JOURNAL OF BACTERIOLOGY, Aug. 1988, p. 3321–3326
0021-9193/88/083321-06$02.00/0

Vol. 170, No. 8

Conditional Impairment of Cell Division and Altered Lethality in *hipA* Mutants of *Escherichia coli* K-12

RENE SCHERRER AND HARRIS S. MOYED*

Department of Microbiology and Molecular Genetics, California College of Medicine, University of California, Irvine, California 92717

Received 19 November 1987/Accepted 19 April 1988

Mutations in *hipA*, a gene of *Escherichia coli* K-12, greatly reduce the lethality of selective inhibition of peptidoglycan synthesis. These mutations have also been found to reduce the lethality that accompanies either selective inhibition of DNA synthesis or heat shock of strains defective in *htpR*. In addition, the mutant alleles of *hipA* are responsible for a reversible cold-sensitive block in cell division and synthesis of macromolecules, particularly peptidoglycan. Recombination between the chromosome of *hipA* mutants and plasmids containing noncomplementing fragments of *hipA*$^+$ revealed that the mutations responsible for both cold sensitivity and reduced lethality were probably identical and, in any case, lay within the first 360 base pairs of the coding region of *hipA*, probably within the first 50 base pairs. We suggest that the pleiotropic effects of mutations in *hipA* reflect the involvement of this gene in cell division.

Mutations in *hipA*, a gene of *Escherichia coli*, suppress the lethal effects of inhibition of peptidoglycan synthesis, wall-less death, caused by an antibiotic or genetic block in the early, middle, or terminal steps (8). A 1,883-base-pair (bp) fragment from the chromosome, the minimum length of fragment capable of *recA*-independent restoration of wall-less death in *hipA* mutants, encodes a weakly expressed peptide of 50,000 M_r which may be toxic when overexpressed (9). Because of the strong temporal linkage of wall-less death to an event of the division cycle, termination of chromosome replication (5), it was suggested that *hipA* might be involved in cell division (8).

We now report that *hipA*, in addition to governing the frequency of wall-less death, also controls death that ordinarily accompanies selective inhibition of DNA synthesis or heat shock of strains incapable of a normal response to heat shock because of mutation in *htpR*. The mutations in *hipA* that uncouple lethality from these inhibitions also cause a reversible cold-sensitive block in cell division. In view of this and earlier indications that *hipA* has a role in cell division, it may be of special interest that the position of *hipA* on the *E. coli* chromosome, min 33.8 (9), places it in or near the terminus of clockwise replication (4).

MATERIALS AND METHODS

Bacterial strains, plasmids, and media. The strains used in this work are listed in Table 1. Plasmids containing the two orientations of a 1,883-bp fragment that includes the 1,321-bp coding region of *hipA*, pHM519 and pHM520, and plasmids lacking either 360 bp from the 5′ end of the coding region, pHM519d, or 225 bp from the 3′ end, pHM520d, were derived from pACYC177 (9). pGK610 was constructed by inserting a 610-bp *Bgl*II-*Hin*dIII fragment from the *hipA* region into a promoter detection vector (11), pKO11. pGK610 contains 250 bp from the 3′ end of a preceding gene, *hipB*, and the first 360 bp of the coding region of *hipA*. pGK300 was constructed by inserting a 300-bp *Bgl*II-*Hpa*I fragment of the *hipA* region into pKO11. It contains 250 bp from the 3′ end of *hipB* and the first 50 bp of the coding region of *hipA*.

The routine culture media were LB broth (7) and TYE agar, which contained 10 g of tryptone, 5 g of yeast extract, 8 g of NaCl, and 15 g of agar per liter. M-9 (7) was the minimal medium. The complete, defined medium was the MOPS medium of Wanner et al. (13). All media used for *dapA* mutants contained 30 to 100 mg of diaminopimelic acid (DAP) per liter; when M-9 was used for these strains, 0.8 mM L-lysine was also added. For the growth of *thyA* mutant strains, LB broth was supplemented with 50 mg of thymidine per liter and TYE agar was supplemented with 10 to 20 mg of thymine per liter. To force retention of plasmids, media for plasmid-bearing strains were supplemented with 100 mg of ampicillin per liter. Transductants were selected in media containing 20 mg of tetracycline hydrochloride per liter.

Tests of the phenotypes of *hipA*$^+$ and *hipA* strains. Qualitative distinction (8) between high frequency, *hipA*$^+$, and low frequency, *hipA*, of lethality due to inhibition of peptidoglycan synthesis and quantification (9) of the frequency of lethality were done by previously described methods. Lethality after starvation for thymine or incubation of strains containing temperature-sensitive (Ts) mutations at nonpermissive temperatures was determined by procedures described in Results. Assessment of cold sensitivity (Cs) was made by a scoring method suitable for handling large numbers of colonies, as follows. Colonies less than 24 h old were picked with flat toothpicks and applied to TYE plates in streaks about 1 cm in length. Each plate could accommodate 50 streaks. Assessment of Cs was made after 24 and 48 h of incubation at 20°C. So far, the results have been the same at both times.

MICs and MBCs of ampicillin, kanamycin sulfate, and nalidixic acid were determined by using smaller increases in concentration of the drugs than in the usual twofold serial dilution. The initial cell density was 10^6 CFU of exponential-phase cultures per ml, and the samples were incubated for 16 h at 37°C. Inhibitory responses were recorded for drug concentrations that prevented visible turbidity, and bacteri-

* Corresponding author.

TABLE 1. *E. coli* K-12 strains

Strain	Relevant genotype	Reference or construction
AT984	*dapA6*	2
CAG456	*htpR165 supC*(Ts)	1
CBK140	*lysA*::Tn*5 thyA36*	12
CP78	*rela+*	3
CP79	*rela*	3
E107	*thyA6 deoC1 dnaB107*(Ts)	14
E177	*thyA6 deoC1 dnaA177*(Ts)	14
HM21	*dapA6 zde-264*::Tn*10 hipA*$^+$	8
HM22	*dapA6 zde-264*::Tn*10 hipA7*	8
HM23	*dapA6 zde-264*::Tn*10 hipA9*	8
HM321	*htpR165 supC*(Ts) *zde-264*::Tn*10 hipA*$^+$	P1·HM22→CAG456
HM322	*htpR165 supC*(Ts) *zde-264*::Tn*10 hipA7*	P1·HM22→CAG456
HM420	*dapA6 thyA*	AT984→*thyA*
HM421	*dapA6 thyA zde-264*::Tn*10 hipA*$^+$	P1·HM22→HM420
HM422	*dapA6 thyA zde-264*::Tn*10 hipA7*	P1·HM22→HM420
HM423	*dapA6 thyA zde-264*::Tn*10 hipA9*	P1·HM23→HM420
HM701	*dapA6 recA56 hipA*$^+$	9
HM721	*dapA6 recA56 hipA7*	9
HM731	*dapA6 recA56 hipA9*	9
HM811	*dnaA177*(Ts) *thyA6 deoC1 zde-264*::Tn*10 hipA*$^+$	P1·HM22→E177
HM821	*dnaA177*(Ts) *thyA6 deoC1 zde-264*::Tn*10 hipA7*	P1·HM22→E177
HM831	*dnaB107*(Ts) *thyA6 deoC1 zde-264*::Tn*10 hipA*$^+$	P1·HM22→E107
HM841	*dnaB107*(Ts) *thyA6 deoC1 zde-264*::Tn*10 hipA7*	P1·HM22→E107
HM871	*dnaC1*(Ts) *thyA47 deoC3 zde-264*::Tn*10 HipA*$^+$	P1·HM22→PC1
HM881	*dnaC1*(Ts) *thyA47 deoC3 zde-264*::Tn*10 hipA7*	P1·HM22→PC1
PC1	*thyA47 deoC3 dnaC*(Ts)	14
RS1	*thyA6 deoC1*	Grow E177 at 42°C
RS2	*thyA6 deoC1 zde-264*::Tn*10 hipA*$^+$	P1·HM22→RS1
RS4	*thyA6 deoC1 zde-264*::Tn*10 hipA9*	P1·HM23→RS1
RS103	*lysA*::Tn*5 thyA36 zde-264*::Tn*10 hipA*$^+$	P1·HM22→CBK140
RS160	*lysA*::Tn*5 thyA36 zde-264*::Tn*10 hipA9*	P1·HM23→CBK140
RS205	*relA zde-264*::Tn*10 hipA*$^+$	P1·HM22→CP79
RS209	*relA*$^+$ *zde-264*::Tn*10 hipA*$^+$	P1·HM22→CP78
RS226	*relA*$^+$ *zde-264*::Tn*10 hipA7*	P1·HM22→CP78
RS235	*relA zde-264*::Tn*10 hipA7*	P1·HM22→CP79

cidal responses were recorded for concentrations which reduced viability 1,000-fold or more.

Measurement of the synthesis of macromolecules. The relative rates of synthesis of DNA, RNA, protein, and peptidoglycan were compared by following the incorporation by log-phase cells of radioactivity from [*methyl*-^{3}H]thymidine, [^{3}H]uridine, L-[4,5-^{3}H]leucine, and DL-(+)-*meso*-2,6-diamino-[G-^{3}H]pimelic acid, respectively. The radioactive compounds were from Amersham. All were of sufficiently high specific activity that the amounts used, 5 to 25 μCi/ml, did not contribute significantly to the concentrations of these compounds in the medium, which was the complete, defined MOPS medium. Synthesis of thymidine and of lysine is blocked in the strains used, RS103 and RS106, and therefore could not confuse the results. This problem was minimized for incorporation of uridine and DAP by long-term prelabeling of the inocula with the same concentrations and specific radioactivity of precursor used during the experiment. Samples (0.1 ml) of the cultures were processed and assayed for incorporation of isotope by standard procedures: precipitation and washing with cold trichloracetic acid with glass fiber filters, drying, and counting in a liquid scintillation spectrometer. Incorporation is expressed in kilocounts per minute per 0.1 ml of culture. Degradation of DNA was inferred from the loss of radioactivity from a *thyA* mutant strain that had been prelabeled by incorporation of [*methyl*-^{3}H]thymidine.

Measurement of cell size. Cells were measured by phase-contrast microscopy with a reticule in the eye piece and uniform polystyrene microspheres as size standards.

RESULTS

Effect of genetic background on Hip phenotypes. It has been shown previously that the characteristic frequencies of wall-less death in both *hipA*$^+$ and *hipA* strains is unchanged over a wide range of growth rates (8). The search for possible interactions of *hipA* with other, recognized regulons was extended to the SOS, stringency, and heat shock systems. A qualitative scoring method indicated that the characteristic wall-less death in both *hipA*$^+$ and *hipA* strains is independent of genetic backgrounds containing mutations in either *recA* or *relA* (see Table 1 for strain descriptions and designations). A quantitative method detected only minor effects of mutations in *recA* and *relA*. These differences were not deemed to be of sufficient size or significance to warrant further examination at this stage of the study.

Interaction of *hipA* and *htpR*. It is impractical to measure the effect of *htpR165* on the frequency of wall-less death because heat shock of *htpR165* strains, the condition which prevents synthesis of the gene product of *htpR*, is itself lethal and lytic (10). In contrast, the effect of *hipA* on *htpR165* was readily measurable. The introduction of *hipA7* into an *htpR165* strain substantially reduced its susceptibility to lethal heat shock (Fig. 1). At the end of 22 h at 42°C, the loss of viability of an *htpR165 hipA*$^+$ strain, HM321, was 19,000-fold but was only 14-fold for the corresponding *htpR165 hipA7* strain, HM322. At earlier times, when killing was still exponential, the differences were even more pronounced. The rates of wall-less death of *hipA*$^+$ *htpR165* and *hipA7 htpR165* strains at a permissive temperature (Fig. 1) were those expected of strains without a mutation in *htpR*.

Effect of *hipA* on thymineless death. Because of the numerous indications that the bacterial envelope and DNA synthesis might be linked (6), it seemed appropriate to determine whether *hipA*, which affects wall-less death, also affects lethality due to selective inhibition of DNA synthesis (DNA-

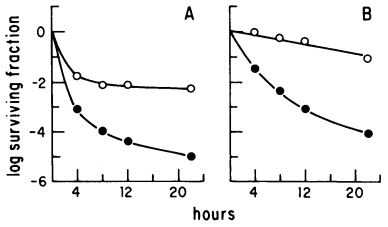

FIG. 1. Effects of ampicillin (A) and elevated temperature (B) on the viability of *hipA*$^+$ *htpR165* and *hip7A htpR165* strains. The log surviving fraction equals log CFU after the designated interval of inhibition by ampicillin or incubation at 42°C minus the log CFU at the start of the experiment. TYE agar was used with (A) or without (B) 100 mg of ampicillin per ml. (A) At designated times of incubation at 32°C, plates were sprayed with solutions of penicillinase, and CFU were counted after 16 to 24 h of further incubation at 32°C. (B) At designated times of incubation at 42°C, plates were shifted to 32°C, and CFU were counted after 24 to 36 h of further incubation at 32°C. Symbols: ●, HM321; ○, HM322.

less death). A comparison of starvation of *hipA*$^+$ and *hipA* mutant strains for DAP or for thymine revealed that both mutant alleles of *hipA* were as effective in preventing thymineless death as in preventing wall-less death (Fig. 2).

An isogenic pair of *hipA*$^+$ and *hipA9* strains, RS2 and RS4, with low thymine requirements were used to study the effect of *hipA* on thymineless death in further detail (data not illustrated). The viability of both strains remained constant for several hours after thymine starvation was initiated. A precipitous drop in the viability of the *hipA*$^+$ strain started after 2 h; a much slower decline of the *hipA9* strain started after 4 h. The absorbance of both cultures increased due to the filament formation often observed during thymine starvation; no difference in the filamentation of the two strains could be detected. The gradual degradation of DNA that occurs with thymine starvation was nearly 10-fold greater in the *hipA*$^+$ strain than in the *hipA9* strain by the end of 24 h. The relative rates of DNA degradation in the two strains may account for the different rates of loss of viability; however, the loss of viability in the *hipA*$^+$ strain was at least 1,000-fold greater than in the *hipA9* strain, whereas the loss of DNA was only 10-fold greater in the *hipA*$^+$ strain than in the *hipA9* strain.

Effect of *hipA* on Ts alleles of *dnaA*, *dnaB*, and *dnaC*. The influence of *hipA* was studied in a series of strains bearing Ts mutations in *dnaA*, *dnaB*, or *dnaC* that are ordinarily lethal at the nonpermissive temperature, 42°C. Mutant alleles of

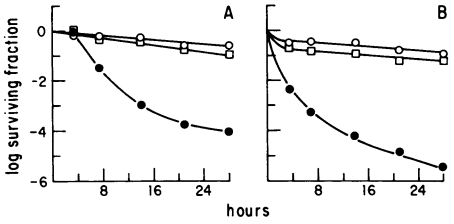

FIG. 2. Effects of starvation for thymine (A) and DAP (B) on the viability of *hipA*$^+$ and *hipA* mutant strains. The log surviving fraction equals log CFU after the designated interval of starvation for DAP or thymine minus the log CFU at the start of the experiment. M9 minimal agar was supplemented with DAP (A) or thymine (B) and with lysine in both cases. At the designated times of incubation at 37°C, the plates were sprayed with solutions of thymine (A) or DAP (B), and CFU were counted after 16 to 24 h of further incubation. Symbols: ●, HM421; ○, HM422; □, HM423.

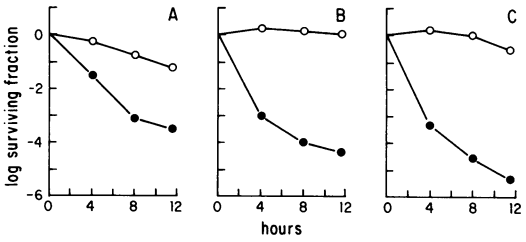

FIG. 3. Effect of *hipA* on survival of strains with temperature-sensitive mutations in DNA synthesis. The log surviving fraction equals log CFU after the designated interval of incubation at 42°C minus the log CFU at the start of the experiment. TYE agar was supplemented with 50 mg of thymidine per liter. At the designated times of incubation at 42°C, the plates were shifted to 32°C, and CFU were counted after 16 to 24 h of further incubation. (A) Symbols: ●, HM811; ○, HM821. (B) Symbols: ●, HM831; ○, HM841. (C) Symbols: ●, HM871; ○, HM881.

hipA greatly suppressed the killing of these strains (Fig. 3). Mutations in *hipA* did not reverse inhibition of DNA synthesis and, in the case of *dnaB*(Ts), did not prevent filamentation at the nonpermissive temperature. Therefore, mutations in *hipA* did not suppress the Ts defects in DNA synthesis; instead, they reduced the lethality that usually accompanies these events.

Effect of *hipA* on other lethal inhibitors and treatments. Neither the high sensitivity of *recA* mutants nor the lower sensitivity of *recA*$^+$ strains to UV light was altered by *hipA*, as seen by the effects of increasing exposure of plates streaked with HM21 (*recA*$^+$ *hipA*$^+$), HM22 (*recA*$^+$ *hipA7*), HM23 (*recA*$^+$ *hipA9*), HM701 (*recA hipA*$^+$), HM721 (*recA hipA7*), and HM731 (*recA hipA9*) (data not shown).

The static and lethal effects of nalidixic acid, kanamycin, and ampicillin on *hipA*$^+$ and *hipA* mutant strains were compared in terms of MIC and MBC (Table 2). As in the case of ampicillin, both strains were equally sensitive to inhibition by nalidixic acid, an inhibitor of DNA gyrase, but the *hipA* mutant was refractory to the bactericidal actions of both drugs. In contrast, the status of *hipA* did not significantly influence either the inhibitory or bactericidal effects of kanamycin.

Cs growth of *hipA* mutants. Reversion of *hipA7* and *hipA9* was encountered in the course of several genetic constructions with cells that had to be grown at 32°C because of Ts mutations in other genes. The growth rates of *hipA* mutant strains were found to be detectably lower than those of *hipA*$^+$ strains at this temperature. Therefore, as a practical matter, experiments at 32°C had to be monitored to determine whether sufficient reversion had occurred to affect the results. The impairment of growth of *hipA* mutant strains increased with decreasing temperature. At the lowest temperature tested, 20°C, those colonies that did develop had lost the original Cs phenotype; most of those colonies

TABLE 2. Drug susceptibilities of *hipA*$^+$ and *hipA* mutant strains

Drug	Susceptibility (μg/ml)			
	HM21 (*hipA*$^+$)		HM22 (*hipA7*)	
	MIC	MBC	MIC	MBC
Ampicillin	20	20	20	>200
Kanamycin sulfate	20	20	10	10
Nalidixic acid	10	10	10	>100

TABLE 3. Effect of plasmids containing fragments of *hipA*$^+$ on reversion of the Cs phenotype of *hipA7* strains

Strain	Plasmid	Growth (CFU/ml)		Efficiency of plating[a] at 20°C (a/b)
		a (20°C)	b (37°C)	
HM22	pACYC177	2.6×10^5	1.1×10^9	2.4×10^{-4}
HM22	pHM519	1.2×10^9	1.3×10^9	0.92[b]
HM22	pHM520	1.2×10^9	1.1×10^9	1.09[b]
HM22	pHM519d	8.4×10^4	6.7×10^8	1.3×10^{-4}
HM22	pHM520d	1.0×10^7	9.5×10^8	1.1×10^{-2}
HM22	pKO11	1.8×10^5	1.2×10^9	1.5×10^{-4}
HM22	pGK300	1.3×10^6	9.3×10^8	1.4×10^{-3}
HM22	pGK610	8.2×10^6	1.0×10^9	8.2×10^{-3}
HM721 (*recA56*)	pACYC177	1.8×10^5	1.1×10^9	1.6×10^{-4}
HM721 (*recA56*)	pHM520d	2.2×10^5	1.1×10^9	2.0×10^{-4}

[a] pACYC177 was the vector used for construction of the pHM plasmids, and pKO11 was the vector used for pGK plasmids.
[b] Complementation of Cs. The efficiency of plating was also 1.0, within experimental error, in a *recA56* background, strain HM721 (Table 1).

consisted of fully reverted cells, but 0.1% were partial revertants. Complete reversion of Cs was invariably accompanied by complete reversion of the Hip$^-$ phenotype; partial reversion of Cs was invariably accompanied by partial reversion of the Hip$^-$ phenotype.

The suggestion from the preceding observations that the Hip$^-$ and Cs phenotypes result from mutation in the same gene was confirmed by genetic analysis. Both phenotypes were 100% cotransducible. Plasmids pHM519 and pHM520 containing the minimal fragment length capable of complementing the Hip$^-$ phenotype of *hipA* mutant strains also complemented the Cs phenotype of these strains.

Testing the effect of plasmids containing fragments of *hipA*$^+$ on the frequency of Cs reversion of a *hipA7* strain (Table 3) showed that mutation in a small region at the 5′ end of *hipA* was responsible for both the Cs and Hip phenotypes. Marker rescue experiments (9) had established that pHM520d, which contains all of the coding region of *hipA*$^+$ except for 225 bp at the 3′ and could not complement *hipA7*, caused a *recA*-dependent increase in reversion to Hip$^+$; whereas pHM519d, which contains all of *hipA*$^+$ except for 360 bp at the 5′ end, could neither complement *hipA7* nor affect the rate of its reversion. A similar set of experiments (Table 3) revealed the parallel effects of these plasmids on the reversion of the Cs of *hipA7* strains. pHM519d did not alter the frequency of reversion of Cs, but pHM520d, which increased reversion of the Hip$^-$ phenotype 100-fold (9), had a quantitatively similar *recA*-dependent effect on reversion of Cs.

The location of the mutation in *hipA7* responsible for Cs was confirmed by the fact that pGK610, which contains the first 360 bp of the coding region of *hipA*$^+$, increased the reversion of Cs as effectively as did pHM520d; the less conclusive result with pGK300 (Table 3) suggested that Cs lay within the first 50 bp of *hipA7*, as the latter plasmid contained only that portion of the coding region of *hipA*$^+$. A similar analysis of the locus of Cs in *hipA9* strains was not feasible because reversion in *hipA9* strains was 10-fold higher than in *hipA7* strains and could be increased only another 10-fold by pHM520d. Marker rescue of *hipA9* with plasmids containing less of the *hipA* sequence, and hence even lower probabilities of homologous recombination, was not attempted.

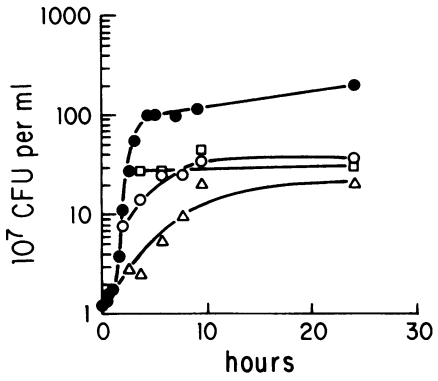

FIG. 4. Cold-sensitive growth of a *hipA* mutant. Cultures of RS4 *hipA9* in LB broth supplemented with 50 mg of thymidine per liter were shifted from 37 to 20°C at 1 h (△), 1.9 h (○), 2.5 h (□), or, in the case of the control, kept at 37°C (●).

Cell division block in *hipA* mutant strains at low temperature. Cell division and the synthesis of macromolecules were compared in *hipA*$^+$ and *hipA7* or *hipA9* strains after a shift from a permissive temperature, 37°C, to a nonpermissive temperature, 20 or 24°C. The experiments were performed in complete synthetic medium or in yeast extract-tryptone medium. No differences in Cs division attributable to the composition of the medium were found. Prolonged incubation at a nonpermissive temperature was not lethal; the efficiencies of plating of *hipA*$^+$, *hipA7*, and *hipA9* strains were unaffected by 24 h of incubation at 20°C prior to incubation at 37°C.

A variably limited number of cell divisions took place in *hipA* mutant strains depending on the age of the culture at the time of the shift to a nonpermissive temperature. This is illustrated for an *hipA7* strain (Fig. 4). The potential for increase in cell number after the shift to low temperature gradually decreased with increasing age of the culture until, in the later stage of exponential growth, inhibition of cell division was immediate. Microscopic measurement revealed that when division ceased at 20°C, the length and volume of *hipA7* cells were twice those of *hipA*$^+$ cells, whereas no differences in size were observed at 37°C.

The effects of a shift to 20°C on macromolecule synthesis and on cell division were compared in an *hipA*$^+$ strain and its isogenic *hipA9* counterpart (Fig. 5). As judged by the rates of incorporation of precursors, the syntheses of RNA, DNA, and protein were less profoundly retarded in the *hipA9* strain at the nonpermissive temperature than was cell division; however, inhibition of peptidoglycan synthesis more closely paralleled inhibition of division.

Discovery of the cold-sensitive block in cell division prompted a reexamination of the earlier conclusion (8) that mutations in *hipA* did not affect growth rates. As before, at 37°C the growth rates of *hipA*$^+$ and *hipA* mutant strains in the exponential phase could not be distinguished. However, the measurements have now been continued into the stationary phase. This showed that *hipA* mutant strains ceased exponential growth one generation earlier than did the corresponding *hipA*$^+$ strain. Over the next several hours, the *hipA* mutants gradually made up this difference in cell density. Slightly early cessation of rapid growth of *hipA* mutants may also account for the few small colonies seen at 16 h but not after 36 h of incubation at 37°C. These

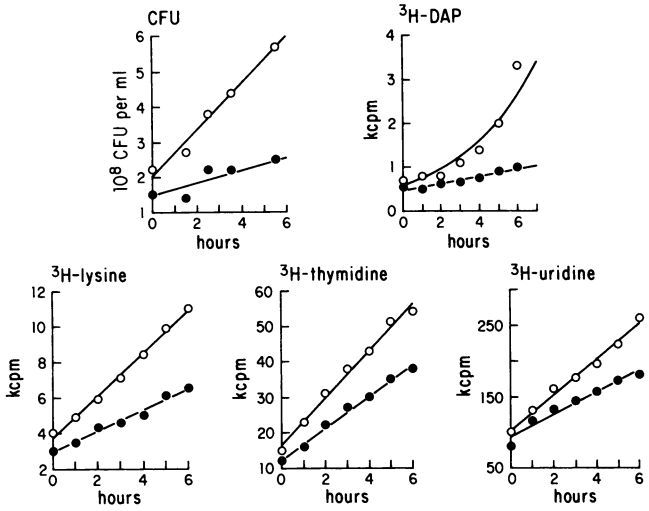

FIG. 5. Effects of low temperature (20°C) on synthesis of macromolecules and on cell division in *hipA*$^+$ and *hipA* mutant strains. At zero time, cultures in the mid- to late log phase of exponential growth at 37°C were shifted to 20°C. The method for measuring incorporation of labeled metabolites is described in Materials and Methods. Symbols: ○, RS103 (*hipA*$^+$); ●, RS160 (*hipA9*).

differences were observed in both complex and minimal media.

DISCUSSION

The suppressions of lethality by mutations in *hipA* are probably related in a significant manner. They do not appear to be indirect results of reduced growth rates, since the vast differences in the death of *hipA*$^+$ and *hipA* mutant strains were observed at doubling times ranging from 0.5 to 1.5 h (8) and since the mutant alleles *hipA7* and *hipA9* had no detectable effect on rates of exponential growth at 37 and 42°C, temperatures at which suppression was demonstrated. Furthermore, the involvement of *hipA* in lethal phenomena is limited. For example, *hipA* did not influence the lethal effects of UV light, nor did it affect the lethal action of kanamycin, a bactericidal inhibitor of protein synthesis. The one consequence of the mutations in *hipA* other than suppression of lethality was a reversible cold-sensitive block in cell division.

This last characteristic of *hipA* mutants offers a clue as to how the pleiotropic effects of these strains might be related: *hipA* is involved in cell division at a step which, when interrupted, is bacteriostatic but not bactericidal. *hipA* mutants are defective in this function at low temperature or, we suggest, when either peptidoglycan or DNA synthesis is selectively inhibited. We also suggest that the lethality of heat shock in strains defective in *htpR* is due to inhibition of either peptidoglycan or DNA synthesis. The consequences for *hipA* mutants would be stasis at low temperature and stasis rather than death when ordinarily lethal inhibitions of peptidoglycan or DNA synthesis are imposed directly or through heat shock.

The element of the preceding explanation suggesting that lethal heat shock involves inhibition of either peptidoglycan or DNA synthesis is based on observation and hypothesis (10), respectively. Heat shock of strains defective in *htpR* causes death and lysis (10), insults for which inhibition of peptidoglycan synthesis should be a prime suspect. Hypothesis about the role of heat shock proteins, such as DnaJ and DnaK, has included the view that they serve as replacements for ordinary proteins that fail at high temperatures (10). If this view is correct, inhibition of DNA synthesis is another candidate for the cause of the lethality of heat shock in *htpR* strains.

Suppression of both wall-less and DNA-less death by mutations in *hipA* might be another reflection of the often suggested linkage between peptidoglycan and DNA synthesis (6), or it could mean that inhibition of each process independently elicits a response in which *hipA* participates. The data bearing on this issue show that there is a pronounced delay in the effect of the mutant alleles on wall-less death (8) (Fig. 1). In contrast, there is no evidence of delay in the effect of the mutant alleles on DNA-less death (Fig. 2 and 3). Thus, the defects in *hipA7* and *hipA9* have a more immediate, perhaps more direct, effect on DNA-less death than on wall-less death.

Peptidoglycan synthesis, as estimated from incorporation of DAP by a *hipA* mutant at 20°C, was the one process found to be as markedly inhibited as cell division (Fig. 5). But even in *hipA* mutants, inhibition of peptidoglycan synthesis by each of four independent, selective methods caused a 100-fold loss of viability (8), whereas there was no loss of viability among the same strains at 20°C. Therefore, if the cold-sensitive block in division is caused by selective inhibition of peptidoglycan synthesis alone, this particular inhibition would have to be uniquely nonlethal.

Because *hipA* is involved in cell division, its location (8) in or near the terminus of clockwise replication of the chromosome (4) is especially interesting. Although this specific position for *hipA* would not appear to be mandatory, since *hipA* is active in *trans* (9), its location near a region of termination could reflect evolution of functionally related genes in this region of the chromosome.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI-20276 from the National Institute of Allergy and Infectious Diseases and by grant MV-387 from the American Cancer Society.

LITERATURE CITED

1. **Baker, T. A., A. D. Grossman, and C. A. Gross.** 1984. A gene regulating the heat shock response in *Escherichia coli* also affects proteolysis. Proc. Natl. Acad. Sci. USA **81**:6779–6783.
2. **Bukhari, A. I., and A. L. Taylor.** 1971. Genetic analysis of diaminopimelic acid- and lysine-requiring mutants of *Escherichia coli*. J. Bacteriol. **105**:844–854.
3. **Fiil, N., and J. D. Friesen.** 1968. Isolation of "relaxed" mutants of *Escherichia coli*. J. Bacteriol. **95**:729–731.
4. **Hill, T. M., and P. L. Kuempel.** 1986. Localization of the replication blocks in the terminus region of the *Escherichia coli* chromosome. J. Cell. Biochem. Suppl. **10B**:175.
5. **Hoffmann, B., W. Messer, and U. Schwarz.** 1972. Regulation of polar cap formation in the life cycle of *Escherichia coli*. J. Supramol. Struct. **1**:29–37.
6. **Kornberg, A.** 1982. DNA replication, p. 451–457. W. H. Freeman & Co., San Francisco.
7. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
8. **Moyed, H. S., and K. P. Bertrand.** 1983. *hipA*, a newly recognized gene of *Escherichia coli* K-12 that affects frequency of persistence after inhibition of murein synthesis. J. Bacteriol. **155**:768–775.
9. **Moyed, H. S., and S. H. Broderick.** 1986. Molecular cloning and expression of *hipA*, a gene of *Escherichia coli* K-12 that affects frequency of persistence after inhibition of murein synthesis. J.

Bacteriol. **166**:399–403.
10. **Neidhardt, F. C., R. A. VanBogelen, and V. Vaughn.** 1984. The genetics and regulation of heat-shock proteins. Annu. Rev. Genet. **18**:295–329.
11. **Rosenberg, M., A. B. Chepelinsky, and K. McKenney.** 1983. Studying promoters and terminators by gene fusion. Science **222**:734–739.
12. **Shaw, K. J., and C. M. Berg.** 1979. *Escherichia coli* K-12 auxotrophs induced by insertion of the transposable element Tn*5*. Genetics **92**:741–747.
13. **Wanner, B. L., R. Kodaira, and F. C. Neidhardt.** 1977. Physiological regulation of a decontrolled *lac* operon. J. Bacteriol. **130**:212–222.
14. **Wechsler, J. A., and J. D. Gross.** 1971. *Escherichia coli* mutants temperature-sensitive for DNA synthesis. Mol. Gen. Genet. **113**:273–284.