Genet. Res., Camb. (1968), **12**, *pp.* 341–354
With 1 *text-figure*
Printed in Great Britain

The sequence of four structural and two regulatory methionine genes in the *Salmonella typhimurium* linkage map

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(*Received* 22 *July* 1968)

1. INTRODUCTION

Genetic and physiological studies of methionine-requiring mutants of *Salmonella typhimurium* have established that at least six structural genes (*metA*, *B*, *C*, *E*, *F* and *H*) mediate the synthesis of this metabolically important amino acid (Glover, 1958; Smith, 1961; Smith & Childs, 1966; J. D. Childs and D. A. Smith, unpublished). The enzyme deficiency of a seventh group of methionine auxotrophs is not known, but they all appear to carry mutations in the same gene, *metG* (Smith & Childs, 1966). Only two pairs of *met* structural genes (*metA* and *H*, and *metB* and *F*) are cotransducible (Fig. 1) (Smith, 1961; Sanderson & Demerec, 1965; J. D. Childs and D. A. Smith, unpublished).

Lawrence, Smith & Rowbury (1968) reported the isolation of mutants for three genes (*metI*, *J* and *K*) involved in the regulation of methionine synthesis. *metI*

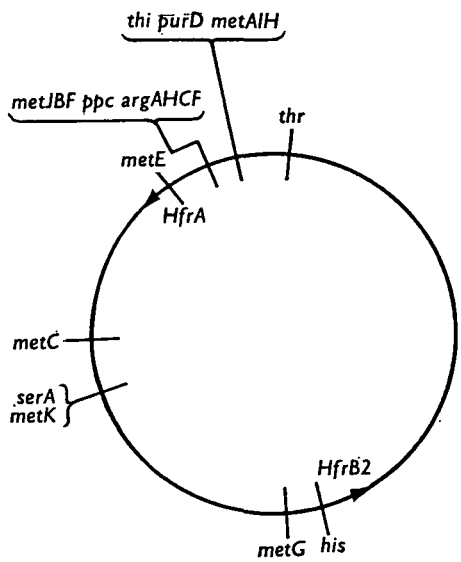


Fig. 1. An abbreviated linkage map of *Salmonella typhimurium* (after Sanderson, 1967). Nutritional requirements: *argA*, *C*, *F*, *H*, arginine; *his*, histidine; *metA*, *B*, *C*, *E*, *F*, *G*, *H*, methionine; *ppc*, aspartate or glutamate; *purD*, adenine + thiamin; *serA*, serine; *thi*, thiamin; *thr*, threonine. Methionine analogue resistance: *metI*, *J*, *K*, HfrA, HfrB2 are the sites of insertion of sex factors in high frequency donors. Bracketed markers are cotransduced.

mutants overproduce methionine, and are not inhibited by the methionine analogue, α-methyl-DL-methionine; they retain sensitivity to two other methionine analogues, DL-ethionine and DL-norleucine. This differential sensitivity to analogues reflects the specific inhibitory effect of α-methyl-methionine on the activity of the first methionine enzyme (Schlesinger, 1967), and it has recently been shown that in *metI* mutants the first enzyme (homoserine-*O*-transsuccinylase) is resistant to α-methyl-methionine or methionine (R. J. Rowbury, K. F. Chater & P. D. Ayling, unpublished). In support of this biochemical evidence, Lawrence *et al.* (1968) found that *metI* was very closely linked (> 95% cotransduction) to *metA*, the gene specifying the first enzyme (Fig. 1).

In contrast with *metI* mutants, *metJ* and some *metK* mutants are derepressed for all the methionine enzymes assayed by Lawrence *et al.* (1968). *metJ* mutants are resistant only to ethionine, whereas *metK* mutants are resistant to α-methyl-methionine, ethionine and norleucine. While *metK* is unlinked to any known *met* gene, *metJ* is > 90% cotransduced with *metB* (Fig. 1) (Lawrence *et al.* 1968). The nature of the *metJ* and *metK* gene products is not yet established.

Tests showing dominance or recessivity of alleles of regulatory genes have been used by many workers, following Jacob & Monod (1961), to investigate the mode of regulation of metabolic pathways. The most satisfactory method yet devised of performing such tests utilizes partially diploid cells in which an F-prime factor carries an allele of the regulatory gene. Isolation of such partial diploids for *metI*, *J* and *K* requires exact knowledge of the location of these regulatory genes and adjacent genes on the *Salmonella* linkage map. In this paper we describe the establishment of the sequence of *metJ*, *B* and *F*, and *metA*, *I* and *H* within their respective transduction fragments by three-point transduction analysis, and the orientation of each fragment with respect to outside markers by analysis of recombinants obtained by Hfr-mediated conjugation.

2. MATERIALS AND METHODS

(i) *Media*

Nutrient agar (NA) and nutrient broth (NB) were supplied by Oxoid Ltd. Minimal agar (MA) was as described by Smith (1961). MA was supplemented as required with the following; DL-allo cystathionine (20 μg/ml); DL-homocysteine thiolactone (100 μg/ml); methionine analogues (1000 μg/ml); all other amino acids (20 μg/ml of the L-isomer); vitamin B_{12} (0·1 μg/ml in growth response tests, 0·01 μg/ml in transduction experiments); and thiamin-HCl (1 μg/ml in growth response tests, 0·1 μg/ml in transduction experiments). The sources of all supplements were as in Lawrence *et al.* (1968).

(ii) *Strains*

The bacterial strains employed are listed in Table 1.

Before use, all strains were purified by two single colony isolations. The nomenclature is that used by Sanderson (1967) and Lawrence *et al.* (1968), following the recommendations of Demerec, Adelberg, Clark & Hartman (1966).

(iii) *Methods*

All incubation was at 37 °C. The maintenance of stock cultures, and propagation, assay and maintenance of transducing phage P22 were as described by Smith (1961). Auxotrophic mutants were induced by diluting an overnight culture 20-fold into NB containing 50 μg/ml of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG), and incubating for 30 min. This suspension was then diluted and spread on to NA so that about 100–200 colonies grew on each plate. Mutant colonies were identified after replication on to suitably supplemented MA. Methionine analogue resistant mutants were isolated and characterized by the methods of Lawrence *et al.* (1968).

Table 1. *List of mutant strains employed*

[1]*argF111*	[4]*metA94*
[5]HfrA*argF111hisD23*	[5]HfrB2*metA746metI706*
[5]*argF111metI708*	[5]HfrB2*metA746metE205metI706*
[5]*argF111metJ741*	[4]*metB23*
[5]*argF111metJ743*	[5]*metB23metI708*
[3]*argF118thi–36*	[5]*metB23metJ744*
[5]*argF111thr*	[5]*metB23thi–36*
[1]HfrA*hisD23metB406*	[1]*metE205metH463*
[2]HfrA*hisD23metI708*	[3]*metE404purD55*
[5]HfrA*hisD23metI749thi–46*	[4]*metF96*
[5]HfrA*hisD23metI750thi–46*	[4]*metF185*
[1]HfrA*hisD23thi–46*	[2]HfrB2*metI706*
[4]*metA15*	

metE404purD55 was derived from *S. typhimurium* LT7. All other strains were derived from LT2. The index numbers indicate the sources of the strains: [1]Dr J. D. Childs, [2]Dr D. A. Lawrence, [3]Dr K. E. Sanderson, [4]Dr D. A. Smith, [5]derivation described in this paper. The gene symbols are as in Fig. 1.

Transduction was carried out by infecting overnight NB recipient cultures (or a concentrated exponential phase culture suspended in NB, containing about 5×10^9 cells/ml) with donor phage at a multiplicity of infection of 5–10. 0·2 ml quantities were spread on to selective medium and plates were incubated for 48 h prior to recombinant analysis. Conjugation was achieved by mixing overnight NB cultures of donor and recipient in the ratio 1:9 by volume, diluting in NB, and spreading 0·1 ml quantities on to selective media so that up to 150 recombinant colonies grew after incubation for 48 h.

Recombinant analysis was carried out by replication on to appropriate media, followed by incubation for 18 h before scoring. Reconstruction experiments for all crosses were performed by plating mixtures of the relevant recombinant genotypes suspended in broth and in saturated cultures of the recipients on to supplemented MA. In no case did the presence of excess recipient cells have any differential effect on the viability of the recombinant types. In all crosses controls showed that none of the markers used reverted significantly.

3. RESULTS

(i) *Derivation of new mutant strains*

For the present studies it was necessary to isolate several strains carrying combinations of markers in the *metA* and *B* transduction fragments. *metI749* and *750* (in HfrA*thi-46hisD23*) and *metJ741* and *743* (in *argF111*) were obtained respectively by the isolation of α-methyl-methionine- and ethionine-resistant colonies of the parent strains, as described by Lawrence *et al.* (1968). The isolation of *metI* and *J* derivatives of *met* auxotrophs was less easily achieved as the auxotrophic requirement (methionine or its precursors) overcomes the inhibitory effect of α-methyl-methionine and ethionine. We thought it possible that for a fixed concentration of analogue there might be a particular concentration of methionine that satisfies the auxotrophic requirement but does not relieve inhibition by the analogue. This was confirmed when ethionine-resistant derivatives of *metB23* were isolated by spreading 0·2 ml of an overnight NB culture of the parent strain on to MA + ethionine, and adding about 0·03 ml of 0·2% methionine solution to a well cut in the agar. Small colonies grew at the outside edge of the halo round the well after 72 h, and after single colony isolation, cultures of two of these were transduced with phage propagated on wild-type LT2. In the case of the strain now designated *metB23metJ744*, three out of 99 transductant clones were resistant to ethionine, as would be expected if the ethionine-resistance of the double mutant was due to a *metJ* mutation. Such a procedure proved ineffective in the isolation of *metI* derivatives of *metA*, so we used NG mutagenesis to isolate *met* auxotrophs from HfrB2*metI706*. One of these responded to cystathionine, a characteristic of *metA* and *B* mutants (Smith, 1961). *metA*, but not *metB*, is linked to *metI* in transduction (Fig. 1) and in the case of the double mutant, seven out of 98 wild-type clones obtained after transduction with phage propagated on wild type LT2 were α-methyl-methionine-resistant, proving the presence of *metI*. We designated the new mutant HfrB2*metA746metI706*. *metA746metE205metI706* was obtained from *metE205metH463* by transduction with phage propagated on HfrB2*metA746 metI706*. A recombinant clone that required homocysteine and vitamin B_{12}, the phenotype predicted for a *metAmetE* double mutant, was identified and purified. The presence of α-methyl-methionine-resistant recombinants in a cross between this strain and *metE205metH463* (Table 5, section IV) confirmed the presence in it of *metI*. Thus it was concluded that the genotype of the double auxotroph was *metA746metE205metI706*.

All the newly isolated *metI* and *J* mutants were tested for linkage to *metA* and *B* respectively in transduction, excretion of methionine at 25 and 37 °C, and resistance to methionine analogues in liquid culture and on plates, and their growth rates in minimal medium measured. *metI749* and *750* closely resembled previous *metI* mutants (Lawrence *et al.* 1968) in all these tests. *metJ741*, *743* and *744* resembled previous *metJ* mutants in all respects, except that in liquid medium they showed slight resistance to α-methyl-methionine and norleucine.

argF111metI708 was derived from *argF111thr*, which had previously been

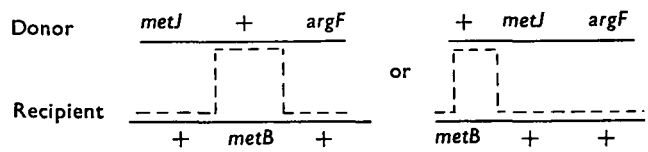
obtained after NG treatment of *argF111*. *argF111thr* was conjugated with HfrA*hisD23metI708* on MA + arginine, and the recombinant colonies were replicated on to MA, MA + arginine + α-methyl-methionine, and MA + arginine. *argF111metI708* was purified from an arginine-requiring, α-methyl-methionine-resistant colony.

As *argF* and *metB* are weakly cotransducible, it was generally simple to exchange one for the other in multiple mutants by transduction. HfrA*argF111hisD23* was derived from HfrA*hisD23metB406*, *metB23metI708* from *argF111metI708*, and *metB23thi–36* from *argF118thi*–36.

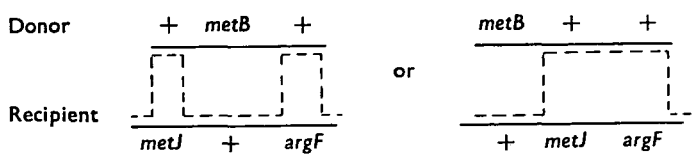
(ii) *Establishment of the sequence of genes in the* metB *region*

It is difficult to determine the sequence of *metB*, *metJ* and *metF* from the cotransduction frequencies: *metB* and *metF*(20–50%), *metF* and *metJ* (30–50%), and *metB* and *metJ* (90–95%). As Armstrong (1967) had clearly established the sequence *-metB-metF-argF-* by two-point cotransduction, reciprocal three-point transduction analysis was carried out on recombinants from two series of crosses between *metB23*, *F96* or *F185* and *argF111metJ741* or *argF111metJ743*. The results are given in Table 2.

The percentage of *arg*$^+$*metB*$^+$*metJ*$^+$ recombinants from crosses Ia, Ib and Ic in Table 2 was expected to be the same whether the sequence was *-metJ-metB-argF-* or *-metB-metJ-argF-*, since they result from two crossovers in each case:



The proportion actually obtained fell within the range 4–9%. In crosses Ib and Ic in Table 2, where *arg* was an unselected marker, the ratio of *argmetB*$^+$*metJ*$^+$ to *argmetB*$^+$*metJ* was very much higher than the 1:10 expected on the sequence *-metJ-metB-argF-* (since *metB* and *metJ* are more than 90% cotransducible). However this sequence was still favoured by these data, because on the alternative sequence *-metB-metJ-argF-*, the ratio would be lower, not higher than 1:10. In the reciprocal crosses IIa and IIb, the class *argF*$^+$*metB*$^+$*metJ*$^+$ would result from four crossovers on the sequence *-metJ-metB-argF-*, or two crossovers on the alternative sequence *-metB-metJ-argF-*:



The extremely low percentage of this class in crosses IIa and IIb (0–0·1%) suggested that it resulted from a four crossover event, and therefore the sequence favoured is *-metJ-metB-argF-*.

https://doi.org/10.1017/S0016672300011927 Published online by Cambridge University Press

Table 2. *The sequence of* argF, metB, metF *and* metJ, *from transduction analysis*

Cross	Markers selected	No. of recombinants	Percentages of recombinant classes			
			arg$^+$*metB*$^+$*metJ*$^+$	*arg*$^+$*metB*$^+$*metJ*	*argmetB*$^+$*metJ*$^+$	*argmetB*$^+$*metJ*
Ia. *metB23* × *argF111metJ741*	*argF*$^+$*metB*$^+$	1197	8·4	91·6	—	—
Ib. *metB23* × *argF111metJ741*	*metB*$^+$	266	7·1	92·1	0·4	0·4
Ic. *metB23* × *argF111metJ743*	*metB*$^+$	852	4·7	93·9	0·5	0·9
			arg$^+$*metB*$^+$*metJ*$^+$	*arg*$^+$*metB*$^+$*metJ*	*arg*$^+$*metBmetJ*$^+$	*arg*$^+$*metBmetJ*
IIa. *argF111metJ741* × *metB23*	*arg*$^+$*metB*$^+$	248	0	100		
IIb. *argF111metJ741* × *metB23*	*argF*$^+$	1679	0·1	97	2·9*	
			arg$^+$*metF*$^+$*metJ*$^+$	*arg*$^+$*metF*$^+$*metJ*	*argmetF*$^+$*metJ*$^+$	*argmetF*$^+$*metJ*
IIIa. *metF96* × *argF111metJ741*	*argF*$^+$*metF*$^+$	173	47·4	52·6	—	—
IIIb. *metF96* × *argF111metJ743*	*metF*$^+$	939	58·4	37·2	4·2	0·3
			arg$^+$*metF*$^+$*metJ*$^+$	*arg*$^+$*metF*$^+$*metJ*	*argmetF*$^+$*metJ*$^+$	*argmetF*$^+$*metJ*
IIIc. *metF185* × *argF111metJ741*	*metF*$^+$	236	56·4	41·9	0·4	1·3
IIId. *metF185* × *argF111metJ743*	*metF*$^+$	586	52·6	43·7	3·2	0·5
			arg$^+$*metF*$^+$*metJ*$^+$	*arg*$^+$*metF*$^+$*metJ*	*arg*$^+$*metFmetJ*$^+$	*arg*$^+$*metFmetJ*
IVa. *argF111metJ741* × *metF96*	*argF*$^+$*metF*$^+$	107	1	99	—	
IVb. *argF111metJ743* × *metF96*	*argF*$^+$	198	2·5	87·9	9·6	
IVc. *argF111metJ741* × *metF185*	*argF*$^+$*metF*$^+$	139	0	100	—	
IVd. *argF111metJ743* × *metF185*	*argF*$^+$	1154	0·6	88·8	10·6	
IVe. *argF111metJ741* × *met F185*	*arg*$^+$*metF*$^+$	108	0·9	99·1	—	
			metB$^+$*metF*$^+$*metJ*$^+$	*metB*$^+$*metF*$^+$*metJ*		
Va. *metB23metJ744* × *metF185*	*metB*$^+$*metF*$^+$	948	94·1	5·9		
Vb. *metF185* × *metB23metJ744*	*metB*$^+$*metF*$^+$	1614	99·3	0·7		

metJ$^+$ colonies were sensitive, and *metJ* colonies were resistant to ethionine. In each cross the recipient is given first. * *metBmetJ*$^+$ and *metBmetJ* (and *metF metJ*$^+$ and *metFmetJ*) double mutants were scored together, since analogue resistance cannot be scored in a methionine requiring strain.

Similarly there should be a large difference in the frequency of the class *argF*$^+$*metF*$^+$*metJ*$^+$ between crosses III and IV of Table 2, if the sequence is *-metJ-metF-argF-*. On the alternative sequence *-metF-metJ-argF-*, this class should occur at a similar frequency in both crosses. In crosses IIIa–IIId, which employ the four combinations of *metF96* and *metF185* with *argF111metJ741* and *argF111metJ743*, the *argF*$^+$*metF*$^+$*metJ*$^+$ class occurred at a frequency of 47–58%, whereas in the reciprocal crosses IVa–IVe, this class was reduced to 0·6–3%. The sequence favoured is therefore *-metJ-metF-argF-*. In crosses IIIb, IIIc and IIId, the ratio of *argFmetF*$^+$*metJ*$^+$ to *argFmetF*$^+$*metJ* was again very high, as in crosses Ib and Ic of Table 2.

Combining the sequences *-metJ-metB-argF-* and *-metJ-metF-argF-* with *-metB-metF-argF-* the overall sequence is *-metJ-metB-metF-argF-*. The position of *metB* between *metJ* and *metF* was confirmed by the analysis of reciprocal three-point crosses between *metF185* and *metB23metJ744* in Table 2 (Va and b). On the sequence *-metJ-metB-argF-*, there should be a difference between reciprocal crosses in the frequency of the unselected marker, *metJ*: when *metBmetJ* is the recipient, recombinants carrying *metJ* arise by two crossovers, whereas when *metBmetJ* is the donor, recombinants with *metJ* are only produced by four crossovers. The percentage of recombinants with *metJ* should be the same in reciprocal crosses if the sequence is *-metB-metJ-metF-*. The results showed a clear difference in the percentage of *metJ*, 5·9% compared with 0·74%, and favoured the sequence *-metJ-metB-metF*.

(iii) *The orientation of the* metB *transduction fragment*

The orientation of a transduction fragment with respect to an outside marker should be most easily determined when markers at opposite ends of the fragment are used. We found that *argF* and *metB* are weakly cotransduced (about 2% in either direction, using *argF111* or *118* and *metB23* or *406*, in agreement with the results of Armstrong, 1967), so must be near opposite ends of their transduction fragment. These two markers were orientated with respect to *thi*, which is in a position clockwise to that of the *metB* region (Fig. 1) (Sanderson & Demerec, 1965), by performing plate mating experiments involving the three chosen markers, and analysing the recombinant colonies by replica plating. The crosses carried out, and the results of the analyses of the recombinants obtained, are presented in Table 3. Reconstruction experiments showed that all recombinant types had equal viability in the conditions of the experiment, but that the generally rather small and translucent *arg* colonies tended to be lost on replication, especially on crowded plates. It is therefore likely that all the *arg* classes in Table 3 are underestimated. *argmet* recombinants were particularly difficult to score, so they were not counted in any cross.

In crosses I(a), (b) and (c) *arg*$^+$*met*$^+$ recombinants were fairly frequent, suggesting that they arose as a result of two crossovers. This would be predicted if the sequence were *-metB-argF-thi-*. If the alternative sequence were true, such recombinants should form a rare four crossover class. In cross II(c) the relative rarity of *arg*$^+$*met*$^+$

Table 3. *The orientation of the* metB *transduction fragment*

Cross	Selective medium	No. of recombinants scored	Percentages of recombinant classes		
			arg$^+$*met*$^+$*thi*$^+$	*argmet*$^+$*thi*$^+$	*arg*$^+$*metthi*$^+$
I. *argF118thi–36* × HfrA*hisD23metB406*	(*a*) MA + ARG	122	63	37	—
	(*b*) MA + MET	202	20	—	80
	(*c*) MA + ARG + MET	205	14·5	7	78·5
(Number of crossovers if sequence is (i)			2	2	2)
(Number of crossovers if sequence is (ii)			4	2	2)
II. *metB23thi–36* × HfrA*argF111hisD23*	(*a*) MA + ARG	145	3	97	—
	(*b*) MA + MET	312	34·5	—	65·5
	(*c*) MA + ARG + MET	221	4·5	78·5	17
(Number of crossovers if sequence is (i)			4	2	2)
(Number of crossovers if sequence is (ii)			2	2	2)

Sequence (i) = *–metB-argF-thi-*; sequence (ii) = *-argF-metB-thi-*; ARG = Arginine; MET = methionine.

recombinants again supports the sequence *-metB-argF-thi-*, as in this case this class would arise as a result of four crossovers.

Unexpectedly, in cross II(c), *arg*$^+$*met* recombinants are almost as rare as wild-type. However, if *argF* and *thi* were very closely linked in conjugation, this would be expected to lower the frequency of this two crossover class, and to give rise to a detectable localized negative interference effect (e.g. Gross & Engelsberg, 1959; Maccacaro & Hayes, 1961); this is observed in the ratio of *arg*$^+$*met*$^+$ to *arg*$^+$*met* recombinants in crosses II(b) and (c) (see also Results, section (v)). We therefore conclude that all results favour the clockwise sequence *-met-argF-thi-* for this region.

Table 4. *Two-point cotransduction data for the* metA *region*

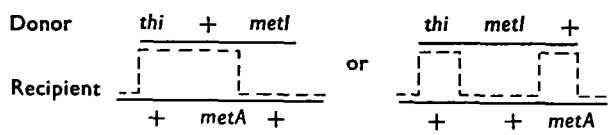
Recipient	Donor	Percentage cotransduction	Source
metH463	*metA94*	35 (215)	P. D. Ayling, unpublished
metA94	*metI*	95–100	Lawrence *et al.* 1968
metH463	*metI*	31–53	
metH463	*purD55*	20 (207)	P. D. Ayling, unpublished
metH463	*thi–46*	1·1 (374)	
purD55	*thi–46*	13·6 (44)	
metA94	*thi–46*	6 (60)	

Figures in parentheses represent the number of colonies scored. In the crosses involving *metI*, the percentage cotransduction summarizes results obtained with 12 *metI* mutants, with each of which 80–1158 colonies were scored.

(iv) *Establishment of the sequence of genes in the* metA *region*

Consideration of the two-point cotransduction values in Table 4 leads to the tentative order *-thi-purD-metA-metI-metH-*. To confirm this sequence, recombinants from reciprocal three-point transduction crosses between *metA94* and *A15*, and the double mutants HfrA*hisD23metI749* (and *I750*) *thi–46* were analysed. The results are shown in Table 5.

In crosses Ia and Ib, the class *metA*$^+$*metI*$^+$*thi* would be produced by two cross-overs on the sequence *-thi-metA-metI-*, or by four crossovers on the sequence *thi-metI-metA-*:



The three other classes would require two crossovers on either sequence. Although the class *metA*$^+$*metI*$^+$*thi* occurred at a comparatively low frequency (0·1%) in comparison with the total number of recombinants, this represented 4% of the *metA*$^+$*thi* class. This number of *metI*$^+$ recombinants would be expected from a two-crossover event since *metA* and *metI* are 95–99% cotransducible.

In cross II, the frequency of transduction for the *thi–46* marker was very low, suggesting that *thi* is very close to the end of the transducing fragment. Therefore

Table 5. *The sequence of* metA, metH, metI *and* thi *from transduction analysis*

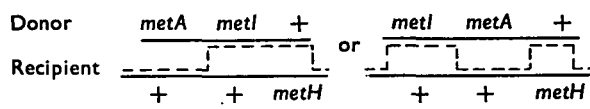
Cross	Markers selected	No. of recombinants	Percentages of recombinant classes			
			metA$^+$*metI*$^+$*thi*$^+$	*metA*$^+$*metIthi*$^+$	*metA*$^+$*metI*$^+$*thi*	*metA*$^+$*metIthi*
Ia. *metA15* × HfrA*hisD23metI749thi–46*	*metA*$^+$	1260	2·4	95·0	0·1	2·5
Ib. *metA94* × HfrA*hisD23metI750thi–46*	*metA*$^+$	308	1·6	89·6	0	8·8
			metA$^+$*metI*$^+$*thi*$^+$	*metA*$^+$*metIthi*$^+$	*metAmetI*$^+$*thi*$^+$	*metAmetIthi*$^+$
II. HfrA*hisD23metI750thi–46* × *metA94*	*thi*$^+$	373	0·5	89·5	9·9*	
			metA$^+$*metH*$^+$*metI*$^+$	*metA*$^+$*metH*$^+$*metI*		
III. *metE205metH463* × HfrB2*metA746metI706*	*metA*$^+$*metH*$^+$ (on MA + B_{12})	207	98·1	1·9		
IV. *metA746metE205metI706* × *metE205metH463*	*metA*$^+$*metH*$^+$ (on MA + B_{12})	117	98·4	1·6		

metI$^+$ colonies were sensitive, and *metI* colonies were resistant, to α-methyl-methionine. In each cross the recipient is given first.

* *metAmetI*$^+$ and *metAmetI* double mutants were scored together since analogue resistance cannot be scored in a methionine-requiring strain.

tenfold concentrated overnight cultures were used. In this cross, it was not possible to decide whether the 0·53% of *metA*$^+$*metI*$^+$*thi*$^+$ recombinants represented a very low two-crossover class, or a high four-crossover one. We were able to resolve the orientation of *metA* and *metI* by analysis of the two transduction crosses (III) *metE205metH463* × HfrB2*metA746metI706 and* (IV) *metA746metE205metI706* × *metE205metH463*. The *metE* and *metH* enzymes control alternative routes for the methylation of homocysteine to give methionine, with the *metH* enzyme being dependent on exogenous vitamin B_{12}. *metE* mutants respond to vitamin B_{12} or methionine, *metH* mutants by themselves are wild type, and the double mutant *metEmetH* responds only to methionine. Transductants in both crosses were selected on MA + B_{12}, and replicated to MA + B_{12}, MA + B_{12} + α-methyl-methionine, and MA. Colonies growing on MA in cross III were omitted from the analysis since these were transduced for the *metE* fragment, not the *metA* fragment. In cross IV, to overcome the leakiness of the *metA746* mutation in the recipient, the cross was performed in broth, and the cells resuspended in saline before plating.

The results of crosses III and IV are presented in Table 5. In cross III, the *metA*$^+$*metH*$^+$*metI* class would require four crossovers on the sequence *-metI-metA-metH-*:



This class occurred at a frequency of 1·9%; since the two-point cotransduction value between *metA* and *metI* ranges from 95 to 99%, it was concluded that *metA*$^+$*metH*$^+$*metI* is a two-crossover class, and therefore the sequence is *-metA-metI-metH-*. In the reciprocal cross, IV, the data would fit either sequence.

We were able to confirm that *purD* is situated between *metI* and *thi* by analysis of the three-point cross *metE404purD55* × HfrA*hisD23metI750thi–46* which gave the results: *metI*$^+$*purD*$^+$*thi*$^+$ = 43; *metIpurD*$^+$*thi*$^+$ = 43; *metI*$^+$*purD*$^+$*thi* = 19; *metIpurD*$^+$*thi* = 10. The high frequency of *metIpurD*$^+$*thi* and *metI*$^+$*purD*$^+$*thi* recombinants argued against the sequences *-purD-thi-metI-*, and *thi-metI-purD-* respectively, because in both cases, these classes would have required four crossovers. Therefore the sequence *-thi-purD-metI-* was indicated. Combining the sequence *-thi-purD-metI-* with *-metA-metI-metH*, the over-all sequence on the *metA* fragment is *-thi-purD-metA-metI-metH-*.

(v) *The orientation of the* metA *transduction fragment*

metI is more than 95% cotransducible with *metA* (Table 4). *metA* and *thi* are about 5% cotransducible in either direction, using *metA15* or *94* and *thi–36* or *–46*. *metI* and *thi* must therefore be near opposite ends of their transduction fragment, and suitable for its orientation. *argF* and *metB*, which are situated in a position anti-clockwise to that of *metI* and *thi* (Fig. 1) (Sanderson, 1967), were the outside markers.

We carried out plate-making experiments involving *metI*, *thi* and *argF* or *metB*,

with selection on MA for prototrophic recombinants. Reconstruction controls (carried out with fewer than 100 colonies per plate) established that both *metI* and *metI*$^+$ organisms were fully viable in the experimental conditions, and distinguishable by replication on to MA and MA + α-methyl-methionine. The crosses performed, and the results of the recombinant analyses, are presented in Table 6. Results originally obtained with a rather larger number of colonies (275–652) from crowded plates (about 300 colonies per plate) were checked in each cross by transferring about 100 colonies to MA templates, whence they were replicated again

Table 6. *The orientation of the* metA *transduction fragment*

Cross	Efficiency of recombination (%)	Numbers of recombinants scored	Percentages of recombinant classes	
			metI$^+$	*metI*
I. *argF118thi–36* × HfrA*hisD23metI708*	0·1	113	6	94
(Number of crossovers if sequence is (i)			2	2)
(Number of crossovers if sequence is (ii)			4	2)
II. *argF111metI708* × HfrA*hisD23thi–46*	0·001	101	10	90
(Number of crossovers if sequence is (i)			4	2)
(Number of crossovers if sequence is (ii)			2	2)
III. *metB23thi–36* × HrfA*hisD23metI708*	0·1	116	10	90
(Number of crossovers if sequence is (i)			2	2)
(Number of crossovers if sequence is (ii)			4	2)
IV. *metB23metI708* × HfrA*hisD23thi–46*	0·01	114	3·5	96·5
(Number of crossovers if sequence is (i)			4	2)
(Number of crossovers if sequence is (ii)			2	2)

Sequence (i) = -(*metB*,*argF*)-*thi*-*metI*-; sequence (ii) = -(*metB*,*argF*)-*metI*-*thi*-. All crosses were made on MA (selection for *thi*$^+$, and *argF*$^+$ or *metB*$^+$). *metI*$^+$ colonies were sensitive, and *metI* colonies were resistant, to α-methyl-methionine. Efficiencies of recombination express the number of recombinants as a percentage of the estimated number of input Hfr cells.

on to MA and MA + α-methyl-methionine. The resulting figures, which are the ones presented in Table 6, showed in all cases about a three-fold increase in the *metI*$^+$ class over the proportion previously obtained from crowded plates. Considering crosses I and III, the results are as predicted if the sequence were -(*metB*,*argF*)-*thi*-*metI*-, as in the alternative sequence the *metI*$^+$ class would require four crossovers, and would therefore be much rarer. In cross IV, the same sequence was favoured, the relative scarcity of *metI*$^+$ recombinants being as expected if *metI*$^+$ were a four-crossover class. However, in cross II, 10% of recombinants were *metI*$^+$; this is high for a four-crossover class, and appears to favour the sequence -*argF*-*metI*-*thi*-. However, a closer examination of the data shows that the over-all efficiency of recombination for this cross is very low, suggesting, as observed in

results section (iii), that the recombination event required between *argF* and *thi* is rare, and that these markers are closely linked. This would also lead to localised negative interference, hence the high *metI*+ class,

We conclude that the clockwise sequence of genes in this region is *-(metB-argF)-thi-metI-*.

4. DISCUSSION

Previous work by Glatzer, Labrie & Armstrong (1966), and Armstrong (1967), who analysed *S. typhimurium*–S. *montevideo* hybrids and recombinants from two-point transduction crosses, provided evidence for the following clockwise sequence: *-metB-metF-ppc-argA-argH-argC-argF-*. The former authors also presented preliminary evidence that the sequence continues *-purD-metA-*.

The present work confirms this sequence, and in addition shows the location of the four genes *metH*, *metI*, *metJ* and *thi*. Therefore the complete known sequence is

-metJ-metB-metF*-ppc*-argA*-argH*-argC**
-argF-thi*-purD*-metA*-metI-metH-*.

Loci which have been asterisked have also been identified in *Escherichia coli*, and show the same sequence as in *S. typhimurium* (Taylor & Trotter, 1967).

The position of *metJ* raises the possibility that it may be an operator gene controlling the expression of *metB* and *metF*. However, two lines of evidence argue against this. First, cotransduction data suggest that *metB* and *F* are not contiguous. Secondly, there is some evidence that the *met*B and *F* enzymes are not co-ordinately controlled. Although the *metB* enzyme, and *metE* and *F* enzymes (assayed together as homocysteine methylase activity) do vary co-ordinately, both in *S. typhimurium* (Lawrence *et al.* 1968) and in *E. coli* (Rowbury & Woods, 1966), the level of methylase activity in *E. coli* is probably determined by the amount of *metE* enzyme, and this does not vary co-ordinately with the *metF* enzyme (Foster Rowbury & Woods, 1963). If *metJ* were an operator gene for *metB*, but not for *metF*, the derepressed levels of the enzymes controlled by the weakly linked genes *metC* and *E* (Fig. 1) (Lawrence *et al.* 1968) could only be explained if a sequential induction process existed. In this connexion it has been suggested that homocysteine may induce homocysteine methylase activity in *E. coli* (Balish & Shapiro, 1967).

There is at present no biochemical evidence to indicate whether the levels of the metA and H enzymes are co-ordinately controlled. This would not be predicted, considering the relatively large distance between these genes (Table 4).

SUMMARY

Three-point transduction analysis was employed to determine the sequence of four structural and two regulatory methionine genes (*metA*, *B*, *F*, *H* and *metI*, *J*, respectively) on two apparently adjacent transduction fragments in *Salmonella typhimurium*. These fragments were subsequently orientated with respect to each other and the rest of the linkage map by the analysis of recombinants obtained in

conjugation experiments. The following clockwise sequence of genes is proposed: *-metJ-metB-metF-argF-thi-purD-metA-metI-metH-*. The bearing of these results on the regulation of the *metB–F* and *metA–H* clusters is discussed.

The Science Research Council supported P.D.A. with a Fellowship and K.F.C. with a Studentship. It is a pleasure to acknowledge the advice and criticism of Dr D. A. Smith during this work and in the preparation of the manuscript. We also profited from discussions with Dr D. A. Lawrence, and from the excellent technical assistance of Messrs T. Allen, M. Oakes and M. Qureshi.

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