JOURNAL OF BACTERIOLOGY, June 1975, p. 1144-1152
Vol. 122, No. 3

Genetic Characterization of the *metK* Locus in *Escherichia coli* K-12

JAMES S. V. HUNTER, RONALD C. GREENE,* AND CHING-HSIANG SU

Veterans Administration Hospital and Department of Biochemistry, Duke Medical Center, Durham, North Carolina 27705*

Received for publication 27 March 1975

Three independently isolated *metK* mutants have been shown to have leisions lying between *speB* and *glc* near 57 min on the *Escherichia coli* chromosome. Two deletions result in a lack of the *metC* gene product but neither extends into the *metK glc* region. The three *metK* mutations are recessive to the wild-type allele carried on the KLF16 episome.

The *metK* locus was first described in *Salmonella typhimurium* as a region lying between *serA* and *metC* concerned with resistance to methionine analogues (20). We subsequently described a class of mutants in *Escherichia coli* K-12 that are resistant to ethionine, derepressed for the methionine biosynthetic enzymes, and deficient in *S*-adenosylmethionine (SAM) synthetase. Since one of these strains produced an altered (unstable) SAM synthetase, we proposed that they contained mutations of the structural gene for the enzyme (11, 12). The behavior of these strains suggested that SAM or one of its metabolites was the co-repressor for regulation of the genes for the methionine biosynthetic enzymes. Since the mutations we have examined lie between *serA* and *metC* (22), we presumed that they were at the *metK* locus. The observation that a *metK* mutant of *S. typhimurium* did not have a low SAM synthetase level (24) called into question the interpretation that the loci were the same in both organisms. A subsequent report (18) has shown that the *metK* strain used by Savin et al. (24) is atypical and that all the other *S. typhimurium* *metK* strains examined have alterations of SAM synthetase. The atypical "*metK*" mutation ("*metK*^{z} *721*") is in a different complementation group from the others. More recently Hobson (17) has shown that extracts from all "*metK*^{z}" strains (including strain *metK721*) degrade SAM more rapidly than other strains of *S. typhimurium*, but the exact nature of the genetic defect of strain *metK721* remains unknown.

The *metK* locus lies in a region of the *E. coli* chromosome that is difficult to map since none of the closely linked markers can be reliably selected; therefore the more remote *serA* has been used as a selective marker in most transductions. The results presented below show that *metK* lies between *speB* (called AUH by W. Maas) and *glc*.

MATERIALS AND METHODS

Materials. Sources of reagents were: glutathione and disodium adenosine 5'-triphosphate from the Sigma Chemical Co., L-methionine from Schwarz/Mann, L-cystathionine and pyridoxal phosphate from Calbiochem, DL-cystathionine from the Cyclo Chemical Co., reduced nicotinamide adenine dinucleotide from P-L Biochemicals, Inc., 5,5'-dithio-bis(2-nitrobenzoic acid) from the Aldrich Chemical Co., beef heart lactic dehydrogenase from the Worthington Biochemicals Corp., [8-^{14}C]adenosine 5'-triphosphate and L-[guanido-^{14}C]arginine from New England Nuclear Corp. Trimethoprim (2,4-diamino-5[-3,4,5-trimethoxybenzyl]primidine) was a gift from J. J. Burchall of the Wellcome Research Laboratories. Sodium glycolate was prepared by neutralization of Fisher "purified" glycolic acid followed by crystallization of the product from water. *O*-succinyl homoserine was synthesized by the method of Flavin and Slaughter (8). Other chemicals were obtained from standard commercial sources.

Media. Defined media are based on the minimal medium of Davis and Mingioli (6) with the omission of sodium citrate and addition of supplements as indicated. In some experiments the amino acid supplement of Maas (22), containing 13 amino acids, was used. The carbon source was 0.5% dextrose except when scoring the *glc* phenotype where it was 1% sodium glycolate. Solid media were prepared by the addition of 1.5% agar (Difco). The P-1 medium of Scott (25) was used for preparation and assay of phage stocks.

Bacterial and phage strains. The bacterial strains used in this work and their sources are given in Table 1. The PL strains were derived from strain SB1804 by a series of manipulations involving the isolation of spontaneous *thy* mutants by the trimethoprim procedure of Stacey and Simson (27), use of the thymine requirement as a selective marker for introduction of *serA25* from strain MA176 and use of the serine

TABLE 1. *Bacterial strains*

Strain	Pertinent genotype	Source or reference
RG62	*metK84*	Greene et al. (11)
RG73	*metK85*	Greene et al. (11)
RG109	*metK86*	Greene et al. (11)
MA176	*thr-1 leu-6 thi-1 serA25 lysA*	W. K. Maas
MA177	*pro trp serA argE speB*	W. K. Maas
RC_1E_1	*glc-1*	W. K. Maas (30)
C600	*leu thr thi*	W. K. Maas
GUC41	*leu thr thi exbB*	W. K. Maas (15)
KS614	$\Delta\lambda^{att}\lambda CI_{857}$ inserted near *metC* (I group lysogen)	K. Shimada (26)
KS940	$\Delta\lambda^{att}$ Δ*metC* (heat stable derivative of KS614)	K. Shimada (26)
HfrC	*metB*	P. Harriman
SB1804	*thr-1 leu-6 thi-1 hisC3 proA2 metG87*	P. E. Hartman (2)
NF28	*his lysA argA321 thi*	N. Fiil (7)
KL16-99	*recA1 thi* Hfr	B. Low
KLF16/110	KLF16/*argG6 metB1 leu-6 recA1 his-1 thy-1*	B. Bachmann
SB1804 strains		
PL8-24	SB1804 *glc-1 serA25*	This paper
PL8-25	SB1804 *glc-1 metK85*	This paper
PL8-26	SB1804 *glc-1 metK84*	This paper
PL8-27	SB1804 *glc-1 metK86*	This paper
PL8-31	SB1804 *glc-1 serA25 metK86*	This paper
PL8-32	SB1804 *glc-1 serA25 metK85*	This paper
PL8-33	SB1804 *glc-1 serA25 metK84*	This paper
NF28 strains		
RG313	*his lysA recA1 thi metK85* F^-	This paper
RG314	*lysA recA1 thi metK84* F^-	This paper
RG316	KLF-16 (*lysA*$^+$ *metK*$^+$)/RG313	This paper
RG317	KLF-16 (*lysA*$^+$ *metK*$^+$)/RG314	This paper
RG330	*his lysA recA1 thi metK*$^+$ F^-	This paper
RG331	*his lysA recA1 thi metK86* F^-	This paper
RG334	KLF16 (*lysA*$^+$ *metK*$^+$)/RG330	This paper
RG335	KLF16 (*lysA*$^+$ *metK*$^+$)/RG331	This paper
RG352	*his lysA recA1 metK*$^+$ *metJ* F^-	This paper
RG353	KLF16 (*lysA*$^+$ *metK*$^+$)/RG352	This paper

requirement to introduce *glc* and the *metK* alleles. This procedure was repeated until all of the desired markers had been introduced. The diploid strains and the corresponding haploids were constructed by first isolating a nitrosoguanidine-induced *serA* mutant of strain NF28 followed by use of the serine requirement to introduce the desired *metK* alleles. The *recA1* mutation was introduced by conjugation with strain KL16-99 and selection of conjugants that were *arg*$^+$ and sensitive to ultraviolet light. The diploids were constructed by conjugation with strain KLF16/110 and selection of *lys*$^+$ conjugants. The diploids were sensitive to the male-specific phage MS2 and could transfer their episomes when cross-streaked against strain MA177. Haploid derivatives isolated after acridine orange treatment (16) required lysine for growth. Strain RG352 was isolated as a spontaneous ethionine-resistant mutant of strain RG330. As well as exhibiting the characteristic *metJ* phenotype (derepressed levels of methionine biosynthetic enzymes when grown on LB broth), the ethionine resistance mutation of strain RG352 is closely linked to *metB* which verifies its identity as a *metJ* mutation (28).

Either of two strains of bacteriophage was used for transduction. Phage 363 (10) was supplied by Werner Maas and phage P1,L4 (3) was given to us by Philip Harriman.

Methods. The procedures for growth of phage stocks and transduction have been described previously (28). Classification of isolated transductants by growth characteristics was done by applying dilutions (1:100) of fully grown cultures to selective plates. *glc* was scored by comparing growth on plates containing either 0.5% dextose or 1% sodium glycolate as carbon source; the arginine sensitivity of *speB* strains was detected on plates supplemented with the amino acid mixture of Maas (22) plus lysine, serine, methionine, and either ornithine or arginine; *metK* was classified by resistance to 0.03 M DL-ethionine or in the case of the *metG* strains by suppression of the methionine requirement. For quantitative enzyme assays, cells were grown in shake flasks in a gyratory air incubator at 37 C and 200 rpm and harvested in late log phase. For determination of *metK* and *speB* phenotypes,

where it was only necessary to distinguish between high and low levels of enzyme activity, overnight cultures were often used. Assays of cystathionine synthetase, cystathionase, SAM synthetase, and protein content have been described previously (11, 19). These assays were usually performed on sonic extracts of cell suspensions [approximately 30 mg of cells per ml of 0.02 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride–0.001 M sodium ethylenedinitrolotetraacetate, pH 7.6], although for some SAM synthetase assays toluene-treated cells were used.

Since all the strains used in this study had arginine decarboxylase, it was possible to screen for *speB* phenotype (agmatine ureahydrolase) by measurement of formation of radioactive urea from L-[guanido-^{14}C]arginine. Reaction mixtures, containing 1.2 mM $MgSO_4$, 25 μM pyridoxal phosphate, 50 μM L-[guanido-^{14}C]arginine (4 μCi/μmol), 0.015 M Tris-hydrochloride (pH 7.6), and 0.1 ml of cell preparation (sonic extract, toluene-treated cells or untreated whole cell suspension) in a total volume of 0.25 ml, were incubated at 37 C for 30 min. The reaction was stopped by pipetting 1 ml of ice cold Tris-hydrochloride (pH 7.6) into each tube and placing it in an ice bath. After all the reaction tubes had been accumulated in the ice bath, the cells were removed by centrifugation in the cold and a 1-ml volume of each supernatant solution was applied to a column (0.6 by 4 cm) of Dowex 50-X2 and each column was washed with 9 ml of water. A 4-ml sample of the combined effluent and wash from each column was mixed with 17 ml of the 2:1 toluene scintillating solution-Triton X-100 counting mixture of Patterson and Greene (23) and counted with a Packard Tri-Carb model 3375 liquid scintillation spectrometer. Initial exploratory experiments showed that [^{14}C]urea was completely removed from these columns by a 5-ml water wash whereas 2 N HCl was required to elute arginine. Much higher rates of urea production were obtained with untreated whole cell suspensions than with sonic extracts or toluene-treated cells. The higher activity of the intact cells is undoubtedly due to their ability to take up arginine from the reaction medium, thus effectively raising the substrate concentrations for arginine decarboxylase and agmatine ureahydrolase. Screening of *speB* phenotype was thus routinely done with untreated cells. Using this procedure the urea production of *speB* cells is less than 1% of that obtained in cells carrying the wild-type allele.

Since it is not possible to screen the *metC* genotype by growth characteristics in some of the strains used in this work, it was necessary to assay cystathionase activity in large numbers of transductants. The *metC* deletion strains have no detectable cystathionase activity, so it is possible to use a qualitative assay for screening purposes. Cultures (0.2 ml) of transductants were grown overnight, cells were sedimented by centrifugation, and the supernatant fluid was discarded. The cell pellets were suspended in 0.05 ml of lysozyme (0.1 mg/ml), 0.02 M Tris-hydrochloride, and 0.001 M sodium ethylenedinitrilotetraacetate. pH 7.6. The cell suspensions were allowed to stand at room temperature for at least 15 min and were disrupted by freezing and thawing three times (31). A cystathionase assay mix (0.1 ml) containing 1 mM L-cystathionine, 1 mM 5,5'-ditho-bis(2-nitrobenzoic acid), 0.16 M potassium phosphate, and 0.125 mM $MgCl_2$ (pH 7.4) was added to each tube, and the tubes were allowed to stand at room temperature. Yellow color was clearly discernable in most of the *metC*$^+$ cultures within 1 h, and its intensity increased as the disrupted cell suspensions were allowed to stand. On the other hand the strains carrying the *metC* deletions showed only a trace of yellow color even when allowed to stand for 24 h.

RESULTS

Nature of the methionine requirement associated with exbB. Two markers used in the location of *metK* are the *exbB* deletion of Guterman and Dann (15) and the *metC* deletion of Shimada et al. (26). In their study of colicin B-resistant mutants, Guterman and Dann (15) isolated a strain that overproduced enterochelin and required methionine for growth. These workers proposed that the *exbB* mutation consisted of a deletion near to but not including *metC* since the strain could grow on medium supplemented with cystathionine (stereoisomer not specified). They therefore suggested that the deletion had removed another previously unknown methionine gene which they named *metL*. Shortly thereafter Thèze et al. (29) designated the genes for the methionine-specific aspartokinase and homoserine dehydrogenase as *metL* and *metM*, thus causing a conflict in the nomenclature. As will be shown below the *exbB* deletion appears to include *metC* since the strain carrying it is devoid of cystathionase activity. Thus there is no evidence for another *met* gene in this region, and the designation *metL* should be reserved for the methionine-specific aspartokinase-homoserine dehydrogenase. The methionine requirement for rapid growth of strain GUC41 is clearly shown in Fig. 1; however, the strain does grow slowly on methionine-free medium. The slow growth in the absence of methionine probably results from the reaction of H_2S with *O*-succinyl homoserine to produce homocysteine directly, a process previously described in *S. typhimurium* by Flavin and Slaughter (9). Supplementation of the growth medium with L-cystathionine does not cause significant increase in the growth rate, but the cells grow markedly faster in medium containing a mixture of the four isomers of cystathionine. The mechanism of utilization of the unnatural isomers of cystathionine is unknown, but the results imply that an enzyme(s) other than cystathionase can convert one or more of the isomers to homocysteine.

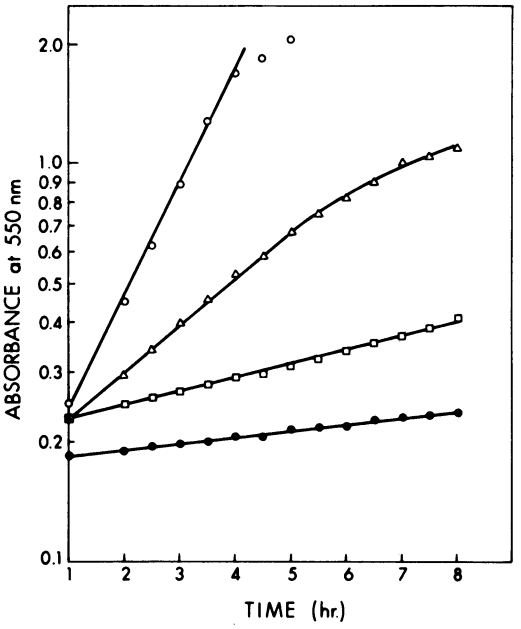


FIG. 1. *Growth of GUC41 (exbB). Minimal salts dextrose medium was supplemented with threonine (0.5 mM), leucine (0.5 mM), thiamine hydrochloride (5 μg/ml), and the indicated sulfur amino acid. Symbols: ●, no further supplement; ○, L-methionine (0.5 mM); □, L-cystathionine (0.5 mM); △, DL-cystathionine (0.5 mM).*

Table 2 shows the cystathionase activities of crude extracts from strains GUC41 (*exbB*) and KS940 (Δ*metC*) and their parent strains (C600 and KS614, respectively). With the extracts from strains GUC41 and KS940 there was no detectable difference in absorbance between reaction mixtures with and without cystathionine during a 5-h incubation period. The maximum specific activities were calculated with the assumption that absorbance differences smaller than 0.01 might have been missed. In the qualitative assay used to classify transductants, several days incubation is required to develop a distinct yellow color in extracts of cells carrying *exbB* or Δ*metC*. Since cystathionine synthetase can catalyze the slow degradation of cystathionine (14), it is probably that the cystathionase levels of these strains are appreciably lower than the maximum values given in Table 2.

Location of metK on the E. coli chromosome. Since Lawrence et al. (20) had shown that the *metK* locus of *S. typhimurium* was near *serA*, we attempted to see whether the SAM synthetase-deficient mutations were located in a similar position. Our first transduction experiments, in which a nitrosoguanidine-induced *serA* derivative of strain NF28 was used as a recipient and the putative *metK* strains as donors, showed about 10% co-transduction of *serA* and *metK* markers. These experiments also showed about 10% co-transduction of *serA* and *lysA*. If the *metK* and *lysA* markers were both on the same side of *serA*, they would be expected to show a high frequency of co-transduction. In subsequent experiments none of the transductants selected for the *lys*$^+$ genotype of the donor received the *metK* allele of the donor showing that *metK* and *lysA* are on opposite sides of *serA*. We then proceeded to locate three *metK* alleles relative to markers clockwise from *serA* (*speB*, *glc*, and deletions including *metC*).

Maas (22) has presented results showing *metK85* to lie further from *serA* than *speB* (clockwise order *serA speB metK*). We have repeated these crosses with three *metK* alleles (*metK84*, *metK85*, and *metK86*). Replica plating is unsuitable for the classification of the *speB* or *metK* phenotypes of the transductants, since the number of cells transferred by replication is variable and the degree of inhibition of sensitive colonies by arginine or ethionine is influenced by the size of the inoculum. To obtain sufficiently small and uniform inocula, dilutions of fully grown cultures of purified transductants were spotted on test plates. Even this procedure was somewhat unreliable in scoring of the *speB*$^+$ phenotype, especially in *metK*$^-$ strains, and some *speB*$^-$ strains grew on the arginine plates. Thus the genotype of all apparent *speB*$^+$ and *metK*$^-$ strains and of a sample of the other transductants was established by assay of SAM synthetase and arginine ureahydrolase. To avoid bias in the picking of colonies, entire transduction plates were scored. As shown in Table 3 the co-transduction frequency of *serA* and *speB* is higher than that of *serA* and *metK*, and all transductants that

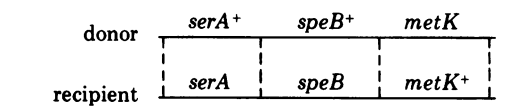
TABLE 2. *Cystathionase activities of metC strains and their parents*[a]

Strain	Pertinent genotype	Cystathionase of (nmol/min × mg protein)
C600	*exbB*$^+$	2.4
GUC41	*exbB*	<0.016
KS614	*metC*$^+$ (λCI$_{857}$)	4.0
KS940	Δ*metC*	<0.012

[a] Cells were grown in minimal salts dextrose medium with required supplements and 0.1 mM L-methionine. C600 and GUC41 were grown at 37 C while KS614 and KS940 were grown at 31 C. Cultures were harvested at cell densities between 1.4 and 2.2 mg/ml.

TABLE 3. *Linkage of metK and speB*[a]

Recipient	Donor	*serA*$^+$ transductants			
		speB metK$^+$	*speB*$^+$ *metK*$^+$	*speB*$^+$ *metK*	*speB metK*
MA177 (*serA speB metK*$^+$)	RG62 (*serA*$^+$ *speB*$^+$ *metK84*)	77	5	8	0
MA177 (*serA speB metK*$^+$)	RG73 (*serA*$^+$ *speB*$^+$ *metK85*)	129	7	20	0
MA177 (*serA speB metK*$^+$)	RG109 (*serA*$^+$ *speB*$^+$ *metK86*)	67	10	13	0



[a] *ser*$^+$ transductants were selected on plates supplemented with B_1, Pro, Trp, and ornithine. Colonies were picked, purified by streaking, grown in liquid medium containing the same supplements, and classified as described in the text.

carry the *metK* allele of the donor also have the *speB* allele of the donor. None of the transductants had the genotype *serA*$^+$ *speB metK* (i.e., the *speB* allele of the recipient and the *metK* allele of the donor). Although the number of transductants tested is not large, the results are consistent with the order *serA speB metK* since all the genotypes can be accounted for by a single crossover on the *speB metK* side of *serA* as shown in the diagram. If the order were reversed, the frequency of *speB metK* transductants would have been higher than that of *speB*$^+$ *metK*$^+$ transductants. These results confirm those of Maas (22) for *metK85* and locate *metK84* and *metK86* in the same relative position.

To ease scoring of *metK* in further crosses, appropriate markers were incorporated into an *E. coli* strain carrying *metG*. The methionine requirement of *metG* strains results from an altered methionyl-transfer ribonucleic acid synthetase which functions adequately only in the presence of high concentrations of methionine (1, 13). Chater et al. (5) have shown that *metK* will suppress the methionine requirement of *metG* mutants in *S. typhimurium*. We find that *metK* mutations will suppress the *metG* phenotype in *E. coli*. The *metG* strains used in this work carry a temperature-sensitive mutation, perhaps in *metG*, since they will grow on methionine-free plates at 25 C and will not grow even on complete medium at 42 C. To avoid difficulties in scoring suppression of the methionine requirement, plates were read as soon as possible after removal from the 37 C incubator. Our original intent was to use *metG* strains as recipients so that we could select for transfer of *metK*; however, too many colonies were found when *metG* cells were spread on methionine-free plates. It is probable that mutation at any of several loci can suppress *metG*. The frequency of occurrence of methionine-independent cells was low enough to allow scoring of *metK* in crosses where *serA*$^+$ cells were selected.

To locate the *metK* alleles relative to the *glc* marker of Vanderwinkle and DeVlieghere (30), a recipient strain carrying *glc*, *serA*, and *metG* was prepared. Using P1 grown on prototrophic *metK* strains, serine-independent transductants were selected. As before, colonies were streaked on serine-free plates, the purified transductants were grown on defined medium, and dilutions of the cultures were spotted on test plates. The frequency of genotypes of transductants given in Table 4 is consistent with the order *serA metK glc* since all the transductants can arise from a single crossover on the *metK glc* side of *serA* as shown on the diagram. If *glc* were between *serA* and *metK*, *metK*$^+$ *glc*$^+$ transductants would be more frequent than *metK glc* transductants, whereas no *serA*$^+$ *metK*$^+$ *glc*$^+$ tranductants were found.

Strains GUC41 (15) and KS940 (26) have been reported to have deletions near the region of interest. Both strains appear to lack the *metC* locus, but the termini of the deletions are unknown. The SAM synthetase activities of strains GUC41 and KS940 are in the normal range and both can utilize sodium glycolate as the sole carbon source so neither *metK* nor *glc* are included in the deletions. Since it is possible to select *metC*$^+$ transductants and since *exbB* and *serA* are co-transducible (15), it appeared that these strains would be good recipients for transductional investigation of the structure of the *metK* region. Thus a series of transductions was performed using strain GUC41 as recipient and strains PL8-25 (*metK84 glc*), PL8-26 (*metK85 glc*), and PL8-27 (*metK86 glc*) as donors. Ninety transductants from each cross

were purified by streaking on methionine-free medium. The purified strains were grown on defined medium and scored on test plates. None of the 270 transductants could utilize glycolate as a carbon source and none was resistant to ethionine. Thus all had the *glc* marker of the donor strain, but none had incorporated the *metK* allele of the donor. A like transduction was done with strain KS940 as the recipient and strain PL8-26 as donor. Ninety transductants from this cross were scored as above. None of the strains would grow with sodium glycolate as the carbon source; thus they had all incorporated the *glc* marker of the donor. Three of the transductants were ethionine resistant having received the *metK* marker of the donor, and the remaining 87 were ethionine sensitive. The scoring of the three *metK* transductants and of 10 of the ethionine-sensitive cultures from each of the transductions was verified by assay of SAM synthetase. In all cases plate classifications and enzyme assays were consistent. The low frequency of co-transduction of *metC*$^+$ and *metK* suggests that the amount of DNA required to replace the deleted material in strains GUC41 and KS940 is so large that the probability of finding a transductional fragment containing both *metK* and the deleted material is low. The failure to observe recombination between *glc* and *metC* is discussed below.

A set of transductions was preformed using strain PL8-31 (*serA glc metK86*) as recipient and strains GUC41 or KS940 as donors, selecting for serine independence. In these experiments the *metK* phenotype was tested by replica plating on medium lacking methionine to detect those colonies which had lost the *metK* marker of the recipient strain.

The frequencies of co-transduction of the *serA* and *metK* markers of the donor shown in Table 5 are rather low. These low frequencies are probably partially due to errors in classification since *metK*$^+$ transductants may be fed by methionine excreted by *metK* transductants, especially when the plates are crowded (KS940 transductions). About 450 transductants (including most of the *metK*$^+$ colonies) were purified by streaking on serine-free medium, and cultures of the purified strains were grown and tested for *metK* and *glc* on selective plates and

TABLE 5. *Transductions of PL8-31 (serA metK glc metC*$^+$*) by strains deleted for metC*

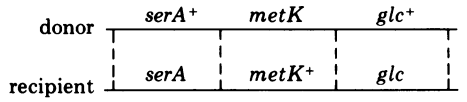
Determinants	Donor	
	GUC41 (*serA*$^+$ *metK*$^+$ *glc*$^+$ *exbB*)	KS940 (*serA*$^+$ *metK*$^+$ *glc*$^+$ Δ*metC*)
A.[a]		
Total *serA*$^+$	831	4,109
serA$^+$ *metK86*	728	3,860
serA$^+$ *metK*$^+$	103	249
serA$^+$ *metK*$^+$/*total serA*$^+$	0.12	0.06
B.[b]		
Total transductants tested	250	449
metK86 glc metC$^+$	139	251
metK$^+$ *glc metC*$^+$	67	100
metK$^+$ *glc*$^+$ *metC*	44	98

[a] Frequency of co-transduction of *serA25* and *metK86*. *ser*$^+$ transductants were selected on plates supplemented with B_1, His, Leu, Pro, and Met. *metK* phenotype was scored by replica plating on the same medium lacking Met.

[b] Distribution of markers among selected transductants. Colonies (including most apparent *metK*$^+$) were purified by streaking and were grown on liquid medium supplemented with B_1, His, Leu, Pro, Thr, and Met with glucose as the carbon source. *metK* and *glc* phenotypes were scored by spotting on test plates and *metC* was scored by cystathionase assay.

TABLE 4. *Linkage of metK and glc*[a]

Recipient	Donor	*serA*$^+$ transductants			
		metK$^+$ *glc*	*metK glc*	*metK glc*$^+$	*metK*$^+$ *glc*$^+$
PL8-24 (*serA metK*$^+$ *glc*)	RG62 (*serA*$^+$ *metK84 glc*$^+$)	383	176	12	0
PL8-24 (*serA metK*$^+$ *glc*)	RG73 (*serA*$^+$ *metK85 glc*$^+$)	375	80	9	0
PL8-24 (*serA metK*$^+$ *glc*)	RG109 (*serA*$^+$ *metK86 glc*$^+$)	421	160	3	0



[a] *ser*$^+$ transductants were selected on plates supplemented with B_1, His, Leu, Pro, Thr, and Met. Transductants were purified by streaking on the same medium and the resultant strains were grown on liquid medium with the same supplements. The cultures were classified for *metK* and *glc* as described in the text.

for *metC* by qualitative cystathionase assay. As shown in Table 5, all of the transductants which retained the *metK* allele of the recipient also carried the *glc* and *metC* alleles of the recipient. About half of the *metK*$^+$ transductants in each cross still retained the *glc* and *metC* markers of the recipient and about half carried the markers of the donor. This is a higher co-transduction frequency of *glc* and *metK* than had been previously observed, but the selection procedure may have been biased in favor of the *metC* deletions. No recombination between *glc* and *metC* was detected in any of the experiments. Since no recombination occurred between *glc* markers and *metC* deletions, no conclusions can be drawn about their order from these experiments. We have been unable to observe any co-transduction of *serA* and a point mutation in *metC* whereas *serA* and *glc-1* are co-transducible. Thus *glc* is located between *serA* and *metC*. The gene order deduced from these transductions is *serA metK glc metC*. The failure to observe any recombination between *glc* and either of the *metC* deletions among a relatively large number of transductants where both characters were transferred implies that *glc* is very close to the termini of the deletions. We have not observed reversion of the *glc-1* mutations, so perhaps it is a deletion that overlaps the termini of the other deletions.

Properties of merodiploids of the metK locus. Since *metK* appears to be the structural gene for SAM synthetase (strains carrying *metK85* make an unstable enzyme), and since the phenotype appears to be related to a reduced SAM pool (11), *metK* mutations are expected to be recessive. Chater (4) showed that several *metK* mutations of *S. typhimurium* are recessive to the wild-type allele, but no studies of the *E. coli* mutations have been reported. We therefore constructed a series of merodiploid strains carrying different *metK* alleles in the chromosome and the F-prime factor KLF16 which contains a portion of the *E. coli* chromosome extending from about 53 min to about 60 min (21). Table 6 shows the specific activities of cystathionine synthetase, cystathionase, and SAM synthetase of several diploids and their parent haploid strains. Extracts from the three *metK* haploid strains have low specific activities of SAM synthetase and elevated activities of cystathionine synthetase and cystathionase compared to an extract of the *metK*$^+$ strain RG330. The KLF16 carrying heterogenotes (*metK*$^+$/*metK*$^-$) all have higher levels of SAM synthetase and lower levels of cystathionine synthetase than the corresponding haploids. The cystathionase activities of these diploid strains are lower than those of the *metK* haploids, but are about twice that of the *metK*$^+$ haploid (RG330). The elevated cystathionase level, which is also present in the *metK*$^+$ diploid (RG334), is due to a gene dosage effect since KLF16 carries the *metC* locus. These results show that the three *metK* mutations are recessive to the wild-type allele, but the expected gene dosage effect for the *metK* locus was not observed. Although there is a twofold effect on the level of cystathionase, the diploid strain with *metK*$^+$ in both the chromosome and episome has only a slightly higher concentration of SAM synthetase ($\times$1.3) than its haploid parent. Since this small effect might be due to partial repression of the *metK* genes, we isolated and characterized a spontaneous *metJ* derivative of strain RG330. A diploid of the *metJ* strain was constructed by conjugation with KLF16/110 and the enzyme activities of the strains were measured. As shown in the lower section of Table 6, the *metJ* strains have higher activities of SAM synthetase, but the ratio of activities of diploid and haploid strains is about the same as that of cells with a functional *metJ* locus (diploid/haploid = 1.4). The reason for the failure to obtain increases of SAM synthetase

TABLE 6. *Enzyme activities of diploid and haploid strains*

Strain	Pertinent genotype	Enzyme activities[a]		
		Cystathionine synthetase	Cystathionase	SAM synthetase
Haploids				
RG313	*metK85*	20.7	61.1	0.19
RG314	*metK84*	34.3	69.0	0.13
RG330	*metK*$^+$	1.8	18.5	5.5
RG331	*metK86*	46.4	91.2	0.23
Diploids				
RG316	KLF16/*metK85*	2.9	42.5	3.1
RG317	KLF16/*metK84*	2.6	30.9	2.5
RG334	KLF16/*metK*$^+$	2.5	34.2	7.1
RG335	KLF16/*metK86*	2.5	40.5	3.8
Haploids				
RG330	*metK*$^+$	2.2	11.2	3.2
RG352	*metK*$^+$ *metJ*	30.0	38.7	8.3
Diploids				
RG334	KF16/*metK*$^+$	1.6	23.8	5.0
RG353	KLF16/*metK*$^+$*metJ*	26.1	101	11.4

[a] Enzyme activities are in nanomoles per minute times milligrams of protein.

comparable to those of cystathionase is unknown, but it is possible that the episomal *metK* gene has a less efficient promoter than that of the chromosome.

DISCUSSION

The three *metK* mutations investigated here lie near 57 min on the *E. coli* chromosome between *speB* and *glc*. Judging from co-transduction frequencies *metK* is closer to *serA* than it is to *metC*. We have isolated a few *metK*$^+$ transductants in crosses between *metK85* and *metK84* strains or between *metK85* and *metK86* strains, but their numbers are too small to demonstrate convincingly that they arose by recombination rather than back mutation. Fine structure mapping of the *metK* locus is made difficult by the lack of a closely linked selective marker and the uncertainty of scoring of the *metK* phenotype by replica plating. Two independently isolated deletions in this region (15, 26) have been shown to include *metC* but not *metK* or *glc*. These deletions have not been well characterized, but the deleted fragments are small enough to be replaced by the deoxyribonucleic acid carried in a P1 phage. Failure to observe recombination between the deletions and *glc-1* suggest that their counterclockwise termini are close to the *glc-1* mutation. As was expected all three *metK* mutations are recessive to the wild-type allele carried on KLF16. In all properties thus far examined the *metK* mutants of *E. coli* and *S. typhimurium* are essentially the same. The loci are in the same general position, the mutant alleles are recessive to the wild type, and all have some type of alteration in SAM synthetase. Thus it is reasonable to conclude that *metK* is the structural gene for SAM synthetase (or for at least 1 subunit if SAM synthetase is a multisubunit enzyme) and that SAM or one of its metabolites is involved in control of the *met* regulon.

ACKNOWLEDGMENTS

We acknowledge the capable technical assistance of Ellen Mack.

This work was supported by program no. 3300-01 from the Veterans Administration Hospital, Durham, N.C. and Public Health Service grant GM 10317 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Ahmed, A. 1973. Mechanism of repression of methionine biosynthesis in *Escherichia coli*. I. The role of methionine, S-adenosylmethionine and methionyl-transfer-ribonucleic acid in repression. Mol. Gen. Genet. **123**:299-324.
2. Blumenthal, T. 1972. P1 transduction: formation of heterogenotes upon cotransduction of bacterial genes with a P2 prophage. Virology **47**:76-93.
3. Caro, L., and C. M. Berg. 1971. P1 transduction, p. 444-458. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 21. Academic Press Inc., New York.
4. Chater, K. F. 1970. Dominance of wild-type alleles of methionine regulatory genes in *Salmonella typhimurium*. J. Gen. Microbiol. **63**:95-109.
5. Chater, K. F., D. A. Lawrence, R. J. Rowbury, and T. S. Gross. 1970. Suppression of methionine transfer RNA synthetase mutants of *Salmonella typhimurium* by methionine regulatory mutations. J. Gen. Microbiol. **63**:121-131.
6. Davis, B. D., and E. S. Mingioli. 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17-28.
7. Fiil, N. 1969. A functional analysis of the *rel* gene in *Escherichia coli*. J. Mol. Biol. **45**:195-203.
8. Flavin, M., and C. Slaughter. 1965. Synthesis of the succinic ester of homoserine, a new intermediate in the bacterial biosynthesis of methionine. Biochemistry **4**:1370-1375.
9. Flavin, M., and C. Slaughter. 1967. Enzymatic synthesis of homocysteine or methionine directly from *O*-succinyl homoserine. Biochim. Biophys. Acta **132**:400-405.
10. Glansdorff, N. 1965. Topography of cotransducible arginine mutations in *Escherichia coli* K-12. Genetics **51**:167-179.
11. Greene, R. C., J. S. V. Hunter, and E. H. Coch. 1973. Properties of *metK* mutants of *Escherichia coli* K-12. J. Bacteriol. **115**:57-67.
12. Greene, R. C., C.-H. Su, and C. T. Holloway. 1970. S-adenosylmethionine synthetase deficient mutants with impaired control of methionine biosynthesis. Biochem. Biophys. Res. Commun. **38**:1120-1126.
13. Gross, T. S., and R. J. Rowbury. 1971. Biochemical and physiological properties of methionyl-s-RNA mutants of *Salmonella typhimurium*. J. Gen. Microbiol. **65**:5-21.
14. Guggenheim, S., and M. Flavin. 1969. Cystathionine-γ-synthase: a pyridoxal phosphate enzyme catalyzing rapid exchanges of β and α hydrogen atoms in amino acids. J. Biol. Chem. **244**:6217-6227.
15. Guterman, S. K., and L. Dann. 1973. Excretion of enterochelin by *exbA* and *exbB* mutants of *Escherichia coli*. J. Bacteriol. **114**:1225-1230.
16. Hirota, Y. 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **46**:57-64.
17. Hobson, A. C. 1974. The regulation of methionine and S-adenosylmethionine synthetase. Mol. Gen. Genet. mutants of *Salmonella typhimurium* with defects in S-adenosylmethionine synthetase. Mole. Gen. Genet. **131**:263-273.
18. Hobson, A. C., and D. A. Smith. 1973. S-adenosylmethionine synthetase in methionine regulatory mutants of *Salmonella typhimurium*. Mol. Gen. Genet. **126**:7-18.
19. Holloway, C. T., C.-H. Su, and R. C. Greene. 1970. Regulation of *S*-adenosylmethionine synthetase in *Escherichia coli*. J. Bacteriol. **104**:734-747.
20. Lawrence, D. A., D. A. Smith, and R. J. Rowbury. 1968. Regulation of methionine synthesis in *Salmonella typhimurium*: mutants resistant to inhibition by analogues of methionine. Genetics **58**:473-492.
21. Low, B. 1972. *Escherichia coli* K-12 F-prime factors, old and new. Bacteriol. Rev. **36**:587-607.
22. Maas, W. K. 1972. Mapping of genes involved in the synthesis of spermidine in *Escherichia coli*. Mol. Gen. Genet. **119**:1-9.
23. Patterson, M. S., and R. C. Greene. 1965. Measurement of low energy beta-emitters in aqueous solution by liquid scintillation counting of emulsions. Anal. Chem. **37**:854-857.
24. Savin, M. A., M. Flavin, and C. Slaughter. 1972. Regula-

tion of homocysteine synthesis in *Salmonella typhimurium*. J. Bacteriol. **111**:547–556.
25. Scott, J. R. 1968. Genetic studies on bacteriophage P1. Virology **34**:564–574.
26. Shimada, K., R. A. Weissberg, and M. E. Gottesman. 1972. Prophage lambda at unusual chromosomal locations. I. Location of secondary attachment sites and properties of the lysogens. J. Mol. Biol. **63**:483–503.
27. Stacey, K. A., and E. Simson. 1965. Improved method for the isolation of thymine-requiring mutants of *Escherichia coli*. J. Bacteriol. **90**:554–555.
28. Su, C.-H., and R. C. Greene. 1971. Regulation of methionine biosynthesis in *Escherichia coli*: mapping of the *metJ* locus and properties of a MetJ$^+$/MetJ$^-$ diploid. Proc. Natl. Acad. Sci. U.S.A. **68**:367–371.
29. Thèze, J., D. Margarita, G. N. Cohen, F. Borne, and J. C. Patte. 1974. Mapping of the structural genes of the three aspartokinases and of the two homoserine dehydrogenases of *Escherichia coli* K-12. J. Bacteriol. **117**:133–143.
30. Vanderwinkle, E., and M. DeVlieghere. 1968. Physiologie et génétique de l'isocitritase et des malate synthases chez *Escherichia coli*. Eur. J. Biochem. **5**:81–90.
31. Weiss, B., and C. Milcarek. 1974. Mass screening for mutants with altered DNases by microassay techniques, p. 180–193. *In* L. Grossman and K. Moldave (ed.), Methods in enzymology, vol. 29. Academic Press Inc., New York.