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A relationship between L-serine degradation and methionine biosynthesis in *Escherichia coli* K12

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While wild-type *Escherichia coli* K12 cannot grow with L-serine as carbon source, two types of mutants with altered methionine metabolism can. The first type, *metJ* mutants, in which the methionine biosynthetic enzymes are expressed constitutively, are able to grow with L-serine as carbon source. Furthermore, a plasmid carrying the *metC* gene confers ability to grow on L-serine. These observations suggest that in these mutants, L-serine deamination may be a result of a side-reaction of the *metC* gene product, cystathionine β-lyase. The second type is exemplified by two newly isolated strains carrying mutations mapping between 89·6 and 90 min. These mutants use L-serine as carbon source, and also require methionine for growth with glucose at 37 °C and above. The phenotypes of the new mutants resemble those of both *met* and *his* constitutive mutants in some respects, but have been differentiated from both of them.

Introduction

Wild-type *Escherichia coli* K12 cannot use L-serine as carbon source (Newman *et al.*, 1982). This is puzzling, since *E. coli* uses a series of related compounds (D-serine, DL-alanine) as carbon source by converting them to pyruvate, and since it synthesizes high levels of the enzyme L-serine deaminase (L-SD), which converts L-serine to pyruvate (Pardee & Prestidge, 1955).

The ability of *E. coli* mutants to grow with L-serine as carbon source is often accompanied by high L-SD activity. For example, the most-studied of the mutants that are able to grow with L-serine, the *ssd* mutants, had very high L-SD activity (Newman *et al.*, 1982). However, others with much less L-SD were also isolated, though they were not studied in detail (Newman *et al.*, 1982).

We recently isolated a new L-SD-deficient mutant, which could not grow with L-serine, glycine and leucine – a combination which the parent strain can use. We cloned a gene, *sdaA*, which, on a multicopy plasmid, conferred on this mutant the ability to grow on L-serine with and without glycine and leucine, and the ability to synthesize high amounts of L-SD (Su *et al.*, 1989). Since this clone contributed only the information for making L-SD, it seems that an elevated level of L-SD is sufficient to allow growth with L-serine as carbon source.

Abbreviations: INT, *p*-iodonitrotetrazolium violet; L-SD, L-serine deaminase.

In this paper, we demonstrate two ways in which growth of *E. coli* with L-serine as carbon source can be achieved without an increase in L-SD. Both of these involve perturbations in methionine metabolism.

Methods

Cultures. The strains used, all derivatives of *E. coli* K12, are described in Table 1, as are plasmids. The minimal medium used, neutralized to pH 7, has been previously described (Newman *et al.*, 1982). L-Serine, when used as carbon source, was added at 0·2%. All derivatives of strain MEW1 carried a deletion of *ilvA*, so L-isoleucine and L-valine were added to all media at 50 μg ml^{-1} each. LB was prepared as described by Miller (1972).

Enzyme assays. L-SD was assayed as previously described, in toluene-treated whole cells (Ramotar & Newman, 1986). One unit of L-SD is the amount of enzyme which catalyses the formation of 1 μmol of pyruvate in 35 min.

Cystathionine β-lyase (EC 4.4.1.8) was assayed in whole cells by the method of Hunter *et al.* (1975), using toluene instead of lysozyme, as suggested to us by R. C. Greene. It was assayed in extracts as described by Uren (1987).

Histidinol dehydrogenase (EC 1.1.1.23) was assayed in crude extracts as described by Martin *et al.* (1971), using both assays: (*a*) based on the use of NADH with phenazinemethosulphate and *p*-iodonitrotetrazolium violet (INT) to form INT-formazan, and (*b*) by spectrophotometric assay of NADH formation.

Mapping. This was done first by conjugation, then by transduction, using strains with conveniently placed Tn*10* insertions as devised by Singer *et al.* (1989).

Table 1. *Bacterial strains and plasmids*

	Genotype and/or relevant characteristics	Source
E. coli K12		
MEW1	*E. coli* K12 Δ*ilvA* Δ*lac*	Su *et al.* (1989)
MEW22	MEW1 *sdaA*::λ*plac* mu9	Su *et al.* (1989)
MEW3	MEW1 derivative able to grow on L-serine as carbon source	This work
MEW5	Independent isolate: like MEW3	This work
CAG5051	HfrH; *nadA*::Tn*10* (17 min) origin 96 min	Singer *et al.* (1989)
CAG5052	KL227 *metB btu*::Tn*10* (89 min) origin 2 min	Singer *et al.* (1989)
MEW6	CAG5052 transduced to *metB*$^+$ with MEW1 as donor	Singer *et al.* (1989)
CAG5054	KL96 *trp*::Tn*10* (27 min) origin 45 min	Singer *et al.* (1989)
CAG5055	KL16 *zed*::Tn*10* (42 min) origin 61·5 min	Singer *et al.* (1989)
CAG8209	KL228 *rpoD*::Tn*10* (63 min) origin 84 min	Singer *et al.* (1989)
18500	*thi*::Tn*10* (90·25 min)	Singer *et al.* (1989)
18477	*zij*::Tn*10* (88·5 min)	Singer *et al.* (1989)
18431	*ilv*::Tn*10* (84·5 min)	Singer *et al.* (1989)
CGSC 1932	*metA28 argH thi-1*	Bachmann & Low (1980)
CGSC 261	P4X *metB1*	Bachmann & Low (1980)
CGSC 4504	*metC69 thi-1*	Bachmann & Low (1980)
CGSC 5096	*metB metD proA3*	Bachmann & Low (1980)
CGSC 2549	*metE46*	Bachmann & Low (1980)
CGSC 3455	*metF63 pro-22*	Bachmann & Low (1980)
CGSC 5521	*metG146 thr-1* Δ(*gpt–proA*) *hisC3*	Bachmann & Low (1980)
CGSC 5850	*metH156 leuB6 thr-1 proC32 purE42 trpE38 lysA23 argH1 thi-1*	Bachmann & Low (1980)
P4X	*metB*	Bachmann & Low (1980)
JJ127A	*metJ*am185 *metF*	P. Marliere
TK4100	MC4100 *metJ*::Cam^r	R. C. Greene
MEW23	MEW1 *metJ*::Cam^r by transduction from TK4100	This work
NU610	*hisT*::Kan^r	Arps & Winkler (1987)
Plasmid		
pIP29	A subclone of pLC4-14 *metC* in pBR322	Belfaiza *et al.* (1986)

Other genetic techniques. Transductions were performed as described by Miller (1972), and transformations as described by Maniatis *et al.* (1982).

Inhibitor sensitivity tests. Sensitivity to 1,2,4-triazole-3-alanine and 3-amino-1,2,4-triazole was tested on plates as described by Rudd *et al.* (1985). Various concentrations of glutamyl-γ-methyl ester were placed on filter discs, and the strains to be tested were streaked radially (Kraus *et al.*, 1979).

Results

Selection of conditional methionine auxotrophs by growth with serine as carbon source

Although *E. coli* K12 does not grow with L-serine as sole carbon source, it can mutate readily to do so (Newman *et al.*, 1982). The mutants we previously described, including the *ssd* mutants, were all isolated at 37 °C (Newman *et al.*, 1982). In this work we used the same selection, i.e. plating cultures of strain MEW1 on medium containing L-serine (2 mg ml^{-1}), and isoleucine and valine (100 μg ml^{-1} each), but incubating the plates at 28 °C rather than at 37 °C. Mutants isolated at 28 °C had a variety of phenotypes (called Gos; *g*rowth *o*n *s*erine), mostly quite different from that of *ssd* mutants (data not shown).

Two such independently isolated Gos_{28} mutants are described in this work. MEW3 (*gos-3*) and MEW5 (*gos-5*) grew slowly with L-serine as carbon source at 28 °C, considerably faster with glucose at 28 °C, but were unable to grow in glucose- or serine-minimal medium at 42 °C unless methionine was added. Since strain MEW1 could grow at 42 °C without methionine, it seemed likely that the mutation which permitted growth on L-serine at 28 °C also caused the conditional methionine requirement.

Although MEW3 and MEW5 did not form colonies on glucose-minimal medium at 37 °C or 42 °C, even after several days incubation, the cells were not killed but could form colonies on return to 28 °C after 24 h incubation at the higher temperature.

Histidine-constitutive (His^c) mutants also have a high-temperature methionine requirement, and are greatly influenced by the addition of adenine to their growth medium (Roth *et al.*, 1966). We therefore tested the effects of adenine on growth of our mutants. Neither strain could grow with adenine at 42 °C. However, when they were incubated for 48 h at 42 °C in the presence of

adenine (but without methionine), both mutants made some extremely long filaments, up to 50 times the original cell length. The parent strain also showed some tendency to filament at high temperature, but the longest cells seen were about four times the normal length.

Map position of the gos-3 and gos-5 mutations

We located *gos-3* and *gos-5* to approximate positions on the *E. coli* linkage map using the series of Hfr Tet^r strains constructed by Singer *et al.* (1989). Using streptomycin-resistant isolates of MEW3 and MEW5 as recipients, and Hfr Tet^r strains as donors, we selected exconjugants at 28 °C on LB plates with streptomycin and tetracyline. These were tested for growth on glucose-minimal medium at 42 °C. Using MEW6, a methionine-independent transductant of CAG5052 [*btu*::Tn*10* (89 min)] as donor, 355 of 500 exconjugants from strain MEW3 and 399 of 500 from MEW5 grew at 42 °C without methionine, showing strong linkage of the *gos* genes to tetracycline resistance at 89 min. We therefore transduced both strains to tetracycline resistance, using P_1 phage grown on strains with Tn*10* inserted at various points in the 89 min region of the genome (Table 1). The *gos* genes were cotransduced with tetracycline resistance at 90·25 min (59%, 32%) and slightly linked to an insert at 88·5 min (4%, 1%). This suggests a map position between 89·6 and 90 min, for both mutations. There are three known methionine biosynthetic loci in this area: the *metJBLF* cluster at 89·0 min, *metA* at 90·5 min and *metH*, which is located in this region but has not yet been accurately mapped.

Growth of known methionine-constitutive mutants on L*-serine*

If the *gos* mutations affect a methionine biosynthetic enzyme, one might expect one or more of the known *met* mutants to grow on L-serine. We therefore tested Met$^-$ mutants affected in the different steps of methionine biosynthesis: *metA*, *metB*, *metC*, *metE* and *metF* (cf. Fig. 1). Cultures grown in glucose-minimal medium at 37 °C were spread on plates containing 0·2% L-serine, and supplemented with isoleucine, valine, methionine and whatever else was required to satisfy any other auxotrophies of the particular strain tested. None of the strains could grow on L-serine (CGSC strains 1932, 261, 4504, 5096, 2549, 3455 and P4X). A similar failure to use L-serine was observed with a *metH* mutant (strain CGSC 5850) lacking the vitamin B_{12}-dependent homocysteine transmethylase but phenotypically Met$^+$ through the *metE* gene product (cf. Fig. 1), and with a *metG* mutant (strain CGSC 5521) in which methionyl-tRNA synthetase is partially deficient.

Table 2. *L-SD activity of various strains of E. coli K12*

Strain	L-SD activity* of cells grown at 28 °C	L-SD activity* of cells grown at 37 °C
MEW1	17	34^m
MEW3 (*gos-3*)	22	27^m
MEW5 (*gos-5*)	21	8^m
TK4100 (*metJ*)	ND	16
MEW23 (*metJ*)	ND	20
JJ127A (*metJF*)	ND	20^m

ND, Not determined.

* Expressed, as described previously (Ramotar & Newman, 1986), in milliunits per 100 Klett units of cells from a mid-exponential phase culture grown in glucose-minimal medium at the same temperature as indicated and assayed at 37 °C. A superscript m indicates that methionine (50 μg ml^{-1}) was added to the culture. Each figure is the mean of two or three different experiments.

In contrast, growth on L-serine was observed with a *metJ* regulatory mutant, constitutive for the methionine biosynthetic enzymes (Su & Greene, 1971), in both a Met$^+$ (strain TK4100) and a *metF* (strain JJ127A) background. To be sure that the ability to grow on L-serine resulted from methionine constitutivity and not from some other difference in strain background, we transduced the *metJ*::Cam^r mutation into our parent strain, MEW1. The resulting strain, MEW23, was able to grow on L-serine as sole carbon source.

We conclude that the constitutive synthesis of methionine biosynthetic enzymes permits growth with L-serine as carbon source.

L-SD activity of gos-3, gos-5 and metJ mutants

The ability of *metJ* mutants to grow with L-serine as carbon source may reflect their altered levels of methionine biosynthetic enzymes. However, they might also for some reason overproduce L-SD, as did our previously described *ssd* mutants which grew with L-serine as carbon source (Newman *et al.*, 1982). We therefore tested MEW3, MEW5, the two *metJ* strains, TK4100 and MEW23, and the *metJ metF* strain, JJ127A, for L-SD activity. Strains MEW3, MEW5 and JJ127A require methionine at 37 °C. Therefore MEW1 was also grown with methionine for comparison (Table 2).

Neither the *gos* mutants nor the *metJ* strains had significantly higher levels of L-SD than the parent strain, whether tested at 37 °C or at 28 °C. Indeed the highest activity seen (34 units) was in the parent strain grown at 37 °C, which does not permit growth on L-serine. Thus, the ability of *gos-3*, *gos-5* and *metJ* mutants to grow with L-serine as carbon source cannot be attributed to high levels of L-SD.

Growth on serine of strains carrying a metC plasmid

The ability of the *metJ* mutant to grow on L-serine might be due to the overproduction of the whole methionine biosynthetic pathway. However, a single enzyme might also be responsible, in which case, a strain overproducing just that enzyme should be able to grow on L-serine. There is considerable evidence that the *metC* gene product, cystathionine β-lyase, can catalyse L-serine deamination (see Discussion). We therefore transformed our wild-type strain MEW1 with a plasmid, pIP29, carrying the *metC* gene.

Strain MEW1(pIP29) grew on plates with L-serine as sole carbon source. All transformants had more cystathionine β-lyase activity than strain MEW1 as judged by the whole-cell assay. The level varied from transformant to transformant, for unknown reasons. Strains which showed the highest cystathionine β-lyase level seemed to grow fastest on L-serine plates.

L-SD activity is not needed for growth on serine

If an excess of the *metC* gene product is sufficient to permit growth on L-serine, then a strain carrying this plasmid should grow on L-serine, even if it cannot make active L-SD. To test this, we introduced pIP29 into strain MEW22 (*sdaA*) which is deficient in L-SD and is thought to carry a mutation in the structural gene for L-SD (Su *et al.*, 1989). Strain MEW22(pIP29) was able to grow with L-serine, although less rapidly than MEW1(pIP29). Some transformants grew faster than others, and these had higher cystathionine β-lyase activity.

We conclude that an excess of the *metC* gene product is sufficient to allow growth on L-serine, even in the absence of L-SD activity.

The gos mutants and histidine constitutivity

Histidine-constitutive strains of *Salmonella typhimurium* resemble the *gos* mutants described here in that they require methionine at 42 °C and form filaments in the presence of adenine (Fink *et al.*, 1967; Murray & Hartman, 1972). We therefore compared the *gos* mutants with an *E. coli hisT* mutant, in which the *his* operon is derepressed (Bruni *et al.*, 1977).

Histidine-constitutive mutants are resistant to 3-amino-1,2,4-triazole and to 1,2,4-triazole-3-alanine (Roth *et al.*, 1964). The *gos-3* and *gos-5* mutants and strain MEW1 were sensitive to triazolealanine tested at low concentration (25 mM) in the presence of aminotriazole (0·8 mM), and to aminotriazole alone (50 mM). The *hisT* strain, NU610, was resistant to these treatments.

We next assessed histidine constitutivity in the *gos* mutants by assaying the level of histidinol dehydrogenase (the *hisD* gene product). We used two assay methods (Martin *et al.*, 1971). Monitoring histidinol-dependent NADH appearance in crude extracts, we were able to demonstrate considerable activity in the *hisT* strain, but very little in the wild-type MEW1 or in the two *gos* mutants (data not shown). In a more sensitive assay, based on converting NADH to a coloured INT-formazan, MEW5 showed somewhat more activity than MEW3 or MEW1, but much less than the *hisT* mutant (Table 3). We concluded that the *gos* mutants are not derepressed for the *his* operon.

Table 3. *Cystathionine β-lyase and histidinol dehydrogenase activity of various strains of E. coli K12*

Strain	Cystathionine β-lyase activity*	Histidinol dehydrogenase activity†
MEW1	4·7	1·6
MEW3	5·5	1·1
MEW5	7·3	2·5
TK4100 (*metJ*)	470	ND
MEW23 (*metJ*)	210	ND
NU610 (*hisT*)	ND	24

ND Not determined.

* Expressed as nmol mercaptide min^{-1} per mg protein of a crude extract incubated with cystathionine, and corrected for activity without added substrate. Crude extracts were made from cells grown at 37 °C in LB.

† Expressed as A_{520} units in a 20 min assay at 37 °C as described by Martin *et al.* (1971). Cells were grown in glucose-minimal medium at 28 °C.

Nevertheless, the striking phenotypic similarity between the *gos* mutants and *Salmonella typhimurium* His^c strains prompted us to test the *hisT* (His^c) strain for its ability to use L-serine as sole carbon source. The *E. coli hisT* strain, NU610, was unable to grow at 37 °C on plates containing 0·2% L-serine, supplemented with isoleucine and valine.

A comparison of cystathionine-β-lyase activity in gos and metJ mutants

The *gos-3* and *gos-5* mutations seem to lie a short distance from the *metJBLF* cluster, but the fact that the *metJ* strains could grow on L-serine induced us to investigate whether the *gos-3* and *gos-5* mutants exhibited other phenotypes associated with *metJ* strains.

We first tested cystathionine β-lyase activity using a whole-cell screening assay (Hunter *et al.*, 1975) on cells grown in LB. The *metJ* strains turned vivid yellow in this assay whereas our parent strain and the *gos* mutants did not.

To be certain that the *gos* mutants do not overproduce cystathionine β-lyase, we also made a quantitative assay of cystathionine β-lyase activity in crude extracts (Table 3). The *metJ* strains had very high activity, 44–100 times more than strain MEW1; the mutants had low levels similar to those of strain MEW1. We conclude that MEW3 and MEW5 are not derepressed for cystathionine β-lyase, and therefore not derepressed for enzymes of the methionine biosynthetic pathway generally.

Sensitivity of the gos-3 and gos-5 mutants to glutamyl-γ-methyl ester

Strains carrying mutations in *metJ* are resistant to glutamyl-γ-methyl ester (Kraus *et al.*, 1979). We therefore tested the *gos* mutants for this phenotype. The two *metJ* strains, TK4100 and MEW23, grew well in the presence of all concentrations tested (0·5–3·0 mg ml^{-1}). Strain MEW1 did not grow as well, but was able to make colonies at all concentrations. However, the *gos* mutants were able to grow slightly at 0·5 mg ml^{-1}, even less at 1·0 and 1·4 mg ml^{-1} and not at all at 3·0 mg ml^{-1}.

We conclude that the *gos-3* and *gos-5* mutants, unlike the *metJ* strains, are hypersensitive to glutamyl-γ-methyl ester.

Discussion

We have previously shown that an increased level of L-SD is sufficient to permit growth of *E. coli* K12 on L-serine, whether the increase is due to an *ssd* mutation or to a high number of copies of the L-SD structural gene (Newman *et al.*, 1982; Su *et al.*, 1989). In this paper, we show that growth on L-serine can be obtained in at least two other ways: (1) by derepression of methionine biosynthetic enzymes, and (2) by an unknown metabolic event causing a conditional requirement for methionine.

Metabolism of L-serine via cystathionine β-lyase

The *metJ* regulatory mutants, which are constitutive for the methionine biosynthetic enzymes, are able to grow on L-serine as sole carbon source. This ability did not depend on active synthesis of L-methionine, as it was also observed in a *metJ metF* methionine auxotroph.

The synthesis of the methionine precursor, homocysteine, actually involves the concomitant conversion of L-serine to cysteine and thence to pyruvate plus NH_3 (Fig. 1). During growth on glucose, therefore, methionine synthesis will result in net conversion of L-serine to pyruvate. However, during growth on L-serine, the methionine biosynthetic pathway does not result in net

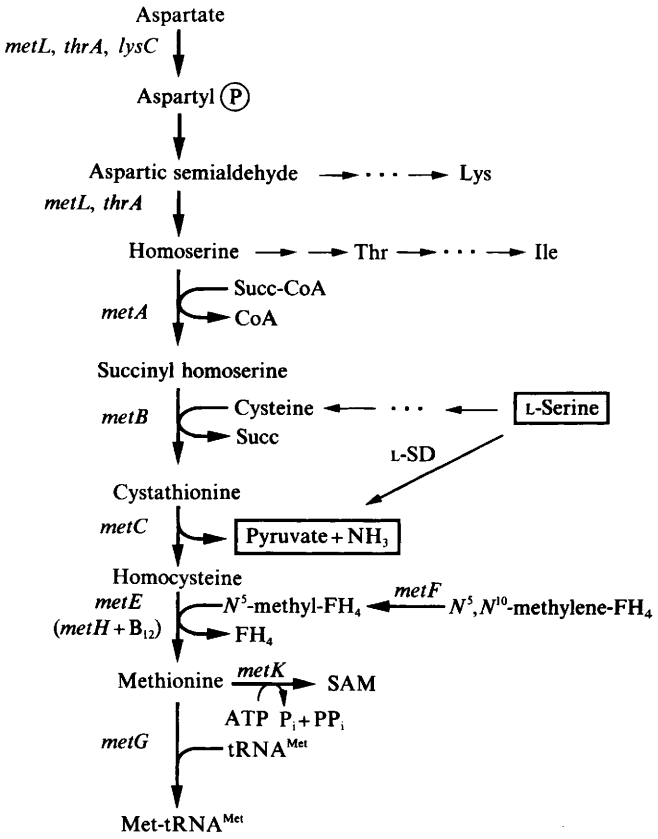

Fig. 1. Biosynthesis of methionine. The steps in the biosynthesis of methionine, and its conversion to *S*-adenosylmethionine (SAM) and methionyl-tRNA are shown, as are the genes coding for the enzymes involved. The reaction catalysed by L-SD is also indicated.

conversion of L-serine to pyruvate, because aspartate, the precursor of methionine, must be made by carboxylation of the pyruvate generated by the pathway.

The ability of methionine-constitutive mutants to grow on L-serine suggests that one of the methionine biosynthetic enzymes might be able to degrade L-serine. We showed that an excess of the *metC* gene product, cystathionine β-lyase, was sufficient to allow growth [strain MEW1(p*metC*)], even in the absence of L-SD [strain MEW22(p*metC*)], suggesting that serine degradation might be a side-reaction of this enzyme. This could involve condensation of L-serine with homocysteine to form cystathionine, mimicking the back-reaction, followed by hydrolysis to homocysteine, pyruvate and NH_3. This hydrolysis might be catalysed by the *metC* gene product. It may even be that the *metC* gene product can bind L-serine and deaminate it in a partial reaction, thus acting as an L-serine deaminase.

Consistent with this hypothesis, the *Neurospora crassa* cystathionine β-lyase has been shown to act as an L-serine deaminase, producing pyruvate from L-serine at

90% of the rate of the lyase reaction, although with 10-fold more substrate (Flavin & Slaughter, 1964). In crude extracts of *E. coli* B, D-serine was deaminated but L-serine was not (Wijesundera & Woods, 1962). The activity purified from *E. coli* K12 catalysed deamination of DL-serine, although the rate of the reaction with serine was only 5% of that with cystathionine, but the isomers were not tested separately (Delavier-Klutchko & Flavin, 1975). In fact, the enzymes involved in cystathionine metabolism have been shown to have considerable flexibility in their substrate requirements (Braunstein & Goryachenkova, 1984; Flavin & Slaughter, 1964, 1967).

In view of these observations, it seems likely that the *E. coli metC* gene product, cystathionine β-lyase, is able to catalyse L-serine deamination.

The nature of the gos-3 and gos-5 mutations

Strains MEW3 (*gos-3*) and MEW5 (*gos-5*) were isolated for their ability to grow on L-serine as carbon source at 28 °C. Their inability to grow at 42 °C without exogenously provided methionine might suggest that their use of L-serine at 28 °C resulted from partial methionine starvation and consequent derepression of *metC*. This is unlikely as judged by (i) their continued ability to grow on L-serine in the presence of methionine, at both 28 and 42 °C, and (ii) their cystathionine β-lyase levels, which were normal in cells grown at 37 °C.

Genetic mapping placed the *gos-3* and *gos-5* mutations between 89·6 and 90·0 min on the genetic map. This location seems to be distinct from that of nearby genes known to code for methionine biosynthetic enzymes: *metH* (near 90·9 min), *metA* (90·5 min), and the *metJBLF* cluster (89·0 min); it is clearly different from that of *metK* (64 min) or *metC* (65 min). Furthermore, known *metA*, *metB*, *metC*, *metE* and *metF* mutants were unable to grow on L-serine.

Many strains of *E. coli* (Ron & Davis, 1971) are naturally methionine-requiring at the upper limits of growth. This seems to be due to an effect on the first enzyme of methionine biosynthesis, homoserine transsuccinylase. However, this defect is unlikely to explain the methionine requirement of the *gos* mutants since the parent strain MEW1 used here, like other *E. coli* strains, grows well at 42 °C without methionine, whereas the *gos* mutants require methionine even at 37 °C. It is possible that the *gos-3* and *gos-5* mutations somehow lower the temperature at which homoserine transsuccinylase becomes limiting.

Strains MEW3 and MEW5 show some characteristics in common with mutants constitutive for histidine biosynthetic enzymes (His^c). This includes a partial septation defect, with the formation of many long filaments when the strains were incubated at 42 °C without methionine. This filamentation was considerably aggravated – in some experiments – in the presence of adenine. When cultures were lightly inoculated, some filaments up to 50 normal cell lengths were observed (data not shown).

Histidine-constitutive mutants of *S. typhimurium* have a phenotype remarkably similar to that of the *gos* mutants: they require methionine at 42 °C and form filaments at 37 °C, and filamentation is exacerbated in the presence of adenine. However, an *E. coli hisT* mutant, known to be constitutive for the histidine operon (Bruni, 1977), was unable to use L-serine as sole carbon source. Furthermore, direct assay of histidinol dehydrogenase activity (product of the *hisD* gene) in the *gos* mutants showed that they were not histidine constitutive.

The cause of the pleiotropic phenotype of the *S. typhimurium* His^c mutants is unknown. It has been shown to result from an excess of the *hisF* and *hisH* gene products (Fink *et al.*, 1967), which catalyse the release of AICAR (5-amino-4-imidazole carboxamide riboside 5′-monophosphate), a by-product of histidine biosynthesis and a normal intermediate in purine biosynthesis. Even though MEW3 and MEW5 are not His^c mutants, the striking similarity of the *E. coli gos* mutants and *S. typhimurium* His^c strains suggests that they may have a similar metabolic defect. One possibility is that in both cases the AICAR pool is affected. Indeed, the *gos* mutants lie suggestively close to the *purH* gene, whose product catalyses the conversion of AICAR to formyl-AICAR in the next step toward formation of inosine monophosphate. The possibility that the mutations are in *purH* is currently under investigation.

A recent report (Matthews & Neidhardt, 1989) suggests that *metK* mutants have an increased capacity to deaminate L-serine, which might suggest that the mutants studied here are of that type. However we have shown that the ability of the *metK* strains to grow on serine is not due to the *metK* mutation, but to a secondary mutation, which we call *rbl* (unpublished work). The *rbl* mutation is different from the previously described *ssd* mutation (Newman *et al.*, 1982), and also from the mutations in MEW3 and MEW5.

Although the actual alteration in the *gos-3* and *gos-5* mutants remains obscure, they are clearly different from any of the other mutants which grow on serine. They are not constitutive for cystathionine β-lyase, unlike *metJ* mutants or strains harbouring a *metC* plasmid. They do not overproduce L-SD, unlike *ssd* mutants or strains harbouring an *sdaA* plasmid. Furthermore, despite a phenotypic similarity, they are not histidine constitutive.

Selection for growth on L-serine seems to reveal intricate metabolic connections. It is particularly inter-

esting that perturbations of this type can affect cell division. We hope that further study will provide insight into the mechanisms coordinating metabolic activity and cell division.

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