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A Relationship between L-Serine Degradation and Methionine Biosynthesis in *Escherichia coli* K-12

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ABSTRACT

A Relationship between L-Serine Degradation and Methionine Biosynthesis in *Escherichia coli* K-12

Elizabeth Alison Brown

Wild type *Escherichia coli* K-12 cannot use L-serine as sole carbon source, but strains with high L-serine deaminase (L-SD) activity, such as those mutant at the *ssd* locus and those carrying many copies of the *sdaA* gene can grow on L-serine. Two classes of mutants were discovered to be able to use L-serine as sole carbon source without having increased L-SD activity.

The first type have altered methionine metabolism. Strains which overproduce the enzyme, cystathionine-ß-lyase, the *metC* product, either because they are derepressed for production of all of the methionine biosynthetic enzymes or because they harbour a multicopy plasmid carrying the *metC* gene, were able to grow on L-serine. These strains were able to deaminate L-serine, even when they had low to normal levels of L-SD activity. This may be due to a side reaction of cystathionine-ß-lyase.

The second type, called Gos mutants, are newly isolated

strains. Gos mutants require methionine for growth at 37° and form long filaments in its absence. These mutants resemble methionine and histidine constitutive mutants in some respects. However, a comparison of Gos strains with these mutants showed that Gos mutants are neither methionine mutants nor histidine constitutive mutants.

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I INTRODUCTION

Escherichia coli K-12 grown in minimal-salts medium can use any of a wide variety of carbon-containing compounds as its sole source of carbon. As carbon source, glucose supports the most rapid growth seen in minimal medium. Some amino acids, such as L- and D-alanine, also support rapid growth. They do so by conversion to pyruvate, which is also used as carbon source, though at a slower rate. A paradox exists in that *E. coli* K-12 cannot use L-serine as carbon source, even though it produces an enzyme, L-serine deaminase (L-SD), which converts L-serine to pyruvate (Pardee and Prestidge, 1955). A study of mutants that upset that paradox, by having the ability to grow on L-serine, may explain why wild type *E. coli* cells cannot use L-serine as sole carbon source.

In the wild type cell, L-SD is made in relatively large quantities (Newman *et al.*, 1985b). When glycine and leucine are included in the growth media, L-SD is induced (Pardee and Prestidge, 1955, Newman and Walker, 1982) and the cell can grow on L-serine (Newman *et al.*, 1981), perhaps because the increased L-SD activity allows enough conversion of L-serine to pyruvate so that it can be used as carbon source.

Though wild type *E. coli* does not grow with L-serine as sole carbon source, it is possible to isolate mutants which do. The most thoroughly studied of these, the *ssd* mutants, have a very complex phenotype, including high L-SD activity

(Newman et al., 1981, Newman et al., 1982a).

Another type of mutant, sdaA, cannot use L-serine even when glycine and leucine are present (Su et al, 1989). Strains which carry the wild type sdaA gene on a plasmid have a lot of L-SD activity and can grow on L-serine.

New mutants have been isolated which can also grow on L-serine and they are characterized in this work. These are called Gos mutants because they grow on L-serine. What is different about Gos mutants is that they are able to grow on L-serine, but they do not have high levels of L-SD activity. If the rate of L-serine deamination is too low to allow the wild type strain to grow with L-serine, the Gos strains must remedy this by increasing L-serine deamination. Since they do not do this by increasing L-SD activity, they must do it in some other way.

The Gos mutants were isolated by their ability to grow on L-serine, but they have other characteristics which do not appear to have any relation to L-serine metabolism. Gos mutants were isolated at 28C and they cannot grow at 37C. At the high temperatures, they form long filaments. This attribute would indicate that the Gos mutants have a defect in cell division. Growth at 37C is restored to these strains if they are supplemented with methionine. This methionine requirement indicates that the gos mutation may affect methionine synthesis or degradation.

If the Gos mutants are in a temperature sensitive class of methionine mutants, then other methionine auxotrophs

should be able to grow on L-serine. Through the analysis of the Gos mutants it was discovered that only strains which were derepressed for methionine biosynthesis were able to grow on L-serine. These strains, however, do not require methionine for growth.

Other known mutants also have pleiotropic effects involving methionine. One class of such mutants includes histidine constitutive strains which are mutant in the regulation of histidine biosynthesis (Murray and Hartman, 1972). These overproduce the histidine enzymes and, like the Gos mutants, require methionine at high temperatures and form filaments. If the Gos mutants fall into this class of strains, then it follows that Gos mutants would be derepressed for the histidine pathway and histidine constitutive mutants would be able to grow on L-serine as carbon source.

The Gos mutants are so interesting because they have traits in common with L-serine-using strains, methionine auxotrophs, methionine regulatory mutants, histidine regulatory mutants and division mutants. Gos mutants may be in a class by themselves that serves to connect all of these areas of metabolism.

In the following sections of the introduction, the experimental evidence related to the preceding paragraphs will be reviewed. This will provide a context for the experiments that were done.

I A L-Serine Deaminase

I A-1 Use of L-serine as Carbon Source

Wild type *E. coli* cannot grow on L-serine alone as carbon source, but it can use it when glycine and leucine are supplemented in the media, perhaps because glycine and leucine induce L-SD (Pardee and Prestidge, 1955, Newman and Walker, 1982). The increase in L-SD activity in the presence of glycine and leucine may enable the cell to convert enough L-serine to pyruvate for the cell to grow. This growth pattern yields two kinds of mutants: those which use L-serine and those which cannot grow on L-serine plus glycine and leucine (SGL).

Escherichia coli K-12 strains which are mutant at the *ssd* locus can use L-serine as sole carbon source (Newman *et al*, 1981). These mutants have very high levels of L-SD activity. However, the mutation is exceedingly pleiotropic. Cells with mutations in *ssd* cannot use succinate as sole carbon source, cannot grow anaerobically, are resistant to certain antibiotics and are very sensitive to ultraviolet irradiation (Newman *et al.*, 1982a).

Strain MEW22 has a mutation that may be in the L-SD structural gene. It is mutant at the *sdaA* locus and it cannot grow on L-serine, even when it is supplemented with glycine and leucine. It also has less than five percent of wild type L-SD activity. When this strain carries a multicopy plasmid containing the wild type *sdaA* gene it can

grow on L-serine and its L-SD activity is very high (Su *et al.*, 1989).

Both the *ssd* mutants (Newman *et al.*, 1982a) and strains carrying many copies of *sdaA*$^+$ (Su *et al.*, 1989) have high L-SD activity and can grow on L-serine. Therefore, high L-SD activity may be sufficient for growth on L-serine. However, the full effects of the products of these two genes is not clear. The *sdaA* gene may not be the structural gene for L-SD and *ssd* mutations are known to be pleiotropic. There remains the possibility that both of these loci code for regulatory genes that affect a variety of processes, aside from L-SD activity.

I A-2 Regulation of L-Serine Deaminase

It is not understood why L-SD activity is important to a glucose-grown cell if it only deaminates L-serine to pyruvate, because no role for that reaction is obvious for cells grown in medium to which L-serine is not added. However, the enzyme is subject to many controls at different levels of cellular activity, so L-SD activity may have an important cellular function in addition to the deamination of L-serine. Otherwise, all of the controls that act upon the gene and on the enzyme would appear to be a waste of energy on the part of the cell.

L-SD activity is induced by glycine and leucine (Pardee and Prestidge, 1955, Newman and Walker, 1982), by UV and other DNA damaging agents, by heat shock (42C) (Newman *et*

al., 1982b) and by anaerobic growth. When a culture is subject to any of the above conditions, L-SD activity is increased as a rapid response.

At the level of the protein, the enzyme is subject to activation by the products of the genes 191 and 128 (Newman et al., 1985a). Strains which are mutant at either the 191 or 128 loci cannot grow on SGL, but are still able to make the L-SD enzyme. However, the 191 and 128 gene products must both be functional for the cell to deaminate L-serine in vivo, since strains mutant in either 191 or 128 do not have any L-SD activity in whole cell assays (in vivo).

L-SD activity can be measured in extracts (in vitro) of these mutants, provided that the enzyme is activated by iron sulfate and DTT (dithiothreitol). Iron and DTT also activate the enzyme of wild type extracts, but not of IK15, another strain which cannot grow on SGL. Since iron and DTT activate L-SD in 191, 128 and in wild type strains, but not in IK15, then strains mutant in 128 or 191, like the wild type, produce an enzyme which responds to activation by iron and DTT, but IK15 does not (Newman et al., 1985a). It is possible that the products of 191 and 128 activate L-SD in vivo much as iron and DTT activate it in vitro.

I B Methionine Metabolism

As previously noted, the selection for growth on L-serine (Gos mutants) resulted in strains that also required

methionine for growth at high temperatures. What is curious is that a mutation which allows growth on L-serine would cause this requirement. A question arises about the connection between L-serine and methionine metabolism. In one set of results presented here, methionine mutants were compared to Gos mutants. In light of that, methionine biosynthesis and its regulation is reviewed below.

I B-1 Methionine Biosynthesis and Metabolism

I B-1.a Genes Coding for Methionine Synthesis

In bacteria such as E. coli and Salmonella typhimurium, methionine is produced by a series of biosynthetic enzymes from homoserine (Figure 1) (Review from Cohen and Saint-Girons, 1987). The genes responsible for producing the methionine enzymes are metA (homoserine succinyl transferase), metB (cystathionine-γ-synthase), metC (β-cystathionase or cystathionine-β-lyase) and metE (homocysteine methylase)/metH (B_{12}-dependent-homocysteine methylase). A mutation in any of these genes results in a requirement for methionine, with the exception of: 1) metH, because the metE gene product can still function in the absence of the metH product and 2) metE, because metH can methylate homocysteine independently of metE, provided that vitamin B_{12} is added to the growth media.

Another enzyme is also necessary for methionine biosynthesis and that is the metF gene product, 5,10-methylene tetrahydrofolate reductase. This enzyme catalyses

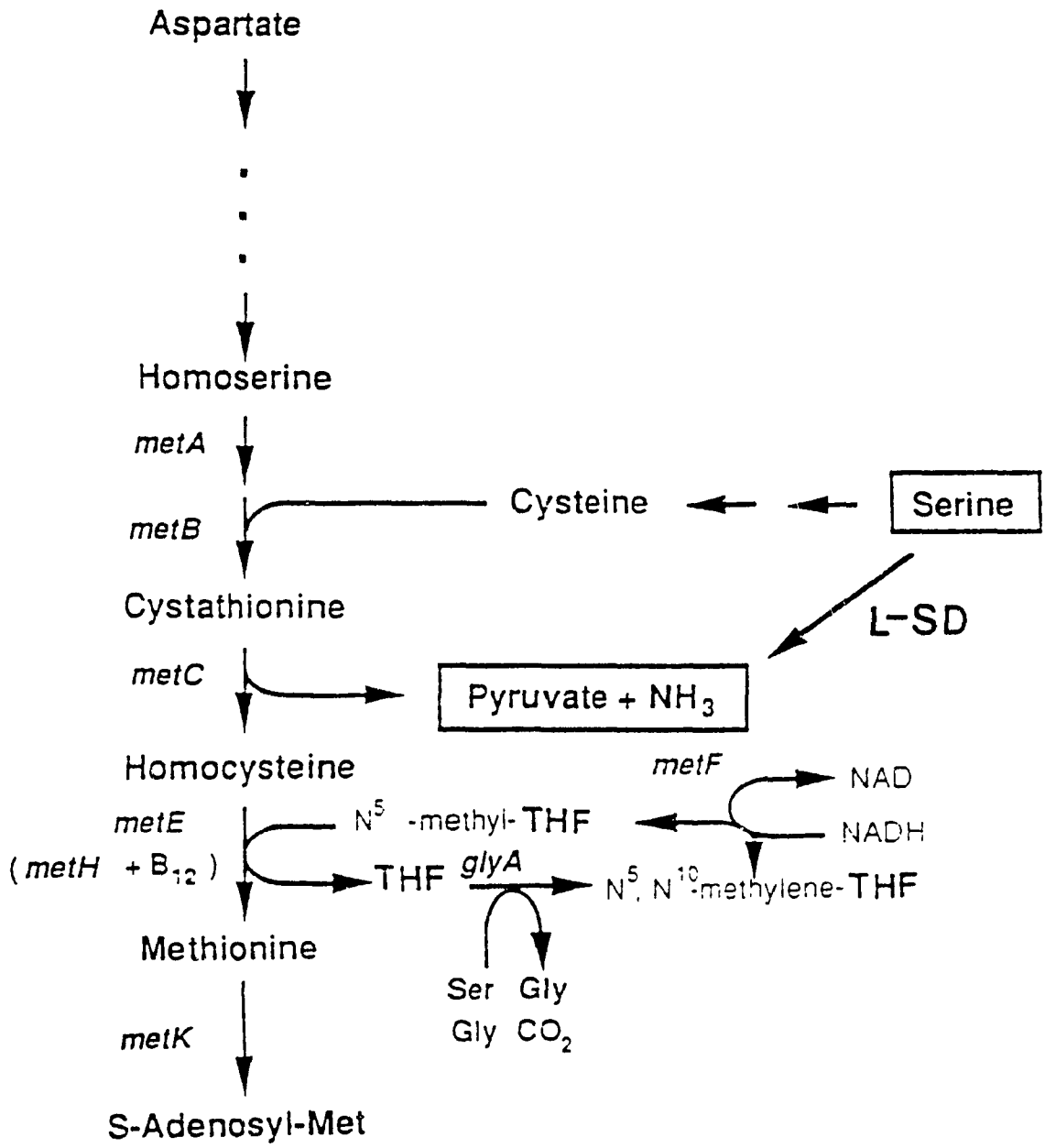

Figure 1. The Methionine Biosynthetic Pathway in E. coli

The synthesis of methionine and the met biosynthetic enzymes are shown. The conversion of L-serine to pyruvate by L-serine deaminase (L-SD) is also presented. Single carbon metabolism with relation to methionine biosynthesis is indicated as well.

the reduction of methylene tetrahydrofolate (THF) to methyl THF. This methyl group, which originally comes from L-serine, is transferred to homocysteine by either of the two homocysteine methylases to make methionine.

The precursor of the methionine pathway, homoserine, which is synthesized from aspartate, is also the precursor of threonine and isoleucine. Production of these compounds is not controlled by the regulators of the methionine pathway. However, it is likely that a disturbance in their production may also affect the production of methionine.

I B-1.b Regulation of the Methionine Pathway

Synthesis of the methionine enzymes is controlled by the metJ product, the methionine repressor in combination with S-adenosylmethionine (SAM), made from methionine by the product of the metK gene (Su and Greene, 1971). The metJ and the metK products are both necessary for the control of the methionine pathway because the repressor and SAM together repress the methionine regulon (Shoeman et al., 1985). When a strain is mutant in either metJ or metK, the methionine pathway is derepressed and all of the methionine enzymes are overproduced (constitutive production). Neither metJ or metK mutants require methionine for growth.

A third regulatory gene, metR, maps beside metE (Plamann and Stauffer, 1987). In fact, these two genes are divergently transcribed and part of their promoter regions overlap. The metR gene is involved in the regulation of metE

and metH (Urbanowski et al., 1987). Because of the shared region between metE and metR, production of metE could be repressed by the action of metR transcription. That is, transcription of metE might be inhibited while metR is being transcribed. This process could also be controlled by metJ because the metR gene is regulated by the metJ product (Urbanowski and Stauffer, 1987).

I B-1.c The Sulfur Steps in Methionine Biosynthesis

Since methionine is a sulfur-containing compound, the biosynthesis of it does not involve only the assembly of carbon units, but sulfur from cysteine is also incorporated into the molecule. This part of methionine biosynthesis, called transsulfuration (Flavin and Slaughter, 1964), is catalysed by the metB and the metC gene products. These reactions are important here because it was found that overproduction of the metC enzyme gave rise to cells with the ability to grow on L-serine. The transsulfuration reactions therefore are delineated below.

I B-1.c.1) Transsulfuration in E. coli

Transsulfuration in E. coli and S. typhimurium is accomplished by the two enzymes which transfer the sulfur group from cysteine to methionine via cystathionine and homocysteine: cystathionine-γ-synthase (metB) and β-cystathionase, also called cystathionine-β-lyase, (metC) (Cohen and Saint-Girons, 1987). The two proteins are very

similar. The metB and metC products are often co-purified in chromatographic systems, share 36% homology and their genes have similar regulatory regions (Belfaiza et al., 1986). The latter is not surprising because they are both under the control of the metJ product.

I B-1.c.2) Transsulfuration in Higher Organisms

Whereas in bacteria the reactions only proceed in the forward direction (O-succinyl-homoserine to cystathionine to homocysteine to methionine, Figure 1), in the mammalian liver only the reverse occurs (Figure 2) (Martel et al, 1987). That is, cysteine is produced from methionine by the enzymes cystathionine-ß-synthase and γ-cystathionase (cystathionine-γ-lyase). In Neurospora crassa and in Saccharomyces cerevisiae, transsulfuration can go in both directions (Martel et al, 1987).

I B-1.c.3) Properties of the Transsulfuration Enzymes

All four of the transsulfuration enzymes (two forward and two reverse) are similar in that they all require pyridoxal-phosphate as a co-factor (Martel et al, 1987) and that they all can act on a wide range of substrates. Because of this lack of specificity, it is feasible that they could perform other, as yet undiscovered functions in the cell.

I B-1.c.3)a) Cystathionine-γ-Lyase

γ-cystathionase from Neurospora can catalyse a number

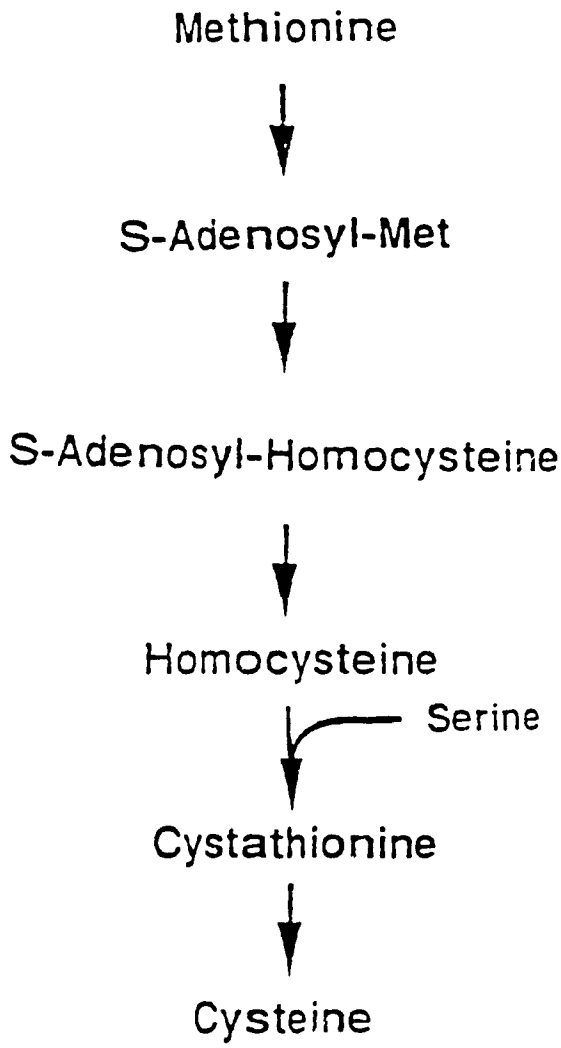

Figure 2. Use of Methionine in Mammalian Cells

Methionine, homocysteine and cystathionine are precursors of cysteine in mammals. This is the reverse of what occurs in E. coli.

of different reactions: β-elimination from other amino acids, such as homocysteine, lanthionine, djenkolic acid and cystine; γ-elimination from hydroxyamino acids; and possibly (very slow) synthesis of cystathionine from cysteine and O-succinyl-homoserine, the reverse of this enzyme's normal function (Flavin and Segal, 1964).

The set of reactions that the mammalian liver γ-lyase can perform includes some, but not all, of the reactions of its Neurospora counterpart (Flavin and Segal, 1964). As well, the γ-cystathionase has some β-lyase activity (Flavin and Slaughter, 1964).

I B-1.c.3)b) Cystathionine-β-Lyase

Cystathionine-β-lyase, which breaks down cystathionine to homocysteine and pyruvate, can also act on other substrates. Delavier-Klutchko and Flavin (1965) purified the enzyme 100-fold from a strain of E. coli that was derepressed for the methionine pathway. This strain yielded five times as much enzyme as did the wild type strain. With this large quantity of enzyme, they found that the enzyme cannot perform any γ-elimination reactions, but it can catalyse β-elimination from thioether-, disulfide-, and hydroxy-amino acids. Pyruvate was formed when L-cystathionine, DL-serine, or DL-alanine were used as substrates for the enzyme (Wijesundera and Woods, 1962).

β-cystathionase can deaminate homoserine and act on lanthionine, on djenkolic acid, on cysteine, as well as on

L-serine. The enzyme can also act on homocysteine, albeit inefficiently (Matsuo and Greenberg, 1959). This indicates that the enzyme has the capability to function in reverse.

Flavin and Slaughter (1964) showed that the Neurospora cystathionine-β-lyase can even act as an L-serine deaminase as it could produce pyruvate from L-serine, provided that the L-serine substrate was present in concentrations which were higher than the concentrations of the cystathionine substrate used.

However, Delavier-Klutchko and Flavin (1964) do not believe that there is a reverse reaction in bacteria. They measured the possible labelled products of cystathionine cleavage (i.e. cysteine or homocysteine) by chromatography and found that only homocysteine was produced. That is, there is no cystathionine-γ-lyase activity. However, they did find very small amounts of α-ketobutyrate produced from cystathionine in E. coli extracts, but attributed it to experimental error because similar amounts of pyruvate were produced by the rat liver γ-cystathionase. Both of these enzymes, β- and γ-cystathionases, are very flexible and it is possible that neither of these occurrences were due to experimental error, but were results of unexpected enzymatic reactions.

I B-2 Methionine Auxotrophy at High Temperatures

Gos mutants and temperature sensitive methionine mutants are not the only categories of mutants that require

methionine for growth at high temperatures. Other classes of mutants which require methionine at high temperatures are described below. It is possible that the Gos mutants fall into one of these classes of mutants.

I B-2.a Histidine Constitutive Mutants

Another class of mutants which have a conditional requirement for methionine are the histidine constitutive (His^C) mutants. S. typhimurium strains which are derepressed for the histidine biosynthetic enzymes cannot grow at 42C without methionine. These cells also are unable to divide at 37C and make very long filaments when incubated at this temperature. In these strains, methionine, or a derivative of it (e.g. SAM), may be involved in the regulation of DNA replication and/or of cell division at high temperatures.

I B-2.a.1) Regulation of Histidine Biosynthesis

The following review of histidine metabolism is derived from Winkler (1987), except where otherwise noted. In both E. coli and in S. typhimurium, histidine is produced in a series of ten steps by eight biosynthetic enzymes (Figure 3). The genes encoding these proteins, [hisGDCBHAF(IE)], map together in the histidine operon. The first step in histidine biosynthesis, catalysed by the hisG product, phosphoribosyltransferase, is subject to inhibition by ppGpp, AMP and ADP and to feedback inhibition by histidine. This controls the flow of metabolites through the histidine

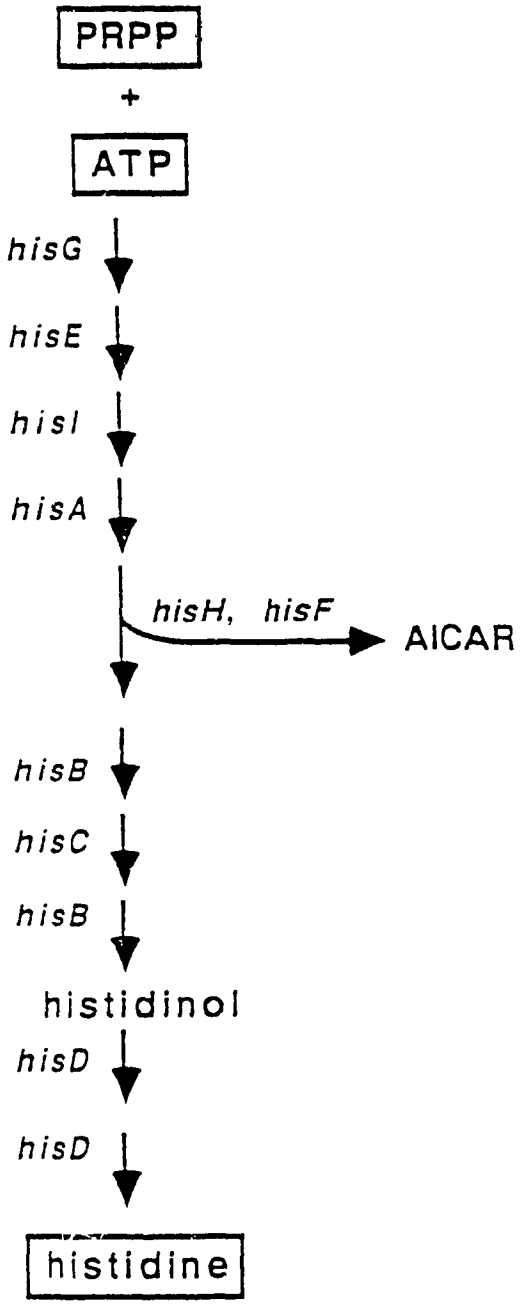

Figure 3. The Histidine Pathway in E. coli

Histidine production and the histidine biosynthetic enzymes are indicated. Also shown is the purine precursor, AICAR (5'-phosphoribosyl-4-carboxamide-5-aminoimidazole), a byproduct of the hisF and hisH enzyme reactions.

pathway.

In addition, an attenuator controls the amount of messenger RNA translated and an array of regulatory genes scattered throughout the genome affect production of histidine enzymes.

The regulatory genes are defined by mutations leading to constitutive production of the enzymes encoded by the *his* operon. They were isolated by virtue of their resistance to the histidine analogue, 1,2,4-triazole-3-alanine (TRA). TRA can be erroneously incorporated into proteins by $tRNA^{His}$ and because it is an amino acid analogue, it probably inactivates those proteins (Roth *et al.*, 1966). Another compound used to isolate His^C mutants is 3-amino-1,2,4-triazole (AT), which inhibits *hisB* dehydratase activity. Often, both of these analogues are used together to isolate His^C strains.

There are six classes of His^C mutants isolated from *S. typhimurium*: *hisO*, *hisR*, *hisS*, *hisT*, *hisU* and *hisW*. *HisO* codes for the operator and maps just upstream of the *his* operon while the other genes map separately, at other locations around the genome.

All of the His^C strains derepress histidine biosynthesis by reducing the pool of charged $tRNA^{His}$ molecules in the cell. When there are fewer charged $tRNA^{His}$ molecules, attenuation of the *his* operon does not function as it should because the ribosomes read through the attenuator, thus giving rise to increased (derepressed)

levels of the *his* enzymes.

I B-2.a.2) Phenotypes of His^C mutants

Some other properties of the histidine constitutive mutants, aside from their increase in histidine production, are that they are temperature sensitive, requiring methionine for growth at 43C, and that *S. typhimurium* His^C mutants form wrinkled colonies at 37C on media with high (2%) glucose (Murray and Hartman, 1972).

The reason that the colonies are wrinkled is because the cells cannot form septa, and so, form very long filaments. That is, they have a block in cell division. Indeed, closer examination reveals that these cells form filaments up to 100 cell lengths at 43C in the absence of methionine. Murray and Hartman (1972) reported that these cells are blocked in DNA synthesis at 43C, when incubated without methionine. Overproduction of both the *hisF* and *hisH* gene products are necessary to produce these filaments at high temperatures.

I B-2.a.3) Reversion of His^C mutants

Smooth, heat resistant revertants of His^C *S. typhimurium* strains isolated by Fink *et al.* (1967) require histidine for growth. This is because the so-called reversion is most often due to a secondary mutation in one of the *his* biosynthetic genes. If one of the histidine enzymes becomes mutated, then histidine is no longer

produced constitutively, even though most of the enzymes are still overproduced. When they (Fink et al., 1967) compared the levels of histidinol phosphate phosphatase in His^C strains and their revertants, they found constitutive levels of the enzyme, indicating that the reversion was not due to a back mutation in the same gene which originally caused the constitutive phenotype.

I B-2.b Temperature Sensitivity at 44C

The Gos requirement for methionine at temperatures above 37C may be related to the fact that many non-wild type strains of E. coli and other Enterobacteriaceae need methionine at temperatures above 44C (Ron and Davis, 1971, Ron, 1975). At this temperature, they cannot grow in the absence of methionine. This condition is due to a defective metA gene product, homoserine transsuccinylase, which does not function well at such high temperatures. At lower temperatures, the enzyme may still be slightly deficient, but this deficiency does not prevent growth. However, this may not be the reason for the Gos mutants' temperature sensitivity because the Gos mutants cannot grow without methionine even at 37C.

I C Purine Metabolism

The possibility that purine metabolism is altered in Gos mutants is suggested by a number of observations. The

gos mutations map around purH and purine metabolism is elaborately intertwined with histidine metabolism. Moreover, in the work reported here, filamentation of Gos strains was increased by adenine (in some experiments). Therefore, purine biosynthesis, its regulation and its relation to histidine biosynthesis are described below.

I C-1 Purine Biosynthesis and Regulation

Purines are synthesized by the purine de novo biosynthetic pathway and are recycled by the salvage pathway. The following description of purine metabolism is reviewed from Neuhard and Nygaard (1987).

I C-1.a The Purine de novo Pathway

In the cell, IMP (inosine monophosphate) is made from PRPP (phosphoribosyl pyrophosphate) by a series of enzymes, encoded by the pur genes. ATP and GTP are then made in divergent pathways from IMP (Figure 4). The part of the de novo pathway that may be affected by L-serine and/or histidine metabolism is in the last steps of IMP production.

AICAR (5'-phosphoribosyl-4-carboxamide-5-aminoimidazole), a byproduct of histidine biosynthesis, is also an intermediate of the purine de novo pathway. To make IMP, AICAR is converted to FAICAR (5'-phosphoribosyl-4-carboxamide-5-formylamidoimidazole) by the purH gene product, and N^{10}-formyl tetrahydrofolate (THF) is the single carbon (C_1) donor for the reaction. FAICAR is subsequently

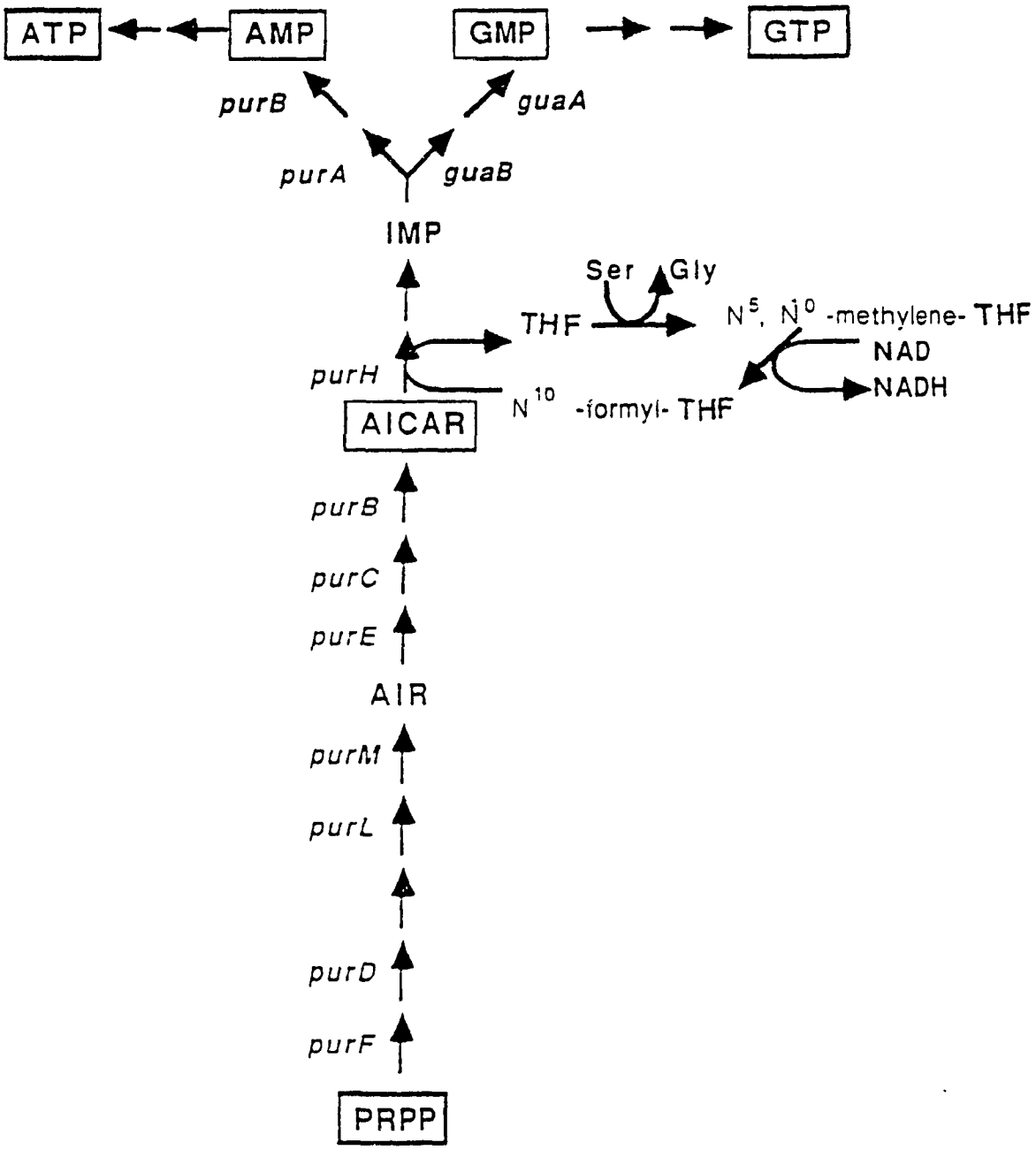

Figure 4. The Purine de novo Pathway

AMP and GMP are synthesized from PRPP (phosphoribosyl pyrophosphate) and AICAR (5'-phosphoribosyl-4-carboxamide-5-aminoimidazole) by pur and gua enzymes. Also indicated is the use of C_1-THF (tetrahydrofolate) by the purH gene product.

converted to IMP by the purJ product in the next step of purine biosynthesis.

I C-1.b Regulation of the de novo Pathway

PRPP amidotransferase, the first enzyme of the de novo pathway, regulates the production of purines. This enzyme is subject to feedback inhibition by AMP and GMP. If this step is inhibited, then purines are not made de novo.

Purine levels in the cell also control transcription of the pur genes. High purine concentrations lead to a repression of the genes. Strains of E. coli and S. typhimurium exist which are derepressed for purine biosynthesis. They are mutant at the purR locus and affect at least eight of the ten IMP-producing enzymes.

I C-1.c The Purine Salvage Pathway

In addition to being produced from the de novo pathway, ATP and GTP can be made from each other. ATP can be converted to GTP if it is first converted to AICAR by the histidine pathway. It is then fed into the purine pathway and made into IMP and thence into GTP. This can only happen if the histidine pathway is not inhibited. If histidine biosynthesis is inhibited, then insufficient AICAR would be made and hence, not enough IMP could be made to produce GTP. Alternatively, ATP can be changed to AMP by adenine phosphoribosyltransferase which can also be converted to IMP.

GTP can also be changed into ATP. This is dictated by the levels of GTP and ATP in the cell. GMP is converted to IMP (which can be made into ATP) by the enzyme GMP reductase. The reaction is inhibited by ATP and is disinhibited by GTP. Therefore, if the cellular concentration of ATP is higher than the concentration of GTP, the cell will not make more ATP.

I C-2 The Roles of THF and L-Serine in Purine Biosynthesis

N^{10}-formyl THF is required for two steps in the de novo pathway: the formation of FAICAR, the direct precursor of IMP and the formation of FGAR (5'-phosphoribosyl-N-formylglycinamide), an earlier intermediate in the pathway. Because of this need of formyl-THF for the biosynthesis of purines, a shortage of C_1 elements in the cell may restrict the synthesis of IMP (Hartman, 1970). This could occur because THF is also required for other pathways in the cell, including methylation of homocysteine to make methionine.

Since L-serine is a methyl donor for THF, the concentration of L-serine could also affect purine metabolism. If L-serine in the cell is exhausted by deamination to pyruvate, the supply of C_1-THF may not be sufficient to support reactions that use C_1-THF. Consequently, this would lead to perturbations in both methionine and purine biosynthesis.

In fact, alterations in the amount of THF affect cellular processes. Insufficient C_1-THF is known to lead to

an accumulation of ZTP in S. typhimurium (Bochner and Ames, 1982). ZTP is an alarmone, signifying a metabolic imbalance to which the cell must adjust itself. If ZTP is an alarmone for THF metabolism, then it may also have an effect on the regulation of C_1 units from L-serine (Stauffer, 1987).

I C-3 The Relationship between Histidine and Purine Biosynthesis

There is a close connection between purine production and histidine biosynthesis in E. coli. PRPP is a precursor of both purines and histidine. ATP, used for histidine biosynthesis, is regenerated by the infusion of AICAR from the histidine pathway into the purine pathway. Also, as mentioned above, histidine biosynthesis is subject to inhibition by AMP and ADP.

Because of this interconnection, regulation of each of these pathways affects the other. For example, if AICAR is not recycled to ATP because of a derepression of the his enzymes, the ATP pool will diminish, leading to a requirement for adenine at 42C (Johnston and Roth, 1979). This can be alleviated with the advent of a hisG mutation, blocking the first step in histidine biosynthesis. Histidine would no longer be overproduced and adenine would not be required by the cell. It follows that a shortage of adenine will inhibit production of histidine, because ATP is a precursor of histidine.

I D Cell Division and Filamentation

Division mutants (*fts*) in *E. coli* are temperature sensitive strains which grow normally at a permissive temperature but are unable to divide and therefore form filaments at a restrictive temperature. Mutants such as Gos and His^c form long filaments at 37C, meaning that they also cannot divide at this temperature. Because the true nature of the Gos mutants is unknown, and because their division is inhibited at 37C, it is possible that the product of *gos* may be involved in division. In addition, one division mutant at an unidentified locus, *fts*"ASH124" (Holland and Darby, 1976), maps very close to *gos*. Cell division is discussed here from a review by Donachie and Robinson (1987), except where otherwise noted.

I D-1 Normal Cell Division

E. coli cells grow exponentially until they reach double their original length and volume, at which time they divide into two daughter cells. Once the cell reaches its dividing size, its DNA has been replicated and the two chromosomes have separated and the cell is both ready and committed to undergo division. Cell division is coordinated with cell growth, protein synthesis, DNA replication and chromosome separation.

In the first step of cell division, a septum, made of a double layer of new cell wall material, is formed in the

centre of the cell, at a specific division site. Next, the outer membrane grows and invaginates, separating the two septal layers. Finally, two daughter cells, each identical to the mother cell's original size and shape, are produced.

I D-2 The Division Genes

Division mutations are classified into three types according to their time of action. The division gene products are involved in the initiation of septation, in septum formation itself or in cell separation. There are also genes whose products control cell growth and chromosome separation, but these are not called division mutants per se because their time of action precedes actual division.

I D-2.a Initiation of Septation

The first class of division mutants, those which are involved in the initiation of septation, carry on dividing for about twenty minutes after they are shifted to the restrictive temperature. This is because they are mutant only at the beginning of division. If septation has already begun, then they can complete a round of division. Strain TOE12 and those strains mutant at ftsH have this phenotype.

I D-2.b Septum Formation

Mutants which stop dividing immediately after the shift to restrictive temperature cannot form septa. These mutations are at the ftsA, ftsD, ftsE, ftsI, ftsQ and ftsZ

loci and in the fts"ASH124" strain. All of these mutants, except ftsA and ftsI, are characterized by making long filaments at the restrictive temperature and by being able to resume division after they are returned to the permissive temperature.

Strains mutant at ftsA also form filaments, but in these strains, the very beginnings of septa are visible as constrictions in the filaments at regular intervals. In ftsI strains, only a single constriction is visible and that is in the middle of the filament. The ftsG gene may also be a division gene, but cells mutant at this locus cannot grow longer at the restrictive temperature and will not resume division upon the shift back to the permissive temperature.

There are at least two stages in septum formation, as determined by resistance to penicillin. Penicillin attacks the cell as new cell wall material is being laid down at the septal sites. Strains mutant in ftsA, ftsD, ftsG, ftsQ and ftsZ are resistant to penicillin at the restrictive temperature. These cells stop dividing before the time of action of penicillin. The products of the ftsI, ftsC or ftsE genes all take effect after the point at which penicillin attacks the cell. Strains mutant at these loci are sensitive to penicillin.

I D-2.c Cell Separation

The third class of division mutants encompasses the envA and cha mutants. The products of these genes are

responsible for increasing the surface area of the cell membrane so that it can grow around and between the newly made septa.

I D-2.d Order of Action

Once the cell has reached double its size and has replicated its DNA, septa form at potential division sites. The first step in septum formation is mediated by the ftsZ gene product. After the initial constriction, the next steps are controlled by ftsA, ftsD and ftsQ. It also is possible that the ftsG gene (which may not be a division mutant per se) is involved at this point.

Cells that have reached this stage of division have the potential to become sensitive to penicillin. The proteins produced from ftsE and ftsI operate after the point of potential sensitivity has been reached. The final stages, before the separation of the two daughter cells, are controlled by the envA and the minB genes which cleave the cell at the septum and inactivate the old potential septal sites, respectively.

I D-3 SOS Response

Cells under stress induced by DNA damage also form filaments. DNA damage triggers the SOS response and cell division is inhibited. The cells do not resume division until the damage has been repaired and the SOS system is returned to its repressed state.

I D-3.a Activating SOS

When the cell is not under stress, a repressor protein, encoded by lexA, blocks transcription of a number of operons which are not expressed unless DNA damage is incurred. After there is damage, an effector molecule, which may be single stranded DNA, resulting from DNA damage, activates the recA protein (D'Ari, 1985). The recA protein attains a proteolytic function and it cleaves the lexA protein so that it can no longer repress the SOS operons. Transcription of the SOS genes follows and continues until the DNA damage is repaired.

The cell returns to its normal state when the recA protein loses its ability to break down the lexA protein, possibly because the effector molecule has been broken down (D'Ari, 1985). More lexA protein is made and repression of the SOS genes is resumed. This may be controlled by the lon gene because mutations at that locus result in a delayed return to normal cell growth after the end of SOS. Heat shock also induces Lon, so Lon could control filamentation under both heat shock and SOS response (Walker, 1987).

I D-3.b Division and the SOS Response

One of the cellular mechanisms under the control of SOS is division, which it inhibits. A mutation in either lexA (tsl) or in recA (tif) will lead to Fts-like phenotypes. That is, cells mutant at these loci will form long, multinucleate filaments at 42C (Huisman and D'Ari, 1981).

However, if either of these mutants have an additional mutation in sfiA/sulA (21 minutes) or in sfiB/sulB (2 minutes, at the ftsZ locus), division will not be affected by the SOS system.

The sfiA gene, under repression by the lexA protein, produces its protein in large quantities when DNA replication is disturbed (Huisman and D'Ari, 1981). The sfiA gene product inhibits or inactivates the ftsZ protein such that septa cannot form. A strain mutant at sfiA does not affect cell division under normal circumstances, when DNA replication is not perturbed (Jaffe et al., 1986). However, it may not be able to inhibit division during the SOS response. When the ftsZ gene has the sulB allele, it becomes resistant to attack by the sfiA product and division is possible during the SOS response.

Division inhibition is also coupled to the SOS response by a protein, encoded by the sfiC gene. The sfiA and sfiC gene products are similar in that they both lead to a rapid constraint of cell division in recA(tif) mutants at 42C. However, the two genes map separately and sfiC is not under direct control of the lexA repressor (D'Ari and Huisman, 1983).

The return to regular growth after DNA repair may be regulated by the lon protein, which has protease activity. This protein may break down the sfiA protein, to rid the cell of it. This could explain why mutations in lon lead to a delayed return to normal growth.

II MATERIALS AND METHODS

II A Bacteria and Plasmids

II A-1 Strains

The bacterial strains used, all derivatives of *Escherichia coli* K-12, are listed in Table 1.

II A-2 Plasmid

The plasmid used was 4.5 kb-long pIP29, constructed by Belfaiza *et al.* (1986). It contains the *metC*$^+$ gene and its flanking regions from plasmid pLC4-14, from the Clarke and Carbon bank, cloned into pBR322.

II B Growth Media

II B-1 Minimal Medium

Liquid minimal medium and minimal medium plates contained 1.25% KH_2PO_4, 0.55% K_2PO_4, 0.2% $(NH_4)_2SO_4$, 0.02% $MgSO_4$, 0.001% $CaCl_2$. Isoleucine and valine, each at 50 µg/ml, were added routinely to the media because MEW1 (Δ*ilvA*) and all its derivatives require isoleucine for growth. The pH of the medium was adjusted to 7.0 with 10% NaOH. In the case of glucose- or L-serine-minimal medium, the carbon source was used at a concentration of 0.2%. SGL medium contained 0.2% L-serine, 650 µg/ml glycine and 150 µg/ml leucine. To make plates from minimal medium, 0.8%

Table 1. *E. coli* K-12 Strains Used

Strain	Relevant Genotype	Source/Reference
MEW1	*ilvA*, Δ*lac*	Newman
GOS3	MEW1, *gos-3*	Newman
GOS5	MEW1, *gos-5*	Newman
MEW22	MEW1, *sdaA*::Δ*plac* Mu9	Su *et al.*, 1989
NU610	*hisJ*::kan^r	Arps and Winkler, 1987a
MEW23	MEW1, *metJ*::cam^r	this study
CAG5051	HfrH, *nadA*::Tn*10* (17')	Singer *et al.*, 1989
CAG5052	KL227 *metB* *btu*::Tn*10* (89')	Singer *et al.*, 1989
CAG5052a	CAG5052 Met$^+$	This study
CAG5054	KL96 *trp*::Tn*10* (27')	Singer *et al.*, 1989
CAG5055	KL16 *zed*::Tn*10* (42')	Singer *et al.*, 1989
CAG8209	KL228 *rpoD*::Tn*10* (63')	Singer *et al.*, 1989
18431	*ilv*::Tn*10* (84.5')	Singer *et al.*, 1989
18477	*zij*::Tn*10* (88.5')	Singer *et al.*, 1989
18500	*thi*::Tn*10* (90.25')	Singer *et al.*, 1989
AB1932	*metA*28, *argH*, *thi-1*	Bachmann
AB2569	*metA*28, proA2, *his4*, *thi-1*, *arg*(E3 or H1)	Marliere
GT344	Gif 881-L, *metB*	Marliere
P4X	*metB*1	Bachmann
P4X8	*metB*	Marliere

Table 1. *E coli* K-12 Strains Used, Continued

Strain	Relevant Genotype	Source
GT346	Gif 881-L, *metC*	Marliere
AT2446	*metC*69, *thi-1*	Bachmann
CD4	*metB*1, *metD*88, *proA*3	Bachmann
AB2549	*metE*46	Bachmann
RC709	*metF*63, *proA*3	Bachmann
CS50	*metG*146, *thr-1*, Δ(*gpt-proA*), *leuB*6, *hisC*3, *thi-1*	Bachmann
RK4536	*metE*70, *metH*156, *leuB*6, *proC*32, *purE*42, *trpE*38, *lysA*23, *argH*1, *thi-1*	Bachmann
Gif 102	*metLM*, *lysC*1004, *thr*AM1005, *thi-1*	Bachmann
RG100	*metJ*97	Bachmann
JAHK9	*metJ*97, *trp-49*	Bachmann
TK4100	MC4100 *metJ*::cam^r	Greene
JJ127A	*metJ*am185, *metF*	Marliere
EWH110	*metK*110, *thi*	Bachmann
GW2586	PL8-31 *metK*86-Tn*5*	Greene
WA_5	JJ127A Met$^+$	this study
WB_2	JJ127A Met$^+$	this study
WC_3	JJ127A Met$^+$	this study
GA_5	JJ127A Met$^+$	this study
GB_2	JJ127A Met$^+$	this study
GC_3	JJ127A Met$^+$	this study

gelrite was dissolved in the liquid before autoclaving.

Except for the experiments testing for AT and TRA resistance, where supplements were added in concentrations suggested by Davis et al (1980), amino acids and vitamins were added in the following concentrations: arginine 50 µg/ml, glycine 100 µg/ml, histidine 20 µg/ml, leucine 20 µg/ml, lysine 50 µg/ml, methionine 20 µg/ml, proline 50 µg/ml, L-serine 100 µg/ml, threonine 50 µg/ml, tryptophan 30 µg/ml, nicotinamide 2 µg/ml, thiamine 1 µg/ml, B_{12} 2 µg/ml.

II B-2 Luria broth

Luria broth (LB) contained 1% bactotryptone, 0.5% yeast extract and 0.5% NaCl. To make plates, 1.8% agar was added to the medium before autoclaving.

II B-3 Antibiotics

Antibiotics were added to either minimal medium or LB in the following concentrations: ampicillin 50 µg/ml, kanamycin 80 µg/ml, streptomycin 100 µg/ml.

II C Isolation of Gos Mutants

A glucose-grown culture of MEW1 was washed, plated onto L-serine-minimal medium plus isoleucine and valine, and incubated at 28C. Single colony isolates that grew on these plates were called Gos strains and they were purified on the same plates.

II D Mapping

II D-1 Mapping by Conjugation

Initial mapping of *gos-3* and *gos-5* was carried out by conjugation with Hfr strains from Singer *et al*. (1989). The CAG5052 strain in the Singer kit carries a *metB* mutation. Because this interfered with mapping of the *gos* mutants, it was transduced to $metB^{+}$ with MEW1 as donor. The $metB^{+}$ strain, CAG5052a, was used in the conjugations. Recipient strains were grown in LB streptomycin and the donor strains were grown up in LB tetracycline media. One ml of the recipient culture was added to 0.2 ml of the donor culture, in a flask containing 7 ml of fresh LB medium. The flask was shaken at ca. 30 rpm at 37C for one hour, on a New Brunswick Scientific Co. gyratory water bath shaker model G76, after which 0.1 ml of the cells were plated onto LB plus tetracycline and streptomycin, and incubated at 37C, to select for exconjugants. Exconjugants were screened for growth on glucose-minimal medium without methionine at 42C.

II D-2 Mapping by Transduction

More accurate mapping was done by P_1-mediated transduction [according to the method described by Miller (1972)] with strains containing Tn*10* insertions at nearby loci, from Singer *et al*. (1989).

II E Testing for Growth

II E-1 Comparative Numbers of Colonies on Different Media

Each culture was grown in glucose-minimal medium, then washed twice and diluted to just visible (ca. 5 x 10^7 cells/ml) in minimal medium. The just visible suspension was further diluted 10^4 fold, in minimal medium, from which 0.1 ml (ca. 500 cells) was plated onto the series of plates.

II E-2 Use of Methionine Intermediates

Single colony isolates of each strain grown on glucose-minimal medium plates at 28C (Gos mutants) or with methionine at 37C (Met mutants) were streaked onto glucose-minimal medium plates containing the necessary growth supplements and one of the following intermediates of the methionine pathway: nothing, 0.5 µM homoserine, 0.5 µM O-succinyl-homoserine, 0.5 µM cystathionine, 0.5 µM homocysteine, or 20 µg/ml methionine. Plates were incubated at 37C or at 42C.

II F Transformation

Transformations were performed according to Maniatis et al. (1982).

II G Enzyme Assays

II G-1 L-Serine Deaminase

L-SD activity was measured in toluene treated glucose grown whole cells, described by Isenberg and Newman (1974). One unit of L-SD activity was taken to be the amount of enzyme that catalysed the formation of 1 nmol of pyruvate per 0.1 ml cells per 35 minutes.

II G-2 Cystathionine-ß-Lyase

II G-2.a Screening Assay

Strains were grown up in 2 ml LB. Whole cells were assayed as follows, previously described by Hunter et al. (1975). From each culture, 0.2 ml cells were centrifuged in a microfuge at high speed for 1 minute and resuspended in 0.1 ml of 20 mM Tris-HCl 1 mM EDTA pH 7.6. To the cells, 5 µl toluene (instead of lysozyme, a change suggested by R. C. Greene) was added and the cells were incubated at 37C for 20 minutes. Fifty µl of the toluene-treated cells were added to 0.45 ml of the cystathionase assay mix [1 part 0.6 M potassium phosphate, ph 7.4, 1 part 1.25 mM $MgSO_4$, 2 parts 5 mM L-cystathionine, 5 parts 2 mM 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB)]. Assay tubes were mixed and incubated at 37C for 20-30 minutes.

II G-2.b Quantitative Assay

Extracts were assayed according to Uren (1987), whereby

10-50 µl of enzyme-containing extract were added to 0.78 ml of 0.1 M Tris-HCl pH 9.0, 0.2 ml of 1 mM L-cystathionine in 0.01 N HCl (where the pH was increased to ca. pH 8), and 20 µl of 10 mM 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB) in 0.1 M potassium phosphate, pH 7.0, in a 1 ml cuvette. The cuvette was kept at 37C and the increase in absorbance at 412 nm was recorded over time. Protein concentrations of the extracts were determined by the method of Lowry et al. (1951). One cystathionine-ß-lyase unit represented 1 µmol of mercaptide produced per minute.

II G-3 Histidinol Dehydrogenase

II G-3.a Assay I

This assay (Martin et al., 1971) measured the amount of NAD reduction in the conversion of histidinol to histidine. Up to 20 µl of crude extract was added to 1 ml of 25C reaction mixture (equal volumes of 0.1 M NAD, 0.015 M L-histidinol-2HCl, 0.005 M $MnCl_2$ and 0.05 M Glycine, pH 9.4, adjusted with NaOH, diluted with 6 volumes of water) in a cuvette with a 1 cm light path. The increase in absorbance at 340 nm was measured over time, at 25C. A unit of histidinol dehydrogenase was the amount that converted 1 µmol of histidinol to histidine per minute at 25C.

II G-3.b Assay II

In this assay (Martin et al., 1971), the NAD reduced by conversion of histidinol to histidine was coupled to the

reaction between PMS (phenazine methosulfate) and INT (2-p-iodophenyl-3-p-nitrophenyl-5-phenyltetrazolium chloride, also called p-iodonitrotetrazolium violet), which produced a red INT-formazan. To between zero and 29 µl of extract dissolved in 0.2 M triethanolamine-HCl, pH 8.6, to a total volume of 0.29 ml, combined with 0.2 ml of dye mix (5 parts 3.2 mg/ml INT, 1 part 0.4 mg/ml PMS, 1 part 0.2% gelatin and 1 part 20 mg/ml NAD), 10 µl of either 0.1 M histidinol-HCl, ca. pH 8, or water (blank) was added. The reaction mixture was incubated for 20 minutes at 37C, and was stopped with the addition of 0.1 ml 0.67 N HCl to each tube.

To be able to read the absorbance of such a small volume of assay mix (0.5 ml), the tubes were diluted 1:1 in the triethanolamine buffer, and the optical density read was doubled. Readings were also corrected for the assay mixes without either histidinol or extract.

II H Inhibitor Sensitivity Tests

II H-1 Glutamyl-γ-methyl-ester

Single colony isolates were streaked onto glucose-minimal medium plates with zero, 0.5, 1.0, 1.4 and 3.0 mg/ml glutamyl-γ-methyl-ester. The plates were incubated at 28C. Growth on the plates indicated resistance to the concentration of glutamyl-γ-methyl-ester tested.

II H-2 3-amino-1,2,4-triazole (AT)

Single colony isolates were streaked onto glucose-minimal medium plates containing nineteen amino acids (no histidine), adenine and thiamine, suggested by Rudd et al., (1985) in concentrations listed in Davis, et al. (1980). Either AT alone (0.8, 25, 50, 75 mM) or 75 mM AT plus 15 µg/ml histidine was included in the media. Because methionine was included in the medium, the plates could be incubated at 37C.

II H-3 1,2,4-triazole-3-alanine (TRA)

Single colony isolates were streaked radially from a central disc to which 1.6 µmol TRA had been added, on plates containing nineteen amino acids (no histidine), adenine, thiamine and 0.8 mM AT. A plate which contained all of the above plus 15 µg/ml histidine and another plate that did not have either histidine or AT, but had TRA, were streaked in the same fashion. Strains were examined for growth after they were incubated at 37C.

II I Filamentation

II I-1 Experiment I

Cultures grown in liquid glucose-minimal medium with and without 40 µg/ml adenine were lightly subcultured into fresh media and shifted to 37C. These cells were examined by removal of a loopful of cells from each culture which were

dried on a microscope slide, heat fixed, stained with methylene blue and observed under a microscope at 1250X magnification.

II I-2 Experiment II

The cells were cultured as in Experiment I but additional cultures were inoculated in which cells were grown in 2% (high) glucose. Cells were subcultured at a higher concentration than they were in Experiment I.

II I-3 Experiment III

This experiment differed from Experiment I in that cultures were grown in glucose-minimal medium with 40 µg/ml adenine, with 20 µg/ml methionine, with both or with neither. Subcultures were allowed to grow for 1.5 hours at 28C and they were shifted to 42C instead of 37C.

II J Selection of Revertants

II J-1 Gos Revertants

Single colony isolates of Gos cells were grown in glucose-minimal medium at 28C and were plated onto glucose-minimal medium with 20 µg/ml histidine. Plates were incubated at 42C. Single colonies that grew up were considered to be heat resistant revertants of gos. Independent isolates were purified on the same medium at 42C and were screened for growth on L-serine-minimal medium

plates at 28C and at 37C, and on glucose-minimal medium plates without histidine at 42C.

II J-2 JJ127A Revertants

Single colony isolates were grown up at 37C in either glucose-minimal medium with 20 µg/ml methionine or in LB. They were washed twice in minimal medium and then plated onto glucose-minimal medium plates and incubated at 37C. Colonies that formed were considered to be <u>metF</u>$^{+}$ revertants.

III RESULTS

Wild type E. coli K-12 cannot use L-serine as its sole carbon source though mutants exist which are able to grow on L-serine (Newman et al., 1981). The most studied of these, the ssd mutants, have high levels of the enzyme L-serine deaminase (L-SD), an enzyme which converts L-serine to pyruvate. A new class of mutants, the Gos mutants (where Gos stands for growth on L-serine), has been isolated. These mutants are unusual because they grew on L-serine even though they showed no increase in L-SD activity.

Gos mutants have some other interesting properties. They require methionine for growth at 37C. When they are incubated without methionine, they form long filaments. The gos gene product may be involved in methionine metabolism and/or cell division. Two of these mutants, gos-3 and gos-5, are characterized in this study.

III A Isolation of Gos Mutants

Mutants that can use L-serine as sole carbon source, such as the ssd mutants, were previously isolated by plating an overnight culture of cells onto 0.2% L-serine-minimal medium plates, and incubating the plates at 37C until colonies formed (Newman et al., 1982a). Gos mutants were isolated from strain MEW1 in the same way, but the plates were incubated at 28C.

III B Initial Characterization of Gos Mutants

III B-1 Temperature Dependent Methionine Requirement

The Gos mutants were tested for growth on 0.2% glucose-minimal media at 28C, at 37C and at 42C. GOS3 and GOS5 grew on glucose at 28C, but they did not grow at 37C or at 42C unless they were provided with 20 µg/ml of L-methionine in the growth medium. This methionine requirement was also seen when L-serine was used as the carbon source.

III B-2 Survival at High Temperatures

GOS3 and GOS5 were unable to grow either at 37C or at 42C in glucose-minimal medium without methionine, though they grew at 28C. These cells might be killed by incubation at the restrictive temperatures (37C or 42C).

To test if the cells were killed at high temperatures, the Gos strains were grown in glucose-minimal medium flasks at 28C. They were then diluted to approximately 5×10^3 cells/ml. From this suspension, 0.1 ml of cells were plated onto glucose-minimal medium plates and the plates were incubated at 28C, at 37C, and at 42C. After twenty-four hours, half of the plates at each of the two higher temperatures were transferred to 28C.

Since each set of plates originally had the same number of cells plated on them, then all the plates that were eventually incubated at 28C should have approximately the same number of colonies. In fact, all the plates incubated

at 28C and those which were transferred to 28C had about five hundred colonies on them. The plates that were left at the higher temperatures did not produce colonies. That is, GOS3 and GOS5 cells remain viable at high temperatures for at least 24 hours, but do not produce colonies even after several days. This may suggest that some Gos mutants are cell division mutants because they are also not killed by incubation at high temperature.

III B-3 L-Serine Deaminase Activity

What is most interesting about GOS3 and GOS5 is though they can use L-serine as their sole carbon source, they do not have high levels of L-serine deaminase activity (L-SD) (Table 2). To show this, L-SD activity was assayed in the Gos strains, and their common parent, MEW1, all grown in glucose-minimal medium. Since the Gos mutants could not grow at 37C without methionine, both MEW1 and the mutants were grown and assayed with methionine at 37C.

The L-SD activities of the Gos strains were very similar to that of their parent, MEW1, at both 28C and at 37C (Table 2). At 28C, both Gos strains had 24 units of L-SD activity, while MEW1 had 16 units. At 37C, when the cells were grown with methionine, the Gos mutants had even less activity than they had at 28C. This is much lower than the level (190 units) seen in an ssd mutant which also grows on L-serine (Newman et al., 1982a). It is therefore unlikely that a change in L-SD accounts for the ability of the Gos

Table 2. L-Serine Deaminase Activity of Gos Mutants

	L-SD Activity of Cells Grown at:	
	A	B
Strain	28C	37C
MEW1	16	13
GOS3	24	21
GOS5	24	7

Cells were grown in glucose-minimal media, with methionine (20 µg/ml) added to cultures in column B. L-SD activity is expressed in nmol pyruvate produced per 0.1 ml cells per 35 minutes at 37C. Cells were grown to mid-logarithmic phase, centrifuged, and resuspended at an optical density of 100 Klett units.

mutants to grow on L-serine.

III C Mapping of *gos-3* and *gos-5*

To see if the *gos-3* and the *gos-5* mutations were at the same locus and if the *gos* mutations lie in an already known locus, the mutations were located on the *E. coli* chromosome. Knowing the map position for these mutations enables one to determine if the growth on L-serine phenotype (Gos) is the result of a new allele of a previously characterized gene or a unique phenotype of an undescribed gene.

III C-1 Initial Mapping by Conjugation

The *gos-3* and *gos-5* mutations were first mapped using Hfr conjugation. This technique uses five different Hfr strains, each of which has an F factor in a different position and/or orientation in the genome (Singer et al, 1989): CAG5051, CAG5052a, CAG5054, CAG5055 and CAG8209. Each Hfr strain also has a Tn*10*, a transposable element conferring resistance to tetracycline, inserted at known sites 13 to 23 minutes from each of the F factor points of entry.

Streptomycin resistant isolates of GOS3 and GOS5 were used in the conjugation so that only exconjugants and not the Hfr donor or the Gos strains would be selected. With both streptomycin and tetracycline in the medium, only exconjugants would be able to grow. The tetracycline would

kill the Gos strains and the streptomycin would kill the Hfr strains.

Exconjugants selected at 28C, resistant to both streptomycin and tetracycline, were screened for growth on glucose-minimal medium without methionine at 42C. The only exconjugants that could grow at 42C on glucose-minimal medium would be those that received the piece of DNA encompassing the wild type *gos* gene from the Hfr donor strain. That is, only strains that were conjugated with an Hfr strain that had its tet^r gene (Tn*10*) around the area of the genome of the *gos* gene would be able to grow at 42C without methionine, provided that sufficient time was allotted for the *gos* gene to be transferred along with the Tn*10*.

Only one Hfr donor strain, CAG5052a which has a Tn*10* at eighty-nine minutes on the *E. coli* chromosome, conferred methionine independence on GOS3 and GOS5 (Table 3). Seventy-one percent of the tetracycline resistant exconjugants from the cross with GOS3 and eighty percent of those with GOS5 were able to grow at 42C without methionine. This suggests that the *gos* gene is located close to the Tn*10* element in *btuB*, i.e. near 89 minutes.

III C-2 Mapping of the Gos Mutants by Transduction with P_1 Phage

The *gos* mutations were more accurately mapped by P_1 phage mediated transduction. The six strains used as donors

Table 3. Location of *gos-3* and *gos-5* by Conjugation

Donor Strain (Hfr) A	F factor Point of Entry B	Tn*10* Position (Selected Marker) C	GOS3 Exconjugants		GOS5 Exconjugants	
			Total Tet^r Str^r D	% Methionine Independent E	Total Tet^r Str^r F	% Methionine Independent G
CAG5051	96'	17'	100	0	100	0
CAG5052a	2'	89'	500	71	500	80
CAG5054	45'	27'	36	0	100	0
CAG5055	61.5	42'	100	0	100	0
CAG8209	84'	65'	9	0	72	0

Streptomycin resistant isolates of GOS3 and GOS5 were conjugated with the CAG strains. The direction of transfer was always such that the Tn10 was transferred within 21 minutes from the point of entry on the *E. coli* chromosome. Exconjugants were selected by resistance to both streptomycin and tetracycline and were screened for growth without methionine at 42C. The number of antibiotic resistant exconjugants is given in columns D and F, and the per cent of those which did not require methionine is given in columns E and G.

for the transductions had Tn*10* inserts at known loci between 89.6 and 90.25 minutes (Singer *et al.*, 1989). P_1 phage grown on each of these six tet^r strains and was used to transduce the donor DNA to GOS3 and to GOS5 (Table 4).

Mapping with P_1 phage is more exact than mapping by conjugation because P_1 transfers only two minutes of the donor DNA. The map distances between *gos* and the Tn*10* insertions can be determined. For example, to find the map position of *gos-3*, the distance between *gos-3* and the Tn*10* insertion in strains 18477 and 18500 can be calculated as follows by $x = l\ (\ 1 - \sqrt[3]{f}\ )$, where x is the distance between the two genes (*gos-3* and Tn*10*), l is the length of the transducing segment (2 minutes) and f is the frequency of Met^+ transductants (Low, 1987). The distance between the Tn*10* insertion in strain 18477 (88.50 minutes) and *gos-3* was 1.3 minutes and the distance between the Tn*10* insertion in strain 18500 and *gos-3* was 0.3 minutes. The *gos-3* mutation therefore mapped between 89.8' and 89.9'. By using similar calculations, *gos-5* mapped between 89.6' and 90.1'. The *gos* mutations are most likely in the same gene, somewhere between 89.6 and 90.1 minutes.

III D Comparisons of GOS3 and GOS5 with Known Methionine Mutants

The *gos* mutants only require methionine for growth at high temperature. There are many mutations known which

Table 4. P_1 Phage-Mediated Transduction of GOS3 and GOS5

Donor Strain	Tn*10* Map Position	GOS3 Transductants		GOS5 Transductants	
		Total Tet^r	% Met Independent	Total Tet^r	% Met Independent
A	B	C	D	E	F
18431	84.50'	318	0	133	0
18477	88.50'	500	4	500	1
18500	90.25'	39	59	25	32

P_1 phage was grown on each of the donor strains. Tn*10* was transduced from the donor strains to the Gos mutants, selecting tetracycline resistance (Tet^r). Tet^r transductants were then screened for growth on glucose-minimal medium without methionine at 42C. The number of tet^r transductants is given in columns C and E, and the per cent methionine independent transductants is given in columns D and F. Linkage between the Tn*10* and the *gos* mutation was determined by the percentage of methionine independent tet^r transductants.

change the gene product only slightly, such that the enzyme can function at 28C, but is unstable at high temperatures. The temperature sensitive phenotype of GOS3 and GOS5 could result from this type of mutation, in a gene coding for one of the enzymes of methionine biosynthesis.

The fact that both gos mutations were mapped within a minute of the metJBLF cluster at 89 minutes suggests that gos might be an allele of one of those methionine genes. It also follows that if GOS3 and GOS5 were mutant in methionine biosynthesis, then some of the methionine mutants should have Gos characteristics. That is, some methionine mutants should be able to grow on L-serine.

III D-1 An Attempt to Identify GOS3 and GOS5 by Growth Requirements

To see if the temperature sensitive methionine requirement in GOS3 and GOS5 arose from a temperature sensitive mutation in one of the genes in the methionine biosynthetic pathway, GOS3 and GOS5 were tested for their ability to use the intermediates of the methionine pathway for growth.

Cells of GOS3 and GOS5 were streaked for single colonies on glucose-minimal medium plates at 28C. The ability of these colonies to grow on plates with the various intermediates of methionine biosynthesis, each at 0.5 μM, was tested. Mutants known to be deficient at various met loci were also tested, as was strain MEW1, in case any of

the intermediates might be toxic (Table 5).

MEW1 should be able to grow on all of the plates because it does not require methionine. Each of the methionine auxotrophs should not be able to grow without any supplement, and should only be able to use those the intermediates that come later in the pathway. For example, since metA catalyses the conversion of homoserine to O-succinyl-homoserine, a metA strain should be able to grow on O-succinyl-homoserine, on cystathionine, on homocysteine and on methionine, but not on homoserine. By comparing the Gos mutants to the methionine mutants, one should be able to determine at which step in the methionine pathway the Gos mutants were blocked.

Because the Gos mutants both grew on cystathionine and on homocysteine, but not on homoserine or on O-succinyl-homoserine, it would the metB gene in which they were mutant if they were mutant at any of the methionine loci.

Unfortunately, it was impossible to distinguish a metA strain from a metB or a metC by use of intermediates. All three types of mutants behaved as would be expected of metB mutants. That is, none of the strains grew on homoserine or on O-succinyl-homoserine, but all grew on cystathionine, homocysteine and methionine. The reason that the metA mutants did not grow on O-succinyl-homoserine could be because they had difficulty in the uptake of intermediates. It is unlikely that it was toxic to the cells as MEW1 was able to grow in the presence of it.

Table 5. Use of Methionine Intermediates by Methionine Mutants

Strain	No supplement	Homoserine	O-succinyl-homoserine	Cystathionine	Homocysteine	Methionine
MEW1	+	+	+	+	+	+
GOS3 (*gos-3*)	-	-	-	+	+	+
GOS5 (*gos-5*)	-	-	-	+	+	+
AB1932 (*metA*)	-	-	-	+	+	+
AB2569 (*metA*)	-	-	-	+	+	+
GT344 (*metB*)	-	-	-	+	+	+
P4X (*metB*)	-	-	-	+	+	+
P4X8 (*metB*)	-	-	-	+	+	+
GT346 (*metC*)	-	-	-	+	+	+
AT2446 (*metC*)	-	-	-	+	+	+
CD4 (*metB,D*)	-	-	-	+	+	+
AB2549 (*metE*)	-	-	-	-	-	+
RC709 (*metF*)	-	-	-	-	-	+
C350 (*metG*)	-	-	-	-	-	+
RK4536 (*metH*)	-	-	-	-	-	+
Gif102 (*metLM*)	-	-	-	+	-	+
RG100 (*metJ*)	+	+	+	+	+	+
JAHK9 (*metJ*)	+	+	+	+	+	+
JJ127A (*metF,J*)	-	-	-	-	-	+
EWH110 (*metK*)	+	+	+	+	+	+

Single colony isolates of each strain were streaked onto glucose-minimal medium plates, containing the necessary growth supplements and one of the following: methionine (20 µg/ml); homocysteine, cystathionine, O-succinyl-homoserine, homoserine (0.5 µM); or no methionine precursor. Strains were incubated at 37C, except for MEW1, GOS3 and GOS5, which were incubated at 42C. + indicates growth and - indicates no growth after seven days.

The metC mutants could have grown on cystathionine either because the cystathionine was broken down into a useable form for the cells or because the cells were not really mutant at the metC locus.

This method therefore could not be used to identify GOS3 and GOS5. These difficulties have been encountered by other investigators (R. C. Greene, personal communication). In an experiment described by Flavin (1975), O-succinyl-homoserine did not support growth of S. typhimurium because it could not enter the cell.

III D-2 Use of L-serine

Because the Gos mutants behaved somewhat like methionine auxotrophs in that they had a requirement for methionine, it seemed possible that some methionine mutants could grow on L-serine. The methionine mutants were tested to see if any of them resembled Gos mutants. For this, the strains' abilities to grow on L-serine were tested and their levels of L-SD were measured.

III D-2.a Growth on L-serine

To test for growth on L-serine by methionine mutants, a variety of methionine mutants collected from various investigators (Table 6) were tested to see if they could use L-serine as sole carbon source.

Of all the methionine mutants tested for growth on L-serine-minimal medium plus methionine at 37C, none of the

Table 6. L-Serine Deaminase Activity in Methionine Mutants

Strain	L-SD Activity	Growth on L-Serine
MEW1	29	-
AB1932 (*metA*)	51[R]	-
GT344 (*metA*)	73[R]	-
P4X (*metB*)	48[R]	-
P4X8 (*metB*)	26[R]	-
AT2446 (*metC*)	27[R]	-
RC709 (*metF*)	40[R]	-
JJ127A (*metF*,*metJ*)	22[R]	+
JAHK9 (*metJ*)	36	+
TK4100(*metJ*)	16	+
MEW23 (*metJ*)	20	+
GW2586 (*metK*)	36	+

L-SD activity is expressed in nmol pyruvate, as in Table 2. [R]methionine (20 µg/ml) was added to the growth media. Single colonies were used both for the L-SD assays and streaking on L-serine plates, though the same single colonies were not necessarily used for both experiments.

strains that required methionine (except JJ127A, see below) were able to grow on L-serine. Even the strains that had the same growth patterns as the Gos mutants (Table 5) were unable to use L-serine as sole carbon source. Only JAHK9 (metJ), TK4100 (metJ) and MEW23 (metJ), JJ127A (metJ metF) and GW2586 (metK) were able to use L-serine as their sole carbon source. Of these, only JJ127A required methionine for growth, because of its metF mutation. While all of these strains tested had different backgrounds, all of the strains that were able to use L-serine had mutations in genes which regulate methionine biosynthesis. It therefore seemed that the deregulation of the methionine pathway resulted in the strain's ability to grow on L-serine.

Since one of the regulatory genes, metJ, is located in the metJBLF cluster near gos, it seemed possible that GOS3 and/or GOS5 might carry a mutation in metJ but metJ does not require methionine. The Gos mutants cannot be mutated in metK since metK maps at 64 minutes (Bachmann, 1987).

III D-2.b L-Serine Deaminase Activity

In addition to testing to see if the methionine mutants were able to grow on L-serine they were also assayed for their levels of L-SD activity. To have the same characteristics as the Gos mutants, these strains would have to be able grow on L-serine and not have high levels of L-SD activity (Table 6).

All of the methionine mutants that were tested,

including the regulatory mutants, had normal levels of L-SD activity. This is consistent with the possibility that the Gos mutants are metJ mutants.

D-3 Comparison of GOS3 and GOS5 with metJ Mutants

The metJ and metK regulatory mutants are derepressed for the methionine biosynthetic enzymes. All methionine gene products under the control of metJ are overproduced in metJ mutants. One particularly easy enzyme to measure is cystathionine-β-lyase, the metC product. Measurement of this enzyme will indicate if any strain, or the Gos mutants in particular, is mutant in the regulation of methionine biosynthesis.

Another characteristic of metJ and metK mutants is their resistance to glutamyl-γ-methyl-ester (Kraus et al, 1979). If GOS3 and GOS5 have mutations in the metJ gene, they, too, should be resistant to this compound.

III D-3.a Cystathionine-β-Lyase Activity

Cystathionine-β-lyase can be measured in more than one way. A quantitative assay on cell extracts compares the enzyme activities of different strains over time. A much quicker whole cell screening assay that has a visible colour change can also be used to determine, within half an hour, whether a strain is derepressed for the enzyme.

The quick screening assay was used routinely, but the quantitative assay was also used to be sure of the

characterization of the Gos mutants.

III D-3.a.1) β-Cystathionase Screening Assay

The cystathionine-β-lyase screening assay (Hunter *et al.*, 1975) measures the activity of the enzyme produced by the *metC* gene. Since this is done with cells grown overnight in rich media, even strains that require methionine can be screened in this way. The cells were grown up in LB because a wild type strain is repressed for cystathionine-β-lyase and a *metJ* strain is derepressed in rich media. Under these growth conditions, a regulatory mutant would be clearly different from one in which methionine biosynthesis is normally controlled.

The amount of activity that a strain has is determined visually by the colour change in the assay mixture. A wi d type repressed strain, like MEW1, or a mutant *metC* strain will have low activity (colourless), while a derepressed strain, such as *metJ*, will have high activity (vivid yellow).

Overnight cultures of strains grown in LB at 37C were centrifuged and assayed according to Hunter *et al.* (1975), at 37C. All of the *metJ* strains that were tested turned bright yellow within thirty minutes, while neither MEW1 nor AT2446 (*metC*) changed colour. The Gos mutants were as colourless as MEW1 and AT2446. This is a good indication that they are not mutant in *metJ* or in the regulation of methionine biosynthesis.

III D-3.a.2) A Quantitative Assay of Cystathionine-β-Lyase

To further ensure that GOS3 and GOS5 were not metJ mutants, a quantitative cystathionine-β-lyase assay was done (Uren, 1987). The amount of mercaptide produced per minute by cystathionine-β-lyase was measured in crude extracts of GOS3, GOS5, MEW1, MEW23 and TK4100 (Table 7).

MEW1, GOS3 and GOS5, showed cystathionine-β-lyase activity between 4.7 and 7.3 nmol/min/mg protein. There was very little difference in enzyme activity between these three strains. The metJ strains, on the other hand, had thirty to one hundred fold more activity than the Gos strains or their parent.

III D-3.b Resistance to Glutamyl-γ-Methyl-Ester

Glutamyl-γ-methyl-ester resistance must be tested in the absence of methionine because methionine reverses its inhibitory effects. GOS3 and GOS5; their parent, MEW1; and two metJ strains, TK4100 and MEW23 (the latter isogenic with GOS3, GOS5 and MEW1, except for the metJ mutation), were purified on glucose-minimal medium. All five strains were then tested for growth at 28C, on glucose plates which contained between zero and 3.0 mg/ml glutamyl-γ-methyl-ester (Table 8).

GOS3 and GOS5 were sensitive to glutamyl-γ-methyl-ester, while MEW1, metJ and metK mutants were resistant to it, albeit to different degrees. With only 0.5 mg/ml of glutamyl-γ-methyl-ester, the Gos mutants grew very poorly

Table 7. Cystathionine-β-Lyase Activity

Strain	Activity[a]
MEW1	4.7
GOS3	5.5
GOS5	7.3
TK4100	470
MEW23	210

[a]Units of activity are expressed in nmoles of mercaptide produced per minute per milligram of protein of a crude extract incubated with cystathionine and corrected for activity without added substrate.

Table 8. Growth on Glutamyl-γ-Methyl-Ester

Strain	Glutamyl-γ-Methyl-Ester (Concentration in mg/ml) 0	0.5	1.0	1.4	3.0
GOS3	+	+/-	+/-	+/-	-
GOS5	+	+/-	-	-	-
MEW1	+	+	+	+	+
TK4100 metJ	+	+	+	+	+
MEW23 metJ	+	+	+	+	+
EWH110 metK	+	+	+	-	-

Single colony isolates, grown on glucose-minimal medium were streaked onto the same plates with glutamyl-γ-methyl-ester added at the concentrations indicated. Plates were incubated up to seven days at 28C.

+ = good growth
+/- = poor growth
- = no growth.

and they did not grow at all with 3.0 mg/ml. MEW1 and the two metJ mutant strains were resistant to at least 3.0 mg/ml glutamyl-γ-methyl-ester and the metK mutant was resistant to at least 1.0 mg/ml, but less than 1.4 mg/ml. Kraus et al (1979) reported that their metK mutant was resistant to up to 1.44 mg/ml.

MEW1 was more resistant to glutamyl-γ-methyl-ester than EWH110 was. This was surprising because MEW1 does not carry a mutation that effects resistance to it and EWH110 has a metK mutation, which does. Also, GOS3 and GOS5 were hypersensitive to glutamyl-γ-methyl-ester, when compared to their parent. The gos mutation gave rise to an increased sensitivity to glutamyl-γ-methyl-ester.

III D-4 The Methionine Pathway as a Possible Route of L-Serine Deamination

Even though neither GOS3 or GOS5 are like the metJ mutants looked at, they affect both L-serine and methionine metabolism. Perhaps L-serine is utilized by the cell through the actions of some enzyme(s) of the methionine pathway, such that some elements of the pathway act as a sort of L-serine deaminase, independent of the L-serine deaminase enzyme that already exists in the cell. This would be a secondary L-serine deaminase.

III D-4.a The Cystathionase Cycle

Cystathionine-β-lyase catalyses the conversion of

cystathionine to homocysteine, releasing pyruvate in the process (Figure 1). In mammalian cells, an enzyme, cystathionine-ß-synthase, operates in reverse, making cystathionine by condensing L-serine with homocysteine (Figure 2). These two enzymes are similar in mechanism and are flexible with respect to substrates handled.

Consequently, it is possible that the E. coli metC enzyme, cystathionine-ß-lyase, in its derepressed state, is responsible for the growth on L-serine. It is conceivable that at high concentration, the metC enzyme in the cell catalyses the reverse reaction in addition to its normal forward reaction. In this way, there would be a cystathionase/L-serine deaminase cycle (Figure 5).

The fact that only metJ and metK mutants can grow with L-serine (D-2.a) suggests that normally regulated strains do not have enough of the enzyme(s) necessary to deaminate L-serine in this way. For the cell to be able to use L-serine this way, the intracellular concentration of cystathionine-ß-lyase must be higher than it would be in a wild type cell. An increase in the enzyme is seen in metJ and metK mutants, but could also occur in a cell that overproduces just the metC enzyme.

To construct strains that overproduce only cystathionine-ß-lyase, the pIP29 plasmid (Belfaiza et al, 1986), carrying the wild type metC gene, was transformed into MEW1, into AT2446 (metC) and into MEW22 (sdaA). Because pIP29 is a multicopy plasmid, each cell would have many

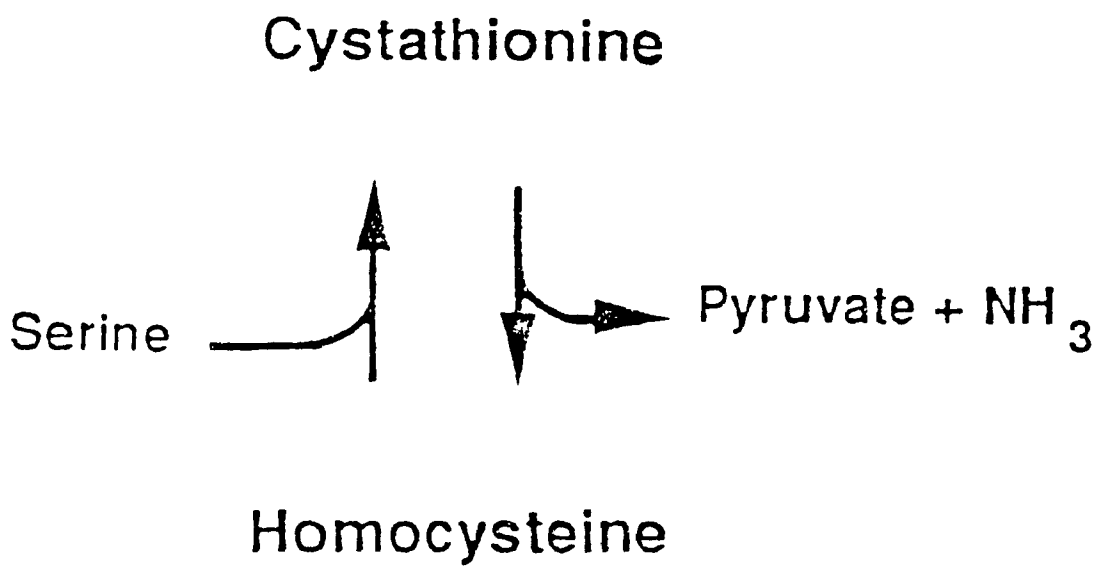

Figure 5. The L-Serine Deaminase Cycle

L-serine is converted to pyruvate via two reactions involving the interconversion of cystathionine and homocysteine.

copies of the metC gene. Thus, those strains might make more of the metC enzyme, and, in fact, they do.

The transformed cells were selected by resistance to ampicillin (due to amp^r carried on pIP29) and then were tested for their abilities to grow on L-serine plates and for their levels of cystathionine-ß-lyase using the screening method.

On L-serine plates, MEW1/pIP29 colonies grew well, but AT2446/pIP29 and MEW22/pIP29 grew poorly. All the transformants that were able to grow on L-serine had more cystathionine-ß-lyase activity than MEW1 but not as much as TK4100 (metJ), as judged by the degree of colour change in the screening assay.

Not all of the pIP29 transformants from any of the three transformation experiments grew equally well on L-serine. However, those that grew best also had the highest levels of cystathionine-ß-lyase activity.

This shows that growth on L-serine, in these strains, is related to cystathionine-ß-lyase activity. That is, the higher the levels of metC enzyme, the better able the cells to use L-serine as a carbon source. This is the case even for MEW22, the strain which may be mutant in the L-SD structural gene, that cannot use L-serine even when L-SD is induced by glycine and leucine. Therefore, growth on L-serine, in derepressed metC strains, is independent of L-SD activity.

III D-4.b L-Serine Deamination in the Absence of L-SD Activity

To see if an increase in the methionine enzymes can effect growth on L-serine in MEW22, MEW22/TK4100 (*sdaA*/*metJ*) was constructed. This strain would not have the usual L-SD activity (Su *et al.*, 1989), but would be derepressed for all of the methionine enzymes, including cystathionine-β-lyase. If the overproduction of β-cystathionase is sufficient to allow growth on L-serine, then this double mutant should be able to grow.

III D-4.b.1) Assays of the Double Mutant *sdaA metJ*

MEW22/TK4100 was constructed by transducing the *metJ*::cam^r element from TK4100 into MEW22. Cystathionine-β-lyase activity was screened in all of the transductants and L-SD activity was measured in some of them.

Out of eighteen chloramphenicol resistant (cam^r) transductants, fourteen had high cystathionine-β-lyase activity, three had moderate levels and only one was indistinguishable from MEW22. Of the eight strains tested for L-SD activity, all had low, but measurable levels of activity. That is, they had between 3 and 6 units of L-SD, with an average of 4.4 units. That is just slightly more activity than MEW22, which has 1 unit of L-SD activity (Su *et al.*, 1989). This confirms that at least fourteen of the transductants show the phenotype expected of an *sdaA metJ* strain.

III D-4.b.2) Growth on L-serine

Overnight cultures of the cam^r transductants, MEW22 (sdaA), TK4100 (metJ), KEC9 (ssd) and MEW1 grown in glucose-minimal medium at 37C were washed and diluted in minimal medium. Approximately 500 cells from each of the cultures were plated onto three sets of plates: 1) L-serine-minimal medium, 2) L-serine-minimal medium plus glycine and leucine (SGL), and 3) glucose-minimal medium. The plates were incubated at 37C.

The sdaA metJ transductants had a very curious growth pattern. After just two days at 37C, about 500 colonies of each of the transductant strains formed on glucose plates. After three days' incubation, there were about half that number of visible colonies on SGL. After five days, about 500 colonies grew up on L-serine plates, but no new colonies formed on the SGL plates.

That is, all the cells that were plated were able to grow on either glucose- or on L-serine-minimal medium, but only half of the cells plated were able to grow on SGL. These cam^r strains were therefore able to grow with L-serine as a carbon source, albeit with some difficulty. The addition of glycine and leucine inhibited growth on L-serine for half the population.

All other strains tested grew as expected from their genotypes. They did not show the unusual growth pattern on SGL that was seen in the sdaA metJ transductants. After three days' incubation, TK4100 and L9 each had about 500

colonies on L-serine, SGL and glucose plates. That is, both of these strains made the same number of colonies on all of the media tested. The MEW22 cells were only able to form colonies on glucose, even after the plates were left at 37C for five days (MEW22 cannot grow on L-serine, even in the presence of glycine and leucine [Su et al., 1989]).

It seems then that a metJ mutation can allow growth on L-serine even in an MEW22 background, devoid of L-SD activity. However, it is strange that the sdaA metJ strain did not grow with L-serine, glycine and leucine.

To further examine their ability to grow with L-serine, the sdaA metJ strains were tested for growth in liquid L-serine-minimal medium. If these strains can use L-serine as sole carbon source on plates, then they should also be able to grow in the same medium in liquid. However, they were unable to grow. This was also true of strains MEW23 and TK4100 (both have the same metJ mutation as the transductants) which were also tested for growth in L-serine liquid and the cells stopped growing after a short time, as if they required something in the medium that had been depleted. MEW23 and TK4100 grew better with the addition of 1 µg/ml thiamine and trace elements to the liquid medium, but the cultures were not as dense as when they were grown in liquid glucose-minimal medium. All agar media, including the gelrite used in these plates, are contaminated by a variety of compounds present in the agar in small amounts (especially vitamins and minerals) Perhaps these strains

grew on L-serine plates by using such a contaminant.

III D-4.b.3 Are the MEW22/TK4100 Strains Really sdaA?

Why the sdaA metJ cells could not grow consistently on SGL is a concern. It is possible that these strains gained a new requirement for a supplement that was not included in the media. Alternatively, it could have been that the cells plated had reverted to $sdaA^+$, enabling them to grow on SGL. This was possible because all the testing for growth on L-serine plates had been done without antibiotics in the media. The sdaA mutation was made with an insert that has a kan^r gene. If cells carrying this insert were plated without the antibiotic, then cells which lost the insert could grow just as well as strains which kept it.

To check if these strains had indeed lost their insertions and thus reverted to $SdaA^+$, two colonies from each of the L-serine and SGL test plates of four different sdaA metJ transductants were repurified on glucose-minimal medium plates. These sixteen strains were then assayed for L-SD activity in glucose-minimal medium. If these strains had reverted to $sdaA^+$, they would all have normal levels of L-SD under these assay conditions.

All of the strains had the low L-SD activity expected (between 2 and 5 units), which means that they were no different from the transductants that were assayed before they were tested for growth on L-serine and repurified. That is, none of these strains had lost their sdaA mutation. So.

it is still unclear why half of the transductants were unable to grow on SGL.

III E Comparisons with Histidine Constitutive Mutants

The gos mutants have attributes in common with histidine constitutive (His^C) mutants. His^C mutants overproduce the histidine biosynthetic enzymes because of a defect in the regulation of histidine biosynthesis. Both the Gos and the His^C cells require methionine for growth at high temperatures and they form long filaments in its absence (Murray and Hartman, 1972). Also, one of the His^C mutants, hisR, maps around 85 minutes (Bachmann, 1987), which is near gos. The His^C cells make filaments because of their inability to make septa and divide at high temperatures without methionine. If GOS3 and GOS5 are really His^C mutants, then they would have other characteristics that are common to those strains, such as having high levels of the histidine biosynthetic enzymes, and resistance to aminotriazole and to triazolealanine.

III E-1 Filamentation

Histidine constitutive strains form filaments in liquid glucose-minimal medium as well as on plates at 42C (Murray and Hartman, 1972). The formation of filaments is even more pronounced when adenine is included in the growth media, though it is not certain why this is the case. If the Gos

mutants also react to these growth conditions by filamenting, then it is possible that they fall into a class of His^c mutants.

III E-1.a Filamentation in Liquid Media

To see if the Gos mutants filament under the same circumstances as the His^c strains, GOS3 and GOS5 were grown in liquid glucose-minimal medium, at the permissive temperature (28C) and were shifted to 37C or to 42C. Samples of the cultures were examined under a microscope at intervals to see whether and when the cells began to form filaments.

III E-1.a.1) Experiment I: Effects of Adenine on Filamentation

GOS3 and GOS5 cultures were grown in liquid glucose-minimal medium with and without 40 µg/ml adenine. Once grown, they were very lightly subcultured to fresh media and shifted to 37C.

After three hours and after thirty-two hours, a loopful of cells from each culture was withdrawn and dried on a microscope slide. The cells were heat fixed on the slides, stained with methylene blue and examined under the microscope at 1250X magnification.

Very few cells were seen under the microscope after three hours of incubation and shaking at 37C. Normal cells would have been in their log phase of growth after that

amount of time and there would have been many cells. It was obvious that the Gos cells were not growing very well.

After thirty-two hours, cultures that did not have any adenine had formed some short filaments. Where adenine had been added, the filaments were very long. Some of the filaments were even as long as the whole microscope field, about 100 cell lengths. This result would be expected in His^c mutants (Murray and Hartman, 1972).

III E-1.a.2) Experiment II: Effects of High Glucose on Filamentation

Experiment I was repeated, but this time, the subcultures were more dense, and another condition was tested. Because S. typhimurium histidine constitutive strains form filaments in high glucose (Murray and Hartman, 1972), the Gos mutants were also tested for filamentation in 2% (high) glucose. As well, MEW1 was inoculated to see if wild type cells filament under these conditions.

After three hours of incubation and shaking at 37C, the Gos cells grown in glucose-minimal medium were about the same size as MEW1. With adenine added, GOS3 and GOS5 formed short filaments (8-10 MEW1 cell lengths). With glucose 2% they were double the size of MEW1 cells. Thus, GOS3 and GOS5 mutants did form filaments at 37C, especially in the presence of adenine.

III E-1.a.3) Experiment III: Measurement of Cell Lengths at 42C

In order to quantify the lengths of the GOS3 and the GOS5 filaments when the growing cells were shifted to higher temperature, the temperature shift experiment was repeated once again. The cells were compared to other Gos cells which were cultured with methionine so that filamenting cells could be compared to Gos cells growing at the same temperature instead of comparing them to MEW1. In this experiment, when the cells were subcultured, they were less heavily inoculated and allowed to grow for 1.5 hours at 28C before they were shifted 42C (Table 9).

Before the shift to 42C, the average length of GOS3 cells was 1.4 µm and GOS5 was 1.8 µm. Neither culture had made very long filaments. Some of the cells were about double their average lengths, but that takes into account cells which were on the verge of dividing. The cultures to which adenine and/or methionine was added also had cells of the same lengths, but all of these cells were not counted. The cultures were examined three hours after the temperature shift, but the exact lengths of the cells, again, was not measured. No filaments had yet begun to form and all the cells were the same lengths as they were at the time of the temperature shift. The only change observed was that the cultures to which methionine was added had begun to grow. That is, they were more dense than the cultures without methionine which looked the same as they did at the time of

Table 9. Length of Cells in Glucose-Minimal Media at 42C

Strain	Addition[a]	Average[b]	Length of Cells (% of population at µm) 0-1.5	1.5-2.5	2.5-3.5	>3.5
0 hours						
GOS3	-	1.4	59.1	40.9	0	0
GOS5	-	1.8	33.0	52.6	14.4	0
8 hours						
GOS3	-	2.8	0	37.5	44.6	17.9
GOS5	-	3.3	0	18.8	42.2	39.0
GOS3	ade	2.8	0	37.5	44.6	17.9
GOS5	ade	2.9	0	33.3	49.7	17.0
24 hours	-	3.0	0	30.4	42.3	27.3
GOS3	-	2.9	0	39.6	41.7	18.8
GOS5	ade	2.8	0	41.9	39.5	18.6
GOS3	ade	2.2	0	42.1	46.5	11.3
GOS5	met	1.5	50.0	48.6	1.4	0
GOS3	met	1.5	48.6	48.6	2.9	0
GOS5	ade met	2.2	5.9	68.6	23.4	2.1

Cells grown in glucose-minimal medium with and without adenine (40 µg/ml) and methionine (20 µg/ml) at 28C were lightly subcultured into fresh media and were left to grow for 1.5 hours. The flasks were transferred to 42C and the cell lengths were measured with a micrometer at 0 hours (the time of the shift), at 8 hours and at 24 hours.

[a] addition: ade = adenine 40 µg/ml, met = methionine 20 µg/ml

[b] Average length of the cells counted

- = no supplement was added

the temperature shift. No growth was observed and the cells had not elongated.

After eight hours at 42C, the cultures were examined again. The cells that were given methionine were growing and dividing, so the cell lengths were about the same as they initially were. Those cells without methionine, either with or without adenine, had all doubled in length. Also, the GOS5 cells had made some filaments that were up to six times their original length.

When the cells were examined after 24 hours at 42C, there was no increase in the average cell length in any of the cultures from what they were at eight hours. The only difference was that some of the filaments had become much longer. One GOS5 cell, grown without adenine, was 25 µm (15 cell lengths) long. None of the cells were as long as the filaments that were seen in Experiment I.

It is obvious that the Gos mutants form filaments at high temperatures if methionine is not added to the growth media. However, it is unclear if adenine enhances the formation of these filaments or what may be the best conditions for inducing long filaments.

III E-1.b Filamentation on Plates

S. typhimurium His^C colonies, grown at 37C without methionine, form wrinkled colonies, presumably due to filamentation. To see if the Gos mutants also filament when they are grown on plates, they were tested in the following

way. MEW1, GOS3 and GOS5 were streaked for single colonies and left to grow on six different plates: 0.2% glucose, 37C and 42C; 2% (high) glucose, 37C and 42C; 0.2% glucose with methionine, 42C; and 2% glucose with methionine, 42C. Representative colonies were suspended in minimal medium and were examined with a microscope.

No Gos colonies formed at 42C in the absence of methionine. With methionine, cells were of normal length. Colonies of GOS3 and GOS5, at 37C with and without adenine, were made up of short filaments. That is, Gos mutants can make filaments when they are grown on plates. This behaviour is very much like that of a histidine constitutive strain (Murray and Hartman, 1972).

III E-2 A Comparison of His^c and Gos Revertants

When His^c strains revert, they usually become His^- because a "reversion" is really a secondary mutation in one the genes in the histidine biosynthetic pathway, alleviating the histidine constitutive phenotype and resulting in a requirement for histidine (Fink et al., 1967 and Arps and Winkler, 1987b).

To see if GOS3 and GOS5 behaved similarly, heat resistant revertants of them were selected as follows. Single colonies of Gos mutants were grown in glucose-minimal medium at 28C. Five ml of cells from each culture were plated onto glucose-minimal medium plus histidine plates and incubated at 42C.

Colonies that formed at 42C were considered to be heat resistant revertants of gos. Three independent isolates of each of GOS3 and GOS5 revertants were purified on the same medium at 42C. They were then screened for growth on L-serine-minimal medium plates at 28C and at 37C, and on glucose-minimal medium plates without histidine at 42C.

None of the GOS3 and GOS5 revertants grew on L-serine, and they all grew on glucose-minimal medium plates at 42C without histidine. Because the revertants no longer were able to grow on L-serine and they could grow at 42C, they were probably true gos revertants. However, it is doubtful that the Gos mutants were His^c mutants because the revertants did not acquire a need for histidine.

III E-3 Resistance to Aminotriazole and to Triazolealanine

Another characteristic of histidine constitutive strains is that they are res tant to 1,2,4-triazole-3-alanine (TRA) and to 3-amino-1,2,4-triazole (AT) (Rudd et al., 1985). To find out if the Gos mutants were also resistant to these compounds, MEW1, NU610 (hisT), GOS3 and GOS5 were tested for their abilities to grow in the presence of these analogues.

III E-3.a Resistance to Aminotriazole

To test for growth on AT, single colony isolates of MEW1, NU610, GOS3 and GOS5 were tested on plates containing only AT (0.8, 25, 50 and 75 mM) and on a plate with a high

concentration of AT (75 mM) to which histidine 15 µg/ml also added. The plates used were glucose-minimal medium containing nineteen amino acids (except histidine, which counteracts the inhibition by AT), adenine and thiamine in concentrations listed in Davis et al. (1980). These are the conditions tested in other work on similar subjects (Rudd et al., 1985). Methionine was also in the medium, so the plates could be incubated at 37C (Table 10).

The plates were first examined for growth after three days, but the hisI strain was a very slow grower and had not formed colonies yet. It was only evident that NU610 was resistant to all concentrations of AT after a week at 37C. Thus, it was at this time that all of the strains were examined for their abilities to grow in the presence of AT.

GOS3, GOS5 and MEW1 grew well with either 0.8 mM or 25 mM AT, poorly with 50 mM AT, and barely at all with 75 mM AT. However, when 15 µg/ml histidine was included with 75 mM AT, all of the strains grew well. The Gos mutants behaved exactly as MEW1 and were at least as sensitive to AT as it was. They were not resistant to as high concentrations of AT as NU610 was, so it is unlikely that the Gos mutants are His^C.

III E-3.b Resistance to Triazolealanine

The Gos mutants were also analysed for their ability to grow in the presence of TRA. TRA prevents de-repression of the his operon in non-constitutive strains (Voll, 1967), so

Table 10. Growth on Aminotriazole

Strain	Concentration of Aminotriazole (mM)				
	0.8	25	50	75	Histidine and 75
GOS3	+	+	+/-	-	+
GOS5	+	+	+/-	-	+
MEW1	+	+	+/-	-	+
NU610	+	+	+	+	+

Single colony isolates were streaked onto glucose-minimal medium plates containing nineteen amino acids (no histidine), adenine and thiamine in concentrations suggested by Davis (1980). Histidine was added at 15 µg/ml. Plates were incubated at 37C for seven days.

+ = growth - = no growth +/- = poor growth

strains that are exposed to TRA will not be able to produce his enzymes, or make histidine. Thus, in the presence of TRA, strains that are not His^c mutants will be unable to grow without histidine.

To test for this, the plates used had nineteen amino acids (no histidine again), adenine, thiamine and AT 0.8 mM. GOS3, GOS5, MEW1 and NU610 were streaked radially on a plate from a central disc to which 1.6 µmol TRA had been added. Two plates, one which contained all of the above plus histidine and another plate that did not have either histidine or AT, but had TRA (to see its effects without AT), were streaked in the same way as the test plate. Strains were examined for growth after they were incubated at 37C.

Only NU610 was able to grow on the test plate. GOS3, GOS5 and MEW1 were all clearly inhibited by TRA. When histidine 15 µg/ml was added to the same medium, all of the strains grew. When TRA was tested alone it was not efficient in inhibiting the growth of the Gos strains, though it did inhibit MEW1.

Thus, TRA is an effective inhibitor of the Gos strains, provided that AT is present, even at as low a concentration as 0.8 mM. GOS3 and GOS5 are less sensitive to TRA than MEW1 is and they are less sensitive to TRA than they are to AT.

III E-4 Histidinol Dehydrogenase

To be absolutely sure that neither GOS3 nor GOS5 was

mutant in a histidine regulatory gene, their levels of histidinol dehydrogenase (the hisD gene product) were measured. In strains where the histidine biosynthetic pathway is derepressed, as in histidine constitutive strains, activity of this enzyme is very high. Two different histidinol dehydrogenase assays of crude extracts (Martin et al., 1971) were performed, and they are both outlined below.

III E-4.a Histidinol Dehydrogenase Assay I

In this histidinol dehydrogenase assay, crude extracts made from NU610, GOS3, GOS5 and MEW1 cells grown in liquid glucose-minimal medium, were monitored for the appearance of histidinol-dependent NADH over time (Table 11).

NU610 had 3 times more activity than GOS3, 8 times more than MEW1 and 28 times more than GOS5. From this experiment, it appeared that the two Gos strains were not the same and that GOS3 had 10 fold more histidinol dehydrogenase activity than GOS5. However, neither of the Gos strains had as much activity as NU610. The amount of activity in them was on the order of the activity exhibited by MEW1.

III E-4.b Histidinol Dehydrogenase Assay II

In this more sensitive assay, the same crude extracts that were made for the first assay were evaluated for their abilities to convert NADH to a coloured INT-formazan compound. The more NADH converted, the higher the activity and the more red colour was produced.

Table 11. Histidinol Dehydrogenase Activity I

Strain	Activity[a]
MEW1	1.4×10^{-2}
GOS3	3.6×10^{-2}
GOS5	0.4×10^{-2}
NU610	11.0×10^{-2}

[a]Units of activity are expressed as absorbance at 340 nm per minute per milligram protein at 25C for crude extracts grown in glucose-minimal medium at 28C. Assays were run in triplicate with and without substrate, and the value given in the table represents the difference between the two averages.

Table 12. Histidinol Dehydrogenase Activity II

Strain	Activity
MEW1	1.6
GOS3	1.1
GOS5	2.5
NU610	24.0

Extracts were made from cells grown in glucose-minimal medium at 28C. A blank was set up whereby optical density was measured in an assay mix that did not have any extract (enzyme) in it. In addition, assays were run with and without substrate. Units of activity were corrected so as not to include activity in assays that were run either without enzyme or without substrate. Activity is expressed as absorbance at 540 nm per mg protein in a twenty minute assay at 37C.

The level of histidinol dehydrogenase in NU610 was ten to twenty fold higher than that in MEW1, GOS3 and GOS5 (Table 12). This shows that the Gos mutants were like their parent in that they were not derepressed for histidine biosynthesis. Therefore, they are not histidine constitutive mutants.

III F Experiments with JJ127A

JJ127A (metJ metF) was able to use L-serine as its sole carbon source (D-2.a). Since this was the only metJ strain in the lab at the time of initial testing, it was not clear if the Gos phenotype of this strain was due to the metJ mutation, to the metF mutation, or to a combination of both mutations. Also, much of the testing that was done on the other metJ strains could not be performed on this strain because JJ127A requires methionine for growth. In order to compare JJ127A with other metJ strains, the metF mutation had to be removed.

III F-1 Attempt to Remove the metF Mutation from JJ127A

In order to determine if the growth on L-serine was due to metJ or metF, JJ127A was transduced by P_1 phage grown on MEW1, selecting for methionine independence on glucose-minimal medium plates. Even though metJ and metF map together in the same gene cluster, this might separate the two mutations such that a $metJ^-$ $metF^+$ strain would be

isolated. The *met* gene cluster is 5.6 to 6.0 kbp long, but *metJ* and *metF* are separated by *metB* and *metL* (Treat *et al.*, 1984). Very unexpected properties of the transductants were observed.

III F-2 Unexpected Phenotypic Diversity in Methionine Independent Transductants of JJ127A

After JJ127A/MEW1 transductants were selected and purified on glucose plates, L-serine deaminase activity was assayed. After two separate transduction experiments, some, but not all, of the transductants had very high levels of L-SD, even higher than the levels observed in *ssd* strains. Even if some of the transductants were *metJ*$^-$ *metF*$^+$ and others were *metJ*$^+$ *metF*$^+$, this was a surprising result. All the transductants had been selected in the same way and both MEW1 and JJ127A had normal levels of L-SD. It was interesting to find out from where so much L-SD activity arose.

The cultures that were assayed were also streaked onto glucose plates at the time of the assays. When these streaks grew, it was obvious that they were composed of mixed populations. Some of the colonies were grey and others were white.

The grey and the white colonies were purified until homogeneous and were assayed again for L-SD. Six strains (WA_5, GA_5, WB_2, GB_2, WC_3, GC_3) were surveyed. There were three grey and three white strains, where each grey-white pair had

been isolated from a single transductant. WA_5, WB_2 and WC_3, the white strains, had normal levels of L-SD while GA_5, GB_2 and GC_3, the grey strains, had the high levels of L-SD that were originally measured (Table 13).

III F-3 Characterization of the Two Populations

Because there was a difference between the L-SD activities of the grey and the white isolates, they were tested to see if they had different growth patterns. All six strains were grown overnight in liquid glucose-minimal medium at 37C. They were washed and diluted in minimal medium such that 5×10^2 cells were plated onto glucose, SGL and L-serine plates.

WA_5, WB_2 and WC_3 were able to form colonies in one day on LB, glucose or SGL plates and in three days on L-serine plates. GA_5, GB_2 and GC_3 were slower growing than the white isolates. Though they grew in one day on LB plates, they took two days to grow on glucose, four days to grow on SGL and five days to grow on L-serine plates. After five days, all the plates had about the same number of colonies on them, showing that all six strains were able to grow on minimal medium plates with either glucose or L-serine as carbon source (Table 13).

The same strains were also tested for growth in liquid L-serine-minimal medium. Overnight cultures of cells grown in glucose were subcultured into L-serine-minimal medium flasks. In liquid, GA_5, GB_2 and GC_3 grew after two days, but

Table 13. L-Serine Deaminase Activity and Growth in JJ127A Transductants

Strain	L-SD Activity	Days for cells to Grow in: Liquid Serine	Form Colonies on: Serine Plates	SGL Plates	Glucose Plates	LB Plates
WA_5	7	4	1	1	1	1
WB_2	11	4	1	1	1	1
WC_3	11	3	1	1	1	1
GA_5	90	2	5	4	2	1
GB_2	105	2	5	4	2	1
GC_3	105	2	5	4	2	1

L-SD activity is expressed in nmol pyruvate, as in Table 2. Growth is expressed in the number of days it took for all of the cells plated to form colonies on plates incubated at 37C.

WA_5, WB_2 and WC_3 took at least three days. The white strains were considered to be unable to grow in liquid L-serine-minimal medium because they took so long to grow. A wild type culture also grows in L-serine-minimal medium after three to five days because mutants able to use L-serine are selected (Newman et al., 1982a). Therefore, the white colonies are not the L-serine users, but the grey ones are.

The growth of the grey and the white strains in L-serine liquid conflicts with their growth on plates. The growth pattern in liquid showed that the grey strains grew on L-serine and the white ones did not grow on L-serine, while on plates they all grew on L-serine.

III F-4 Isolating Revertants

It was not known what was causing the high levels of L-SD activity in GA_5, GB_2 and GC_3. It is possible that something such as a suppressor was transduced to JJ127A from MEW1 along with the wild type metF gene. In order to determine if this was the case, Met^+ revertants were isolated from JJ127A to see if there would be the same effect on L-serine deaminase levels as there was in the transductants. If a suppressor had been transduced from MEW1, then the revertants would not have such high levels of L-SD activity because they would not have the suppressor.

To get revertants, JJ127A was grown in liquid glucose-minimal medium with methionine and then was washed in minimal medium and plated onto glucose plates without

methionine. Colonies that grew up were considered to be methionine independent revertants of metF.

Two types of revertants were isolated. One type of revertants formed normal colonies and was able to grow on L-serine. The other type was mucoid and did not grow on L-serine. Since none of the revertants showed high L-SD activity, it is unlikely that the high L-SD activity in the previous experiment was due to the selection of revertants on the transduction plates. Therefore, a gene was transduced from MEW1 to JJ127A which gave the transductants high L-serine deaminase activity, and strains MEW1 and JJ127A differ in more than the metJ and the metF loci studied in this transduction.

III F-5 Acquisition of an Isoleucine Requirement

JJ127A, unlike MEW1 which carries a deletion in ilvA, does not require isoleucine for growth. Routinely, isoleucine and valine are included in media in the lab because all the standard lab strains require isoleucine. The transductants and the revertants were isolated on plates that had 50 µg/ml each of isoleucine and valine. When they were cultured without isoleucine and valine, all of the JJ127A/MEW1 transductants as well as the JJ127A revertants could not grow without them. In the transductants, this could be explained if the piece of DNA from MEW1 that was transferred to JJ127A contained the ilvA deletion, though in fact, this is too far to be cotransduced. However, since

this phenotype manifested also in the revertants, it must be due to the reversion of the metF gene.

The transductants and the revertants were all tested to see if their requirement was indeed for isoleucine or for one of its precursors such as threonine, lysine, aspartate or homoserine. Some representative mucoid and normal revertants, the three white and the three grey strains were streaked onto a series of glucose plates, each containing one of the test compounds, nothing or isoleucine.

After two days at 37C, all the revertants and the transductants grew on plates that had been supplemented with either threonine or isoleucine. Therefore, the requirement picked up by these metF$^+$ strains was for threonine and was not due to the transfer of the ilvA deletion from MEW1.

III F-6 Cystathionine-β-Lyase Activity

The recipient strain in the transduction, JJ127A, carried both metJ and metF mutations. In order to grow without methionine, the transductants had to acquire metF$^+$, but did not necessarily also acquire metJ$^+$. To test whether one or both genes were transduced, the level of cystathionine-β-lyase in the JJ127A transductants was determined. All these strains (WA_5, WB_2, WC_3, GA_5, GB_2 and GC_3) had the low activity expected of wild type E. coli, not a metJ mutant. One would assume, therefore, that the transductants must have been metJ$^+$ metF$^+$.

However, the methionine independent revertant strains

of JJ127A were also assayed for β-cystathionase activity, and expressed it at wild type levels. They must therefore have been revertant both for metJ and metF. If this was the case, then one cannot be certain of the genotype of either the transductants or the revertants.

If the strains that had been isolated by transduction or reversion were not metJ, the question remains as to why they were able to grow on L-serine and from whence they attained such high levels of L-serine deaminase activity.

Because this was a particularly interesting problem, the transduction was repeated at least five times. However, these grey and white colonies were seen only after two consecutive transductions. It is not known why this is, but it will be studied further by other members of the lab.

IV DISCUSSION

Certain mutants of E. coli can use L-serine as sole carbon source without an increase in L-serine deaminase activity. These are: (1) Gos mutants, selected for their ability to grow on L-serine-minimal medium at 28C and (2) mutants with high levels of cystathionine-β-lyase, either due to metJ and metK mutants or due to an overproduction specified by a plasmid, pIP29. In this study it has been shown that the second group could be using methionine biosynthetic enzyme(s) to deaminate L-serine and though the Gos mutants have been studied in detail, their method of using L-serine is as yet undetermined.

Gos mutants are pleiotropic in that they affect L-serine and methionine metabolism and cell division. Though the exact nature of the Gos mutants was not elucidated, the fact that they exist showed that L-serine metabolism, methionine metabolism, cell division and possibly purine metabolism are interrelated.

L-serine and methionine metabolism are already known to be related since L-serine is necessary for methionine biosynthesis and because single carbon units for C_1-THF reactions originate from L-serine. The latter is required for both methionine and purine biosynthesis. A further connection between L-serine and methionine metabolism was revealed by the suggestion that methionine pathway enzymes can deaminate L-serine.

IV A Growth on L-Serine in Derepressed Methionine Mutants

Before now, the best understood strains that were known to be able to use L-serine as sole carbon source had high levels of L-serine deaminase activity. These are the *ssd* mutants and strains carrying many copies of the *sdaA* gene. In this work, other strains have been studied that do not have high levels of L-SD activity, but can nonetheless, grow on L-serine as sole carbon source. In these strains, methionine biosynthetic enzyme(s) convert(s) L-serine to pyruvate, which the cell uses as carbon source.

One type of mutant that deaminates L-serine via the methionine pathway is mutant in the regulation of methionine biosynthesis. Mutations in either the *metJ* or the *metK* genes give rise to strains that are derepressed for the methionine biosynthetic pathway. All the *metJ* and *metK* mutants that were tested grew on L-serine and had levels of L-SD activity that were comparable to the wild type levels.

When the *metJ* mutation was transduced into MEW1, making MEW23, it also was able to confer growth on L-serine without elevating the level of L-SD activity. Even when the *metJ* mutation was transduced into MEW22, which had no L-SD activity and could not grow on even SGL medium, *metJ* conferred growth on the *sdaA*/*metJ* transductants. A *metJ* mutation in *E. coli*, therefore enables the cell to grow on L-serine, even if it has no L-SD activity.

Whereas, MEW22 cannot grow at all on either L-serine or

on SGL, the MEW22/TK4100 (sdaA metJ) transductants were able to grow on L-serine plates and half of the population could grow on SGL. It is not understood why this occurred. Perhaps glycine and leucine had an inhibitory affect on some colonies, but it is not clear why some colonies were different from others since the metJ character was directly selected in the transduction. This may be a threshold problem in that the plates might contain small amounts of some compound which the double mutants require. This is supported by the fact that the strains grew much better on L-serine plates than in liquid medium.

IV B The Effects of pIP29 on MEW1 and MEW22

IV B-1 Growth on L-Serine with pIP29 Plasmid

To find out if the metC gene product, cystathionine-β-lyase, was responsible for growth on L-serine, the multicopy pIP29 plasmid, carrying a wild type copy of metC was transformed into E. coli. This should increase production of only cystathionine-β-lyase without overproducing the other methionine biosynthetic enzymes. When pIP29 was transformed into MEW1 it conferred the ability to grow on L-serine onto the transformant cells. The transformants that carried the plasmid (MEW1/pIP29) did not have high levels of L-SD activity. More striking than MEW1/pIP29's ability to use L-serine as sole carbon source was that of MEW22/pIP29 because MEW22 has only one unit of L-SD activity (Su et al., 1989),

compared to 29 units of L-SD activity in MEW1 (Table 6).

IV B-2 MEW22/pIP29 Grows Slowly on L-Serine

It is clear that increased cystathionine-ß-lyase activity is sufficient to allow growth on L-serine. However, MEW22/pIP29 grew notably slower than the metJ strains or an ssd mutant. This could be because the metJ strain had two alternatives in using L-serine and used both cystathionine-ß-lyase and L-SD to deaminate L-serine. It might also be that metJ strains use both the metC product, cystathionine-ß-lyase, and the metB product, cystathionine-γ-synthase. In that case, MEW22/pIP29 would then be limited to overproduced cystathionine-ß-lyase and one copy of cystathionine-γ-synthase, and might therefore grow more slowly. This could be tested by cloning metB alone and together with metC and putting them into MEW22.

IV B-3 Not All MEW1/pIP29 or MEW22/pIP29 Were the Same

MEW1 and MEW22 were transformed with pIP29 by selecting ampicillin resistant colonies on LB plates. The transformants were purified on the same medium and then on glucose-minimal medium. Single colonies were streaked onto L-serine and glucose-minimal media.

Since the strains with the most cystathionine-ß-lyase activity grew the best, it would appear that the amount of metC enzyme is directly proportional to the cell's ability to use L-serine as carbon source. It is most likely that

this enzyme is responsible for the cell's ability to grow on L-serine. The metB enzyme may also be required, but clearly, cells that have a lot of ß-cystathionase can grow on L-serine.

It is possible that overproduction of cystathionine-ß-lyase is not healthy for the cell, and strains with compensating mutations are selected during the purification. This might lead to variations in the ability of the strains to grow on L-serine.

One might think that pIP29 somehow caused overproduction of L-SD. However, transformants were assayed for both cystathionine-ß-lyase and L-SD activity. They had the high levels of cystathionine-ß-lyase expected, and usual wild type levels of L-SD in MEW1/pIP29 and low mutant levels of L-SD in MEW22/pIP29.

IV C Other Methionine Enzymes May Be Required for Growth on L-Serine

While it is clear that the metC gene product is involved in L-serine deamination, it is not clear whether it is the only methionine biosynthetic enzyme involved. This could be studied by making double mutants with metJ. Consider, for example, a strain which is metJ metB (unfortunately the hardest one to construct due the proximity of the two genes). This strain would overproduce all methionine biosynthetic enzymes except the metB product.

If the metB product is necessary for growth on L-serine, the metJ metB mutant would not grow. If more than one double mutant could grow on L-serine, then L-serine deamination could be alternately performed by more than one of enzymes of the methionine pathway. If all but one of the proposed double mutants grow on L-serine, metJ metC, for example, then it is possible that only cystathionine-ß-lyase is required to deaminate L-serine.

One might also wonder if flow of carbon through the methionine biosynthetic pathway is required for L-serine deamination, by synthesis of a precursor from homoserine. By testing the double mutants described above for growth on L-serine, one could ascertain whether a block in methionine biosynthesis would inhibit metJ cells from growing on L-serine.

Alternatively, one could test for growth of MEW1/pIP29 on L-serine in the presence of methionine. Methionine acts as a feedback inhibitor, so it would block its own synthesis. If MEW1/pIP29 uses only cystathionine-ß-lyase to deaminate L-serine and is not dependent on any intermediate of the methionine pathway, then it should be able to grow on L-serine. However, growth of MEW1/pIP29 on L-serine plus methionine would not be an absolute indication that cystathionine-ß-lyase deaminates L-serine independent of the other components of the methionine pathway. Feedback inhibition is not complete and sufficient precursor could be made, if necessary, for L-serine deamination.

IV D Possible Routes of L-Serine Degradation

IV D-1 L-Serine Deamination by the Transsulfuration Enzymes

Another possible way for L-serine deamination to take place would be if cystathionine-β-lyase was to bind L-serine and, on its own, deaminate it to pyruvate. This is feasible because all of the cystathionine synthase and lyase enzymes show a wide range of substrate specificity. In this way, cystathionine-β-lyase could substitute as a secondary L-SD.

IV D-2 Reverse Activity of the Transsulfuration Enzymes

IV D-2.a The L-Serine Deaminase Cycle with Overproduced Enzymes

Alternatively, one or more of the methionine enzymes could have the capacity to work in both the forward and reverse directions. That is, L-serine could condense with homocysteine to form cystathionine as it does in the mammalian liver and in fungi, catalysed by cystathionine-β-synthase. This is the reverse function of β-cystathionase in *E. coli*. In the forward reaction, cystathionine would be cleaved to form homocysteine again, and the products of the reaction would be pyruvate and ammonia. When the enzymes are overproduced, enough substrate (L-serine and cystathionine) could be converted back and forth, thus creating an L-serine deaminase cycle.

If one or more of the *E. coli* methionine biosynthetic

enzymes has the capacity to function in reverse, then L-serine deamination by those enzymes could take place. This does not occur in measurable amounts in the wild type *E. coli* cell, possibly because the Km for the forward reaction is much greater than that of the reverse.

If a strain had an exceptionally high concentration of cystathionine-β-lyase, as it would in a *metJ* strain or in a strain containing the pIP29 plasmid, the Km of the reactions would not be important. The high level of the overproduced enzyme, together with a high concentration of substrate exogenously provided, should permit a significant amount of the back reaction to take place. Even if only a small percentage of the enzyme activity is reverse activity, more enzyme will still be working backwards. This would be physiologically significant (i.e. allow growth on L-serine) even if the enzyme normally functions in the other direction.

IV D-2.b L-Serine Deaminase Cycle with Altered Enzyme Function

If the preceding argument is correct, it should be possible to isolate a mutant in *metC* such that the Km in the reverse direction is greatly increased. This might lead to a deficiency in the forward reaction, that is, requirement for methionine. In this case, the amount of cystathionine-β-lyase made would not increase, but its substrate specificity would be altered. The Gos mutants could be of that type,

were it not for the fact that they are not metC mutants because they map far from metC and because they grow on cystathionine, whereas metC mutants cannot.

IV E Gos Mutants Are Not Methionine Mutants

IV E-1 Gos Mutants are Mutant in Methionine Biosynthesis

That the Gos mutants map near the metJBLF cluster and that these strains require methionine for growth at 42C, suggested that gos-3 and gos-5 might be methionine auxotrophs, mapping in one of the methionine genes at that locus. However, gos-3 and gos-5 were mapped to a position that was at least half a minute away. This is far enough away from the cluster for gos to be distinct from it. As well, if the Gos mutants indeed were methionine auxotrophs, one would expect that at least one of the methionine requiring strains would have the ability to use L-serine as sole carbon source. Since this is not the case, it is not likely that the mutations in the gos mutants are in methionine biosynthetic genes.

IV E-2 Gos Mutants Are Not Methionine Regulatory Mutants

The possibility remains that the Gos mutants have mutations in one of the methionine regulatory genes. Again, this is unlikely due to their map position. Also, Gos strains do not have increased cystathionine-β-lyase activity, an indication that they are not derepressed for

methionine biosynthesis. They are also sensitive to glutamyl-γ-methyl-ester, a compound to which metJ and metK mutants are both resistant.

Gos mutants are even more sensitive to glutamyl-γ-methyl-ester than MEW1 is. One explanation for this takes into account that glutamyl-γ-methyl-ester is a methionine analogue. Gos mutants could be hypersensitive to it if the methionine pool is decreased in the cell at 28C. Since the Gos mutants might cause a decrease in methionine synthesis by increasing L-serine deamination, the cellular concentration of methionine could be lowered. This could then lead to a greater ratio of glutamyl-γ-methyl-ester to methionine in the Gos cell compared to the wild type cell and consequently cause a hypersensitivity to the compound.

IV E-3 The Gos Mutation Is Not a Result of Deficient metA Product

Other strains of E. coli have a methionine requirement at higher temperatures (Ron and Davis, 1971), but this is due to a defect in metA gene product, where the cell cannot grow at 44C without methionine in the media. The Gos mutants, however, cannot grow even at 37C without methionine. It is possible that one of the effects of Gos is that it lowers the temperature at which the metA product ceases to function properly. This is unlikely because gos maps about half a minute away from metA (90.5).

IV F The Relationship between His^c and Gos Mutants

IV F-1 Similarities and Differences between His^c and Gos Mutants

Histidine constitutive mutants also have a temperature sensitive requirement for methionine and form filaments at high temperatures, especially in the presence of adenine. It was this that led to the consideration that GOS3 and GOS5 might be His^c mutants.

Gos mutants are not likely to be His^c mutants. They do not have high levels of histidinol dehydrogenase, so they do not seem to be derepressed. They are sensitive to aminotriazole and to triazolealanine and their revertants are not histidine dependent. However, there are still some common features of His^c mutants and Gos mutants that cannot be overlooked.

Knowing that Gos mutants are not His^c mutants does not explain why these two types of mutations are so similar phenotypically. It is curious why a mutation that derepresses the histidine pathway and a mutation that allows growth on L-serine through the methionine pathway both require methionine at 42C and form filaments in its absence, especially in the presence of adenine.

IV F-2 A Reason Why Gos Mutants Require Methionine

Part of this riddle is easily solved. If Gos mutants usurp the methionine biosynthetic enzymes in order to

deaminate L-serine for use as carbon source, this would lead to difficulty producing methionine. As proposed above, if the gos mutation has somehow altered the Km for the back reaction of cystathionine-ß-lyase, then homocysteine would repeatedly be converted to cystathionine. The cells might be able to tolerate this at 28C. However, at 37C and above, they might not be able to generate enough methionine. Moreover, the enzyme itself might function differently at high and low temperatures. Also, the cells would require methionine even when grown on glucose because L-serine would more frequently be converted to pyruvate than to methionine by the methionine enzymes.

IV F-3 Why His^c Mutants Require Methionine

The reason that histidine constitutive mutants need methionine for growth at high temperatures is more difficult to establish. A mutation resulting in a His^c phenotype is pleiotropic, and each of its effects could affect the others. Since ATP is required to make histidine, the cell's supply of purines would diminish and it would have trouble making DNA. Without chromosome replication, the cell cannot divide and thus will form filaments.

IV F-4 The Implications of C_1 Metabolism

In trying to increase the concentration of purines, the cell will require C_1-THF. Then, the cell will also be unable to make methionine, due to a decrease in available THF. If

it is THF that controls purine and methionine production, then L-serine may play a role in both methionine and purine biosynthesis, since L-serine is a C_1 donor of THF. Therefore, both the methionine requirement and filamentation with adenine in Gos and His^c mutants is tied in with the need for C_1 units.

In His^c mutants, L-serine would be depleted through successive conversion to C_1-THF for both purine and methionine biosynthesis. In Gos mutants, L-serine would be used up as the cell breaks it down for carbon source. In addition, the deamination of L-serine by the methionine enzymes will inhibit them from making methionine, causing an even greater methionine requirement than one due to C_1 exhaustion. Whether the cell is a His^c or a Gos mutant, it will have trouble making both methionine and purines, leading to a methionine requirement and filamentation.

IV G Effects of *metJ* on Threonine and Isoleucine Production

JJ127A methionine independent strains, selected either by reversion or transduction, showed an unexpected requirement for threonine or isoleucine. This could be explained as a deficiency in threonine due to the uncontrolled conversion of homoserine to methionine, leaving little homoserine to make threonine. In the *metE* strain, methionine is always provided to overcome the effects of the mutation, and this, by feedback inhibition of the first step

in the methionine pathway, would prevent the drain of threonine to methionine.

However in the revertant, methionine is not provided, and the cell might be deficient in any compound made from threonine, e.g. isoleucine. This would imply either that all metJ strains should require threonine, or that the revertant is not the same as the wild type, due to a functionally incomplete reversion at metF or due to a suppressor mutation.

The problem remains even more unclear because the methionine independent strains were not immediately tested for cystathionine-ß-lyase. They were tested considerably later and did not have a high level of the enzyme. Thus it is not known whether they retained the metJ mutations once they had become methionine independent. Also, metJ revertants might be selected in this background.

IV H Summary

Gos mutants, like other strains that affect L-serine metabolism, are pleiotropic. In addition to enabling the cell to grow on L-serine as sole carbon source without an increase in L-serine deaminase activity, they caused the cell to require methionine at high temperatures and effected filamentation in its absence. In this way, Gos mutants were unlike any other known mutants.

Strains mutant at gos are not like ssd mutants or

strains carrying many copies of sdaA, which also grow on L-serine, because they have high L-SD activity. The Gos mutants were comparable to methionine auxotrophs in that they needed methionine, and they were like methionine regulatory mutants because those strains were also able to use L-serine as sole carbon source. They were also similar to division mutants because they formed filaments at high temperatures, but this phenotype was alleviated with the addition of methionine to the growth medium. Gos mutants were most like histidine constitutive strains because they required methionine and formed filaments at high temperatures.

In order to understand why Gos mutants were able to grow on L-serine, methionine regulatory mutants were studied, even though Gos mutants did not fall into this class of mutants. Derepressed strains of methionine could deaminate L-serine via the methionine biosynthetic enzymes. This may also be the case for Gos mutants. Even though their mutations do not map within any of the methionine genes, Gos mutants could affect methionine enzymes indirectly by producing a factor that changes the Km of the enzymes.

To discern why Gos mutants made filaments, His^c mutants were also analysed. Strains that are derepressed for histidine biosynthesis may inhibit purine metabolism, prevent DNA synthesis and thus make filaments. If purines are not made as a result of altered C_1 metabolism, this could be why Gos strains also make filaments.

It is also possible that Gos mutants affect purine metabolism more directly, since gos maps very close to purH and the two genes may be allelic. Experiments have been initiated to determine if this is the case.

Gos mutants are dissimilar to all other types of mutants that require methionine, that form filaments or that grow on L-serine. Since the affects of the Gos mutants touch all of these processes, gos could turn out to be an important regulatory gene of cellular metabolism.

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