Genetic Analysis of Temperature-Sensitive Lethal Mutants of *Salmonella typhimurium*

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Manuscript received January 13, 1989
Accepted for publication August 30, 1989

ABSTRACT

We have isolated 440 mutants of *Salmonella typhimurium* that show temperature-sensitive growth on complex medium at 44°. Approximately 16% of the mutations in these strains have been mapped to 17 chromosomal locations; two of these chromosomal locations seem to include several essential genes. Genetic analysis of the mutations suggests that the collection saturates the genes readily mutable to a ts lethal phenotype in *S. typhimurium*. Physiological characteristics of the ts lethal mutants were tested: 6% of the mutants can grow at high temperature under anaerobic conditions, 17% can grow when the medium includes 0.5 M KCl, and 9% of the mutants die after a 2-hr incubation at the nonpermissive temperature. Most ts lethal mutations in this collection probably affect genes required for growth at all temperatures (not merely during high temperature growth) since Tn*10* insertions that cause a temperature-sensitive lethal phenotype are rare.

CONDITIONAL lethal mutants have provided the means to genetically study mutations in essential genes. Extensive temperature-sensitive lethal mutant collections exist in bacteriophage T4 (WOOD and REVEL 1976), *Escherichia coli* (KOHIYAMA *et al.* 1966; SEVASTOPOULOS, WEHR and GLASER 1977), and *Saccharomyces cerevisiae* (KABACK *et al.* 1984; PRINGLE and HARTWELL 1981), while recessive lethal collections exist in *S. cerevisiae* (GOEBL and PETES 1986), *Drosophila melanogaster* (LEFEVRE and WATKINS 1984; JUDD, SHEN and KAUFMANN 1972) and *Caenorhabditis elegans* (BRENNER 1974). The mutations in these collections have allowed the genetic analysis of essential functions such as DNA replication, the cell cycle and cell division, membrane biogenesis, transcription, translation and development.

We have collected 440 temperature-sensitive lethal mutants of *Salmonella typhimurium*. This enteric bacterium is a particularly favorable organism for genetic analysis of such mutations, because there exists a large collection of mapped transposable elements (SANDERSON and ROTH 1988), an efficient generalized transducing phage P22 (SCHMIEGER 1972), and a library of partially mapped cloned fragments (KUKRAL *et al.* 1987). These tools allow the rapid mapping of mutations, as well as the easy re-isolation of mutations in unmutagenized backgrounds. Many genes capable of yielding ts lethal mutations are known in *E. coli*, as well as in *S. typhimurium*. The virtual identity of the genetic maps of these two related enteric bacteria allows the use of genetic information from both bacteria in the preliminary analysis of these ts mutations.

Only a specific subset of bacterial genes can mutate to a temperature-sensitive lethal phenotype. The gene products must be required for growth on a solid complex medium and mutable to thermolability. A large subset of enteric genes encode proteins for the biosynthesis of metabolites, utilization of nutrients for carbon or nitrogen sources and formation of surface structures or features and are not required for growth on a rich medium in the laboratory (BACHMANN 1983; SANDERSON and ROTH 1988). In addition, genes whose role or function is required, but for which another gene can substitute (for example in transport functions, see FURLONG 1987), will not yield mutations showing a conditional lethal phenotype.

The conditional lethal collection described here should be a useful starting point for relatively labor-intensive screens to identify mutations in essential functions of enterics. We estimate that the existing set of mutants comes close to saturating the potential targets for such mutations in *S. typhimurium*. In several other organisms, the number of such genes also appears to be surprisingly small, with estimates of 250 and 1200 for *S. cerevisiae*, 5000 for *D. melanogaster*, and 2000 for *C. elegans*. Although a large number of ts mutations have been previously collected in *E. coli* (ISONO, KRAUSS and HIROTA 1976), no estimate of the degree of genetic saturation in this collection has been documented.

MATERIALS AND METHODS

Strains: All bacterial stains are derivatives of *Salmonella typhimurium* LT2. Temperature-sensitive mutants in the range SE5026 through SE5261 are derived from LT2, and thus should be prototrophic. Temperature-sensitive mutants

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SE5292 through SE5497 were derived in the auxotrophic strain *leu-485*.

Media: Solid complex medium was LB medium (15 g agar, 5 g NaCl, 5 g yeast extract, 10 g Bacto-tryptone). Liquid complex medium is identical to solid medium, without the agar. Minimal medium E was used (VOGEL and BONNER 1956), supplemented with 0.2% glucose, and leucine when strain *leu-485* was used. In addition, minimal E plates were supplemented with methionine for use at 44°, since the wild-type strains show this requirement at 44° (though not at 42°). Green plates (CHAN *et al.* 1972) were used to score sensitivity to bacteriophage P22HT*105/1 int-201*. The NCE medium was used for diethyl sulfate (DES) mutagenesis (BERKOWITZ *et al.* 1968). Tetracycline was added at 20 μg/ml to LB plates. Bacterial strains are stored in 8% DMSO at −70°.

Anaerobic growth: Anaerobic growth conditions were created for solid media in a sealed chamber using a GasPak-Plus (BBL) system. LB plates were supplemented with 0.2% glycerol and 20 mM $NaNO_3$ for anaerobic growth.

Mutagenesis: DES mutagenesis was performed as described by ROTH (1970). Individual clones of the wild-type strain (either LT2 or *leu-485*) were inoculated into liquid LB medium and grown overnight with shaking at 37°. A 0.1-ml aliquot of the overnight culture was placed into 5 ml of NCE medium (lacking any carbon source); 50 μl of DES were added, the culture was vortexed briefly and incubated at 37° for 30 min. After this incubation, 0.1 ml of the mutagenized culture was diluted into 5 ml of LB broth, and incubated 18–36 hr at room temperature (20–23°). The mutagenized culture was then diluted and plated onto LB plates, generally with 50–300 colonies per plate. The plates were incubated at room temperature (18–23°) for 3–4 days, then replica printed to LB plates for incubation at 44° and room temperature. The replica prints were compared, and colonies showing weak or no growth at 44° were purified at least twice and retested for temperature sensitivity. To measure the extent of mutagenesis, the amount of cell death and the frequency of auxotrophic mutations were measured. Cell death varied between 90 and 99%, while the frequency of auxotrophic mutants varied between 1 and 6%. At this level of mutagenesis, between 0.01 and 0.25% of all colonies plated were identified as temperature-sensitive lethal mutants.

Mutant strains SE5026 through SE5144 contain some nonindependent ts mutants; mutant strains SE5145 through SE5497 are independently derived; each independent mutant arose from the mutagenesis of a culture grown from an individual colony.

The ts mutants were then grown in liquid LB broth at room temperature, and stored at −70° in 8% DMSO. The mutations are designated *clm*, for *c*onditional *l*ethal *m*utation, until their identity is further determined.

MNNG mutagenesis: Mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (Sigma) was performed by inoculating individual clones of *leu-485* into LB broth to form overnight cultures, then diluting 1:100 into LB broth to a density of OD_{600} = 0.2–0.5. MNNG (2.5 mg/ml in ethanol) was added to a final concentration of 62.5 μg/ml and incubation was continued at 37° for 10 min. After mutagenesis, cells were pelleted and washed twice with an equal volume of 50 mM Tris (pH 6) and resuspended in a fivefold excess of LB broth. Incubation with shaking continued overnight at room temperature. The cultures were diluted, plated onto LB plates to achieve 100–200 colonies per plate, incubated at room temperature and then replica printed at 44° and room temperature to identify ts mutants.

Isolation of linked Tn*10* insertions. Tn*10* insertions linked to ts mutations were identified by cotransduction of tetracycline resistance and thermoresistance. The protocol for generating a pool of 2000 strains carrying independent Tn*10* insertions is described by DAVIS, BOTSTEIN and ROTH (1980). Lysates of generalized transducing phage P22 were grown on the pooled Tn*10*-carrying strains. These lysates were used to transduce the ts mutants to tetracycline resistance. Replica prints identified strains showing coinheritance of tetracycline resistance and thermoresistance. Linkage of the Tn*10* insertion and ts mutation was verified in all cases by backcross experiments.

Transduction crosses: In all cases, transduction crosses and P22 phage sensitivity tests were performed with the P22 *HT105/1 int-201* derivative described by SCHMIEGER (1972) and ANDERSON and ROTH (1978). Transduction crosses were performed at multiplicities of infection between 0.1 and 10, with lower MOIs generally used for full plate transductions, and higher MOIs for spot tests.

Spot test transductions were achieved by transferring phage from microtiter dish wells with a multipronged inoculator onto a plate spread with an overnight culture of a ts mutant. Plates were incubated at either 30° (to assure the relative transducibility of strains) or at 44° to test cotransduction of temperature resistance and tetracycline resistance. All positive results from spot tests were repeated with full plate transductions. Using these methods, linkages of about 15% were the apparent limits of the resolution; such weakly linked Tn*10* insertions were sometimes positive and sometimes negative in spot tests, although by full plate transduction, such weak linkages were always detected. This places the calculated distance limits of these transduction crosses as 16 kb between the Tn*10* insertion and linked ts mutation (see below).

Modification of WU's model for genetic-physical distance correlation: WU (1966) provided a means to estimate the physical distance between two genetic markers, given cotransduction data. However, this model is valid for crosses involving two point mutations and must be modified for these experiments in which a point mutation and an insertion mutation are cotransduced. Thus, for cases in which the insertion is the selected marker, the probability of coinheriting the point site is given by:

$$\text{P(coinheritance)} = \frac{(k - s - m)^3}{(k - m)^3}$$

where k is the size of the transduced fragment (44 kb for phage P22), m is the size of the insertion (9.5 kb for Tn*10*), and s is the distance between the site of insertion and the site of the point mutation (SANDERSON and ROTH 1988).

Phenotype testing of ts mutants: Temperature sensitive lethal mutants were characterized for additional phenotypes that they might possess. Auxotrophic mutants failed to grow on E (or E + leu) plates at 30°. P22 resistance was scored by cross-streaking the strains against phage P22*HTint* on green plates (CHAN *et al.* 1972) at 30°, and scoring the color reaction indicative of phage sensitivity. Osmotic remedial mutants grew significantly better at 44° when streaked on LB + 0.5 M KCl than on LB plates. Anaerobically remedial mutants grew significantly better at 44° under anaerobic conditions (generated by a Gas Pak-Plus) than under standard aerobic conditions. The "killer" phenotype was scored by growing overnight cultures of the ts mutants at 21° in LB, diluting these cultures, and plating onto LB. "Killers" were strains showing greater than 70% loss of viability by incubation on LB plates at 44° for 2 hr (or 6 hr) compared with a control plate incubated at 30°. The "snake" morphology was determined by phase microscopy of cells after a shift from exponential growth in liquid LB at 30° to 44° for 2 hr. Cells longer than about four cell lengths were termed "snakes."

The leakiness of strains was scored by streaking the ts mutants from stab cultures onto LB plates, and incubating at 44°. Strains were scored non-leaky if no growth was seen after 24-hr incubation; slightly leaky if very weak growth; quite leaky if significant, but less than wild-type (9.3%), or approximately wild-type (6.7%) growth by this test.

Cytology of ts mutants: Phase contrast microscopy of temperature-sensitive mutants was performed using a Zeiss Photomicroscope III. This microscopy included staining with the fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) to observe the bacterial nucleoid. Detailed results will be described elsewhere. Cells were grown in LB broth at 30° for 1.5 hr to an approximate OD_{600} of 0.2. The temperature was shifted for 2–2.5 hr to 44°. Cells were treated with 3% toluene for 15 min at 37°, then 0.1 μg/ml DAPI was added, and microscopy of cells was performed. Microscope slides were pretreated with 1% polylysine for 15 min at room temperature, then rinsed with water. This treatment was found to slow movement of the cells significantly. Photographs of cells were made, and from these, cells longer than about four cell lengths were termed "snakes" for the purposes of Table 1.

TABLE 1

Characteristics of ts lethal mutants

Additional Phenotypes	Percent of ts mutants
Auxotrophic (415)	26.9
P22 resistant (195)	5.9
Osmotically remedial (440)	17.1
Anaerobically remedial (440)	6.3
"Killer"–6 hr (372)	31.8
"Killer"–2 hr (372)	8.7
"Snake" morphology (440)	16.9
Leakiness of mutants (420)	
Nonleaky	76.6
Slightly leaky	7.4
Quite leaky	16.0

[a] The total number of ts lethal mutants tested for each phenotype is given in parentheses. Phenotypes were tested as described in MATERIALS AND METHODS.

RESULTS

Characteristics of the temperature-sensitive mutants: Independent cultures of *S. typhimurium* LT2, or a strain carrying the auxotrophic mutation *leu-485*, were mutagenized with DES. To identify strains carrying temperature-sensitive mutations, a mutagenized culture was diluted and plated on LB plates at room temperature, then replica printed to LB plates at 44°. Strains unable to grow on LB plates at 44° were purified, retested, and saved, as described in MATERIALS AND METHODS. Increased numbers of colonies per plate decreased the frequency of both auxotrophic and ts mutants isolated, a phenomenon previously described by HOROWITZ and LEUPOLD (1951). The protocol used to isolate these mutants did not demand recovery from a temperature shift. Thus, mutations that cause death of the cells upon a temperature shift (rather than just cessation of growth) were recovered, and exist in the collection (see below).

In order to understand the general characteristics of the mutations that cause temperature sensitivity in this collection of mutants, these strains were tested for a variety of physiological traits. Some of these (auxotrophy and P22 phage resistance) seem likely to result from a mutation in addition to that causing temperature sensitivity. Other physiological traits seem likely to arise as a result of the particular temperature sensitive mutation. These results are listed in Table 1, and described below.

Auxotrophic mutations occur in about 27% of the ts lethal mutants. In a survey of 25 such strains, P22-mediated transduction to prototrophy did not remove the ts lethal mutation (data not shown). Thus, most of these mutants must carry two unlinked mutations, one causing the ts lethal phenotype, another causing the auxotrophy. The frequency of auxotrophs among the ts lethal mutants suggests that the mutants have suffered several lesions. Assuming that the number of genes capable of causing auxotrophy is about 200, and that about 4000 genes exist in *S. typhimurium*, the frequency of auxotrophs suggests that each strain must carry about 5 mutations in addition to the one causing the ts lethal phenotype.

Bacteriophage P22 resistance occurs in approximately 6% of the ts mutants. Most of the mutations causing P22 resistance probably do not cause thermolability, since many P22-resistant mutations are known to arise as null mutations in genes not required for growth in LB medium.

Some ts lethal mutations cause death of cells upon shift to the nonpermissive temperature. While a nonpermissive temperature shift causes some cell death in cultures of most ts mutants, the rate of cell death at the nonpermissive temperature varies greatly among different mutants (KOHIYAMA *et al.* 1966). Approximately 9% of the temperature-sensitive mutants show extensive cell death (>70%) after 2 hr at 44° ("2-hr killer" phenotype); 32% of the ts mutants show extensive cell death after a 6-hr incubation at 44° ("6-hr killer" phenotype) (Table 1) (A. SPRINGER and A. LUTTINGER, unpublished observations). However, the majority of the mutations in this collection cause bacteriostatic defects that are reversible after 2–6 hr of incubation at the nonpermissive temperature.

Growth conditions are known to suppress some conditional mutations. Previous studies in *S. cerevisiae* have shown that most temperature-sensitive nutritional mutants are phenotypically corrected by the addition of NaCl to the medium (HAWTHORNE and FRIIS 1964). In *S. typhimurium*, KOHNO and ROTH (1979) showed that all ts mutations in the *hisG* enzyme were also correctible by the addition of 0.2 M KCl or NaCl to the medium. In the collection of ts lethal mutants, only 17% show significantly improved growth at 44° when grown on LB plates containing 0.5 M KCl. Even fewer showed improved growth on 0.2 M KCl or NaCl. In addition, anaerobic growth was

found to suppress 6% of the ts lethal mutants. These mutants show significantly stronger growth at high temperature under anaerobic conditions than under aerobic conditions. The mutations in these strains may affect cellular functions required only for growth in aerobic environments. Alternatively, cell physiology under aerobic and anaerobic conditions may differ enough that some mutant proteins may function during anaerobic growth.

The mutant strains have been characterized cytologically, both through phase-contrast microscopy and fluorescence microscopy of DAPI-stained cells after 2 hr at the nonpermissive temperature. Approximately 17% of the mutants appear as elongated cells after this nonpermissive temperature incubation. These mutants probably harbor mutations similar to those in the *fts* genes of *E. coli*, which affect cell division processes (RICARD and HIROTA 1973). In addition, mutations that cause round, rather than rod-shaped cells, and other mutations that apparently cause altered nucleoid shapes and positions exist within this collection.

Finally, the relative growth of mutant strains on LB plates at 44° was tested. The colony sizes shown by mutants strains were scored on a relative scale as described in Table 1. Most of these strains (77%) appear to have severe growth defects when tested in this manner.

Linkage relationships among the ts mutations: Genetic linkage between mutations present in the ts collection can suggest whether the collection saturates the genes mutable to a temperature-sensitive phenotype. To determine saturation, a set of 19 ts mutants was chosen at random, and Tn*10* insertions were isolated nearby the temperature-sensitive mutations (see MATERIALS AND METHODS). P22-mediated transductional linkage was tested between these 19 Tn*10*s and 229 of the ts lethal mutations. Preliminary results were obtained by spot tests, and confirmed with full plate crosses to establish the linkage between the Tn*10* and ts mutations. These results are listed in Table 2. The data show that many mutations in the ts collection are linked to the same Tn*10* insertion. Overall, 28 conditional lethal mutations (*clm*) cluster into two linkage groups (*clmC* and *clmF*), and 34 mutations fall into 12 other linkage groups. Using a modification of WU's model for converting transductional linkage to physical distance (WU 1966; SANDERSON and ROTH 1988; see MATERIALS AND METHODS), the distance between each of the ts mutations and the corresponding linked Tn*10* insertion was calculated. Any two ts mutations that were calculated within 3 kb of each other were considered to affect the same gene. Using this criterion, the histogram in Figure 1 shows the distribution of the number of mutations that affect the same (or very closely linked) genes. The average of this distribution (excluding the two locations with large numbers of mutations) is 2.3. These results suggest that the 229 tested mutations saturate the genes readily mutable to a ts lethal phenotype in *S. typhimurium*.

Relative frequencies of mutant phenotypes: To achieve an independent estimate of the proportion of chromosomal genes mutable to ts lethal, the relative frequencies of temperature-sensitive lethal mutations and temperature-sensitive auxotrophic mutations were compared. DES mutagenesis of 15 independent cultures led to the identification of 75 mutants able to grow on minimal medium at 21°, but not on minimal medium at 44°. These mutants included both temperature-sensitive auxotrophs (able to grow in LB at 44°) and temperature-sensitive lethal mutants (unable to grow on LB at 44°). Of the 75 mutants, 26 ts lethal mutants (in 11 independent cultures) and 49 temperature-sensitive auxotrophs (in 13 independent cultures) were identified. From the relative frequencies of these mutation types, these experiments suggest that 1.9-fold more genes are mutable to ts auxotrophy than are mutable to ts lethality.

Alternative mutagenesis schemes: Mutagenesis with nitrosoguanidine yielded a separate collection of 60 temperature-sensitive mutants. Transductional linkage between these mutations and the set of 19 mapping Tn*10* insertions was tested. These results suggest that MNNG targets the same set of genes as DES mutagenesis (data not shown).

In order to determine whether the growth medium changes the genes that serve as targets for mutagenesis, 40 ts lethal mutants were isolated on minimal medium (rather than LB broth) and the mutations in these strains were tested for linkage with the set of 19 mapping Tn*10* insertions. These crosses also showed that the diversity of mutations generated by this alternative protocol are similar to those obtained by the standard protocol (data not shown).

Map locations of ts mutations: Using the mini-Tn*10* collection of KUKRAL *et al.* (1987), *F′* mapping, and Hfr crosses (CHUMLEY, MENZEL and ROTH 1979), the genetic map positions of 72 of the ts mutations were determined. The ts mutations with known map locations are shown in Table 3. The mnemonic "*clm*," for *c*onditional *l*ethal *m*utation, has been given to the mapped ts lethal mutations.

The map locations of the two large groups of linked mutations are known. One group, *clmC*, maps at 69 min, linked to *argG*, and contains seventeen ts mutants, while the other, *clmF*, maps at 64 min linked to *metC*, and has 16 ts mutants within the group. Several genes with potential mutability to a ts lethal phenotype map at 69 min in *E. coli* (see Figure 2 and DISCUSSION).

Tn*10* insertions do not cause ts lethal mutations: Temperature-sensitive lethal mutations often arise as missense mutations that cause thermolability of a protein required for growth at all temperatures. However, temperature-sensitive mutations could also result from a null mutation in a gene only required for

TABLE 2

Linkage relationships among ts mutants

Tn*10* donor strain	Strain with linked ts mutation	Other mutant strains (% cotransduction)
SE7310 (*z--2384*)	*SE5026* (*70*)	*SE5207* (*66*) SE5073 (20), SE5187 (35), SE5191 (15)
SE7311 (*zie-2385*)	*SE5044* (*10*) (*clmA44*)	*SE5152* (*15*), *SE5294* (*20*), SE5117 (85), SE5341 (25)
SE7312 (*zgj-2386*)	SE5049 (55) (*clmC49*)	SE5086 (75), SE5089 (100), SE5182 (40), SE5079 (75), SE5208 (55), SE5214 (60), SE5222 (85), SE5247 (50), SE5307 (65), SE5323 (80), SE5360 (100), SE5391 (85), SE5405 (65)
SE7313 (*z--2387*)	SE5053 (75)	SE5306 (25)
SE7314 (*zid-2388*)	*SE5057* (*85*) (*clmD57*)	*SE5129* (*100*), *SE5236* (*75*), *SE5257* (*75*)
SE7315 (*z--2389*)	*SE5125* (*75*)	*SE5197* (*80*), *SE5305* (*95*), *SE5325* (*100*), SE5369 (30)
SE7316 (*zhb-2390*)	*SE5134* (*85*) (*clmE134*)	SE5143 (55)
SE7317 (*zie-2391*)	*SE5152* (*15*) (*clmA152*)	*SE5044* (*15*), *SE5294* (*15*), *SE5341* (*15*), SE5117 (60)
SE7318 (*zai-2392*)	SE5179 (95) (*clmB179*)	(None)
SE7319 (*zge-2393*)	SE5202 (70) (*clmF202*)	SE5171 (45), SE5174 (70), SE5206 (5), SE5213 (70), SE5219 (70), SE5229 (85), SE5232 (15), SE5253 (5), SE5311 (50), SE5309 (10), SE5359 (65), SE5372 (20), SE5375 (75)
SE7320 (*zge-2394*)	SE5206 (55) (*clmF206*)	SE5171 (20), SE5174 (10), SE5202 (50), SE5213 (30), SE5219 (25), SE5229 (10), SE5232 (60), SE5253 (30), SE5311 (20), SE5375 (95), SE5309 (70), SE5359 (10), SE5372 (55)
SE7321 (*zge-2395*)	SE5229 (35) (*clmF229*)	SE5171 (20), SE5174 (10), SE5202 (15), SE5206 (55), SE5213 (20), SE5219 (20), SE5232 (40), SE5253 (30), SE5311 (20), SE5375 (95), SE5309 (20), SE5359 (10), SE5372 (60)
SE7322 (*z--2396*)	SE5260 (85)	SE5404 (65)
SE7323 (*z--2397*)	*SE5292* (*14*)	*SE5216* (*21*)
SE7324 (*zgj-2398*)	SE5307 (75) (*clmC277*)	SE5049 (35), SE5079 (35), SE5086 (50), SE5089 (100), SE5182 (55), SE5208 (55), SE5214 (70), SE5222 (90), SE5247 (60), SE5323 (90), SE5360 (100), SE5405 (65), SE5391 (70), SE5261 (90)
SE7325 (*z--2399*)	*SE5308* (*75*)	*SE5352* (*85*), *SE5353* (*85*)
SE7326 (*zge-2400*)	SE5311 (40) (*clmF281*)	SE5171 (35), SE5174 (20), SE5202 (45), SE5206 (40), SE5213 (45), SE5219 (35), SE5229 (10), SE5232 (70), SE5253 (5), SE5309 (10), SE5372 (70), SE5375 (85), SE5359 (25)
SE7327 (*z--2401*)	*SE5326* (*4*)	*SE5183* (*5*), SE5119 (15)
SE7328 (*z--2402*)	SE5080 (100)	(None)

The strains listed in column 1 carry Tn*10* insertions that were isolated by their linkage to the temperature sensitive mutations in the strains of column 2. The percent cotransduction is given in parentheses. In column 3 are other strains within the ts collection that carry mutations linked to each of the Tn*10* insertions. In all cases, the cotransduction results arise from the selection for tetracycline resistance, and subsequent scoring of thermosensitivity or thermoresistance in at least 20 transductants. Mutant strains with a ts defect calculated within 3 kb of each other (SANDERSON and ROTH 1988) are listed in italics.

growth at high temperatures. To test the frequency of such genes, Tn*10* insertions showing a temperature-sensitive phenotype were sought. Using P22 phage grown on a pool of over 2000 independent Tn*10* insertions, the wild-type strains (both LT2 and *leu-485*) were transduced to tetracycline resistance at 30°. Of over 2000 transductants in each strain, none appeared temperature sensitive for growth on LB plates at 44°. Thus, Tn*10* insertions do not readily yield a temperature-sensitive lethal phenotype, and the collection of ts mutants must consist predominantly of missense mutations in genes required for

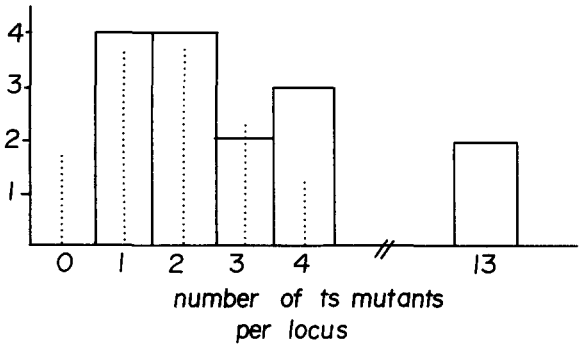

FIGURE 1.—Distribution in the number of temperature-sensitive mutations showing close transductional linkage within the ts collection. Among the linked mutants identified in Table 2, only those calculated within 3 kb of one another are plotted. These data show that, among the 229 tested mutants, an average of 2.0 mutants exist at each of the loci mutable to ts lethality (see DISCUSSION). The dashed lines indicate the expected number of linked mutants in a Poisson distribution with an average of 2.0 mutants per locus.

TABLE 3

Map locations of temperature-sensitive mutations

Map location	Gene	Linked Tn*10*Δ16Δ17	Ts strains
6	*clmI*	zag-3262	SE5155 (6K), 5235, 5319 (2K), 5374, 5399
8	*clmB*	zai-3030	SE5179
11	*clmG*	zbb-3089	SE5046
25	*clmR*	zcf-3233	SE5199 (6K), 5329 (6K)
28	*clmJ*	zci-3314	SE5147 (6K)
44	*clmN*	zee-3061	SE5243
51	*clmT*	zfb-3310	SE5212 (2K), 5354 (6K)
55	*clmQ*	zff-3028	SE5314, 5393
61.5	*clmK*	zgc-3121	SE5159
64	*clmF*	zge-3017	SE5150, 5178, 14 others (see Table 2)
69	*clmC*	zgj-3163	SE5310, 5346, 5397, 14 others (see Table 2)
71	*clmE*	zhb-3195	SE5134, 5143
82	*clmP*	zic-3068	SE5125 (2K), 5197 (2K), 5305 (2K), 5316, SE5320 (6K), 5325 (2K), 5335, 5369
83	*clmD*	zid-3265	SE5057, 5129, 5236, 5257
84	*clmA*	zie-3305	SE5044, 5117, 5152, 5294, 5341
84	*clmH*	zie-3161	SE5115, 5139
96	*clmS*	zjg-3290	SE5437
99	*clmM*	zjj-3116	SE5242 (2K)

Transductional linkage was established between the mini-Tn*10* insertions in the collection of KUKRAL *et al.* (1987) and ts lethal mutations in the strains listed. The map locations of the mini-Tn*10* insertions are known (KUKRAL *et al.* 1987). The designations (2K) and (6K) indicate strains with a 2-hr or 6-hr killer phenotype, as described in MATERIALS AND METHODS. Some of the map locations were initially identified by *F'* complementation or Hfr crosses. Other ts alleles at these locations may exist within the collection (except at the sites listed in Table 2).

growth at all temperatures. [However, null mutations with a ts lethal phenotype do exist, see PAEK and WALKER (1987) and LIPINSKA *et al.* (1989)].

DISCUSSION

The collection of 440 temperature-sensitive mutants of *Salmonella typhimurium* should provide a useful set of mutants to study cell functions that are essential for bacterial growth under standard laboratory conditions. Most of the temperature-sensitive mutants were isolated after chemical mutagenesis with diethyl sulfate. In the collection, 27% of the ts mutants also have an auxotrophic mutation, indicating that the level of mutagenesis was quite high.

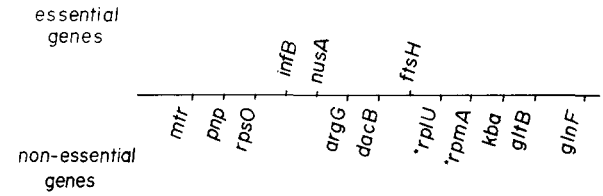

FIGURE 2.—The "essential" and "nonessential" genes identified in *E. coli* at 69 min. For "nonessential" genes, null mutations remain viable. The essential or nonessential nature of *rplU* and *rpmA*, two ribosomal proteins has not been determined. Thus, between 3 and 5 "essential" genes exist in this region. Fourteen ts mutations map to this region.

The collection of ts lethal mutants has been partially characterized for genetic and physiological relatedness. Growth conditions that suppress the temperature-sensitive phenotype of certain mutants have been found. Some of the mutants (17%) show salt-remedial growth, which may arise by stabilization of the thermosensitive protein (HAWTHORNE and FRIIS, 1964; KOHNO and ROTH 1979) or by suppression of membrane defects (BILSKY and ARMSTRONG 1973). Anaerobic growth was found to suppress 6% of the ts mutations. This may indicate that some essential functions are served by different gene products in aerobic and anaerobic growth, or are non-essential under anaerobic growth conditions. Previously BOLING, ADLER and MASKES (1984) showed that the ts phenotype of a *polA* mutation is suppressed by anaerobic growth, and recently, we have found DNA gyrase ts mutations that are suppressed by anaerobic growth (M. SCHMID, unpublished observations). Further investigation is required to determine whether the genes suppressed by anaerobic growth all share a common function affecting DNA. The sets of salt remedial and anaerobic remedial mutants do not overlap significantly.

Mutations that cause bacteriocidal effects seem to alter a specific subset of indispensible functions. Mapping data indicate that the bacteriocidal phenotype is characteristic of particular genes. For example, most of the mutations in *clmP*, *clmR* and *clmT* seem to cause irreversible defects. These mutations may include those severely affecting protein synthesis, cell wall or membrane integrity, DNA integrity, or ATP pools, which lead, respectively, to jammed translation machinery, to cell lysis, to DNA degradation or to severe energy depletion. In addition, such mutations may affect large multicomponent structures such as the nucleoid. Such large structures, once disassembled or incorrectly assembled, might be unable to correctly

reassemble when permissive growth conditions are reestablished.

The mutations in this collection seem to affect known essential genes as well as unknown genes. Two loci, *clmC* and *clmF* harbor large numbers of conditional lethal mutations. The *clmC* linkage group is linked to *argG* at 69 min. This region is genetically well-characterized, and three to five genes encode indispensible proteins in this region, as shown in Figure 2. We suspect, from physiological and cytological characteristics, that the 17 mutations in this region affect several of these genes. For example, most of these mutants have normal sized cells after incubation at 44°, however, SE5361 forms filaments, and its mutation may alter the *S. typhimurium ftsH* gene. Four of these *clmC* mutations cause irreversible effects after a 2-hr (SE5261, SE5391) or 6-hr (SE5079, SE5405) temperature shift, while the others seem reversible. The *clmF* mutations linked to *metC* at 64 min, may affect undescribed essential genes. From the frequency of ts mutations, the large differences in linkage to Tn*10* insertions, and the cytological differences among the mutants, we believe that these mutations probably also affect several genes. The current *E. coli* and *S. typhimurium* maps show only the *ecfA* gene as an essential gene in this region (BACHMANN 1983; SANDERSON and ROTH 1988); these *clmF* mutants should help to define other essential genes in this region.

Two independent estimates of the number of genes mutable to a temperature-sensitive lethal phenotype were made. First, temperature-sensitive auxotrophs are 1.9-fold more frequent than temperature-sensitive lethal mutations. If these two groups of genes can mutate to a ts phenotype at the same frequency, and estimating that 200–400 genes can mutate to give auxotrophy, the ratio of ts lethal to ts auxotrophic mutations suggests that only 100–200 genes are mutable to a temperature-sensitive lethal phenotype. Similar data from HOROWITZ and LEUPOLD (1951) showed that temperature-sensitive auxotrophs were 3.3-fold more common than temperature-sensitive lethals, which suggests even fewer genes mutable to a ts lethal phenotype. Second, the extent of genetic saturation in the collection of ts lethal mutations was measured. If the *clmC* and *clmF* data are excluded, but the rest of the data are considered to represent a single distribution, the average number of closely linked mutations in the collection is 2.3. This number averages a truncated distribution, since it ignores the number of genes that statistically are missing from the collection. By assuming a Poisson distribution and iteratively solving for the number of genes in this missing class, the average was recalculated. The recalculated average, 2.0 mutations per gene, and the 229 mutants tested, suggest that only 115 genes are mutable to a ts lethal phenotype.

These calculations rely on the premise that all genes are equally mutable to temperature sensitivity, which is certainly an oversimplification. When differential mutability of genes is included, the lower end of the distribution—those genes mutated less frequently than average—will increase the number of genes that were missed in this collection. While the negative binomial distribution could more accurately predict the number of target genes, it requires knowledge of the distribution of mutabilities of different genes and the current data do not give enough information to use this distribution. However, in the bacteriophage T4, when collections of temperature-sensitive and amber mutants were compared, temperature-sensitive mutations were found in 70% of the genes defined as essential by amber mutations (WOOD and REVEL 1976). Thus, while individual proteins may pose problems, most proteins seem mutable to thermolability.

In addition, the genetic analysis used to calculate these numbers is based on close linkage, not complementation, and two closely linked genes will behave as one "gene" in these calculations. Thus, if most essential genes reside in clusters, these experiments will overestimate the saturation of the ts lethal collection, and thus underestimate the number of genes that serve as targets for ts lethal mutations. While some clustering of essential genes emerges from the current *E. coli* and *S. typhimurium* genetic maps, many genes required for DNA replication, transcription and cell division are widely separated on the genetic map. In addition, the ratio of ts lethal and ts auxotroph mutants will not be affected by the genetic locations of the genes. These two estimates give remarkably similar numbers, and suggest that the collection of 440 ts lethal mutants includes mutations that saturate the 100–200 target genes by two- to fourfold. In this range of saturation, the Poisson distribution predicts that 2–13% of the target genes are not represented in the current collection.

Enteric bacteria have a genome size of about 4700 kb, most of which seems capable of encoding proteins (see DNA sequencing of large regions such as *oriC*, WALKER *et al.* 1984). Several factors probably contribute to the small number of target genes that seem to yield ts lethal mutations. First, most of these mutants show little or no growth at 44°. Thus the mutations in this collection must cause a severe deficiency of growth at the nonpermissive temperature. Secondly, a large number of genes in the enteric genome may be truly dispensible for growth in the relatively constant environment in the laboratory. Natural environments pose changing and varied conditions, and evolutionary survival may require a large arsenal of genes required only under specialized conditions (KOCH 1971). In addition, redundant gene products may serve some of the functions essential for growth in the laboratory. In eukaryotic genomes, redundancy arises

from gene duplication, but in prokaryotes, only a few cases of duplicated genes are known. However, prokaryotes may harbor a significant number of functionally redundant, but structurally unrelated genes. For example, the prokaryotic ribosome consists of 52 proteins, at least 9 of which are individually dispensable either *in vivo* or *in vitro* (NOLLER and NOMURA, 1987). The prokaryotic ribosome must contain the structural and functional redundancy that allows translation, even in bacteria missing these ribosomal proteins. Many metabolic pathways, for example glycolysis, show evidence of enzymatic redundancy (FRANKEL 1987) and many transport functions have overlapping specificities (FURLONG 1987). Evidence for other functional redundancies comes from the cases in which combinations of mutations are found lethal, while the individual mutations show no effects (for example, see SUZUKI, NISHIMURA and HIROTA 1978).

This collection of temperature-sensitive mutants of *S. typhimurium* will allow further analysis of some of the poorly understood essential functions in enterics. Genetic analysis suggests that the collection of 440 mutants includes lesions in most of the genes that can mutate to a temperature-sensitive lethal phenotype. The relatively small size of this set allows repeated screening procedures that should provide an overview of the cellular functions that serve as targets for ts lethal mutations in enteric bacteria.

JUNE FUKAYAMA's excellent technical assistance contributed greatly to these experiments. We are grateful for TOM SILHAVY's critical reading of this manuscript. This work was supported by U.S. Public Health Service grant GM 36485 and Searle Scholar's Award 86-E103.

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Communicating editor: N. KLECKNER