieved by the construction of merodiploid strains (2, 15, 31). Duplications with predetermined endpoints were obtained by using a strategy adapted from reference 33.

β-Galactosidase assays. Levels of β-galactosidase were assayed as described by Miller (40), using the $CHCl_3$-sodium dodecyl sulfate permeabilization procedure. To measure *his* operon expression by using transcriptional *lac* fusions at *hisC* and *hisIE*, cultures were grown in E medium containing 0.2% glucose and 0.1 M histidine; overnight cultures were diluted into fresh medium to reach an optical density at 540 nm (OD_{540}) of 0.4 to 0.6.

Osmosensitivity assays in liquid medium. All assays were carried out at 37°C with shaking. Cultures were preadapted for >8 h in E medium containing 0.3 M NaCl; the cultures were then diluted to obtain an initial OD_{540} of 0.05. Growth was monitored by nephelometric measurements with a Spectronic 200 spectrophotometer. Osmoprotection was achieved in the presence of 0.5 mM proline (17).

Thermosensitivity assays. All assays were carried out in E medium. After overnight preincubation at 30°C, the cultures were diluted to an OD_{540} of 0.05 and transferred to a shaking water bath prewarmed at 42°C. Growth was monitored by nephelometric measurements as described above.

UV survival assays. Overnight cultures made in NB were diluted 1/10 in the same medium. When the cultures reached an OD of 0.5, the cells were harvested and resuspended in E buffer (E medium without glucose). Five-milliliter aliquots were transferred to sterile, empty petri dishes. Irradiation was achieved by opening the plates under a 15-W Sylvania UV lamp at a distance of 30 cm in the absence of daylight illumination. Cell suspensions were stirred during irradiation. After serial dilution in foil-covered tubes, irradiated cultures were plated on NB.

Microscopic observation of cultures. Aliquots of exponentially growing cells in E medium containing 2% glucose were extracted and transferred to slides coated with a thin layer of agar (21). Cells were photographed with a Zeiss Ultraphot microscope under phase-contrast optics, using a Kodak Pan film. Photographs were developed with Kodak Technidol liquid developer.

RESULTS

Suppression of salt sensitivity by insertion of mini-Tn*10* elements. After mutagenesis with either Tn*10d*Tet or Tn*10d*Cam, salt-resistant derivatives of the His^c strain TR6753 were isolated on 3× E plates containing either tetracycline or chloramphenicol. Salt-resistant isolates appeared at a frequency of 1 per ~3,000 inserts tested. To be classified as suppressor mutations, the insertions were required to pass the following tests.

(i) After more than two rounds of purification, the isolates carrying putative suppressors were lysed with phage P22 HT; the lysates were then used to transduce the parental strain TR6753, selecting either Tet^r or Cam^r. Antibiotic-resistant transductants were replica plated to 3× E plates. Complete (100%) linkage between the transposon-encoded antibiotic resistance and the suppressor phenotype (salt resistance) was taken as evidence that suppression was caused by the insertion mutation.

(ii) The lysates described above were also used to transduce strain *his*Δ*3050*. His$^+$ transductants were replica plated to 3× E plates; salt sensitivity indicated inheritance of the mutation *hisO1242*.

(iii) To confirm that the suppressor phenotype was caused by the transposon insertion (and not by another mutation located elsewhere in the genome), reconstruction experiments were carried out. First, the transposon insertion was transduced to strain *his*Δ*3050*, selecting the transposon-encoded antibiotic resistance. The resulting transductants were then transduced to His$^+$ with a lysate grown on the His^c strain TR6753. The presence of the suppressor was confirmed because all of the

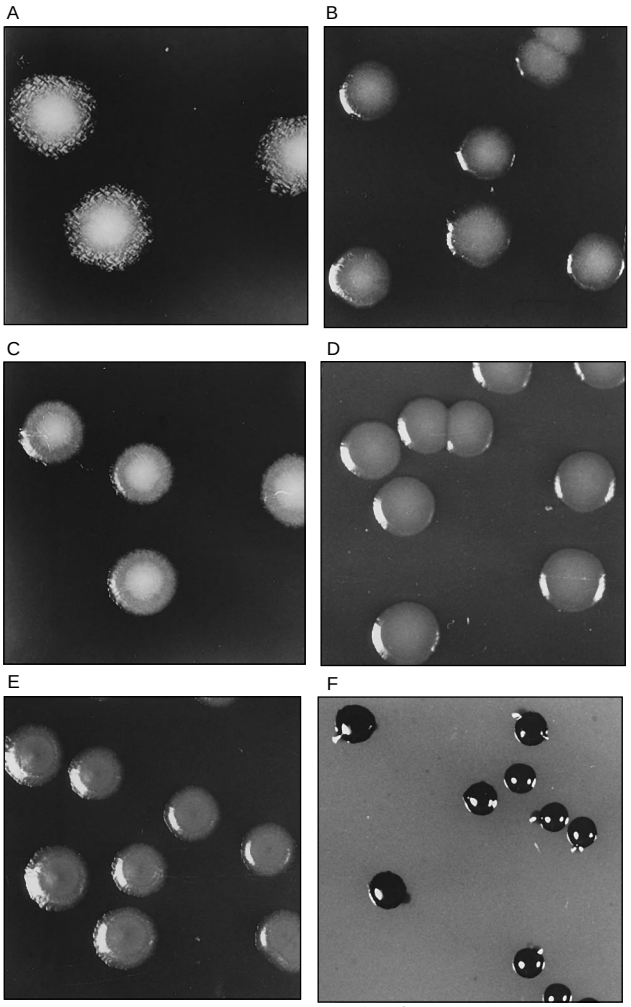

FIG. 1. Colonies of *S. typhimurium* HisO$^+$, HisO^c, and suppressor-containing derivatives of the latter, all grown on green plates. (A) TR6753 (*hisO1242*); (B) LT2 (*hisO*$^+$); (C) SV2064 (*hisO1242 osmH3*::Tn*10d*Tet); (D) SV2069 (*hisO1242 sfiX1*::Tn*10d*Tet); (E) SV2071 (*hisO1242 sfiY1*::Tn*10d*Tet); (F) SV2068 (*hisO1242 sfiW1*::Tn*10d*Tet).

His$^+$ Tet^r or His$^+$ Cam^r transductants were salt resistant. As a control, when the same lysates were used to transduce strain *his*Δ*3050*, all of the transductants were salt sensitive.

Eleven (seven Cam^r and four Tet^r) salt-resistant isolates of independent origin were used for further study. All proved to be thermoresistant on E plates incubated at 42°C, suggesting that osmosensitivity and thermosensitivity had been simultaneously suppressed by a single insertion mutation. Moreover, these salt-resistant isolates were smooth on green plates (although their colony morphology was slightly different from that of the parental strain: compare Fig. 1B and C). Microscopical observation of salt-resistant isolates grown in E liquid cultures containing 2% glucose confirmed that the insertions studied were able to suppress cell division inhibition, although short filaments were still formed (see Fig. 4C).

Suppression of thermosensitivity by insertions of Tn*10d*Tet. To ascertain whether suppression of thermosensitivity was always associated with high-salt resistance, thermoresistant derivatives of strain TR6753 were isolated by mutagenesis with Tn*10d*Tet and plating on E medium at 42°C (prewarmed). Thermoresistant isolates appeared at a frequency of 1 per 4,000 Tet^r inserts. All (four of four, independent) proved to be salt resistant, thereby confirming that osmosensitivity and thermosensitivity had been simultaneously suppressed. These isolates were smooth on green plates, like the salt-resistant isolates described above.

Insertions that suppress osmosensitivity, thermosensitivity, and cell division inhibition are alleles of a single locus. To ascertain whether the suppressor alleles belonged to one or more loci, recombination tests were carried out by transduction and replica printing. To assay every allele combination, transposon substitutions were performed. Two insertions were considered allelic whenever the resident allele was excluded from the recombinants (e.g., when >99% of the Tet^r transductants were no longer Cam^r). These tests indicated that all general suppressors that abolish salt sensitivity, thermosensitivity, and cell division inhibition are alleles of a single locus. Henceforth, this locus will be called *osmH* (for osmosensitivity caused by HisH and HisF overproduction). Further work was carried out with the following alleles: *osmH1*::Tn*10d*Cam, *osmH2*::Tn*10d*Cam, and *osmH3*::Tn*10d*Tet (isolated as salt resistant) and *osmH4*::Tn*10d*Tet (isolated as thermoresistant).

Suppression of cell division inhibition by Tn*10d*Tet insertions. For the detection of insertions suppressing cell division inhibition, Tn*10d*Tet inserts were obtained on strain TR6753. Tet^r transductants were replica plated to green plates supplemented with tetracycline, to give 300 to 500 colonies per plate. The plates were scored for the presence of smooth colonies; these appeared at a frequency of 1 per ~2,000 Tet^r isolates. The initial sample included 47 independent smooth isolates; 14 proved to be P22 resistant and were discarded. The remaining putative suppressor-containing isolates were subjected to tests similar to those described above, to correlate the presence of the insertion with the suppressor phenotype and to prove that the parental strain still contained the mutation *hisO1242*.

None of the smooth isolates formed filaments in E liquid medium containing 2% glucose. However, these isolates belonged to three classes, on the basis of cell and colony morphology, nutritional requirements, and the ability to suppress other manifestations of the pleiotropic response.

Class I isolates form small, smooth, dark colonies on green plates (Fig. 1F). They are unable to grow on minimal medium (either E or NCE). This inability to grow in minimal medium hampers the study of thermosensitivity and osmosensitivity, because HisO^c strains grown in rich medium are osmoresistant and thermoresistant (data not shown). If insertions of this class are transferred to the wild-type strain LT2, the inability to grow in liquid medium and the abnormal colony shape are likewise observed. Thus, class I insertions cause these defects on their own, irrespective of the genetic background of the strain (His$^+$ or HisO^c). The growth defect of SfiW$^-$ mutants is not relieved (i) with any single component of an auxanography pool, (ii) with any pairwise combination of the nutrients of the auxanography test of Davis et al. (19), (iii) with any concentration of Casamino Acids (0.2, 0.4, or 0.6%), or (iv) with fumarate as a carbon source alternative to glucose.

Class II isolates form large, smooth, light colonies on green plates (Fig. 1E). They are prototrophic and thermoresistant but osmosensitive.

Class III isolates form large, smooth, light colonies on green plates (Fig. 1D). They are prototrophic, thermosensitive, and osmosensitive.

Suppressors of cell division inhibition are alleles of three loci. Tn*10d*Tet insertions suppressing cell division inhibition were replaced with Tn*10d*Kan; allelic insertions were detected by transductional recombination as described above. These tests defined three groups of insertions, each corresponding to one of the classes described above (which, in turn, are not

allelic with *osmH* insertions). Thus, each class of insertions defines a distinct locus. These loci will henceforth be called *sfiW* (class I), *sfiY* (class II), and *sfiX* (class III). The original collection contained 10 *sfiW* alleles, 16 *sfiY* alleles, and 7 *sfiX* alleles; representative alleles of each class are listed in Table 2.

Genetic mapping of suppressors. For mapping, all Tn*10* insertions characterized as suppressors of one or more manifestations of the pleiotropic response were transferred to strain LT2; the resulting strains are not included in Table 1.

(i) Mapping of *osmH* alleles. Transductions with the locked-in lysate collection generated patches of Tet^s transductants when strains TT15258 and TT15253 were used as donors. The Mud-P22 prophages of these strains map at min 50 and 49, respectively. Lysates from TT15258 are packaged clockwise; the packaging direction of TT15253 is unknown. Cotransductional mapping confirmed that *osmH* is 1% linked to *cysA* and 5% linked to *zej-3271*::Tn*10*. In turn, *cysA* and *zej-3271*::Tn*10* are 92% linked. These results indicate that *osmH* lies on min 49; the marker order is *osmH-zej-3271*::Tn*10-cysA*.

(ii) Mapping of *sfiW* alleles. Dense patches of Tet^s transductants were obtained with locked-in lysates of strain TT15240, whose Mud-P22 prophage maps at min 21.5 and packages counterclockwise. Cotransductional mapping indicated that *sfiW* is 95% cotransducible with *aroA* and 7% cotransducible with *aspC*. The linkage between *aroA* and *aspC* is 3%. Thus, *sfiW* maps on min 19, and the marker order is *aroA-sfiW-aspC*.

(iii) Mapping of *sfiX* alleles. Tet^s transductants were obtained with lysates from TT15249 and TT17163, which package clockwise from min 40.5 and 42, respectively. Cotransductional mapping indicated that *sfiX* is 11% linked to *zee-78*::Tn*10* and 3% linked to *metG*. In turn, *zee-78*::Tn*10* and *metG* are 77% cotransducible. Thus, *sfiX* maps on min 44, and the marker order is *sfiX-zee-78*::Tn*10-metG*.

(iv) Mapping of *sfiY* alleles. Lysates from TT15268 and TT15269, mapping at min 83 and packaging clockwise and counterclockwise, respectively, gave thick patches of Tet^r transductants. A lysate from TT15271 (min 84, counterclockwise) gave a faint transduction patch. These data indicated that the *sfiY* locus maps around min 83 on the *S. typhimurium* chromosome. Cotransductional mapping confirmed that *sfiY* is 25% linked to *ilvA* and 72% linked to *cya*. The linkage between *ilvA* and *cya* is 6%; thus, *sfiX* lies between *ilvA* and *cya* on min 83.

Effect of osmolytes on the growth of a His^c strain and its mutant derivatives carrying suppressor mutations. The salt-resistant phenotype of suppressor-containing derivatives of TR6753 is not observed only on 3× E plates. With other osmolytes tested (NaCl, D-xylose, and D-sorbitol), clear differences were observed between the parental His^c strain and its *osmH* derivatives.

(i) When osmosensitivity was assayed on NCE plates, the following MICs were found for the His^c strain: 0.3 M NaCl, 0.6 M D-xylose, and 1 M D-sorbitol. To prevent the use of D-xylose and D-sorbitol as carbon sources (29), the plates contained 0.2% glucose. The HisO$^+$ strain and the His^c strains carrying an *osmH* suppressor still grew (slowly) on 1 M NaCl, >1 M D-xylose, and >1 M D-sorbitol.

(ii) Growth curves obtained in the presence of 0.3 M NaCl are shown in Fig. 2A. Growth of the HisO^c strain was severely inhibited, while the growth curves of *hisO1242 osmH* derivatives were nearly identical to that of the HisO$^+$ strain. Thus, mini-Tn*10* insertions at *osmH* completely suppress the osmosensitivity of His-constitutive strains. In Fig. 2A, one of the strains shown carried an *osmH* allele (*osmH4*::Tn*10d*Cam) isolated as a thermoresistant suppressor, while another strain carried the allele *osmH3*::Tn*10d*Tet, isolated as osmoresistant;

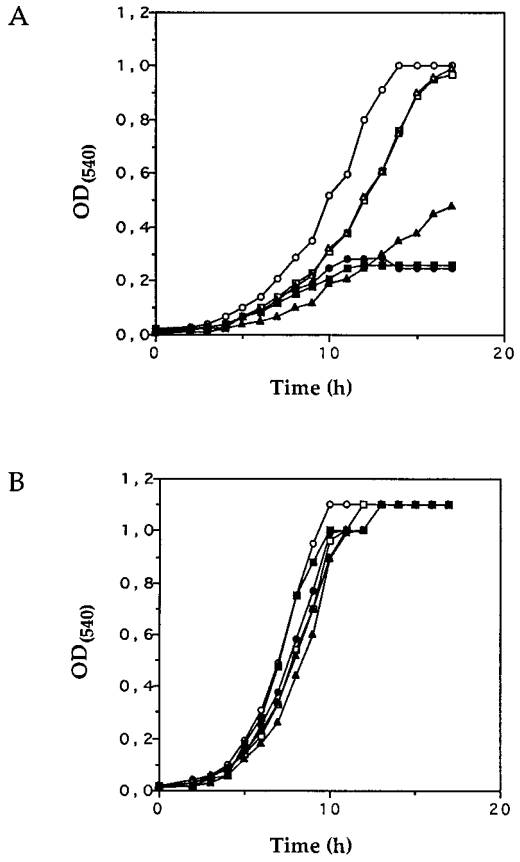

FIG. 2. Growth curves of *S. typhimurium* HisO$^+$, HisO^c, and suppressor-containing derivatives of the latter, all grown in 3× E liquid medium (A) and in 3× E liquid medium supplemented with 1 mM proline (B). ○, LT2 (*hisO*$^+$); ●, TR6753 (*hisO1242*); △, SV2082 (*hisO1242 osmH4*::Tn*10d*Tet); □, SV2064 (*hisO1242 osmH3*::Tn*10d*Tet); ▲, SV2069 (*hisO1242 sfiX1*::Tn*10d*Tet); ■, SV2071 (*hisO1242 sfiY1*::Tn*10d*Tet).

both curves are identical. Osmosensitivity was not suppressed by *sfiX* and *sfiY* alleles. Because HisO^c strains are not osmosensitive in rich media (data not shown), *sfiW* alleles could not be tested for suppression of osmosensitivity. As an internal control, we tested the ability of the HisO^c strain TR6753 and its derivatives carrying *osmH*, *sfiX*, or *sfiY* alleles to grow under low-osmolarity conditions (E liquid medium). All growth curves were similar to that of the wild-type (data not shown). These experiments confirmed that *osmH* alleles specifically suppress the inability of HisO^c strains to grow in high-osmolarity media.

(iii) Osmosensitivity of the HisO^c strain and its derivatives containing *sfiX* and *sfiY* alleles was relieved by the presence of proline 1 mM (Fig. 2B). The alleviating effect of proline on the osmosensitivity of HisO^c strains has been previously reported by Csonka (18).

Growth of the His^c strain and its suppressor-containing derivatives at 42°C. The HisO^c strain and its derivatives carrying *osmH*, *sfiX*, or *sfiY* alleles grew normally in E medium at 30°C, and their growth curves were similar to that of an isogenic HisO$^+$ strain (data not shown). In contrast, growth of the HisO^c strain was severely inhibited at 42°C, while its derivatives containing *osmH* or *sfiY* alleles were thermoresistant (Fig. 3). HisO^c SfiX$^-$ strains grew poorly, confirming that *sfiX* mutations do not suppress thermosensitivity (Fig. 3). Addition of proline to E medium did not relieve the thermosensitive phe-

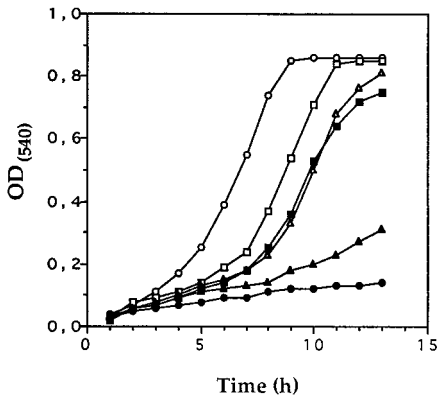

FIG. 3. Growth curves of *S. typhimurium* HisO$^+$, HisO^c, and suppressor-containing derivatives of the latter, all grown in E liquid medium at 42°C. ○, LT2 (*hisO*$^+$); ●, TR6753 (*hisO1242*); △, SV2082 (*hisO1242 osmH4*::Tn*10d*Tet); □, SV2064 (*hisO1242 osmH3*::Tn*10d*Tet); ▲, SV2069 (*hisO1242 sfiX1*::Tn*10d*Tet); ■, SV2071 (*hisO1242 sfiY1*::Tn*10d*Tet).

notype of the HisO^c strain and its HisO^c SfiX$^-$ derivatives (data not shown). Thus, thermosensitivity and osmosensitivity are two independent manifestations of the pleiotropic phenotype (and not two features of the same phenomenon).

Suppression by *osmH* alleles is unrelated to changes in expression of the histidine operon. All phenotypes of the pleiotropic response are relieved or abolished by *osmH* mutations. Thus, the effect of *osmH* mutations is formally similar to that of *cis*-acting mutations (e.g., p_1 promoter mutations) that reduce *his* transcription (12). OsmH$^-$ mutations cannot be active in *cis* because they are unlinked to the *his* operon and recessive (see below). Although the possibility that a recessive mutation could lower *his* transcription seemed a priori unlikely because *trans*-acting positive regulators of the p_1 promoter are not known to exist (53), we used transcriptional β-galactosidase fusions to measure *his* transcription levels in *osmH* mutants. Suppressor *osmH* alleles were introduced in isogenic *hisO*$^+$ and *hisO1242* strains that contained a MudJ insertion in *hisC*. While the presence of the attenuator deletion *hisO1242* caused a 10- to 15-fold difference in the β-galactosidase activity of the *lac* fusion *hisC9968*::MudJ, no significant difference was observed when the strains contained an *osmH* suppressor (Table 3). These results indicate that *osmH* mutations do not lower transcription from the p_1 promoter.

However, HisH and HisF are also produced from transcripts originating at the internal p_2 promoter (48). The weakness of this promoter allows suppression of the pleiotropic effects of HisH and HisF overexpression by polar mutations upstream p_2 (12), indicating that high levels of HisH and HisF products are not obtained in the absence of constitutive expression from p_1. However, processed mRNA species derived from the main *his* transcript are known to have increased stability (1). Thus mutations affecting the production and/or the stability of processed mRNAs containing the distal part of the operon can be expected to affect HisH and HisF levels. As a consequence, these (hypothetical) mutations might suppress the pleiotropic response. Since all processed mRNA species known include *hisIE* (1), we investigated the effect of *osmH* mutations on the activity of *hisIE9969*::Mud1-8 *lac* fusions. The results (Table 3) indicate that *osmH* mutations do not affect *hisIE* expression levels. Thus, the possibility that *osmH* mutations mimic suppression by lowering HisH and HisF expression seems unlikely.

TABLE 3. Activities of transcriptional *hisC*::*lac* and *hisIE*::*lac* fusions in OsmH$^+$ and OsmH$^-$ backgrounds

Strain	Genotype	β-Galactosidase activity (Miller units)
SV2090	*hisO*$^+$ *hisC9968*::MudJ	493
SV2091	*hisO1242 hisC9968*::MudJ	5,953
SV2092	*hisO*$^+$ *hisC9968*::MudJ *osmH1*::Tn*10d*Tet	445
SV2093	*hisO1242 hisC9968*:MudJ *osmH1*::Tn*10d*Tet	6,028
SV2137	*hisO*$^+$ *hisIE9969*:Mud1-8	52
SV2138	*hisO1242 hisIE9969*:Mud1-8	494
SV2139	*hisO*$^+$ *hisIE9969*::Mud1-8 *osmH1*::Tn*10d*Tet	55
SV2140	*hisO1242 hisIE9969*::Mud1-8 *osmH1*::Tn*10d*Tet	471

Effect of suppressor mutations on cell division. Strains LT2 (HisO$^+$), TR6753 [HisO^c], SV2064 [HisO^c OsmH$^-$], SV2069 [HisO^c SfiX$^-$], and SV2071 [HisO^c SfiY$^-$] were grown in E medium containing 2% glucose; strain SV2068 [HisO^c SfiW$^-$] was grown in NB containing 2% glucose. Photographs obtained from late-exponential-phase cultures are shown in Fig. 4. Division of the parental HisO^c strain is severely inhibited. Filamentation is not observed in its suppressor-containing derivatives, although short filaments seem to coexist with normal cells in strain SV2064 [HisO^c OsmH$^-$]. None of the *sfi* mutants forms filaments, but all show abnormal cell morphology; lysis might be occurring in the strains containing *sfiX* and *sfiY* suppressors, while HisO^c SfiW$^-$ cells are small and dark.

Suppression of cell division inhibition in HisO^c strains does not cause UV sensitivity. To explore the possibility that mutations that suppress HisO^c division inhibition can affect genes related to the SOS pathway, we determined the UV sensitivity of strains carrying *osmH*, *sfiX*, *sfiW*, and *sfiY* insertion mutations. No differences were observed between the suppressor-containing isolates and the parental His^c strain TR6753; in turn, the UV sensitivity of TR6753 was similar to that of the parental strain LT2 (data not shown). These results agree with earlier observations indicating that the division inhibition pathway triggered by HisH and HisF overexpression is unrelated to the SOS response (28).

Complementation of suppressor alleles and dominance studies. Although most insertion mutations can be expected to be recessive (7, 36), Tn*10* outward promoters can also activate adjacent genes (16, 50). Thus, we carried out complementation tests to ascertain whether the suppressor mutations studied are dominant or recessive.

(i) For complementation analysis of *sfiW* mutants, we constructed a duplication with endpoints at min 12 and 25, using a strategy adapted from that of Hughes and Roth (33). Two strains, each carrying a Mud-P22 prophage in the same orientation in *purE* (TT15235) and *purB* (TT15241), were lysed with P22 HT. The lysates were mixed to transduce strain TR6753, selecting Cam^r on minimal plates. Prototrophic Cam^r transductants can be expected to contain a duplication of the *purE-purB* region, with an hybrid locked-in Mud-P22 prophage in the middle. This assumption was confirmed because segregation in the absence of antibiotic selection yielded only Cam^s prototrophs (see reference 33). One isolate carrying one such Mud-held duplications between min 12 and 25 was propagated as strain SV2155. This strain was rough on green plates and showed all the additional manifestations of the pleiotropic response.

To examine whether *sfiW* mutations are dominant or recessive, a *sfiW*::Tn*10d*Tet allele was introduced into strain SV2155 by P22 HT transduction, selecting Tet^r. To maintain the duplication, chloramphenicol was also added to the selective plates. The resulting Cam^r Tet^r transductants contain one copy of the *sfiW*$^+$ allele and one *sfiW*::Tn*10d*Tet insertion. All Tet^r

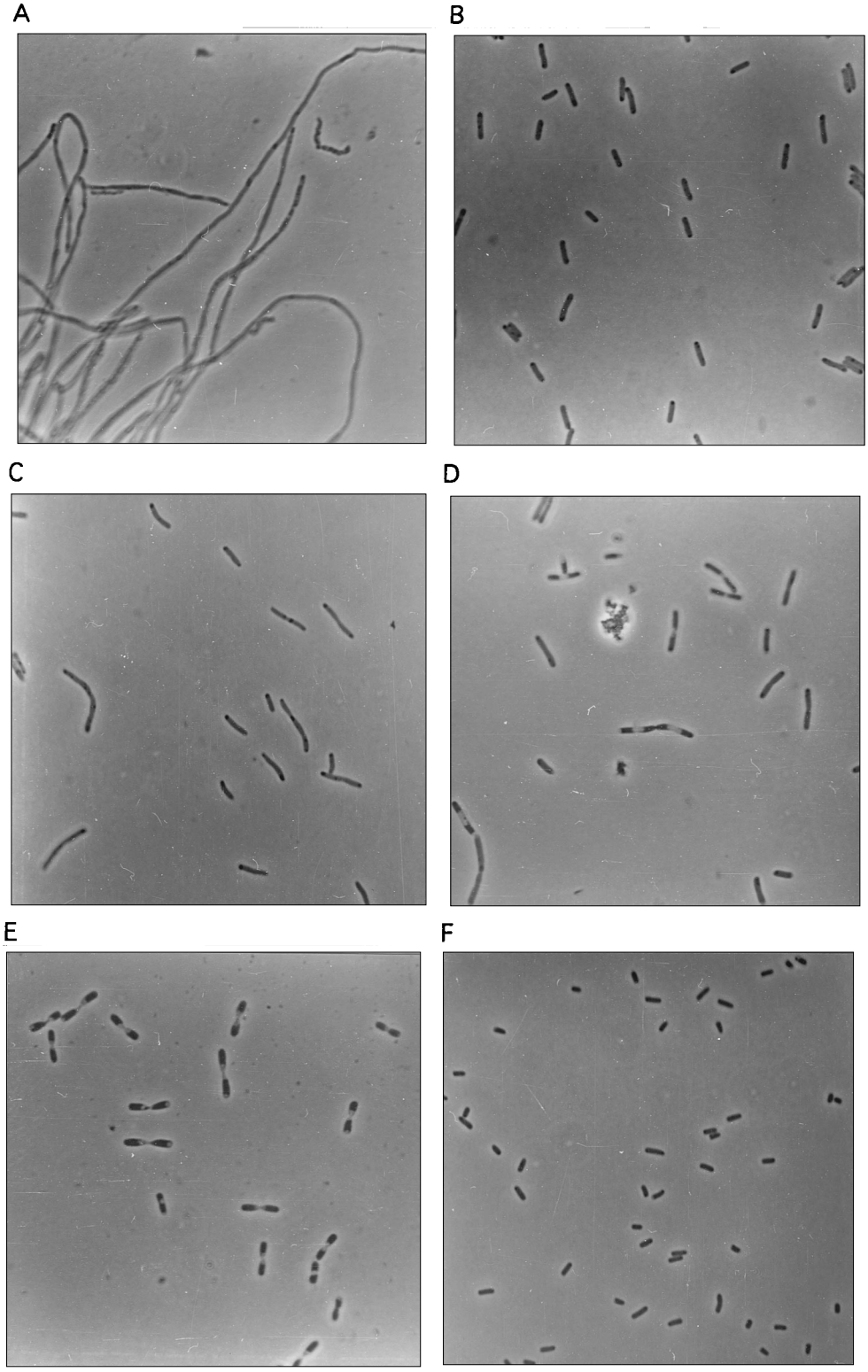

FIG. 4. Phase-contrast micrographs of late-exponential-phase cultures of *S. typhimurium* HisO$^+$, HisO^c, and suppressor-containing derivatives of the latter, all grown in liquid E medium containing 2% glucose. (A) TR6753 (*hisO1242*); (B) LT2 (*hisO*$^+$); (C) SV2064 (*hisO1242 osmH3*::Tn*10d*Tet); (D) SV2069 (*hisO1242 sfiX1*::Tn*10d*Tet); (E) SV2071 (*hisO1242 sfiY1*::Tn*10d*Tet); (F) SV2068 (*hisO1242 sfiW1*::Tn*10d*Tet).

Cam^r transductants were rough and light green, indicating that *sfiW* mutations are recessive. The presence of both *sfiW*$^+$ and *sfiW* alleles in the strain was confirmed by segregation: upon nonselective streaking on green plates, Cam^s isolates were obtained. These belonged to two types: rough, light green, Tet^s and smooth, dark, Tet^r.

(ii) For complementation analysis of *sfiX* mutants, we constructed a Mud-held duplication with endpoints in *hisH* (min 42) and *cysA* (min 50). The resulting strain (SV2150) was then used as the recipient of a *sfiX*::Tn*10d*Tet allele. All the Tet^r transductants were rough, indicating that *sfiX* mutations are recessive. The merodiploid nature of these transductants was confirmed by segregation as described above.

(iii) Complementation analysis of *osmH* and *sfiY* mutants was analyzed with a slightly different strategy. Since *osmH* is linked to *cysA*, both the suppressor and a linked *cysA*::MudP insertion can be cotransduced to a His^c recipient (TR6753). Simultaneous selection of prototrophy and the two incoming markers allows the isolation of rare transductants in which the transduced fragment has recombined with one copy of a preexisting duplication. The OsmH$^+$/OsmH$^-$ merodiploids obtained proved to be salt sensitive, thermosensitive, and rough on green plates, indicating that *osmH* mutations are recessive. Segregation of the merodiploids provided an internal control for the experiment.

Since *sfiY* is linked to *ilvA*, an analogous procedure was used to obtain SfiY$^+$/SfiY$^-$ merodiploids: the HisO^c strain TR6753 was transduced with a P22 HT lysate grown on a donor carrying an *sfiY* suppressor and a linked *ilvA*::MudQ insertion, selecting Ilv$^+$ Cam^r Tet^r transductants. The SfiY$^+$/SfiY$^-$ merodiploids obtained formed wrinkled colonies on green plates (and filaments in liquid medium) and were thermosensitive, indicating that *sfiY* mutations are recessive.

Combinations of suppressor mutations. Taking advantage of the allele variants harboring different mini-Tn*10* insertions (Tn*10d*Tet, Tn*10d*Cam, and Tn*10d*Kan), we carried out transductional crosses to construct His^c strains containing more than one suppressor mutation. The relevant conclusions from these experiments are as follows.

(i) All allele combinations are viable.

(ii) The hierarchy of phenotypes defined by single suppressors remains unaltered in double- and triple-suppressor mutants; for instance, *osmH sfiX*, *osmH sfiY*, and *osmH sfiX sfiY* strains are osmoresistant and thermoresistant and do not form filaments. In turn, *sfiX sfiY* mutants are thermoresistant and do not form filaments.

(iii) The cell shape of *osmH sfiX* and *osmH sfiY* mutants resembles that of *osmH* strains (compare Fig. 5 with Fig. 4C). Thus, the cell morphology conferred by *osmH* alleles is epistatic over those of *sfiY* and *sfiX*. The same conclusion applies to colonial morphology (data not shown).

(iv) When *sfiY* and *sfiX* alleles are combined, both the colony shape and the cell morphology of the double-suppressor mutants remain like those of the parental strains, which are in turn hard to distinguish (Fig. 1 and 4D and E). These results do not clarify whether the phenotypes conferred by *sfiX* and *sfiY* mutations are epistatic or additive. However, the fact that *sfiX sfiY* mutants are thermoresistant indicates that *sfiX* mutations are not epistatic over *sfiY*.

Altogether, these observations suggest a model in which the pleiotropic response involves the hierarchical expression of *osmH*, *sfiY*, and *sfiX* (Fig. 6). The observation that *sfiX sfiY* mutants are thermoresistant places *sfiX* below *sfiY* (and not in a parallel position).

The existence of *sfiW* suppressors complicates the situation. When combined with *osmH*, *sfiX*, or *sfiY* alleles, the resulting double- and triple-suppressor mutants do not grow in minimal medium and show the colony and cell shape typical of SfiW$^-$ mutants. Thus, the *sfiW* effects on growth pattern, cell shape, and colony morphology are epistatic over those of *osmH*, *sfiX*, and *sfiY*. The growth defect caused by *sfiW* alleles does not allow testing HisO^c OsmH$^-$ SfiW$^-$ and HisO^c SfiX$^-$ SfiW$^-$ strains for suppression of thermosensitivity and osmosensitivity; thus, the consequences (if any) of combining *sfiW* alleles with *osmH* and *sfiY* cannot be analyzed.

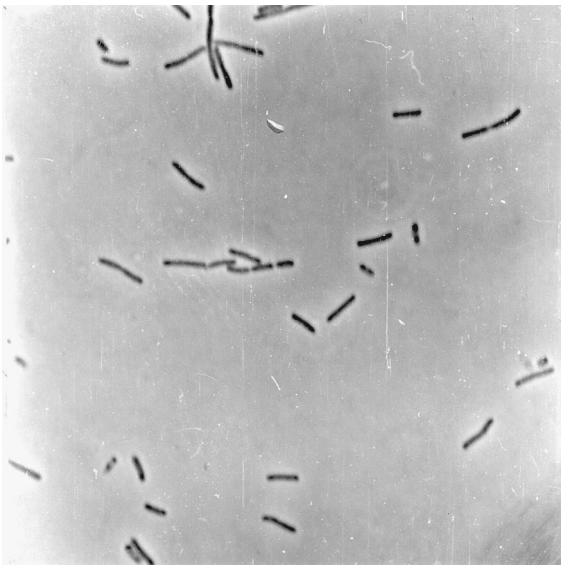

FIG. 5. Phase-contrast micrograph of a late-exponential-phase culture of strain SV2129 (*hisO1242 osmH3*::Tn*10d*Cam *sfiX2*::Tn*10d*Tet) grown in liquid E medium containing 2% glucose.

DISCUSSION

This report describes insertion mutations that suppress one or more manifestations of the His^c pleiotropic response. All of the suppressor mutations are recessive; therefore, they identify genes that trigger the pleiotropic response when HisH and HisF are overproduced. With the exception of *sfiW* alleles,

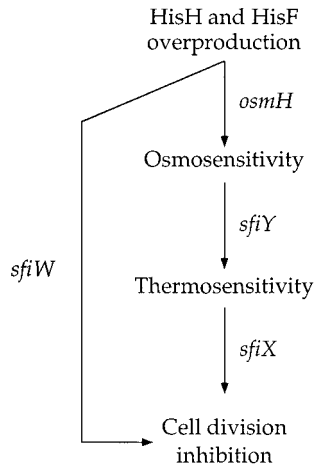

FIG. 6. A pathway-like model for the His^c pleiotropic response, based on the hierarchy of suppressor mutations and on the epistatic effects observed among certain suppressor phenotypes.

none of the suppressors studied confers a recognizable phenotype to the wild type.

Insertions in *osmH* (min 49) suppress osmosensitivity, thermosensitivity, and cell division inhibition. Insertions in *sfiY* (min 83) suppress thermosensitivity and cell division inhibition (but not osmosensitivity). Insertions in *sfiX* (min 44) suppress cell division inhibition only. The general suppression exerted by *osmH* insertions might indicate that the pleiotropic response triggered by HisH and HisF overexpression has a single cellular target, the product of the *osmH* gene. However, the existence of partial suppressors, the hierarchy of their phenotypes, and the existence of epistatic effects among the suppressors suggest that the pleiotropic response is a pathway which can be interrupted at different stages (Fig. 6).

The existence of a fourth type of suppressor, *sfiW*, adds further complexity to the picture. Certain phenotypes of *sfiW* mutations are epistatic over those of *osmH*, *sfiY*, and *sfiX*, suggesting that *sfiW* might be placed above *osmH* in the tentative pathway of Fig. 6. Alternatively, *sfiW* might suppress filamentation by a mechanism unrelated to the pathway defined by *osmH*, *sfiY*, and *sfiX*. The latter explanation seems more reasonable because the distinct phenotypes conferred by *sfiW* mutations (bizarre morphology of cells and colonies, inability to grow in minimal medium) are likewise observed in a $HisO^+$ background. On this rationale, *sfiW* has been placed outside the *osmH-sfiY-sfiX* pathway drawn in Fig. 6.

None of the suppressors studied causes UV sensitivity; thus, the corresponding wild-type genes must be involved in an SOS-independent process of filament formation. This assumption agrees with the previous observation that the pleiotropic effects of HisH and HisF overproduction are SOS unrelated and *sulA* independent (28). In *E. coli*, SOS-independent division inhibitors are encoded by the *minB* operon (8, 20). The map positions of the *osmH*, *sfiW*, *sfiX*, and *sfiY* loci on the *S. typhimurium* chromosome are all different from that of the *E. coli minB* operon (4). Thus, the cellular functions that cause filamentation upon HisH and HisF overproduction seem to identify novel loci involved in an SOS-independent process of cell division inhibition. The uniqueness of the His^c pleiotropic response (and thus of the scores used for suppressor isolation) may explain why novel genes putatively related to cell division have been detected in our hunts.

The list of suppressors of the His^c pleiotropic response described in this report may be incomplete, first because all of the suppressors described are insertion mutations, and thus any essential genes that might be involved in the His^c response would be overlooked in our hunts, and second because use of ATS transposase can be expected to broaden Tn*10* target specificity, but target preferences must still exist (5). This may explain why one type of mutation which has been previously shown to suppress the pleiotropic response of His^c mutants (*envB* [3]) has not been found among the suppressors described in this work.

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