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# Modulation of the Heat Shock Response by One-Carbon Metabolism in *Escherichia coli*

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**A genetic screen designed to isolate mutants of *Escherichia coli* W3110 altered in the ability to induce the heat shock response identified a strain unable to induce the heat shock proteins in a rich, defined medium lacking methionine after exposure to 2,4-dinitrophenol. This strain also grew slowly at 28°C and linearly at 42°C in this medium. The abnormal induction of the heat shock proteins and abnormal growth at both high and low temperatures were reversed when methionine was included in the growth medium. The mutation responsible for these phenotypes mapped to the *glyA* gene, a biosynthetic gene encoding the enzyme that converts serine and tetrahydrofolate to glycine and 5,10-methylenetetrahydrofolate. This reaction is the major source of glycine and one-carbon units in the cell. Because fixed one-carbon units, in the form of methionine, allowed mutant cells to induce the heat shock response after exposure to 2,4-dinitrophenol, a one-carbon restriction may be responsible for the phenotypes described above.**

The heat shock regulon in *Escherichia coli* consists of approximately 18 proteins whose rates of synthesis are increased when cells encounter stressful changes in the environment (42). Many of these proteins are highly conserved phylogenetically (12). The *E. coli* heat shock proteins DnaK, GroEL, and HtpG have homologs in eukaryotes, which are referred to as hsp70, hsp60, and hsp90, respectively. The *E. coli* heat shock proteins ClpB and ClpP together make up an ATP-dependent protease (17, 18, 35), which has a homologous counterpart in eukaryotic cells (13, 21). The functions of many of the proteins in this regulon have come to light in recent years. The heat shock proteins Dnak, DnaJ, GroEL, GroES, and GrpE are members of a class of proteins called chaperones (12), which modulate protein-protein interactions of many kinds. These interactions include the assembly and disassembly of oligomeric protein complexes, such as those associated with the initiation of DNA synthesis in *E. coli* (31) and bacteriophage λ (1, 2, 8); the solubilization of protein aggregates (34); and the binding of proteins destined for export across or insertion into membranes (3, 47).

The conditions known to induce some or all of the heat shock proteins are many and include temperature upshifts, carbon or phosphate starvation, and exposure to ethanol or heavy metals (42). A number of antibiotics which interfere with ribosome function also induce the expression of this regulon (41). It is known that induction of the heat shock proteins is dependent on a positive activator, $\sigma^{32}$, the product of the *rpoH* gene. This protein is a sigma factor which binds core RNA polymerase and allows the polymerase to recognize heat shock promoters (14, 19). The increase in the level of $\sigma^{32}$ following exposure to high temperature is thought to be rapid enough to fully account for the induction of the heat shock proteins (38). This increase in the level of $\sigma^{32}$ is brought about by increasing the production of *rpoH* message, by increasing translation of existing message, and by stabilizing existing $\sigma^{32}$, which normally has a half-life of 1 min (38, 39). The rapid turnover of $\sigma^{32}$ is thought to be regulated, in part, by the heat shock proteins DnaK, DnaJ, and GrpE (11, 39, 40). How *E. coli* senses the conditions which ultimately lead to increased transcription and translation of the *rpoH* gene remains largely unknown. How, or even if, cell physiology acts to modify or modulate the magnitude of this response, through genetic elements other than $\sigma^{32}$, also remains unknown. The *E. coli* mutant described in this work was isolated in a screen designed to find mutations in genes other than *rpoH* which affected the expression of the heat shock proteins.

## MATERIALS AND METHODS

**Bacterial strains.** The strains used in this study were derivatives of the wild-type *E. coli* strain W3110. Strains are described in Table 1. Mutagenesis was done by growing strain W3110 for 2 h in the presence of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (3 μg/ml) by the method of Miller (22).

**Media and bacterial growth.** All cultures were grown aerobically in rotary-action waterbath shakers at 28 or 42°C. The cultures were grown in a 3-(*N*-morpholine)propanesulfonic acid (MOPS) medium supplemented with 0.4% glucose, 19 amino acids, and four bases (45). The vitamins thiamine, calcium pantothenate, *p*-aminobenzoic acid, *p*-hydroxybenzoic acid, and 2,3-dihydroxybenzoic acid were included at 0.01 mM each. When experiments required labeling in the presence of methionine, it was included in the medium at 0.02 mM. The amino acid, base, and vitamin stock solutions were made fresh monthly because it was noted that cells carrying the *glyA* mutation described in this work would not achieve steady-state growth in older medium. Cultures used for experiments were inoculated from an overnight culture into fresh, warm medium so that the optical density at 420 nm ($OD_{420}$) or $OD_{520}$ was about 0.05. Growth was monitored by measuring optical density at either 420 or 520 nm. The latter wavelength was used when 2,4-dinitrophenol (DNP) was to be added to the growth medium. The culture was then grown to an $OD_{420}$ or $OD_{520}$ of 1.0 or 0.8, respectively, diluted at least 1:15 into fresh, warm medium, and grown to an $OD_{420}$ or $OD_{520}$ of 0.30 or

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TABLE 1. *E. coli* strains and plasmids

| Strain or plasmid | Relevant genotype or characteristics | Source or reference |
|---|---|---|
| *E. coli* | | |
| W3110 | Wild type | This lab |
| Ds127 | MNNG-mutagenized W3110, *tolC glyA127* | This work |
| CR1 | Ds127 Tn*10*dCam *glyA127* | This work |
| DG127 | W3110 Tn*10*dCam *tolC* | This work |
| DG231 | W3110 *zff-208*::Tn*10* | This work |
| DG232 | W3110 *zff-208*::Tn*10 glyA127* | This work |
| CAG18481 | MG1655 *zff-208*::Tn*10* | 33 |
| Plasmids | | |
| pGS29 | *glyA* gene, truncated *hmp* gene, Amp$^r$ | 37 |
| pFN476 | Low-copy-number vector, Amp$^r$ | This lab |
| pFN600 | *glyA* gene, truncated *hmp* gene in pFN476, Amp$^r$ | This work |
| pFN601 | Truncated *glyA* gene and truncated *hmp* in pFN476, Amp$^r$ | This work |

0.20, respectively, before being exposed to DNP or ethanol or shifted to 42°C. For DNP shocks, DNP was made as a 10 mM stock solution in culture medium, and an appropriate amount was added to cells in order to give a 0.5 mM final concentration.

**Two-dimensional gel electrophoresis.** Sample preparation and two-dimensional polyacrylamide gel electrophoresis were done by the methods of O'Farrell (25) with modifications (4, 41).

**Labeling of cells and measurements of rates of synthesis of individual proteins.** At the indicated times, a sample (1 ml) of culture was removed and transferred to a warm scintillation vial containing approximately 50 μCi of [$^{35}$S]methionine (1,200 Ci/mmol, 11 mCi/ml) for a 5-min pulse (10-min pulses were used in determining the DNP and ethanol induction curves). If the culture medium contained methionine, cells were pulsed with 150 μCi of [$^{35}$S]methionine. Following the pulse, the labeled cells were chased with 0.085 ml of a 0.2 M solution of unlabeled methionine. A sample (0.5 ml) of [$^3$H]leucine-labeled cells was added to the [$^{35}$S]methionine-labeled cells. Labeling with tritium was done by growing wild-type cells in 10 ml of glucose-rich MOPS medium (GRM) without methionine, containing 0.08 mM leucine, in the presence of 1.5 mCi of [$^3$H]leucine from a starting $OD_{420}$ of 0.05 to a final $OD_{420}$ of 1.0. The mixed sample was prepared for electrophoresis as described previously (43). An autoradiogram of the two-dimensional gel was prepared for each sample and served to permit visualization of the proteins whose rate of synthesis was to be quantitated. Protein spots were excised from the two-dimensional gel and solubilized, and the $^{35}$S and $^3$H contents were quantitated by scintillation counting as described previously (27). The differential rate of synthesis of a protein was defined as the $^{35}$S/$^3$H ratio in that protein divided by the $^{35}$S/$^3$H ratio of total cellular, trichloroacetic acid-precipitable protein. The relative differential rate of synthesis of a protein was defined as the differential rate of synthesis of a protein at a given time after an experimental treatment divided by the differential rate of synthesis of that protein during steady-state growth before the experimental treatment.

**Plasmid construction.** Plasmid pGS29 contains the *glyA* gene and about half of a divergently transcribed gene, *hmp*, inserted into pBR322 (29, 36, 37, 44) (Fig. 1). In order to clone *glyA* into a low-copy vector, the *Stu*I-*Sal*I fragment containing the *glyA* gene and 527 bp of *hmp* was cut from pGS29 and subcloned into the *Sal*I and *Sma*I sites of pFN476. This plasmid was designated pFN601. A control plasmid containing a truncated *glyA* gene and the 527 bp of *hmp* was constructed by cutting the *Eco*RV-*Sal*I fragment from pGS29 and subcloning it into the *Sal*I and *Sma*I sites of pFN476. This plasmid was designated pFN600. Plasmid pFN476 and its derivatives, pFN600 and pFN601, contain a pSC101 origin of replication and are present at one to five copies per cell (32).

## RESULTS

**Isolation of an *E. coli* mutant unable to adapt to low levels of DNP.** A genetic screen done with the uncoupler of oxidative phosphorylation DNP was designed to isolate mutants altered in their expression of heat shock proteins in response to this agent. DNP causes precursor proteins normally destined for export or insertion into the cell membrane to accumulate in the cytoplasm (9) and induces 52 proteins, including the heat shock proteins.

*E. coli* K-12 strain W3110, mutagenized with MNNG by a protocol designed to minimize the production of linked mutations (22), was replica plated from LB plates onto LB plates containing 0.5 mM DNP and incubated at 28°C. Of 36,000 colonies screened, 106 failed to grow or grew poorly in the presence of DNP.

Two-dimensional gels of these strains, labeled before and after exposure to 0.5 mM DNP, were examined in order to assess whether any of the mutants had defects in expression of the heat shock proteins. One strain, designated Ds127, was chosen for further study. Strain Ds127 exhibited several abnormal phenotypes. It was unable to form colonies on LB plates containing 0.5 mM DNP at 28°C. It failed to induce 33 of the 52 proteins induced in the parental strain, W3110, following exposure to DNP. The 33 uninduced proteins included 13 of the 14 heat shock proteins normally induced by exposure to DNP. The strain also had a glycine requirement.

**Genetic characterization of strain Ds127.** In order to map the mutation responsible for the DNP sensitivity of strain Ds127, this strain was transduced back to DNP resistance by using a P1 lysate made from a strain of W3110 containing random Cam$^r$ transposons. The DNP-resistant transductants were then tested for resistance to chloramphenicol. With a P1 lysate made on one of these DNP-resistant, chloramphenicol-resistant strains, strain CR1, it was shown that the Cam$^r$ transposon Tn*10* was linked with a cotransduction frequency

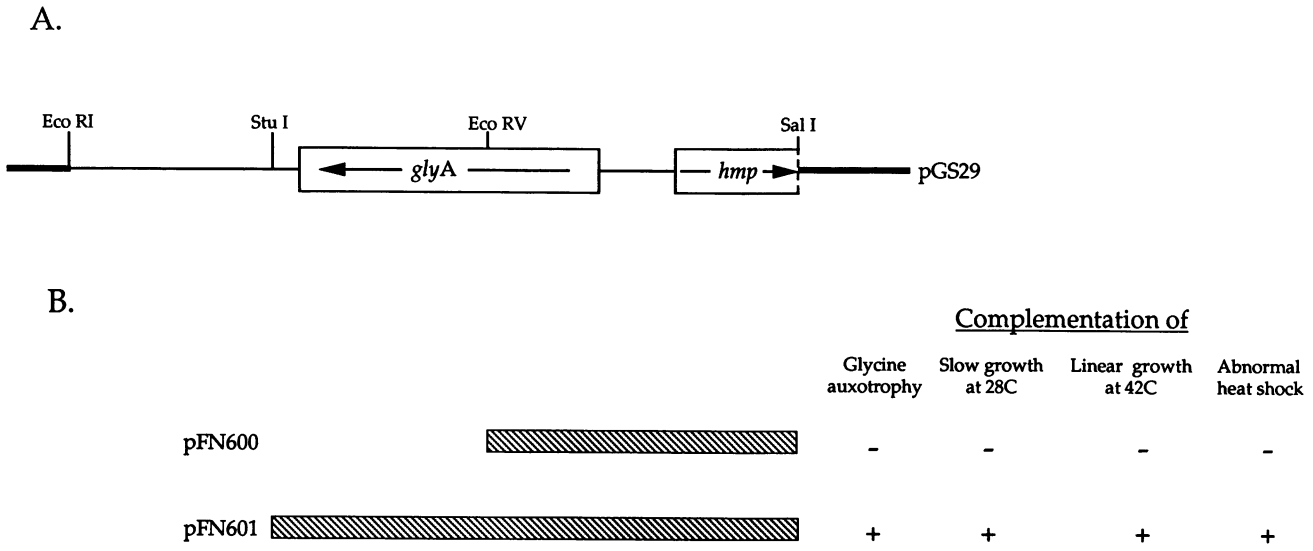


FIG. 1. Cloning the *glyA* gene into a low-copy-number vector. (A) Schematic diagram of the *glyA* gene in pGS29. Thick lines represent pBR322 DNA. (B) Hatched bars represent DNA subcloned from pGS29 and inserted into the low-copy-number vector pFN476 to give plasmids pFN600 and pFN601. The ability of pFN600 and pFN601 to complement the phenotypes of a *glyA127* mutant is also shown.

of 95% to the gene responsible for the DNP sensitivity in strain Ds127. This Cam$^r$ Tn*10* was then used to map the linked mutation causing DNP sensitivity to about 66 min on the *E. coli* chromosome. In addition to being sensitive to DNP, strain Ds127 was sensitive to other hydrophobic agents, such as bile salts and sodium dodecyl sulfate. It was also resistant to colicin E1. These are phenotypes associated with mutations in the *tolC* gene (26, 46), which maps to 65.5 min on the chromosome. The phenotypes listed above, together with the map location of the mutation responsible for the DNP sensitivity, make it likely that the DNP sensitivity of strain Ds127 results from a *tolC* mutation. Genetic analysis indicated that the sensitivity of strain Ds127 to hydrophobic chemicals, including DNP, and its resistance to colicin E1 could be separated from the other phenotypes of this strain. When the mutation responsible for DNP sensitivity was crossed out of strain Ds127 by P1 transduction, the resulting strain, CR1, still exhibited an abnormal induction of proteins after exposure to 0.5 mM DNP and was still auxotrophic for glycine. In addition, the mutation causing DNP sensitivity was transduced from strain Ds127 into strain W3110, and this strain, DG127, exhibited a normal induction of all proteins following exposure to DNP. Thus, the DNP sensitivity of strain Ds127 was likely due to a *tolC* mutation, and this mutation was distinct from the mutations in this strain which resulted in glycine auxotrophy and the abnormal induction of proteins in response to DNP.

**Strain Ds127 contains a *glyA* mutation.** Auxanography (7) of strain Ds127 indicated that it had a glycine requirement which was fulfilled by glycine alone. The fact that serine could not alleviate the glycine requirement suggested that the strain was mutated in the *glyA* gene, which encodes the enzyme serine hydroxymethyltransferase (SHMT) (28, 36). SHMT catalyzes the conversion of serine and tetrahydrofolate to glycine and 5,10-methylenetetrahydrofolate. This reaction is considered to be the major source of glycine and 5,10-methylenetetrahydrofolate (one-carbon units) used in the synthesis of methionine, histidine, purines, thymine, and formyl-methionyl-tRNA (36) (Fig. 2). P1 transductional mapping placed the mutation responsible for glycine auxotrophy near 55 min on the *E. coli* chromosome, close to the *glyA* gene. In addition, examination of two-dimensional gels of strain Ds127 revealed that the GlyA protein was missing from its normal position and had shifted to a position 0.15 pH units more acidic than the wild-type protein (10). Revertants selected for glycine prototrophy had a GlyA protein which ran normally on two-dimensional gels. In addition, the mutation responsible for the auxotrophy could be complemented by the low-copy-number plasmid pFN601 (Fig. 1),

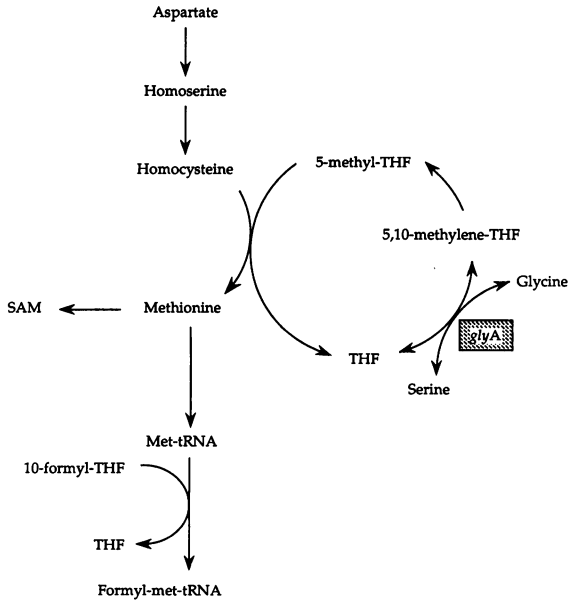


FIG. 2. Synthesis of glycine, methionine, and one-carbon units. Shown are the pathways concerned with glycine synthesis and the synthesis, interconversion, and utilization of one-carbon units in the synthesis of methionine. Abbreviations: THF, tetrahydrofolate; SAM, *S*-adenosylmethionine; *glyA*, the gene for serine hydroxymethyltransferase.

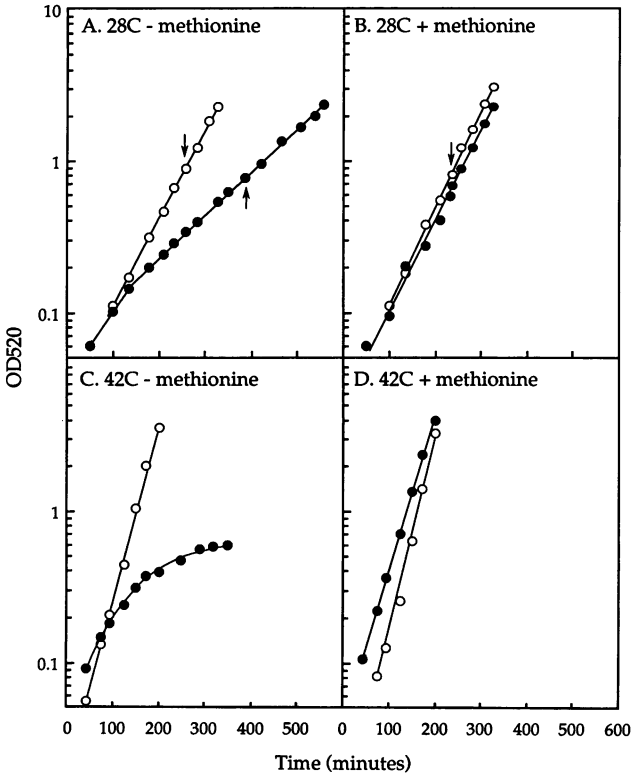


FIG. 3. Growth of strains DG231 (○) and DG232 (●) in (A) GRM without methionine at 28°C, (B) GRM with methionine at 28°C, (C) GRM without methionine at 42°C, and (D) GRM with methionine at 42°C. Arrows indicate when cultures were diluted 1:15. The growth curves were normalized to allow for the dilution.

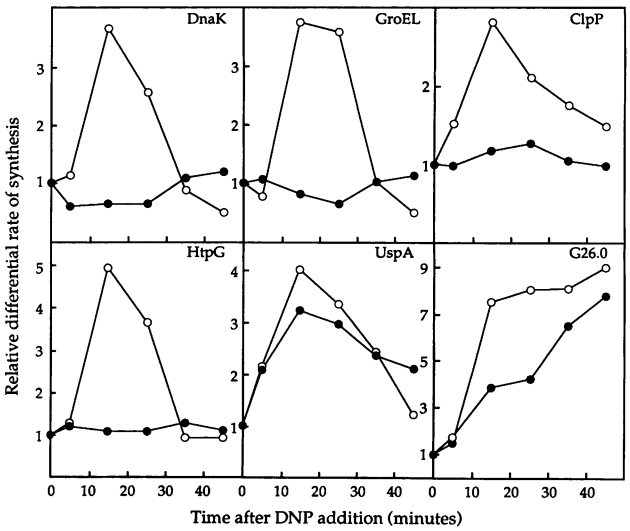


FIG. 4. Relative differential rates of synthesis of heat shock proteins in strains DG231 and DG232 following the addition of DNP. Strains DG231 (○) and DG232 (●) growing in GRM without methionine at 28°C were pulse labeled at the indicated times after a challenge with 0.5 mM DNP. The relative differential rates of synthesis of the heat shock proteins DnaK, GroEL, ClpP, and HtpG are shown. The proteins UspA and G26.0, which are inducible by DNP but are not part of the heat shock regulon, are included as positive controls.

which carried the *glyA* gene. Together, these observations indicated that Ds127 was auxotrophic for glycine because of a mutation in the *glyA* gene. The *glyA* allele in Ds127 is referred to as *glyA127* throughout this article. This allele was transduced into strain W3110 by using a linked Tn*10* (*zff-208*::Tn*10*) (33) to give strain DG232. This was done in order to study the *glyA127* mutation separately from the other mutations present in Ds127.

**Growth phenotypes of a strain containing the *glyA127* allele.** Strain DG232 grew more slowly at 28°C in GRM without methionine (Fig. 3) than the isogenic wild-type strain DG231. Under these conditions, strain DG231 grew with a doubling time of 55 min, whereas strain DG232 exhibited biphasic growth. The growth rate of strain DG232 was close to that of strain DG231 after an overnight, stationary-phase culture grown in GRM without methionine was subcultured in fresh GRM without methionine. After 1.5 generations, the doubling time of strain DG232 had slowed to 90 min and remained at this rate, even after a log-phase culture was diluted into fresh medium. The fact that the doubling time of DG232 remained at 90 min following dilution into fresh medium indicated that the slower growth of DG232 than of strain DG231 was not due to depletion of a critical nutrient from the medium. Rather, this result suggested that strain DG232 accumulated some compound during the stationary phase which supported a high rate of growth for a short time after the stationary-phase culture was first diluted into fresh medium. Strain DG232 was unable to attain steady-state growth at 42°C in GRM without methionine. Its growth was linear, not logarithmic, under these conditions.

The slow growth of strain DG232 at 28°C and its linear growth at 42°C were seen when cells were grown in GRM lacking methionine. These growth deficiencies were alleviated when cells were provided with exogenous methionine. When strain DG232 was grown in GRM with methionine, its growth rate at 28 or 42°C was virtually indistinguishable from that of strain DG231 grown in the same medium (Fig. 3).

**Strain DG232 shows an abnormal heat shock response following exposure to DNP or ethanol in GRM without methionine.** When strain DG232 growing at 28°C in GRM without methionine was exposed to DNP (0.5 mM), it was unable to induce 13 of the 14 heat shock proteins that were induced in the isogenic wild-type strain DG231 under identical conditions. However, unlike strain Ds127, strain DG232 induced 32 of the remaining 38 non-heat shock proteins normally induced by 0.5 mM DNP. Thus, the *glyA127* allele affected the expression of the heat shock regulon, and six other proteins, after exposure to DNP.

Figure 4 shows the induction of four of the heat shock proteins in strains DG231 and DG232 after exposure to 0.5 mM DNP. With the exception of HtpH, which is induced by DNP in *glyA127* mutant strains, the response of these four proteins in strain DG232 is typical of the remaining heat shock proteins. Also shown are two control proteins, UspA (24) and G26.0 (41), which are induced by DNP but are not part of the heat shock regulon. The fact that the induction of these control proteins was unaffected by the presence of the *glyA127* mutation shows that the decreased expression of the heat shock proteins in strain DG232 following exposure to DNP was probably not caused by the *glyA127* mutation restricting entry of DNP into the cells in some manner or rendering its action less effective. It cannot, however, be ruled out that the control proteins might be induced in

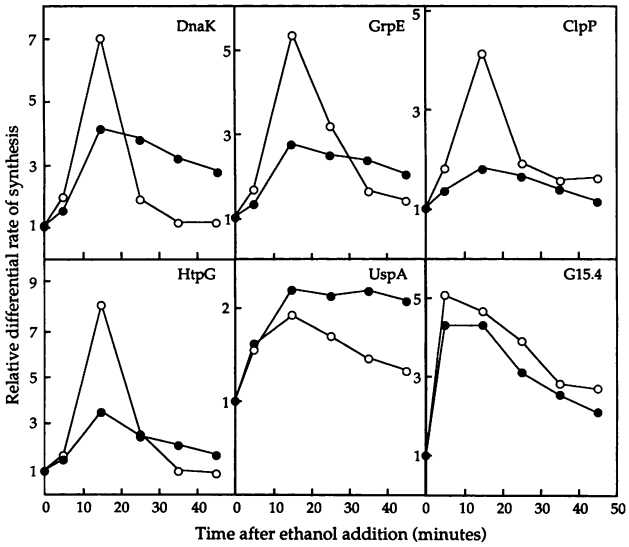


FIG. 5. Relative differential rates of synthesis of heat shock proteins in strains DG231 and DG232 following the addition of ethanol. Strains DG231 (○) and DG232 (●) growing in GRM without methionine at 28°C were pulse labeled at the indicated times after a challenge with 3% ethanol. The relative differential rates of synthesis of the heat shock proteins DnaK, GroEL, ClpP, and HtpG are shown. The proteins UspA and G15.4, which are inducible by ethanol but are not part of the heat shock regulon, are included as positive controls.

response to low concentrations of DNP, concentrations which could be achieved in both strains even if the *glyA127* mutation did restrict DNP entry into cells.

In order to test whether the inability of strain DG232 to induce the heat shock regulon after exposure to DNP was a special case unique to this agent, strains DG231 and DG232 growing in GRM without methionine at 28°C were challenged with ethanol, an agent previously shown to induce the heat shock response in *E. coli* (42). Figure 5 shows the induction of four heat shock proteins and two control proteins in strains DG231 and DG232 after exposure to ethanol. The *glyA127* mutant strain was altered in its ability to induce the heat shock proteins but not the control proteins after exposure to ethanol. The inability of strain DG232 to induce the heat shock proteins was more pronounced following a DNP challenge than it was following an ethanol challenge. When strain DG232 was grown in GRM without methionine, the heat shock proteins were often repressed (Fig. 5 and Table 2; also see Table 4) following exposure to DNP. The relative rate of synthesis of the heat shock proteins in strain DG232 was 20 to 60% of the rate in DG231 following exposure to ethanol (Fig. 5). While the *glyA127* mutation did not nullify the heat shock response following an ethanol challenge, it did attenuate it, indicating that the effects of the *glyA127* mutation on the heat shock response are not seen solely after a DNP challenge.

Because the abnormal growth phenotypes of strain DG232 could be alleviated by providing the cell with exogenous methionine (Fig. 2), we tested whether the abnormal heat shock phenotype following exposure to DNP could also be alleviated in the same way. Table 2 summarizes the results from such an experiment. It was clear that exogenous methionine did restore to strain DG232 the ability to induce the heat shock proteins in response to DNP exposure.

**Strain DG232 is able to induce the heat shock regulon following a temperature shift.** In view of the fact that strain DG232 showed an abnormal heat shock response following exposure to DNP and ethanol and grew linearly at 42°C, we compared the rates of synthesis of heat shock proteins following a shift from 28 to 42°C in strains DG231 and DG232 growing in GRM without methionine. Surprisingly, induction of the heat shock proteins was equivalent in both strains (Table 3). It is unlikely, therefore, that the linear growth of *glyA127* mutant strains in GRM without methionine at 42°C is due to an inability of this strain to increase the synthesis of the heat shock proteins at high temperature.

**Abnormal phenotypes of strain DG232 can be complemented by a low-copy-number plasmid containing the *glyA* gene.** Revertants of a *glyA127* strain selected for glycine prototrophy grew at a wild-type rate in GRM without methionine at 28°C, had a GlyA protein which ran normally on two-dimensional gels, and were able to induce the heat shock proteins following exposure to DNP (10). These results suggested that the *glyA127* mutation was likely responsible for all of the phenotypes of strain DG232 described above.

In order to show that the *glyA127* mutation was responsible for these phenotypes, we tried to complement these deficiencies with a high-copy-number plasmid, pGS29, containing the *glyA* gene (37), but were unsuccessful. In order to rule out the possibility that the failure of pGS29 to complement the abnormal phenotypes of strain DG232 was due to toxic effects which may have come from having the *glyA* gene present on a high-copy-number plasmid, the *glyA* gene was subcloned from pGS29 and inserted into the low-copy-number plasmid pFN476 to give pFN601. This plasmid contains the *glyA* gene, upstream sequences, and 527 bp of the divergently transcribed gene *hmp*. A control plasmid, pFN600, containing a truncated *glyA* gene and the upstream DNA, including the 527 bp of *hmp*, was also constructed (Fig. 1).

Figure 6 shows growth curves for strain DG232 containing either pFN476, pFN600, or pFN601 in GRM without methionine at 28 and 42°C. Plasmid pFN601, carrying the *glyA* gene, was able to complement the slow growth of strain DG232 strain at 28°C in GRM without methionine and the linear growth of this strain at 42°C in this medium. The doubling time of DG232/pFN601 was 55 min at 28°C and 25 min at 42°C. These doubling times were indistinguishable from those of the wild-type strain DG231 growing under the same conditions. Neither the vector pFN476 nor the control plasmid pFN600, which carries the truncated *glyA* gene, were able to alleviate the abnormal growth phenotypes at either 28 or 42°C.

Table 4 shows that strain DG232 recovered the ability to induce the heat shock proteins after exposure to 0.5 mM DNP when it carried plasmid pFN601. As with the growth rate, neither the vector nor the control plasmid pFN600 allowed strain DG232 to induce the heat shock regulon after a DNP challenge. These experiments show that in strain DG232, linear growth at 42°C, slow growth at 28°C, and the lack of a heat shock response following exposure to DNP are the result of a mutation in the *glyA* gene.

## DISCUSSION

In this article, we have described the phenotypes associated with a *glyA* mutation in *E. coli*. This mutation caused cells to grow slowly in GRM without methionine at 28°C and to grow linearly in the same medium at 42°C. In addition,

TABLE 2. Rates of synthesis of heat shock proteins in strains DG231 (*glyA*$^+$) and DG232 (*glyA127*) before and after exposure to DNP[a]

| Protein[b] | Methionine in medium | Strain | Differential rate during steady-state growth[c] | Differential rate after DNP addition[c] | Induction[d] |
|---|---|---|---|---|---|
| ClpB (hsp) | − | *glyA*$^+$ | 1.61 ± 0.25 | 3.72 ± 0.74 | 2.31 ± 0.58 |
| | | *glyA127* | 1.04 ± 0.01 | 0.88 ± 0.07 | 0.85 ± 0.06 |
| | + | *glyA*$^+$ | 1.37 ± 0.47 | 5.53 ± 1.33 | 4.05 ± 1.69 |
| | | *glyA127* | 1.08 ± 0.04 | 3.61 ± 0.52 | 3.34 ± 0.50 |
| ClpB* (hsp)[e] | − | *glyA*$^+$ | 0.92 ± 0.04 | 2.18 ± 0.11 | 2.37 ± 0.16 |
| | | *glyA127* | 0.88 ± 0.03 | 0.93 ± 0.05 | 1.06 ± 0.07 |
| | + | *glyA*$^+$ | 0.75 ± 0.04 | 2.11 ± 0.20 | 2.83 ± 0.29 |
| | | *glyA127* | 0.75 ± 0.09 | 1.54 ± 0.09 | 2.05 ± 0.27 |
| ClpP (hsp) | − | *glyA*$^+$ | 1.70 ± 0.32 | 6.89 ± 0.55 | 4.06 ± 0.83 |
| | | *glyA127* | 1.49 ± 0.11 | 2.19 ± 0.19 | 1.47 ± 0.17 |
| | + | *glyA*$^+$ | 2.00 ± 0.64 | 10.85 ± 0.36 | 5.43 ± 1.74 |
| | | *glyA127* | 2.37 ± 0.33 | 11.73 ± 1.78 | 4.96 ± 1.03 |
| DnaK (hsp) | − | *glyA*$^+$ | 0.75 ± 0.08 | 2.63 ± 0.26 | 3.48 ± 0.50 |
| | | *glyA127* | 1.03 ± 0.06 | 0.81 ± 0.03 | 0.79 ± 0.06 |
| | + | *glyA*$^+$ | 0.72 ± 0.06 | 3.30 ± 0.22 | 4.58 ± 0.48 |
| | | *glyA127* | 0.73 ± 0.10 | 2.10 ± 0.12 | 2.88 ± 0.44 |
| GroEL (hsp) | − | *glyA*$^+$ | 1.64 ± 0.11 | 4.78 ± 0.32 | 2.92 ± 0.28 |
| | | *glyA127* | 1.42 ± 0.03 | 1.25 ± 0.06 | 0.88 ± 0.04 |
| | + | *glyA*$^+$ | 1.56 ± 0.14 | 5.86 ± 0.18 | 3.76 ± 0.37 |
| | | *glyA127* | 1.64 ± 0.02 | 4.02 ± 0.05 | 2.45 ± 0.05 |
| GroES (hsp) | − | *glyA*$^+$ | 1.81 ± 0.46 | 3.27 ± 0.06 | 1.81 ± 0.46 |
| | | *glyA127* | 1.94 ± 0.35 | 1.08 ± 0.03 | 0.56 ± 0.10 |
| | + | *glyA*$^+$ | 2.22 ± 0.12 | 4.67 ± 0.31 | 2.11 ± 0.18 |
| | | *glyA127* | 2.46 ± 0.12 | 5.20 ± 1.11 | 2.11 ± 0.46 |
| GrpE (hsp) | − | *glyA*$^+$ | 1.83 ± 0.63 | 4.41 ± 0.18 | 2.41 ± 0.83 |
| | | *glyA127* | 2.09 ± 0.08 | 2.18 ± 0.23 | 1.04 ± 0.12 |
| | + | *glyA*$^+$ | 2.62 ± 0.21 | 7.46 ± 1.57 | 2.85 ± 0.64 |
| | | *glyA127* | 2.53 ± 0.16 | 5.69 ± 0.27 | 2.25 ± 0.18 |
| HtpH (hsp) | − | *glyA*$^+$ | 1.11 ± 0.13 | 6.05 ± 0.57 | 5.45 ± 0.83 |
| | | *glyA127* | 0.95 ± 0.03 | 3.59 ± 0.36 | 3.77 ± 0.40 |
| | + | *glyA*$^+$ | 1.59 ± 0.04 | 11.92 ± 2.53 | 7.49 ± 1.60 |
| | | *glyA127* | 2.07 ± 0.40 | 9.23 ± 03.23 | 4.46 ± 1.78 |
| HtpI (hsp) | − | *glyA*$^+$ | 0.99 ± 0.07 | 2.71 ± 0.32 | 2.73 ± 0.38 |
| | | *glyA127* | 1.06 ± 0.05 | 1.16 ± 0.09 | 1.09 ± 0.10 |
| | + | *glyA*$^+$ | 1.01 ± 0.11 | 4.11 ± 0.04 | 4.07 ± 0.43 |
| | | *glyA127* | 0.95 ± 0.05 | 2.44 ± 0.21 | 2.56 ± 0.26 |
| HtpK (hsp) | − | *glyA*$^+$ | 1.12 ± 0.11 | 3.44 ± 0.27 | 2.84 ± 0.35 |
| | | *glyA127* | 1.70 ± 0.23 | 1.98 ± 0.65 | 1.17 ± 0.41 |
| | + | *glyA*$^+$ | 2.72 ± 0.40 | 5.40 ± 0.40 | 1.98 ± 0.32 |
| | | *glyA127* | 2.29 ± 0.18 | 5.70 ± 1.05 | 2.49 ± 0.50 |
| HtpO (hsp) | − | *glyA*$^+$ | 0.72 ± 0.08 | 2.20 ± 0.29 | 3.08 ± 0.55 |
| | | *glyA127* | 0.91 ± 0.04 | 0.81 ± 0.09 | 0.88 ± 0.10 |
| | + | *glyA*$^+$ | 1.18 ± 0.36 | 4.45 ± 1.04 | 3.78 ± 1.46 |
| | | *glyA127* | 0.98 ± 0.14 | 3.03 ± 0.18 | 3.08 ± 0.49 |

*Continued on following page*

TABLE 2—*Continued*

| Protein[b] | Methionine in medium | Strain | Differential rate during steady-state growth[c] | Differential rate after DNP addition[c] | Induction[d] |
|---|---|---|---|---|---|
| Lon (hsp) | − | *glyA*$^+$ | 1.14 ± 0.15 | 3.97 ± 0.31 | 3.49 ± 0.52 |
| | | *glyA127* | 1.10 ± 0.06 | 0.93 ± 0.12 | 0.85 ± 0.12 |
| | + | *glyA*$^+$ | 0.99 ± 0.19 | 4.02 ± 0.33 | 4.06 ± 0.84 |
| | | *glyA127* | 0.99 ± 0.14 | 2.50 ± 0.21 | 2.53 ± 0.42 |
| G26.0 (c) | − | *glyA*$^+$ | 0.94 ± 0.05 | 7.13 ± 0.55 | 7.57 ± 0.73 |
| | | *glyA127* | 1.34 ± 0.16 | 6.73 ± 1.09 | 5.02 ± 1.01 |
| | + | *glyA*$^+$ | 0.63 ± 0.06 | 7.87 ± 0.44 | 12.49 ± 1.47 |
| | | *glyA127* | 0.71 ± 0.09 | 8.64 ± 0.93 | 12.24 ± 2.05 |
| UspA (c) | − | *glyA*$^+$ | 1.16 ± 0.09 | 7.09 ± 1.11 | 6.09 ± 1.06 |
| | | *glyA127* | 1.13 ± 0.12 | 6.59 ± 0.55 | 5.81 ± 0.77 |
| | + | *glyA*$^+$ | 0.75 ± 0.05 | 7.78 ± 0.71 | 10.41 ± 1.14 |
| | | *glyA127* | 0.82 ± 0.09 | 8.57 ± 0.73 | 10.51 ± 1.47 |

[a] Cells growing in GRM with or without methionine, as indicated, were pulse labeled for 5 min with [$^{35}$S]methionine, starting 15 min after exposure to DNP, and chased with an excess of cold methionine for 3 min. Rates of synthesis were determined by the double-label method on spots punched from two-dimensional gels. The growth temperature was 28°C for all cultures.

[b] Abbreviations in parentheses indicate whether the protein is a heat shock protein (hsp) or a control protein (c) which is induced by DNP but is not part of the heat shock regulon. G26.0 is a protein spot for which the corresponding gene is unknown; therefore, an alpha-numeric based on its migration on a two-dimensional gel is shown.

[c] Values shown are averages from triplicate gels ± standard deviation. See text for explanation.

[d] Values shown are the differential rate of synthesis after exposure to DNP divided by the differential rate of synthesis during steady-state growth before the addition of DNP ± the standard deviation of the ratio.

[e] ClpB* is encoded by the same reading frame as ClpB. It was previously known as HtpS (35, 41).

cells carrying this mutation failed to induce the heat shock proteins in a normal fashion following exposure to DNP or ethanol. This was a surprising result because mutations in amino acid-biosynthetic genes are not usually thought to be capable of producing such pleiotropic effects. The phenotypes seen in strain DG232 are not due to a lack of glycine or serine, amino acids synthesized by SHMT, because these amino acids are supplied in the medium. Rather, the phenotypes must arise because strain DG232 cannot synthesize a key metabolite or because mutant SHMT protein poisons the cell. We believe that the former possibility is more likely, because the abnormal phenotypes associated with DG232 can be alleviated simply by providing cells with exogenous methionine, and because if the latter were true, the *glyA127* mutation should be dominant, which it is not.

SHMT participates indirectly in the final step of methionine synthesis by providing the one-carbon unit of 5-methyltetrahydrofolate, which is used to methylate homocysteine (Fig. 2). The fact that both the growth rate inhibition and the abnormal heat shock phenotype of strain DG232 can be phenotypically suppressed by exogenous methionine can be explained by postulating that one-carbon units, which are normally provided by the conversion of serine to glycine by

TABLE 3. Rates of synthesis of heat shock proteins in strains DG231 (*glyA*$^+$) and DG232 (*glyA127*) after a temperature shift from 28 to 42°C[a]

| Protein[b] | Relative differential rate of synthesis after temp shift[c] | |
|---|---|---|
| | DG231 (*glyA*$^+$) | DG232 (*glyA127*) |
| ClpP (hsp) | 1.54 | 1.97 |
| DnaK (hsp) | 7.69 | 5.11 |
| GroEL (hsp) | 3.33 | 5.01 |
| GrpE (hsp) | 2.71 | 3.36 |
| HtpG (hsp) | 7.47 | 7.35 |
| UspA (c) | 3.48 | 2.79 |

[a] Cells growing in GRM without methionine were pulse labeled for 5 min with [$^{35}$S]-methionine, starting 3 min after a shift from 28 to 42°C, and chased with an excess of cold methionine for 3 min. Rates of synthesis were determined by the double-label method on spots punched from two-dimensional gels.

[b] Abbreviations in parentheses indicate whether the protein is a heat shock protein (hsp) or a control protein (c) which is induced by high temperature but is not part of the heat shock regulon.

[c] Values shown are the differential rate of synthesis after the temperature shift divided by the differential rate of synthesis during steady-state growth at 28°C. Values are averages from duplicate gels.

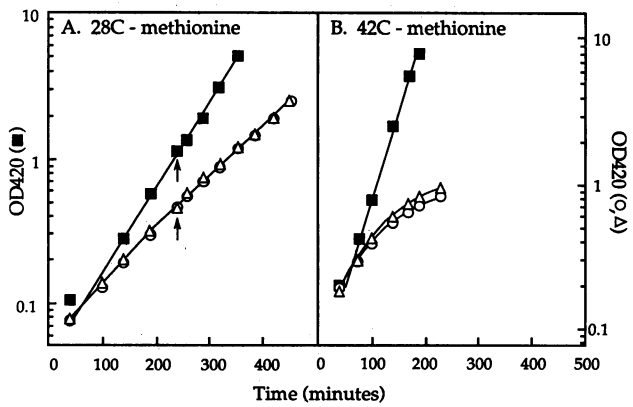


FIG. 6. Abnormal growth phenotypes of strain DG232 can be complemented by a low-copy-number plasmid carrying a wild-type *glyA* gene. Strain DG232 carrying pFN476 (○), pFN600 (△), or pFN601 (■) were grown in GRM without methionine at (A) 28°C or (B) 42°C. Arrows indicate when cultures were diluted 1:15. The growth curves were normalized to allow for the dilution.

TABLE 4. Complementation of the abnormal heat shock phenotype of strain DG232 (*glyA127*) by a low-copy-number plasmid containing a wild-type *glyA* gene[a]

| Protein[b] | Relative differential rate of synthesis after DNP addition[c] | | | | | |
|---|---|---|---|---|---|---|
| | DG231 (*glyA*$^+$)/pFN476 | DG232 (*glyA127*)/pFN476 | DG231 (*glyA*$^+$)/pFN600 | DG232 (*glyA127*)/pFN600 | DG231 (*glyA*$^+$)/pFN601 | DG232 (*glyA127*)/pFN601 |
| ClpP (hsp) | 2.80 | 1.84 | 2.84 | 1.62 | 2.95 | 3.28 |
| DnaK (hsp) | 3.58 | 0.72 | 3.70 | 0.66 | 3.34 | 3.07 |
| GroEL (hsp) | 2.43 | 0.82 | 2.35 | 1.02 | 2.42 | 2.43 |
| GrpE (hsp) | 2.24 | 1.11 | 2.04 | 1.02 | 2.31 | 2.33 |
| HtpG (hsp) | 5.24 | 2.58 | 4.64 | 2.50 | 4.63 | 4.58 |
| UspA (c) | 5.72 | 6.13 | 6.77 | 5.91 | 6.15 | 6.31 |

[a] Cells were pulse labeled for 5 min in GRM without methionine with [$^{35}$S]-methionine, starting 15 min after exposure to DNP, and chased with an excess of cold methionine for 3 min. Rates of synthesis were determined by the double-label method on spots punched from two-dimensional gels. The growth temperature was 28°C for all cultures.

[b] Abbreviations in parentheses indicate whether the protein is a heat shock protein (hsp) or a control protein (c) which is induced by DNP but is not part of the heat shock regulon.

[c] Values shown are the differential rate of synthesis after exposure to DNP divided by the differential rate of synthesis during steady-state growth before the addition of DNP. Values shown are the averages for duplicate gels. Plasmid pFN476 is vector only, plasmid pFN600 contains a truncated *glyA* gene, and plasmid pFN601 contains a complete *glyA* gene.

SHMT, are limiting in strain DG232. Providing exogenous methionine would help relieve this limitation because endogenously synthesized one-carbon units would be spared for use in other reactions. Consistent with the hypothesis that one-carbon units are rate limiting for growth, we have found that strain DG232 grows more slowly than the wild-type strain DG231 in GRM containing methionine but missing the purine bases guanine and adenine, which also require one-carbon units for their synthesis (10).

In what ways might a one-carbon restriction result in the abnormal heat shock response seen in strain DG232 following a DNP challenge? One explanation is that the lack of induction is the consequence of a slow growth rate. Perhaps the levels of heat shock proteins present in strain DG232 during steady-state growth are able to keep up with the production of misfolded proteins or the accumulation of precursor proteins following exposure to DNP, because the total rate of protein synthesis is relatively low. We do not believe this to be the case because we have found that wild-type cells growing in glucose minimal medium at 28°C with a doubling time of 120 min induce the heat shock regulon in response to DNP. Strains that have mutations in genes other than *glyA* and grow slowly in GRM without methionine are also able to induce the heat shock proteins after exposure to DNP (10).

It was surprising that a mutation in an amino acid-biosynthetic gene could affect the expression of a large regulon, the primary purpose of which is to mediate interactions among polypeptides. Strain DG232 growing in GRM without methionine and limited for one-carbon units would have many cellular processes altered under these conditions. A restriction in the pool of one-carbon tetrahydrofolate adducts would affect the synthesis of purines, histidine, thymine, and methionine and the formylation of aminoacylated initiator tRNA. If cells were restricted for methionine as the result of a *glyA* mutation, the cells would also likely be restricted for *S*-adenosylmethionine. *S*-Adenosylmethionine is a methyl donor used in methylation of DNA (20), the modification of tRNA (23), the methylation of proteins involved in chemotaxis (30), and the methylation of other cytoplasmic proteins (15). All of the above are candidates for processes which may, when altered, affect the expression of the heat shock proteins.

However, an additional observation we have made opens a very different possibility. We have noted that strain DG232 behaves under some circumstances as if it were relaxed with respect to ribosomal protein synthesis, much like a *relA* mutant (10). This phenotype has been seen in cells that were restricted in their ability to initiate protein synthesis either by exposure to kasugamycin, which inhibits translational initiation by interfering with the binding of the 30S ribosomal subunit to mRNA (16), or by artificial limitation for initiation factor 2 (6). Because the initiator tRNA formyl-methionyl-tRNA requires one-carbon units for the synthesis of both the formyl group and the methionine, we measured formyl-methionyl-tRNA levels in the *glyA* mutant and found them to be only 5 to 10% of the levels found in *glyA*$^+$ strains (10). For this reason, we are currently exploring the role that formyl-methionyl-tRNA, initiation of protein synthesis, and the stringent network might play in the regulation of the heat shock response.

While strain DG232 was deficient in inducing the heat shock proteins following a DNP or ethanol challenge at 28°C, it was able to induce these proteins to the same extent as the isogenic *glyA*$^+$ strain DG231 following a shift from 28 to 42°C. The reason for this difference is not known. It may be that the signal, or signal transduction pathway, for inducing the heat shock regulon is qualitatively different for these two classes of stress, and the *glyA127* mutation affects a key process uniquely required for the induction of these proteins following exposure to DNP or ethanol. Alternatively, it may be that the steps required for the induction of the heat shock regulon are the same for both classes of stress and the difference between the induction by DNP and the induction by heat in the mutant is a quantitative difference between the importance of these steps. For example, increased translation of existing *rpoH* message may be of critical importance for induction of the heat shock proteins after a DNP challenge, while stabilization of the RpoH protein is of minor importance. Following a temperature shift, increased translation of the existing *rpoH* message may be less important for heat shock protein induction than stabilization of the RpoH protein. If the *glyA127* mutation causes poor translation of the *rpoH* message under all conditions, exposure to DNP will result in poor induction of the heat shock regulon, whereas the induction after a temperature shift will be relatively unaffected.

Much remains to be learned about the ways in which cells integrate information from disparate biochemical levels in order to grow and remain viable in both constant and

changing environments. This mutant appears to offer an experimental approach to studying this type of integration.

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