JOURNAL OF BACTERIOLOGY, Apr. 1988, p. 1582–1588
0021-9193/88/041582-07$02.00/0

Vol. 170, No. 4

Abnormal Induction of Heat Shock Proteins in an *Escherichia coli* Mutant Deficient in Adenosylmethionine Synthetase Activity

ROWENA G. MATTHEWS[1] AND FREDERICK C. NEIDHARDT[2]*

Department of Microbiology and Immunology[2] and Department of Biological Chemistry and Biophysics Research Division,[1] The University of Michigan, Ann Arbor, Michigan 48109-0620

Received 31 August 1987/Accepted 5 January 1988

Most prototrophic strains of *Escherichia coli* become restricted for methionine at 44°C. A mutant strain (RG62 *metK*) in which the level of *S*-adenosylmethionine synthetase activity is only 10 to 20% of normal shows constitutive expression of one of the heat shock proteins, the *lysU* gene product, lysyl-tRNA synthetase form II, at 37°C. These findings suggested a possible linkage between methionine metabolism and heat shock. We examined the induction of heat shock polypeptides in strain RG62 (*metK*) and in its parent, RG (*metK*$^+$), from which it was derived by spontaneous mutation. Exponential-phase cultures of the two strains were pulse-labeled with [^{3}H]leucine shortly after a shift from 37 to 44°C, and the total cellular polypeptides were examined by two-dimensional electrophoresis. The results confirmed the constitutive production of the *lysU* gene product previously reported for strain RG62, but also revealed that the induction of 2 of the 17 heat shock polypeptides, C14.7 and G13.5, was markedly depressed. Otherwise the heat shock induction pattern was similar in timing and magnitude in the two strains. Transformation of the mutant strain with a plasmid, pK8, containing the *metK* coding sequence and promoter region as a 1.8-kilobase insert into pBR322 restored normal induction of C14.7 and G13.5, but did not prevent constitutive expression of the *lysU* gene product in the medium required for growth of this strain. The three heat shock polypeptides abnormally controlled in strain RG62 are the three polypeptides which are not induced when rapid synthesis of the *htpR* gene product is induced by isopropyl-β-D-thiogalactopyranoside at 28°C (R. A. VanBogelen, M. A. Acton, and F. C. Neidhardt, Genes Dev. 1:525–531, 1987). We postulate that induction of these three polypeptides involves metabolic signals in addition to the synthesis of the *htpR* gene product and that strain RG62 (*metK*) fails to produce the signals involved in induction of C14.7 and G13.5 on a shift-up in temperature and produces the signal related to *lysU* induction even at 37°C.

With the demonstration that induction of the heat shock proteins at 28°C does not protect cells against exposure to lethal temperatures (17), one is left without a role for these proteins in *Escherichia coli*. Their induction appears to be required for adaptation of *E. coli* to growth at elevated but sublethal temperatures since mutants defective in the heat shock regulatory protein, the *htpR* gene product, are unable to grow normally at temperatures above 30°C (16). This suggests that attention should be directed to the metabolic alterations in cells which have been subjected to a temperature shift since some heat shock proteins may be induced in response to these alterations.

Seminal studies by Ron and Davis (14) established that the growth of prototrophic strains of *E. coli* at temperatures between 40 and 45°C leads to methionine limitation and that this limitation is associated with decreased activity of the first enzyme in the methionine biosynthetic pathway, homoserine transsuccinylase, the *metA* gene product. If some change in methionine metabolism after a temperature shift generates one of the signals for the heat shock response, strains altered in methionine metabolism might exhibit an abnormal pattern of induction of the heat shock proteins. Indeed, it has been established that strain RG62 (*metK*) shows constitutive induction of one of the heat shock proteins, the *lysU* gene product, lysyl-tRNA synthetase form II, at 37°C (7).

Strain RG62 (*metK*) has deficient *S*-adenosylmethionine (AdoMet) synthetase activity. Since AdoMet, rather than methionine, serves as a corepressor for the methionine regulon (4), *metK* mutants have elevated levels of the enzymes involved in methionine biosynthesis (6) and of serine hydroxymethyltransferase (the *glyA* gene product) (15). The current work demonstrates that RG62 (*metK*) shows markedly depressed induction of two other heat shock polypeptides, C14.7 and G13.5, after a shift from 37 to 44°C and that this strain also shows abnormal nutritional requirements at 44°C.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains used in this study are listed in Table 1. Strain DM25(pK8) (kindly provided by G. Douglas Markham) contains the *metK* coding sequence and promoter as a 1.8-kilobase *Pst*I fragment inserted into the *amp* site of pBR322 (10).

Media and growth conditions. All cultures were grown aerobically in rotatory action shakers at either 37 ± 0.1 or 44 ± 0.2°C. The growth of cells was monitored at 420 nm, and the cells were grown to an optical density of 0.3 (~3 × 10^7 cells per ml) before the temperature was shifted by transfer from a bath at 37°C to one at 44°C. Cells were grown in 3-[*N*-morpholine]propanesulfonic acid (MOPS) minimal medium (11), supplemented with 10 μM thiamine, 0.4% glucose, and amino acids as indicated. The concentrations of amino acid supplements are those used in defined rich medium (18) unless noted otherwise. Supplements to glucose minimal medium for growth of RG62(pK8) were serine, methionine, leucine, isoleucine, and valine.

Construction of RG62(pK8) and RG62(pBR322). Plasmid

* Corresponding author.

TABLE 1. *E. coli* K-12 strains used in this work

Strain	Description	Source
RG	*thi metK*$^+$	R. Greene
RG62	*thi metK hsd*$^+$	R. Greene
RG62(pBR322)	*thi metK* Tet^r	This work
RG62(pK8)	*thi metK*$^+$ Tet^r	This work
DM25(pK8)	*thi metJ* Tet^r *proC leu hsdS* (*lac gal ara rpoB*)[a]	G. D. Markham
DH1	F$^-$ *thi recA1 hsdR17* (*gyrA96 endA1 supE44* λ^-)[a]	W. Dunnick

[a] Markers in parentheses are irrelevant to the present work and are included for strain description.

DNA was isolated from DM25(pK8) by the method of Maniatis et al. (9). Strain DM25 carries *hsdS*, and the plasmid DNA is not methylated. The isolated plasmid DNA was used to transform competent DH1 cells (strain DH1 carries *hsdR*) prepared by $CaCl_2$ treatment (9). The transformed cells were selected for resistance to tetracycline, and the methylated plasmid DNA was isolated and used to transform competent RG62 cells, also prepared by $CaCl_2$ treatment. The transformed strain RG62(pK8) was selected for tetracycline resistance on Luria broth plates. Competent RG62 cells were also transformed with pBR322 plasmid DNA isolated from DH1(pBR322). To maintain the plasmids, RG62(pK8) and RG62(pBR322) were propagated on plates containing 30 μg of tetracycline per ml of Luria broth or in liquid media containing 10 to 30 μg of tetracycline per ml.

Radioactive labeling of protein and resolution on two-dimensional gels for autoradiography. Cultures of exponentially growing cells ($A_{420} \cong 0.3$) were pulse-labeled for 5 min at 37°C and then 5, 30, and 60 min after a shift to 44°C. If labeling with leucine was to be performed, the growth medium contained 80 μM leucine (1/10th the usual supplement) and 120 and 80 μM valine and isoleucine, respectively (1/5th the usual supplements) to prevent isoleucine restriction during the labeling. Portions (2 ml) were transferred to preheated vials containing L-[^{3}H]leucine (140 mCi/mmol; 100 μCi/ml) or L-[^{3}H]lysine (83 Ci/mmol; 100 μCi/ml) and labeled for 5 min. Unlabeled leucine (8 mM) or lysine (8 mM) was added for a 3-min chase. Extracts were prepared and processed for resolution on two-dimensional polyacrylamide gels (12) as previously modified (1). Gels were treated with 2,5-diphenyloxazole (PPO) after dehydration in dimethyl sulfoxide, dried, and exposed to Kodak XAR film for 2 to 16 days at −70°C (2).

RESULTS

Growth characteristics of the *metK* mutant strain and its parent strain. RG62 was derived from RG, a prototrophic K-12 strain, by spontaneous mutation to ethionine resistance (6). The growth of the parent strain before and after a shift from 37 to 44°C is shown in Fig. 1, and the doubling times characterizing growth of this strain before and after temperature shifts in various media are given in Table 2. Shifting the temperature from 37 to 44°C resulted in a 1.8-fold decrease in growth rate in glucose minimal medium. Supplementation of glucose minimal MOPS medium with methionine resulted in very little change in growth rate immediately after the shift, but the growth rate at 44°C slowly decreased until it was 1.5-fold slower than that at 37°C. These growth patterns are similar to those originally described by Ron and Davis (14). When grown in glucose-MOPS supplemented with 20 mM bicarbonate, thiamine, and all 20 amino acids, the parent strain showed a small decrease in growth rate on a shift from 37 to 44°C (Fig. 1B). When methionine was omitted from the supplemented medium, the growth rate at 37°C was unaffected, but a shift to 44°C resulted in a marked growth lag and subsequent recovery. In contrast to the recovery of growth rate seen in amino acid-supplemented medium lacking methionine after a shift to 44°C, no recovery was seen in glucose minimal medium and the doubling time of 103 min was close to the doubling time of 120 min seen immediately

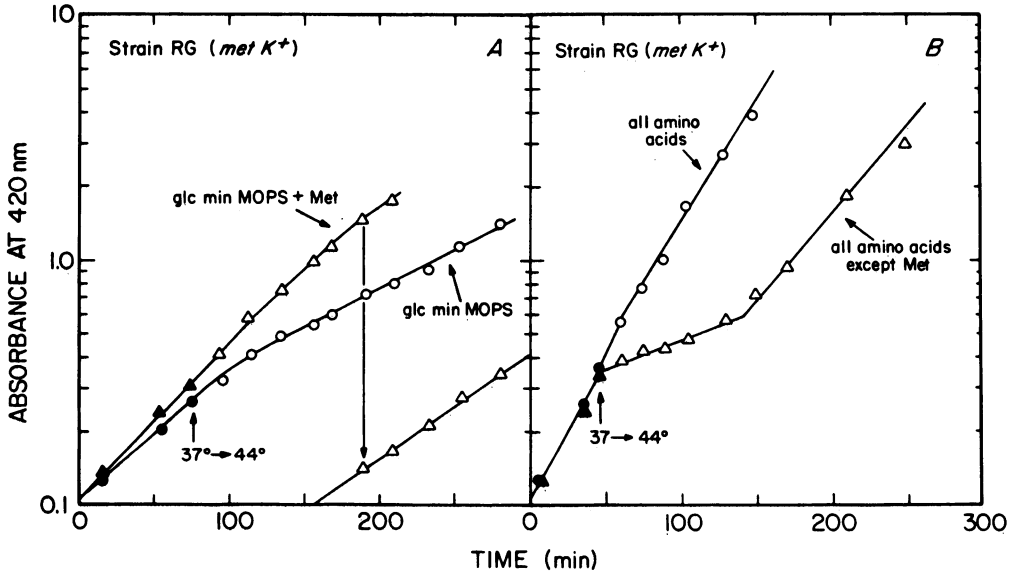

FIG. 1. Growth characteristics of a prototrophic *E. coli* K-12 strain (RG *metK*$^+$) before and after a shift from 37 to 44°C. Cultures were grown aerobically in glucose minimal MOPS medium with or without supplementation with 0.2 mM methionine (A) or glucose-MOPS medium supplemented with all 20 amino acids or with 19 amino acids (lacking methionine) (B). When the cultures reached an A_{420} of 0.3, they were shifted to 44°C. The arrow in panel A indicates the time at which this culture was diluted 10-fold into the same medium (glucose minimal MOPS supplemented with methionine).

TABLE 2. Doubling times for strain RG (*metK*$^+$) and RG62 (*metK*) before and after shifts from 37 to 44°C

Addition(s) to glucose minimal MOPS	Doubling time (min) of strain:			
	RG		RG62	
	37°C	44°C	37°C	44°C
Thiamine	57	103	65	260
Thiamine + Met	48	73	53	290
Thiamine + Ser + Ile	58	132	71	75
Thiamine + 20 amino acids + HCO_3^-	27	30	59	32
Thiamine + 19 amino acids (no Met) + HCO_3^-	27	120[a], 43	59	40

[a] Doubling time immediately after the shift from 37 to 44°C.

after a shift to 44°C in supplemented medium lacking methionine. For unknown reasons, methionine synthesis in an amino acid-supplemented medium can support rapid growth at 44°C, but remains rate limiting in minimal medium.

Figure 2 shows the growth characteristics of the *metK* mutant strain, and the generation times associated with these growth curves are given in Table 2. When these cells were shifted from 37 to 44°C in glucose minimal MOPS medium, the growth rate decreased fourfold, and supplementation with methionine did not increase the growth rate after a shift. Instead, there was an unexpected growth stimulation by serine and isoleucine at 44°C but not at 37°C. From these results, we conclude that derepression of the methionine regulon by reduction of AdoMet synthetase activity in the *metK* mutant is not sufficient to sustain the growth rate of RG62 in glucose minimal MOPS medium after a shift from 37 to 44°C. The *metK* strain grew considerably more slowly than its parent at 37°C in a medium supplemented with all amino acids, and after a shift to 44°C the growth rate increased to a value close to that of the parent strain (Fig. 2B). Omission of methionine from the supplemented medium caused no detectable change in growth rate at 37°C and only a small decrease in growth rate at 44°C. Note that the rate of growth at 44°C in the absence of methionine was close to that of the parent strain after recovery from the growth lag and that no growth lag was observed in the mutant strain after the shift.

When the parent strain was shifted from 37 to 44°C in supplemented medium lacking methionine, the temperature shift resulted in a diauxic lag, apparently owing to methionine limitation. Since the methionine biosynthetic enzymes are already derepressed in the *metK* mutant, no diauxie is observed after a shift in this medium and the growth rate at 44°C is close to that of the derepressed parent strain. In glucose minimal medium, the parent strain did not show diauxie after a shift from 37 to 44°C, either because it failed to derepress the methionine regulon under these conditions or because it required amino acid precursors for methionine biosynthesis. As already noted, rapid growth of the mutant strain in glucose minimal medium at 44°C requires supplementation with serine and isoleucine, but these two amino acids do not support recovery of the parent strain at 44°C in glucose minimal medium.

Induction of heat shock proteins after a temperature shift of parent and mutant strains. In Fig. 3, fluorograms are shown of the two-dimensional gel electrophoresis pattern of total cellular polypeptides isolated from *E. coli* after pulse-labels (5 min) with tritiated leucine. Samples of the cultures were labeled at 37°C and 5 min after a shift to 44°C. The fluorograms shown are from cultures grown in glucose minimal MOPS medium supplemented with methionine and leucine (80 μM), isoleucine (80 μM), and valine (120 μM). The parent strain exhibited a typical heat shock induction pattern. In contrast, the mutant strain showed marked abnormalities in induction of heat shock proteins. As previously observed (7), the *metK* strain showed increased synthesis of the *lysU* gene product at 37°C as compared with its parent (the upper spot in box 9, the *lysU* gene product, is more intense in the mutant at 37°C, while the lower spot in box 9, the *lysS* gene product, is more intense in the parent at this temperature). Upon a shift to 44°C, the intensity of the *lysU* spot increased in both mutant and parent strains and an

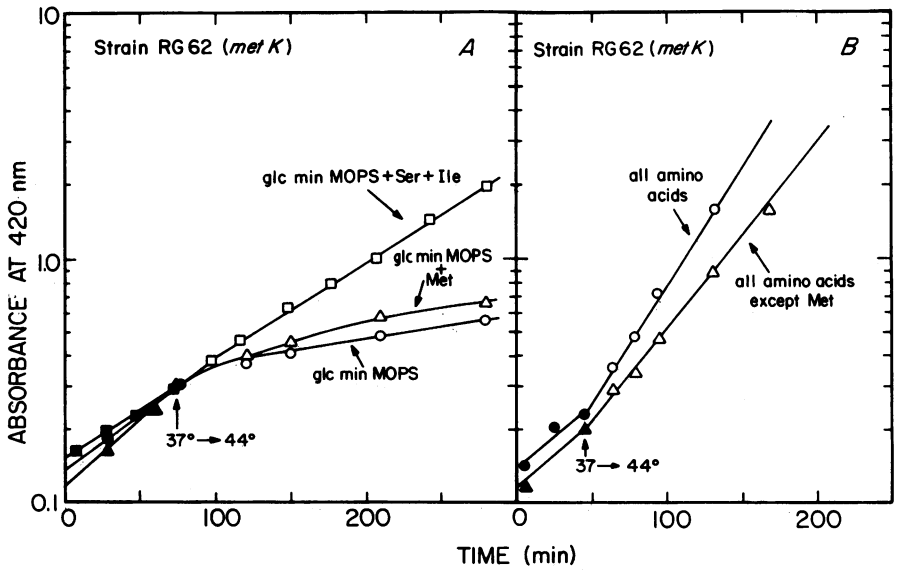

FIG. 2. Growth characteristics of strain RG62 (*metK*) before and after a shift from 37 to 44°C. Cultures were grown aerobically in glucose minimal MOPS medium either unsupplemented or containing 0.2 mM methionine or 10 mM serine and 0.4 mM isoleucine (A) or in glucose-MOPS supplemented with all 20 amino acids or with 19 amino acids (lacking methionine) (B).

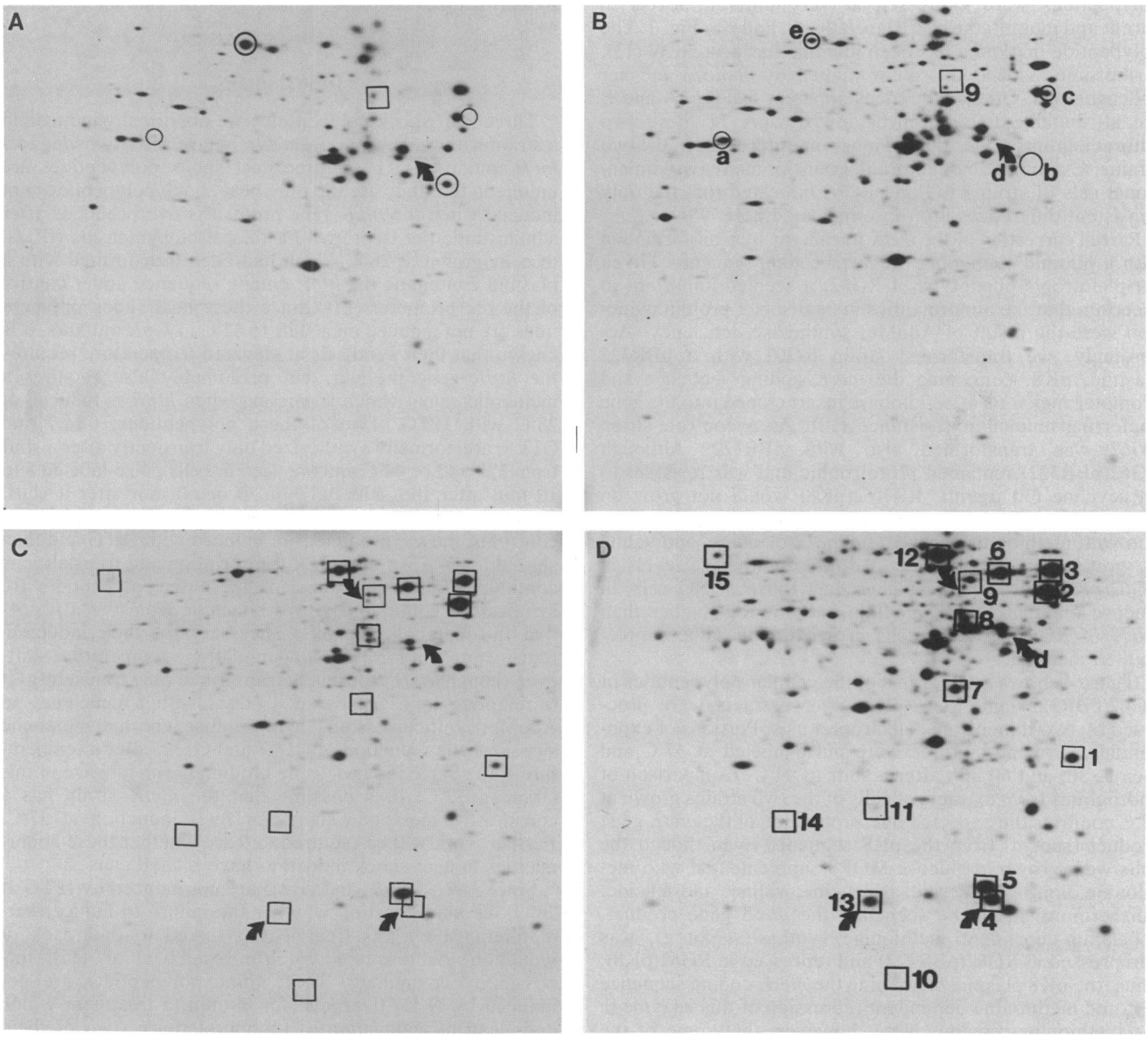

FIG. 3. Synthesis of individual proteins in strains RG62 (*metK*) and RG (*metK*$^+$) before and after a shift from 37 to 44°C. (A) Strain RG62, 37°C; (B) strain RG, 37°C; (C) strain RG62, 44°C; (D) strain RG, 44°C. The arrow marked d in panels B and D indicates the position of the *metK* gene product, AdoMet synthetase, in fluorograms of proteins from the parent strain. No spot is visible in this position in fluorograms of proteins of the mutant strain (A and C). Other differences between parent and mutant strains at 37°C are indicated by the circles; proteins migrating in positions a and e were identified as ribosomal protein W and cobalamin-independent methionine synthase, respectively (see the text). The boxes surround heat shock proteins, which are numbered in panel D as follows: 1, B25.3; 2, B56.5 (*groEL*); 3, B66.0 (*dnaK*); 4, C14.7; 5, C15.4 (*groES*); 6, C62.5; 7, D33.4; 8, D48.4; 9, D60.5 (*lysU*); 10, F10.1; 11, F21.5; 12, F84.1; 13, G13.5; 14, G21.0; 15, H94.0 (*lon*).

additional strong spot, which is probably not a *lysU* isopeptide, was also induced. In addition, two heat shock polypeptides which are normally transiently induced after a shift from 37 to 44°C, C14.7 and G13.5, were produced in markedly diminished amounts after the temperature shift. The magnitude and timing of induction of the other heat shock proteins were similar in mutant and parent strains. Similar differences in the heat shock induction pattern were observed for mutant and parent strains in the absence of methionine and in cultures labeled with tritiated lysine in the absence of added leucine, isoleucine, and valine (data not shown). The differences seen in the heat shock protein induction patterns cannot be attributable to differences in methionine pool sizes in the two strains since the addition of methionine to the medium fails to affect the heat shock induction pattern of the parent strain (RG) despite its effect on the intracellular methionine pool size.

In addition to changes in the lysyl-tRNA synthetase pattern, strain RG62 showed several other differences in the fluorograms of cell polypeptides isolated after labeling at 37°C. The *metK* gene product, AdoMet synthetase (spot d), did not appear in the normal position on the two-dimensional gels, and one of the gene products of the methionine regulon, the *metE* gene product, cobalamin-independent methionine synthase (spot e), remained derepressed in the presence of methionine. Besides these expected differences, three other

polypeptides were expressed at very different levels in parent and mutant; these are labeled a, b, and c in Fig. 3. The polypeptide marked a has been identified as protein W (13), a ribosome-associated protein studied by Ganoza and her colleagues (5). Other differences apparent in Fig. 3A and B are attributable to differences in exposure of these two autoradiograms rather than to inherent differences in the two strains. Examination of autoradiograms of many two-dimensional gels of strains RG and RG62 indicated that the only consistent differences are those indicated here.

Partial correction of the *metK* phenotype by transformation with a plasmid containing the *metK* coding sequence. Given the pleiotropic phenotype of RG62, it seemed important to ascertain that the abnormalities in heat shock protein induction were the result of AdoMet synthetase deficiency. Accordingly, we transformed strain RG62 with a pBR322 plasmid, pK8, containing the *metK* coding sequence and promoter region on a 1.8-kilobase insert cloned into the gene conferring ampicillin resistance (10). As a control, strain RG62 was transformed also with pBR322. Although RG62(pBR322) remained prototrophic and was resistant to tetracycline (30 μg/ml), RGK62(pK8) would not grow on glucose minimal MOPS medium and required supplementation with methionine, serine, leucine, isoleucine, and valine for growth. In this medium, AdoMet synthetase levels in RG62(pK8) were 17-fold higher than those of RG cells in glucose minimal MOPS medium and 130-fold higher than those of RG62(pBR322) cells grown in the same supplemented medium.

Figure 4 shows fluorograms of the cellular polypeptides of RG62(pBR322) and RG62(pK8) after extracts were processed by two-dimensional electrophoresis. Portions of exponentially growing cultures were pulse-labeled at 37°C and then 5, 30, and 60 min after a shift to 44°C. Comparison of fluorograms from extracts of cells of the two strains grown at 37°C confirms the expected overproduction of the *metK* gene product (spot d) from the pK8 sequence even though the cells were grown in glucose-MOPS supplemented with methionine along with serine, isoleucine, valine, and leucine. Furthermore, it can be seen that the *metE* gene product, cobalamin-dependent methionine synthase (spot e), was derepressed in RG62(pBR322) and repressed in RG62(pK8). Thus, the pK8 plasmid containing the *metK* coding sequence restored methionine-dependent repression of this enzyme in the methionine regulon. Note, however, that some of the other abnormalities seen with strain RG62 remain, particularly the constitutive induction of the *lysU* gene product at 37°C. After a shift from 37 to 44°C, induction of C14.7 and G13.5 was restored to normal in strain RG62(pK8) in contrast to the diminished response in strain RG62(pBR322). The timing and magnitude of induction of the other heat shock proteins were unaffected by the presence or absence of the *metK* coding sequence. Control experiments (data not shown) indicated that supplementation of glucose minimal medium with serine, methionine, leucine, isoleucine, and valine did not significantly alter the two-dimensional gel pattern of the parent strain (RG) at 37°C and did not lead to changes in its heat shock induction pattern after a shift from 37 to 44°C.

Since strain RG62(pK8) requires supplementation with serine, isoleucine, methionine, valine, and leucine for growth in glucose minimal medium, the ability of the pK8 plasmid to restore rapid growth of RG62 in glucose minimal MOPS medium supplemented with methionine could not be tested in the absence of the amino acids required for growth of RG62 (serine and isoleucine), and both RG62(pK8) and RG62(pBR322) showed similar growth characteristics at 44°C.

DISCUSSION

Three heat shock polypeptides are abnormally induced in a strain with reduced AdoMet synthetase activity owing to a *metK* mutation. These three heat shock polypeptides are unique in that they are the only heat shock polypeptides not induced when the *htpR* gene product is overproduced after administration of isopropyl-β-D-thiogalactopyranoside (IPTG) to cells grown at 28°C which had been transformed with a plasmid containing the *htpR* coding sequence under control of the *tac* promoter (17). Since these heat shock polypeptides are not induced on a shift to 42°C in *htpR* mutants, it is known that their synthesis at elevated temperature requires the *htpR* gene product, but presumably also requires a metabolic signal which is missing when *htpR* is induced at 28°C with IPTG. Two of these polypeptides, C14.7 and G13.5, are normally synthesized only transiently after a shift from 37 to 42 or 44°C and are seen in cells pulse-labeled 5 to 10 min after the shift but not 30 or 60 min after a shift. However, when cells containing the *htpR* plasmid under control of the *tac* promoter are induced with IPTG and then shifted to 42°C, so that *htpR* transcription and translation is continuous rather than transient, these two polypeptides are synthesized continuously. We conclude from these results that the metabolic signal(s) necessary for their induction remains present for at least 60 min after a temperature shift, even though *htpR* is normally transcribed only transiently. It is tempting to suggest that a strain with a deficiency in AdoMet synthetase is unable to produce a normal metabolic signal for the induction of C14.7 and G13.5 after a temperature shift. Since the *lysU* gene product is constitutive in this strain at 37°C, it is possible that the *metK* strain has a constitutive metabolic signal for *lysU* induction at 37°C. Further work will be required to learn whether these abnormalities in heat shock induction have related causes.

Since *lysU*, C14.7, and G13.5 are not induced by IPTG at 28°C, we may question whether the failure to induce thermotolerance by this treatment is linked to their lack of induction. As discussed by VanBogelen et al. (17), this possibility is unlikely. These three polypeptides are not induced by H_2O_2 treatment for 60 min, a treatment which does lead to acquisition of thermotolerance; two of them (C14.7 and G13.5) are induced by puromycin treatment and the third by nalidixic acid, neither of which treatments produce thermotolerance. Furthermore, strain RG62, when shifted to 44°C in glucose minimal MOPS medium, acquires thermotolerance toward a subsequent shift to 52°C; this acquisition of thermotolerance occurs under conditions in which C14.7 and G13.5 are not induced at 44°C (data not shown).

RG62 (*metK*) has a pleiotropic phenotype. Intracellular AdoMet pools are lowered in the mutant (6), and reactions requiring AdoMet, such as methylations of nucleic acids, proteins, and small cellular metabolites and biosynthesis of polyamines and cyclopropane fatty acids, may be impaired when the cellular demand for AdoMet is elevated. In addition, the methionine regulon is derepressed and methionine is excreted into the medium (6). This report documents additional metabolic abnormalities associated with growth at 44°C, specifically a requirement for serine and isoleucine rather than the normal requirement for methionine. It is not yet clear whether the abnormal nutritional requirements for growth of this strain at elevated temperatures are related to

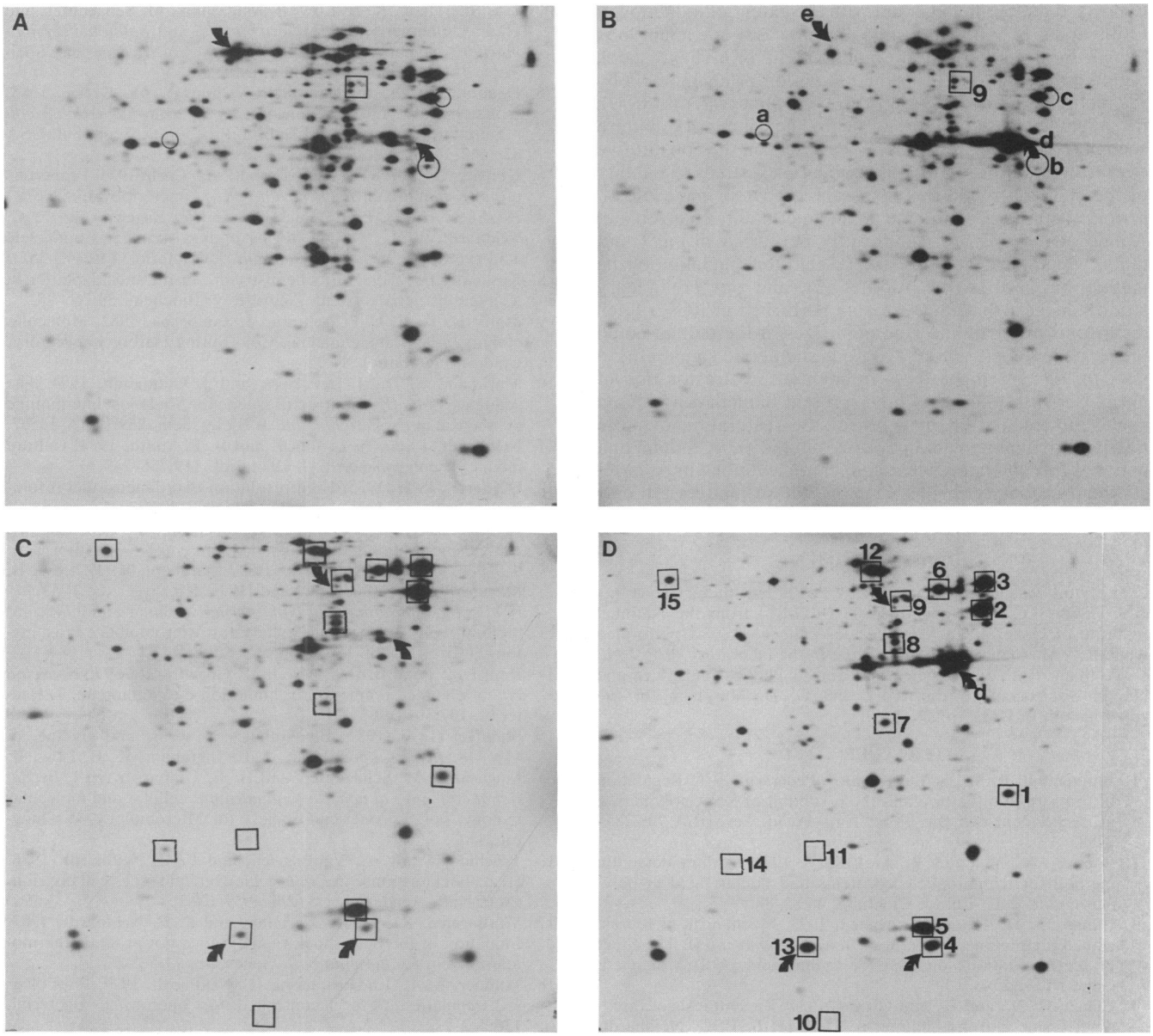

FIG. 4. Partial restoration of a normal heat shock response in RG62 (*metK*) by transformation with a plasmid, pK8, containing the *metK* coding sequence and promoter region as an insert in pBR322. The fluorograms show the incorporation of [^{3}H]lysine during a 5-min period either before a shift from 37 to 44°C or from 5 to 10 min after the shift. (A) Strain RG62 (*metK*) transformed to tetracycline resistance with pBR322, 37°C; (B) strain RG62 transformed to tetracycline resistance with pK8, 37°C; (C) RG62(pBR322), 44°C; (D) RG62(pK8), 44°C. Labeled spots a through e and 1 to 15 are those identified in Fig. 3.

the abnormalities in heat shock polypeptide induction, but our preliminary experiments do suggest such a linkage.

Supplementation of glucose minimal MOPS medium with all the amino acids except methionine and lysine (which was the amino acid used for labeling) results in rapid growth of strain RG62 after a shift to 44°C and is also associated with almost normal levels of induction of C14.7 and G13.5 (data not shown). Similarly, supplementation of glucose minimal MOPS medium with serine (and isoleucine to prevent the isoleucine restriction resulting from catabolism of high levels of serine) accelerates the growth rate of strain RG62 at 44°C and leads to partial restoration of the induction of C14.7 and G13.5 (compare, for example, the intensity of C14.7 and G13.5 in Fig. 3C [RG62 grown in glucose minimal MOPS medium supplemented with methionine, isoleucine, leucine, and valine] and in Fig. 4C [RG62(pBR322) grown in glucose minimal MOPS medium supplemented with serine, methionine, isoleucine, leucine, and valine]).

These preliminary results suggest that induction of G13.5 and C14.7 will be observed under conditions that permit growth of the *metK* mutant at 44°C. If this prediction is borne out, then the metabolic requirements for growth after a shift to 44°C may provide a clue to the nature of the metabolic signal for induction of these two proteins and perhaps to their function in the cell. If their induction requires AdoMet itself, then the rate of growth of strain RG62 in any defined medium at 44°C should be highly correlated with the level of AdoMet synthetase activity

expressed in that medium and with the extent of induction of G13.5 and C14.7 after the shift. In that case, one might look for an AdoMet-dependent modification of a heat shock regulatory protein, akin to the regulation of chemotaxis by the methylation of the chemotactic receptor proteins (8). On the other hand, the abnormal heat shock induction pattern seen in strain RG62 may instead derive from the constitutive overproduction of methionine, which may stress the ability of the cell to provide the necessary precursor molecules—serine, aspartate, cysteine, and methyltetrahydrofolate. Amino acid pools are significantly perturbed in *metJ* mutants, which lack the repressor protein for regulation of the methionine regulon by AdoMet (3), and our preliminary studies suggest that they are also perturbed in RG62. Such distortions in the amino acid pool sizes of the mutant could prevent the generation of a normal metabolic signal after a shift to higher temperature. In such a case, restoration of normal growth and heat shock protein induction by amino acid supplementation may reflect the restoration of more nearly normal amino acid pool sizes in the *metK* mutant and may not be correlated with significant elevations in AdoMet synthetase activity in these media. These matters are currently under active investigation in our laboratories.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants GM 17892 (F.C.N.) and GM 24908 (R.G.M.) from the National Institute of General Medical Sciences. The work was performed while R.G.M. spent a sabbatical leave in the laboratory of F.C.N.

We thank Ruth VanBogelen and Teresa Phillips for advice and helpful discussions of this work and G. D. Markham for the generous gift of DM25(pK8).

LITERATURE CITED

1. **Blumenthal, R. M., S. Reeh, and S. Pedersen.** 1976. Regulation of transcription factor ρ and the α subunit of RNA polymerase in *Escherichia coli* B/r. Proc. Natl. Acad. Sci. USA **73**:2285–2288.
2. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acid in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
3. **Clandinin, M. T., and A. Ahmed.** 1973. Mechanism of repression of methionine biosynthesis in *Escherichia coli* II. The effect of *metJ* mutations on the free amino acid pool. Mol. Gen. Genet. **123**:325–331.
4. **Cohen, G. N., and I. Saint-Girons.** 1987. Biosynthesis of threonine, lysine and methionine, p. 429–444. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
5. **Ganoza, M. C., C. Cunningham, and R. M. Green.** 1985. Isolation and point of action of a factor from *Escherichia coli* required to reconstruct translation. Proc. Natl. Acad. Sci. USA **82**:1648–1652.
6. **Greene, R. C., J. S. V. Hunter, and E. H. Coch.** 1973. Properties of *metK* mutants of *Escherichia coli*. J. Bacteriol. **115**:57–67.
7. **Hirshfield, I. N., P. L. Block, R. A. Van Bogelen, and F. C. Neidhardt.** 1981. Multiple forms of lysyl-transfer ribonucleic acid synthetase in *Escherichia coli*. J. Bacteriol. **146**:345–351.
8. **Koshland, D. E.** 1981. Biochemistry of sensing and adaptation in a simple bacterial system. Annu. Rev. Biochem. **50**:765–782.
9. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
10. **Markham, G. D., J. DeParasis, and J. Gatmaitan.** 1984. The sequence of *metK*, the structural gene for *S*-adenosylmethionine synthetase in *Escherichia coli*. J. Biol. Chem. **259**:14505–14507.
11. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture media for enterobacteria. J. Bacteriol. **119**:736–747.
12. **O'Farrell, P. H.** 1975. High resolution two-dimensional resolution of proteins. J. Biol. Chem. **250**:4007–4021.
13. **Phillips, T. A., V. Vaughn, P. L. Block, and F. C. Neidhardt.** 1987. Gene-protein index of *Escherichia coli* K-12, edition 2, p. 919–966. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
14. **Ron, E. Z., and B. D. Davis.** 1971. Growth rate of *Escherichia coli* at elevated temperature: limitation by methionine. J. Bacteriol. **107**:391–396.
15. **Stauffer, G. V.** 1987. Biosynthesis of serine and glycine, p. 412–418. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 1. American Society for Microbiology, Washington, D.C.
16. **Tsuchido, T., R. A. VanBogelen, and F. C. Neidhardt.** 1986. Heat shock response in *Escherichia coli* influences cell division. Proc. Natl. Acad. Sci. USA **83**:6959–6963.
17. **VanBogelen, R. A., M. A. Acton, and F. C. Neidhardt.** 1987. Induction of the heat shock regulon does not produce thermotolerance in *Escherichia coli*. Genes Dev. **1**:525–531.
18. **Wanner, B., R. Kodaira, and F. C. Neidhardt.** 1977. Physiological regulation of a decontrolled *lac* operon. J. Bacteriol. **130**:212–222.