Dominance of the Wild-type Alleles of Methionine Regulatory Genes in *Salmonella typhimurium*

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SUMMARY

F' elements carrying wild-type alleles of the methionine regulatory genes *metJ* and *K* have been transferred from *Escherichia coli* to *metJ* and *K* mutants of *Salmonella typhimurium*, and the resulting heterogenotes tested for resistance to methionine analogues and for repressibility of cystathionase (one of the methionine biosynthetic enzymes). The wild-type alleles of both genes were dominant in both tests. Increased *metK* gene dosage was found to have no effect on the rate of uptake of [¹⁴C]methionine by cell suspensions. Possible roles for the *metJ* and *K* gene products in the regulation of methionine synthesis are considered.

INTRODUCTION

The six structural genes controlling methionine biosynthesis in *Salmonella typhimurium* are not contiguous (Fig. 1; Smith, 1961; Sanderson & Demerec, 1965; Childs & Smith, 1969), and the repression of their expression by exogenous methionine appears not to be co-ordinate (Lawrence, Smith & Rowbury, 1968). Two kinds of mutants in which expression of these genes was constitutive were found among mutants resistant to methionine analogues (Lawrence *et al.* 1968). *metJ* mutants were resistant to DL-ethionine, but not to α -methyl-DL-methionine or DL-norleucine, while *metK* mutants were resistant to all three analogues. Some *metK* mutants were also isolated in which regulation was normal, and, unlike constitutive *metK* mutants, these did not show a reduced growth rate in minimal medium compared with the wild-type. *metJ* mutations were about 95% cotransduced with *metB*, and *metK* mutations about 1% with *serA* (Fig. 1).

In the present work F' episomes carrying, respectively, the *metB* and *serA* regions of the *Escherichia coli* genome (K. Brooks Low, personal communication) have been transferred into *Salmonella typhimurium* strains, and the dominance of various *metJ* and *K* alleles tested in the resulting hybrid heterogenotes. The possibility that *metK* may specify a component required for methionine uptake has also been tested, by examining the effect of increasing the *metK* gene dosage on the rate of uptake of radioactive methionine.

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Fig. 1. Linkage map of *Salmonella typhimurium*. The map and nomenclature are based on those of Sanderson (1967). Bracketed markers are cotransduced. Arrows represent the sites of insertion of sex factors in high frequency donors. KLF 10 and KLF 16 represent F' episomes derived from *Escherichia coli* by K. Brooks Low, and carrying regions of the *E. coli* genome homologous with the regions of the *S. typhimurium* genome covered by the arcs.

METHODS

Media. Nutrient agar (NA) and nutrient broth (NB) were supplied by Oxoid Ltd. The minimal medium (MM) was as described by Smith (1961). Minimal agar (MA) was obtained by adding 1.5% (w/v) Oxoid agar no. 1 to MM. MM was supplemented as required with the following: L-arginine, 20 µg./ml.; DL-ethionine, 1000 µg./ml.; α-methyl-DL-methionine, 1000 µg./ml.; L-methionine, 20 µg./ml.; L-serine, 200 µg./ml.; uracil, 20 µg./ml.

Organisms. The nomenclature is that used by Sanderson (1967). Two *Escherichia coli* F' donor strains were kindly provided by K. Brooks Low: a JC1553 (*argEhisleu metB lac mal xyl str-rrecA1*) donor of KLF 10, an episome carrying the *malB* and *metB* genes (Fig. 1), and a JC1553 *thy* donor of KLF 16, an episome carrying the *serA* and *thy* genes. All other strains were derived from *Salmonella typhimurium* strain LT2. A list of the regulatory mutants is given in Table 1, and Table 2 shows the derivation of multiple mutants.

Techniques. Cultures were grown at 37° unless otherwise stated. The maintenance of stock cultures, and the propagation, assay and maintenance of transducing phage P 22 or its non-lysogenizing variant L 4 (Smith & Levine, 1967; supplied by H. O. Smith) were as described by Smith (1961). Growth experiments in MM were essentially

Table 1. *Methionine regulatory mutants of Salmonella typhimurium*

All strains were derived from *Salmonella typhimurium* LT2. Tests of resistance to the methionine analogues α -methyl-DL-methionine (AMM), DL-ethionine (ETH) and DL-norleucine (NOL), and detection of methionine excretion by cross-feeding of a methionine auxotroph, were as described by Lawrence *et al.* (1968). The nomenclature is that of Sanderson (1967).

Structural gene mutations	Methionine regulatory gene mutations	Resistance (R) or sensitivity (S) to			Excretion of methionine
		AMM	ETH	NOL	
<i>argFIII</i>	—	S	S	S	—
<i>argFIII</i>	<i>metJ713</i>	S	R	S	+
<i>argFIII</i>	<i>metJ743</i>	S	R	S	+
<i>argFIII</i>	<i>metK747</i>	R	R	R	+
<i>serAII</i>	—	S	S	S	—
<i>serAII</i>	<i>metK747</i>	R	R	R	+
<i>serAII</i>	<i>metK751</i>	R	R	R	—
<i>serAII</i>	<i>metK752</i>	R	R	R	+
<i>serA13</i>	<i>metJ744</i>	S	R	S	+

Table 2. *The derivation of Salmonella typhimurium multiple mutants*

Spontaneous (spont) methionine regulatory mutants were obtained as colonies growing on MA containing ethionine (Lawrence *et al.* 1968). *N*-methyl *N'*-nitro *N*-nitrosoguanidine treatment (NG), conjugation (conj) and transduction (trans) were as described by Ayling & Chater (1968). Temporary strain numbers are for use only in the context of this table, recipient numbers preceding those of donors in descriptions of crosses. Bracketed strains were used only in deriving strains and are not referred to in the text. A linkage map including the markers used is given in Fig. 1.

Temporary strain number	Genotype	Mode of origin, or source
1	<i>argFIII</i>	J. D. Childs
2	(<i>argFIII</i> ilv)	From 1 by NG
3	(<i>argFIII</i> metB406)	2 × 18 (conj)
4	<i>argFIII</i> metJ713	3 × 10 (trans)
5	<i>argFIII</i> metJ743	From 1 by spont. mutation
6	<i>argFIII</i> metK747	2 × 21 (conj)
7	(<i>argFIII</i> rec-301)*	From 1 by NG
8	<i>argFIII</i> rec-301pyr*	From 7 by NG
9	(<i>argFIII</i> serA13)	2 × 20 (conj)
10	(<i>metJ713</i>)	D. A. Lawrence
11	<i>serAII</i>	D. A. Lawrence
12	<i>serAII</i> metK747	From 11 by spont. mutation
13	(<i>serA13</i>)	K. E. Sanderson
14	<i>serA13</i> metJ744	9 × 18 (conj)
15	(<i>serA13</i> rec-302)*	From 13 by NG
16	<i>serA13</i> rec-302met-754*	From 15 by NG
17	(<i>metK747</i>)	12 × wild-type LT2 (trans)
18	(<i>HfrAhisD23</i> metB406)	J. D. Childs
19	(<i>HfrK3</i> serA13)	K. E. Sanderson
20	(<i>HfrK3</i> serA13his)	From 19 by NG
21	(<i>HfrK3</i> metK747his)	20 × 17 (trans)

* Recombinant formation in conjugation and transduction was greatly reduced with *rec-301* or *-302* recipients, though F' merodiploid formation was normal. The *rec* mutations also resulted in hypersensitivity to ultraviolet irradiation and, in the case of *rec-301*, to NG (Chater, 1969).

as described by Lawrence *et al.* (1968). Episome transfer was carried out by the spot method of Fink & Roth (1968). F' heterogenotes were purified by subculturing the crude isolates in selective MM to stationary phase, and spreading a 10^{-6} dilution on selective MA. After incubation of such plates for 48 h., discrete colonies were obtained. Haploid segregants from heterogenote cultures were detected, after subculture in NB to stationary phase (sometimes followed by subculture in NB + acridine orange (20 μ g./ml.; Hirota, 1960)), by spreading a suitable dilution on NA, incubating for 24 h., and replicating to appropriately supplemented MA, so that auxotrophic segregants and prototrophic heterogenotes could be distinguished.

Assay of cystathionase activity. Late logarithmic phase MM cultures grown at 25° in randomized positions in a shaking water-bath were harvested by centrifugation, washed in culture volumes of 10 mM potassium phosphate buffer (pH 7.4), and finally resuspended in the same buffer. The optical density of each suspension was measured at 650 nm., and usually adjusted to 1.5 units (1 unit = 0.375 mg. dry wt./ml.), though less dense suspensions were sometimes used. Toluene was added (to 1% of the total volume), and the suspension emulsified using a Whirlimixer, prior to incubation at 30° for 20 min. Such suspensions were then used immediately, or after storage at 0° for up to 2 h. The method for assaying cystathionase activity was adapted from that of Rowbury & Woods (1964) with cell-free extracts. Incubation was at 30°. The reaction mixture contained, in a final volume of 1 ml. (μ moles): potassium phosphate buffer, pH 7.4, 100; pyridoxal phosphate, 0.0025; DL-allocystathionine, 10; and toluene-treated cells, 0.04 to 0.3 mg. dry wt. Cystathionine was added to the pre-incubated mixture to start the reaction, which was stopped after measured time intervals of up to 40 min. by the addition of 0.33 ml. of a 1% solution of 2,4-dinitrophenylhydrazine in 2 M-HCl. After a further 15 min., 2 ml. of 2 M-KOH was added. The extinction at 445 nm. was determined after 10 min. had elapsed, against a blank from which both cystathionine and toluene-treated cells had been omitted. Corrections to this reading were made by subtraction of readings obtained with controls lacking, respectively, cystathionine and toluene-treated cells. Under the conditions of the assay 1 μ mole of pyruvate 2,4-dinitrophenylhydrazone gave an extinction rate at 445 nm. of 5.0. The formation of pyruvate from cystathionine by toluene-treated cells proceeded at a constant rate for at least 40 min. This rate was shown to be proportional to the quantity of cells added to the reaction mixture.

Assay of methionine permease activity. Cultures were grown overnight (with shaking) in 10 ml. MM + 0.4% glucose, then added to 40 ml. fresh medium and incubated for a further 75 min. Chloramphenicol (200 μ g./ml.) was added, and incubation continued for 30 min. The cells were then harvested by centrifugation, washed in 50 ml. ice-cold MM + glucose + chloramphenicol, and finally resuspended in 5 ml. of the same medium. The extinction at 650 nm. of each suspension was determined, and the volume required to give a final concentration of 50 μ g. dry wt./ml. was added to a 25 ml. beaker containing a suitable quantity of MM + glucose + chloramphenicol, in a shaking water-bath at 25°. After 1 min. 50 sec., shaking was stopped and aeration continued through the rest of the experiment by the operation of a spring-loaded syringe with its tip in the reaction mixture. Methyl-labelled [14 C]methionine (Radiochemical Co., Amersham, Bucks.) (10.7 μ Ci/ μ mole, final concentration 5 μ M) was added 10 sec. later, and 1 ml. samples removed by means of the syringe to Millipore filtration units so that filtration took place at 10, 25, 40, 55 and 70 sec. Filtration was

immediately followed by washing twice with 5 ml. MM + glucose + chloramphenicol at room temperature. In all experiments, a final sample was taken into 1 ml. 10% trichloroacetic acid at 85 sec. The insoluble material was filtered and washed twice with 5 ml. trichloroacetic acid. All filters were then dried under a lamp and added to 5 ml. of scintillation fluid (xylene Pop-op Ppo) prior to assaying in a liquid scintillation counter.

The method is based on that of Piperno & Oxender (1968) as modified by P. D. Ayling & E. S. Bridgeland (manuscript in preparation). I am indebted to Dr Ayling for his collaboration in the performance of these experiments.

RESULTS

Dominance tests with metJ mutants

The properties of KLF 10 in Salmonella typhimurium. The episome to be used in the *metJ* dominance tests, KLF 10, was first transferred from its *Escherichia coli* JC1553 host into *Salmonella typhimurium argFIIIrec-301pyr*. As the episome carried the *argH* gene (i.e. the *E. coli* homologue of *argF* in *S. typhimurium*) but not the *pyr*⁺ allele, the required heterogenotes were selected on MA + uracil. The episome could then be freely transferred to further *S. typhimurium argFIII* recipients, selecting for prototrophic growth, without loss of efficiency due to restriction (Okada, Watanabe & Miyake, 1968). KLF 10 heterogenotes segregated frequent arginine-requiring organisms (10 to 50%) when subcultured into NB. Of more than 300 such segregants from cultures of *FIIImetJ/argKLF10* heterogenotes examined during this investigation none was ethionine-sensitive, showing that recombination between the episome and the host's chromosome was rare.

Ethionine sensitivity of metJ heterogenotes. Ethionine sensitivity was tested by streak tests on MA + ethionine and by growth experiments in MM + ethionine. Typical results of the latter experiments (each of which was carried out at least twice) are shown in Fig. 2; essentially similar results were obtained from the streak tests. Possession of either of two mutations *metJ713* and *J743* resulted in resistance of the haploid host cell to ethionine (Fig. 2*b, c*). When such cells were made heterozygous for *metJ* by the introduction of KLF 10, they became sensitive to the analogue (Fig. 2*f, g*). The degree of sensitivity was not the same in the two heterogenotes: *metJ713/KLF10* was as sensitive as a wild-type (*metJ*⁺/*metJ*⁺) homogenote (Fig. 2*e*), whereas *metJ743/KLF10* was slightly more resistant.

The sensitivity of the two *metJ/metJ*⁺ strains could have reflected either dominance of *metJ*⁺ over *metJ*, or some other, independent, effect on ethionine resistance of the presence of KLF 10. The latter possibility was unlikely, because the presence of KLF 10 in the ethionine-resistant strain *metK747*, in which the lesion resulting in resistance was located outside the region covered by the episome, did not result in ethionine sensitivity (Fig. 2*d, h*).

Repressibility of the methionine enzymes in metJ heterogenotes. Lawrence *et al.* (1968) showed that the ability of *metJ* and some *metK* mutants to cross-feed a methionine-requiring strain in streak tests on MA resulted from constitutive synthesis of the methionine biosynthetic enzymes. Haploid strains possessing *metJ713* or *J743* mutations gave such cross-feeding (Table 1). However, the introduction into these strains of *metJ*⁺, on the episome KLF 10, resulted in loss of the ability

to cross-feed, while in control experiments the wild-type (*metJ*⁺) failed to cross-feed, and a *metK*₇₄₇ (*metJ*⁺*metK*) strain succeeded in doing so, both with and without the episome. Thus repressibility of the methionine biosynthetic enzymes appeared to be dominant to their constitutive synthesis in *metJ*/*metJ*⁺ heterogenotes. To confirm and measure this effect, one of the enzymes was assayed in the appropriate strains grown in the presence and absence of methionine. Cystathionase, the enzyme specified by *metC* (Fig. 1), was selected because of the convenience of the assay method, but it had

Fig. 2. Growth of *metJ* heterogenotes and control strains in the presence of ethionine. All the parental haploid strains had the *argF111* mutation (see Table 1). Heterogenotes were obtained by transferring the *Escherichia coli* episome KLF 10 (Fig. 1) into these parental strains. Inocula were in the exponential phase of growth. Cultures were grown in MM (Δ) and MM + ethionine, 1000 μg./ml. (○). The relevant genotypes (i.e. with respect to *metJ* and *K*) are indicated on the graphs: where merodiploids were used, relevant episomal markers are given after a diagonal stroke.

the disadvantage of relatively low sensitivity to regulation. For this reason, the experiments were carried out with cultures grown in groupings that permitted appropriate analyses of variance to be made on results obtained in essentially identical conditions. The results of these experiments are given in Table 3. Before analysis, all results were transformed into logarithms to ensure that, for example, a twofold difference between untransformed readings was given equal weighting in the analysis whatever the absolute value of the readings.

In Expts 1 and 2 the activity of cystathionase in the *metJ*/*metJ*⁺ heterogenotes was compared with the mean of the values for the wild-type and mutant haploids. In the absence of dominance of either *metJ*⁻ or *metJ*⁺, no significant difference would have been expected. However, *metJ*₇₁₃/KLF 10 (*J*/*J*⁺) closely resembled the haploid wild-type (*J*⁺), and differed significantly ($P = 1$ to 0.1 %) from the mean of the two haploids

(Expt 1). This suggested that *metJ713* was completely recessive to the episomal *metJ*⁺. In contrast, the second *J/J*⁺ strain (*metJ743*/KLF 10; Expt 2) differed only marginally ($P = 10$ to 5%) from the mean of the two haploids, and thus *metJ743* appeared not to be fully recessive to *metJ*⁺. In confirmation of this, the *metJ743*/KLF 10 heterogenote was found to differ significantly ($P = 5$ to 1%) from a *J*⁺/*J*⁺ strain (Expt 3). Finally, the effect of KLF 10 on the regulation of cystathionase synthesis was tested in a wild-type (*metJ*⁺*metK*⁺) strain and the constitutive mutant *metK747* (*J*⁺*K*) in which the regulatory mutation was situated outside the region duplicated by the episome (Expt 4). No significant effect of the episome was detected in these strains, showing that the effect of KLF 10 on regulation observed in *metJ* mutants was due to a true dominance effect, and not to some other, nonspecific, regulatory effect of the episome.

Table 3. *Cystathionase activity in metJ heterogenotes and control strains*

Each value is the mean of two or more replicate experiments in which cystathionase activity was determined by the toluene-treated cell procedure described in Methods. The activity is given as μ moles pyruvate produced from cystathionine in 1 h. by 1 mg. (dry wt) of cells, at 30°. All strains possessed a chromosomal *argF111* mutation which was expressed only in haploid strains. Arginine (20 μ g./ml.) was therefore added to haploid, but not to partially diploid cultures. In the column 'Pertinent genotype', chromosomal methionine regulatory gene mutations precede the diagonal stroke, and regulatory genes carried by the episome KLF 10 follow it. Arginine-requiring segregants were obtained from all heterogenote cultures used, following subculture into nutrient broth, proving that the parental cultures had been heterogenotic.

The results of statistical analysis of these experiments are given in the text.

Experiment	Regulatory mutations	Possession of KLF 10	Pertinent genotype	Cystathionase activity		Number of replicates
				Methionine absent from growth medium	10 mM-methionine present in growth medium	
1	None	—	<i>J</i> ⁺	0.83	0.45	2
	<i>metJ713</i>	—	<i>J</i>	1.92	1.79	
	<i>metJ713</i>	+	<i>J/J</i> ⁺	0.85	0.45	
2	None	—	<i>J</i> ⁺	0.91	0.51	3
	<i>metJ743</i>	—	<i>J</i>	1.41	1.49	
	<i>metJ743</i>	+	<i>J/J</i> ⁺	0.91	0.69	
3	None	+	<i>J</i> ⁺ / <i>J</i> ⁺	0.64	0.37	2
	<i>metJ743</i>	+	<i>J/J</i> ⁺	0.85	0.61	
4	None	—	<i>J</i> ⁺ <i>K</i> ⁺	0.61	0.28	2
	None	+	<i>J</i> ⁺ <i>K</i> ⁺ / <i>J</i> ⁺	0.53	0.21	
	<i>metK747</i>	—	<i>J</i> ⁺ <i>K</i>	1.36	1.09	
	<i>metK747</i>	+	<i>J</i> ⁺ <i>K/J</i> ⁺	1.01	1.01	

Dominance tests with metK mutants

The properties of KLF 16 in Salmonella typhimurium. As the episome KLF 16 extended from *thy* on one side of *serA* to a point well to the other side (Fig. 1; K. Brooks Low, personal communication), it was considered that it might carry the *metK* gene (*metK* and *serA* being cotransduced; Lawrence *et al.* 1968), and therefore be suitable for use in *metK* dominance tests. The episome was transferred from its *Escherichia coli* JC1553 *thy* host into *Salmonella typhimurium serA13rec-302met-754*, with selection of the required heterogenotes on MA + methionine. One of these was purified and used for transfer of KLF 16 to further *S. typhimurium serA* recipients, the resulting heterogenotes being selected as prototrophs. KLF 16 was found to be more stable than

KLF 10 in *S. typhimurium* hosts: fewer than 1% serine-requiring segregants were usually obtained after growth of KLF 16 heterogenotes in NB. However, acridine orange treatment (Hirota, 1960) increased the proportion of segregants. No ethionine-sensitive recombinants were detected among more than 100 serine-requiring segregants from various *metK*/KLF 16 cultures, so that, as with KLF 10 heterogenotes, recombination between the episome and the host's chromosome was rare.

Fig. 3. Growth of *metK* heterogenote and control strains in the presence of methionine analogues. All the parental haploid strains possessed *serA* mutations (see Table 1). Heterogenotes were obtained by transferring the *Escherichia coli* episome KLF 16 (Fig. 1) into these parental strains. Inocula were in the exponential phase of growth. Cultures were grown in MM (Δ), MM+ethionine, 1000 $\mu\text{g./ml.}$ ($\circ$), and MM+α-methylmethionine, 1000 $\mu\text{g./ml.}$ ($\blacktriangle$). The relevant genotypes (i.e. with respect to *metJ* and *K*) are indicated on the graphs: where merodiploids were used, relevant episomal markers are given after a diagonal stroke.

Methionine analogue sensitivity of metK heterogenotes. As with *metJ* heterogenotes, the results of streak tests of the analogue resistance of *metK* heterogenotes were confirmed by growth experiments in liquid media. Examples of the latter results are shown in Fig. 3. Possession of any of the three mutations *metK747*, *K751* and *K752* by a haploid strain resulted in its resistance to α-methylmethionine and ethionine (Fig. 3b). The introduction into these strains of *metK*⁺, on KLF 16 (Fig. 3e), made them as

sensitive to both analogues as the wild-type (*metK*⁺) haploid and homogenote (Fig. 3*a,d*). The absence of a nonspecific effect of KLF 16 on analogue resistance was confirmed (Fig. 3*c,f*) by the observation that ethionine resistance in a *metJ* mutant was independent of the presence or absence of the episome, which did not cover the *metJ* region (Fig. 1). Thus the analogue sensitivity observed in *metK/metK*⁺ heterogenotes reflected a true dominance of the wild-type over the mutant alleles.

Repressibility of the methionine enzymes in metK heterogenotes. Cross-feeding tests showed that the possession of KLF 16 by *metK747* and *K752* mutants prevented the excretion of methionine observed in the parental haploid strains, while excretion by strain *metJ744* was unaffected by possession of the episome. Thus repressibility of the methionine biosynthetic enzymes was apparently dominant to their constitutive synthesis in *metK/metK*⁺ heterogenotes. As in the previous experiments with *metJ*

Table 4. *Cystathionase activity in metK heterogenotes and control strains*

Each value is the mean of two replicate experiments in which cystathionase activity was determined by the toluene-treated cell procedure described in Methods. The unit of cystathionase activity is given in Table 3. All strains possessed a chromosomal *serA* mutation which was expressed only in haploid strains. Serine (200 µg./ml.) was therefore added to haploid, but not to partially diploid, cultures. In the column headed 'Pertinent genotype', chromosomal methionine regulatory gene mutations precede the diagonal stroke, and regulatory genes carried by the episome KLF 16 follow it. Serine-requiring segregants were obtained from all heterogenote cultures used, following subculture into nutrient broth containing acridine orange (Hirota, 1960). This proved that the parental cultures had been heterogenotic.

The results of statistical analysis of these experiments are given in the text.

Experi- ment	Regulatory mutations	Possession of KLF 16	Pertinent genotype	Cystathionase activity	
				Methionine absent from culture medium	10 mM-methionine in culture medium
1	None	—	<i>K</i> ⁺	0.75	0.37
	<i>metK747</i>	—	<i>K</i>	1.36	1.15
	<i>metK747</i>	+	<i>K/K</i> ⁺	2.56	1.25
2	None	—	<i>K</i> ⁺	0.32	0.16
	<i>metK751</i>	—	<i>K</i>	0.96	0.35
	<i>metK751</i>	+	<i>K/K</i> ⁺	2.03	0.72
3	None	—	<i>K</i> ⁺	0.56	0.16
	<i>metK752</i>	—	<i>K</i>	1.47	1.28
	<i>metK752</i>	+	<i>K/K</i> ⁺	2.18	1.04
4	None	+	<i>K</i> ⁺ / <i>K</i> ⁺	2.35	0.93
	<i>metK747</i>	+	<i>K/K</i> ⁺	2.18	0.75
5	None	—	<i>J</i> ⁺ <i>K</i> ⁺	0.53	0.24
	None	+	<i>J</i> ⁺ <i>K</i> ⁺ / <i>K</i> ⁺	2.42	1.01
	<i>metJ744</i>	—	<i>JK</i> ⁺	2.24	1.60
	<i>metJ744</i>	+	<i>JK</i> ⁺ / <i>K</i> ⁺	12.05	9.30

heterogenotes, this effect was examined quantitatively by assaying cystathionase activity in the appropriate strains grown in the presence and absence of methionine (Table 4). The experiments were again designed to permit statistical analysis of the results.

In Expts 1 to 3 three *metK/metK*⁺ heterogenotes were compared with the wild-type (*metK*⁺) and the appropriate *metK* haploid strain. One of the strains (*metK751*) was

of the nonexcreting type (Table 1), and this was the only one of the three *metK* mutants to show repressibility in the haploid state. However, all three *K/K*⁺ heterogenotes showed repressibility, but in each case the total activity was several times greater than in the haploid wild type (*metK*⁺) and was indeed significantly greater in two cases than in the *metK* haploid strain ($P = 5\%$ with *metK747*, $<0.1\%$ with *K751*, in non-orthogonal comparisons).

A simple hypothesis to explain these results was that KLF 16 carried the *metC* gene, leading to a gene dosage effect on cystathionase activity in the heterogenotes. To test this, strain *serA13rec-302met-754/KLF16* was crossed with the mutant *metC104* (Smith & Childs, 1966) on MA. Confluent growth was obtained. A purified subculture of this prototrophic growth was treated with acridine orange, and methionine-requiring segregants were obtained, confirming that KLF 16 carried the *metC* gene. In addition, the cystathionase activities of *metC104/KLF16* (*C/C*⁺) and the wild-type homogenote (*C*⁺/*C*⁺) were compared. The values obtained for cells grown in the absence and presence, respectively, of 10 mM methionine were: 1.23 and 0.51 units for *C/C*⁺, and 2.05 and 0.80 units for *C*⁺/*C*⁺. The two strains differed significantly ($P = 1$ to 0.1%), and by about the amount which would be predicted if the *C/C*⁺ strain possessed one copy fewer of the *metC* gene than did the *C*⁺/*C*⁺ strain. Clearly, then, a gene dosage effect was responsible for the high activities found in KLF 16 heterogenotes. (It was not possible to estimate the number of copies of the episome per chromosome from the increase in cystathionase activity when KLF 16 was present, because the enzymes from *Escherichia coli* and *Salmonella typhimurium* may differ in their repressibility and catalytic activity.)

The activity of cystathionase observed in the latter experiment with the wild-type homogenote (*metC*⁺*K*⁺/*C*⁺*K*⁺) was very similar to that of the *K/K*⁺ strains (Table 4, Expts 1 to 3). A further experiment confirmed this: no significant difference was detected between *metK747/KLF16* (*K/K*⁺) and the *K*⁺/*K*⁺ strain (Table 4, Expt 4). Thus the *metK* phenotype was not expressed in a strain possessing KLF 16. Finally, the effect of KLF 16 was examined in a normally regulated (*J*⁺*K*⁺) strain, and a *metJ* (*JK*⁺) mutant in which constitutivity resulted from a mutation outside the diploid region (Table 4, Expt 5). The effect of the *metJ* mutation was highly significant ($P < 0.1\%$), regardless of the presence of the episome; similarly, the effect of the episome was also highly significant ($P < 0.1\%$), regardless of which *metJ* allele was present. However, no significant difference could be detected between the effect of the episome on the two strains (*J*⁺*K*⁺ and *JK*⁺): in each case it caused four or five times more cystathionase activity than was found in the corresponding haploid, due to the *metC* gene dosage effect. Thus KLF 16 affected the *regulation* of cystathionase synthesis only in constitutive *metK* mutants. This specific effect of the episome could only be interpreted to mean that *metK*⁺ was dominant to *metK*.

The absence of effects of metK mutation and metK gene dosage on the rate of methionine uptake by cell suspensions

Lawrence *et al.* (1968) found that growing cultures of *metK* mutants incorporated less radioactivity from ¹⁴C-labelled ethionine or methionine, measured over 30 min. intervals, than did *metJ* or wild-type strains. This observation, coupled with the cross-resistance of *metK* mutants to three methionine analogues that all inhibit the growth of *Salmonella typhimurium* in different ways, indicated that a methionine uptake

system (permease) might be defective in *metK* mutants. A permease specific for methionine has been detected in *Escherichia coli* (K_m (methionine), $2.27 \pm 0.39 \mu\text{M}$; Piperno & Oxender, 1968), and preliminary results (P. D. Ayling & E. S. Bridgeland, personal communication) indicate that a transport system having a rather similar K_m (methionine) also exists in *S. typhimurium*. Therefore any gross differences in the rate of methionine uptake between *S. typhimurium* strains should have been revealed by measuring the initial rate at which cells incorporated radioactivity from ^{14}C -labelled methionine at an extracellular concentration of $5 \mu\text{M}$, i.e. above the K_m value. This method was used not only to detect differences between wild-type and *metK* strains, but also to test the effect of *metK* gene dosage on the rate of uptake. This was undertaken following the observation of Ames & Roth (1968) that the presence of an episome

Table 5. *The effects of metK mutation and gene dosage on the rate of uptake of [^{14}C]-L-methionine by cell suspensions*

Uptake of radioactivity by cells was measured as described in Methods, at 15 sec. intervals during the first 75 sec. after addition of [^{14}C]-L-methionine to cell suspensions maintained at 25° . The rates of uptake were calculated from these data by the method of least squares. In each experiment, determinations were made with all four strains, so that valid comparisons could be made between strains.

Strain	Pertinent genotype	Rate of uptake of [^{14}C]-L-methionine (nmoles/mg. dry wt cells/min.)			
		Expt 1	Expt 2	Expt 3	Mean
<i>serA11</i>	<i>metK</i> ⁺	0.74	1.31	0.83	0.96
<i>serA11metK752</i>	<i>metK</i>	0.52	1.36	0.85	0.91
<i>serA11/KLF16</i>	<i>metK</i> ⁺ / <i>K</i> ⁺	0.30	1.22	0.82	0.78
<i>serA11metK752/KLF16</i>	<i>metK</i> / <i>K</i> ⁺	0.26	1.09	0.81	0.72

carrying the histidine permease gene resulted in a two- to threefold increase in the rate of histidine uptake by *S. typhimurium*. Thus if *metK* specified methionine permease, the presence of KLF 16 (which carried *metK*⁺) in a cell should have resulted in a two- to threefold increase in the rate of methionine uptake. (It is assumed that the V_{max} of the *E. coli* and *S. typhimurium* permeases is the same; this appears to be so for histidine permease (Ames & Roth, 1968), and unpublished preliminary results obtained by P. D. Ayling and E. S. Bridgeland suggest that it is also true for methionine permease.) Triplicate experiments were therefore carried out with *metK*⁺, *K752*, *K*⁺/*K*⁺ and *K752/K*⁺ strains (in collaboration with Dr P. D. Ayling). The results are given in Table 5. Although the absolute rates of uptake varied between experiments, it was clear that the rate of uptake was neither decreased by *metK* mutations nor increased when several copies of the *metK* gene were present (i.e. in KLF 16 merodiploids): indeed, the presence of KLF 16 appeared slightly to reduce the rate of uptake, though this effect was not statistically significant. It was concluded that the *metK* gene did not specify a high affinity methionine permease, though the possibility that it could specify a low affinity one having a K_m (methionine) greater than $5 \mu\text{M}$ was not excluded by these results.

DISCUSSION

To what was previously known about *metJ* and *K* mutants from the work of Lawrence *et al.* (1968) (see Introduction) it can now be added that representative mutant alleles of both loci were recessive to the respective wild-type alleles derived from *Escherichia coli* (Fig. 2, 3; Tables 3, 4).

The complete dominance of *metJ*⁺ to the *metJ*713 allele indicated that the *metJ*⁺ product was active, and the *metJ*713 product inactive. That the gene product is a rather large molecule is suggested by the similarity in the lengths of the *metB* and *metJ* genes, deduced from the finding that the variation in cotransduction frequencies of various mutations in the two genes with particular *metF* mutations was similar (*metB*-*metF*31, 21.6 to 34.1 %; Smith, 1961: *metJ*-*metF*96, 31 to 52 %; Lawrence *et al.* 1968): since the molecular weight of the *metB* polypeptide (the subunit of cystathionine-γ-synthetase) is about 40,000 daltons (Kaplan & Flavin, 1966), the *metJ* gene product is likely to be comparable in size. More direct evidence that the *metJ* gene product is indeed a protein has been provided by the recent isolation and identification of a *metJ* mutant suppressible by nonsense suppressors (A. J. Minson, personal communication).

At this point it is suggested that the *metJ* protein may be an apo-repressor of methionine synthesis. If so, the observation that the *metJ*743 allele, unlike *metJ*713, was not completely recessive to *metJ*⁺ (Fig. 2; Table 3), could be explained if the *metJ* product normally forms an oligomeric protein, in which case inactive mixed protein molecules containing mutant and wild-type subunits might have formed in the *metJ*743/KLF 10 heterogenote. Thus the *metJ* gene product, if an apo-repressor, may resemble the *lac* apo-repressor of *Escherichia coli* in being an oligomer of high molecular weight (Muller-Hill, Crapo & Gilbert, 1968).

Recently, evidence that *metJ* specifies an apo-repressor has been obtained with a strain carrying a *metJ* mutation isolated by D. A. Lawrence (unpublished) as a suppressor of a methionine auxotroph. In four determinations, this strain consistently gave very similar, *partially* derepressed cystathionase activity whether grown in the presence or absence of methionine (10 mM) (mean values, 0.56 and 0.63 units, respectively: Chater, 1969). It is difficult to envisage any explanation for this observation other than that in this strain the *metJ* product is a methionine apo-repressor whose conformation is unaffected by the presence of co-repressor, but which has some regulatory activity.

Ethionine inhibits the growth of bacteria by replacing methionine in protein synthesis (Spizek & Janecek, 1969): it has no effect on *S*-adenosylmethionine synthesis (Cox & Smith, 1969; Gross, 1969) and little effect on the activity of the methionine first enzyme (D. A. Lawrence, personal communication). Thus the ethionine resistance of *metJ* mutants is probably the result of reduced incorporation of the analogue into proteins, attributable to the high intracellular methionine level.

The complete dominance of *metK*⁺ to the three *metK* mutations tested indicated that the *metK*⁺ allele produced active, and the *metK* mutant alleles inactive, product, with respect both to methionine analogue resistance and (where relevant) to regulation of methionine synthesis. This eliminated a positive role for the *metK* product in the regulation of methionine synthesis. We may also be certain that the *metK* mutations did not result in the acquisition of novel properties by the mutant gene product such

that the methionine analogues were actively prevented from reaching their sites of inhibition. The resistance of *metK* mutants to α -methylmethionine, which acts specifically by inhibiting the activity of the methionine first enzyme (Schlesinger, 1967; Rowbury, 1968), could not have been the result of an altered component of the first enzyme, because α -methylmethionine resistance would then have been dominant to sensitivity; moreover, this enzyme was sensitive to feedback inhibition by methionine in a *metK* strain (Chater & Rowbury, 1970).

The possibility that the *metK* phenotype might result from an altered specificity or activity of methionine permease was eliminated by the finding that the rate of uptake of radioactive methionine was not influenced by *metK* mutation or by changes in *metK* gene dosage. This conclusion has recently been confirmed by the identification of a gene specifying methionine permease, which is located far from *metK* on the linkage map (P. D. Ayling & E. S. Bridgeland, personal communication).

Lawrence *et al.* (1968) were able to reject the possibility that the *metK* gene product was an apo-repressor; and Gross & Rowbury (1969) showed that methionyl-tRNA was unlikely to have any effect on regulation, while methionyl-tRNA synthetase was specified by the *metG* gene. Thus conventional explanations for the *metK* phenotype all appear inadequate, and it is necessary to look for a feature of methionine biosynthesis that is not shared by other well-established biosynthetic systems. The most obvious such feature is the role of methionine as the immediate precursor of *S*-adenosylmethionine, which is the universal donor of methyl groups in transmethylation reactions (Cantoni, 1965). *S*-Adenosylmethionine is known to be involved in feedback inhibition of the first enzyme of methionine biosynthesis in *Escherichia coli* (Lee, Ravel & Shive, 1966) and *Salmonella typhimurium* (D. A. Lawrence, unpublished data); it is reasonable to suppose that it might also be involved in repression. *S*-Adenosylmethionine deficiency would then be expected to cause release from both feedback inhibition and repression, which might in turn result in the resistance to both α -methylmethionine and ethionine which is one of the features of the *metK* phenotype. The release from control of methionine biosynthesis would be most marked in strains with the most serious *S*-adenosylmethionine deficiencies, and such strains would also be expected to be somewhat slow-growing. Methionine-excreting *metK* strains may illustrate this situation.

Some difficulties arise in the application of this hypothesis to *metK* strains. Chater & Rowbury (1970) have demonstrated that feedback inhibition by methionine occurred in a *metK* strain, though it could be argued that sufficient methionine was accumulated by the cells in the conditions of the experiment to overcome a K_m barrier to *S*-adenosylmethionine synthesis. Secondly, A. J. Minson (personal communication) has obtained viable *metK* mutants suppressible by nonsense suppressors: although this is evidence that the *metK* gene product is a protein, it suggests that the function of the protein is not indispensable, as it would be expected to be if it were required to maintain a pool of *S*-adenosylmethionine.

Thus our present working hypothesis may be summarized as follows: the central factor in regulating methionine biosynthesis is the intracellular level of *S*-adenosylmethionine, which is dependent upon the activity of the *metK* gene product (on the simplest model, this would be the enzyme *S*-adenosylmethionine synthetase). *S*-Adenosylmethionine participates both in feedback inhibition, by interaction with the enzyme homoserine-*O*-transsuccinylase, and in repression, by activating a (possibly oligomeric)

protein apo-repressor coded for by the *metJ* gene. The active repressor acts in an unspecified way to reduce further synthesis of the enzymes of methionine synthesis.

A recent report by Green, Su & Holloway (1970) describes *S*-adenosylmethionine synthetase deficient mutants of *Escherichia coli* which were ethionine-resistant, and possessed high levels both of the methionine biosynthetic enzymes and of methionine. No further data were presented that would permit comparison of the *E. coli* mutants with the known kinds of ethionine-resistant mutants of *Salmonella typhimurium* (Lawrence *et al.* 1968).

Some interesting incidental points arise from the use of F' factors derived from *Escherichia coli* in this study. The rarity of recombination observed between the episomes and the chromosomes of the *Salmonella typhimurium* host cells suggested the absence of fine structure homology of the two types of DNA. Despite this, the *E. coli* regulatory elements efficiently controlled the expression of *S. typhimurium* genes. This is therefore another example of the evolutionary preservation of those parts of proteins concerned with quaternary interactions (Balbinder, 1964; Ito, 1969). Similar conclusions may be drawn from the work of Fink & Roth (1968). Finally, the regulation of methionine synthesis in *E. coli* appears to be similar to that in *S. typhimurium*.

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