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Derivation of Glycine from Threonine in *Escherichia coli* K-12 Mutants

J. FRASER AND E. B. NEWMAN*

Department of Biological Sciences, Sir George Williams University, Montreal, Quebec, Canada

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Escherichia coli AT2046 has been shown previously to lack the enzyme serine transhydroxymethylase and to require exogenous glycine for growth as a consequence. Strains JEV73 and JEV73R, mutants derived from strain AT2046, are shown here to be serine transhydroxymethylase deficient, but able to derive their glycine from endogenously synthesized threonine. Leucine is shown to be closely involved in the regulation of biosynthesis of glycine, to spare glycine in strain AT2046T, to replace glycine in strain JEV73, and to increase threonine conversion to glycine in a representative prototroph of *E. coli*. An interpretation of strains JEV73 and JEV73R as regulatory mutants of strain AT2046 is given. A hypothesis as to the role of leucine as a signal for nitrogen scavenging is suggested.

The biosynthesis of glycine in *Escherichia coli* K-12 involves the conversion of serine to glycine by serine transhydroxymethylase (STHM) (8, 15). This has been considered to be the only pathway for glycine biosynthesis in *E. coli* K-12 since a STHM-deficient strain requires glycine for growth (12). Similarly a mutant deficient in serine biosynthesis requires exogenous serine or glycine and has no alternative source of glycine (18).

However, some indications point to a second pathway of glycine biosynthesis. A substrain of a serine/glycine auxotroph was shown to grow with exogenous threonine (18). A prototroph has also been shown to convert exogenous threonine to glycine, but little conversion of endogenous threonine to glycine (as judged from incorporation of exogenous aspartate) was seen (14). This pathway from threonine is thought to be used only in the presence of exogenous threonine, and then only by certain strains.

In *E. coli* K-12, in any case, only the first pathway can normally be of quantitative importance since mutants deficient in it require glycine for growth. In *Clostridium pasteurianium*, glycine is derived principally through the second pathway, i.e., threonine cleavage, by an enzyme, threonine aldolase, that produces acetaldehyde and glycine (1). This enzyme has also been described in mammalian tissue (4, 16).

In this paper, a prototrophic strain of *E. coli*, JEV73R, is described. This strain derives its glycine from threonine and is totally lacking in STHM. The derivation of this strain via leucine-dependent strain JEV73 from STHM-deficient strain AT2046T and the characteristics that enable the use of the threonine pathway will be discussed.

MATERIALS AND METHODS

Cultures. *E. coli* K-10 is a prototrophic strain of *E. coli* K-12 obtained from A. Garen, Yale University. Strain Cu1008 is an isoleucine-requiring strain of *E. coli* K-12 obtained from M. Levinthal, Purdue University. Strain HM100 is a serine/glycine auxotroph described previously (7). Strain AT2046 is a glycine-requiring mutant obtained from L. Pizer (12). This strain was reported to grow slowly without supplement, and this was ascribed to the conversion of endogenous threonine to glycine. However, our isolate of AT2046 grew very little without supplement and could not use exogenous threonine as a source of glycine. From it, clones that could use exogenous threonine were isolated by plating on threonine-supplemented medium. One such strain is referred to here as AT2046T.

Strain JEV73 was isolated from cultures of AT2046, incubated with low amounts of glycine (3 μg/ml) and leucine (100 μg/ml). When such a culture reached a high optical density, it was subcultured into medium with only leucine and plated after growth for single colonies on minimal medium supplemented with leucine. A colony from such a plate, strain JEV73, was shown to be unable to grow on minimal medium without leucine, but to grow with leucine, threonine, or glycine.

Strain JEV73R was isolated by plating strain JEV73 at high density on minimal medium plates. Single colonies were isolated and restreaked a second time for single colonies. Strain JEV73R originated from one such colony.

Media and methods for following growth have been described previously (8). All cultures were grown in synthetic medium with such additions as noted in the text. Threonine deaminase was assayed according to a slight modification (3) of the method of Pardee and

Prestidge (10). STHM was assayed by the method of Taylor and Weissbach (17) as modified by Folk and Berg (2). The leucine used for studies of inhibition of threonine deaminase was purchased from Sigma Chemical Co. and shown to be essentially isoleucine free, as judged by its inability to stimulate uptake of [^{14}C]glycine by an isoleucine-starved, isoleucine-requiring auxotroph strain Cu1008.

Chemical determinations. Methods for the isolation of purines and determination of their specific activity have been described previously (8). Protein hydrolysates were prepared, chromatographed in two dimensions, and exposed for radioautography by the methods of Roberts et al. (14). Proteins were determined by the method of Lowry et al. (5), with trypsin as a standard.

Chemicals. Radioactive compounds were purchased from Amersham/Searle. All other chemicals were obtained from Schwarz/Mann. Orangeburg, N.Y., or Sigma Chemical Co., Saint Louis, Mo.

RESULTS

Effects of leucine in strain AT2046T and derivative strains. Strain AT2046T depends for growth on an exogenous supply of either threonine or glycine. Leucine could not replace these. However, the addition of L-leucine did decrease the amount of glycine needed to produce a given quantity of cell material. To show this, strain AT2046T was grown with 100 μg of glycine per ml, subcultured into triplicate cultures containing 3 μg of glycine per ml with (100 μg/ml) or without leucine, and allowed to grow until successive optical density readings were identical. The optical density and protein per milliliter of culture were then determined.

The presence of leucine allowed the synthesis of about 2.5-fold more protein and optical density units than were made by cells grown in its absence. Growth was still limited, however, by nutrient availability.

The relationship between glycine and leucine became even more obvious with the isolation of a strain, JEV73, in which leucine entirely replaced glycine as exogenous nutrilite. This strain had an alternative requirement for leucine, glycine, or threonine, but did not grow in unsupplemented medium. Various other substances tested did not support growth. These included the amino acids alanine, arginine, aspartate, glutamate, histidine, isoleucine, valine, methionine, lysine, phenylalanine, proline, tryptophane, and tyrosine, tested at 300 μg/ml, as well as xanthine at 20 μg/ml and formate at 500 μg/ml.

Strain JEV73 reverted to prototrophy much more frequently than its parent AT2046T. It was not possible, therefore, to perform the standard dose response curves as have been reported for strain AT2046 (9), it being impossible to limit growth with a nutrilite and not accumulate revertants. An estimate of the amount of nutrilite needed to permit growth could be obtained, however, by determining the lowest amount of nutrilite that would sustain growth without the accumulation of revertants. This turned out to be 150 μg of threonine per ml (1.25 μmol/ml), 50 μg of glycine per ml (0.67 μmol/ml), or 5 μg of leucine per ml (0.04 μmol/ml). This corresponds to about 250 μg of threonine per ml or 75 μg of glycine per ml needed by strain AT2046.

The low amount of leucine required would indicate that the leucine is not acting directly as a precursor of another compound. More likely, the presence of leucine allows the activation of a metabolic pathway that cannot be used in its absence. A similiar conclusion is indicated by experiments in which the ability of strain JEV73 to make transitions between media was examined. Strain JEV73 made almost all transitions smoothly and rapidly (Fig. 1). Cells pregrown in glycine could adapt to growth with either threonine or leucine, and strains pregrown with leucine could adapt to either glycine or threonine. However, cells pregrown in threonine could adapt to glycine but not leucine. It seems that strain JEV73 growing with leucine supplies its glycine by a leucine-dependent pathway, the synthesis of which is repressed during growth with threonine. Cells grown with threonine would have little or none of the required enzyme(s) and, when shifted to leucine, would be starved for glycine and unable to begin to grow within the relatively short period of the experiment.

To examine this further, strain JEV73 was plated in minimal medium, and a typical revertant was isolated. This strain, JEV73R, had no organic requirements (other than glucose). When grown in minimal medium, it adapted without difficulty to growth in leucine, glycine, or threonine (Fig. 2). However, once grown in threonine, the cells could adapt to glycine but not to leucine or minimal medium.

STHM levels in strains JEV73 and JEV73R. The characteristics of the mutants described above are consistent with the idea that in strain JEV73 glycine is supplied by a pathway activated in the presence of leucine and repressed by growth in threonine. In strain JEV73R, the pathway would be leucine independent but threonine repressible. In neither case would STHM be reactivated.

To test this, STHM was assayed in strains JEV73, JEV73R, and K10, in all cases grown with 10 μg of leucine per ml. STHM could be detected as expected in the typical *E. coli* K-10

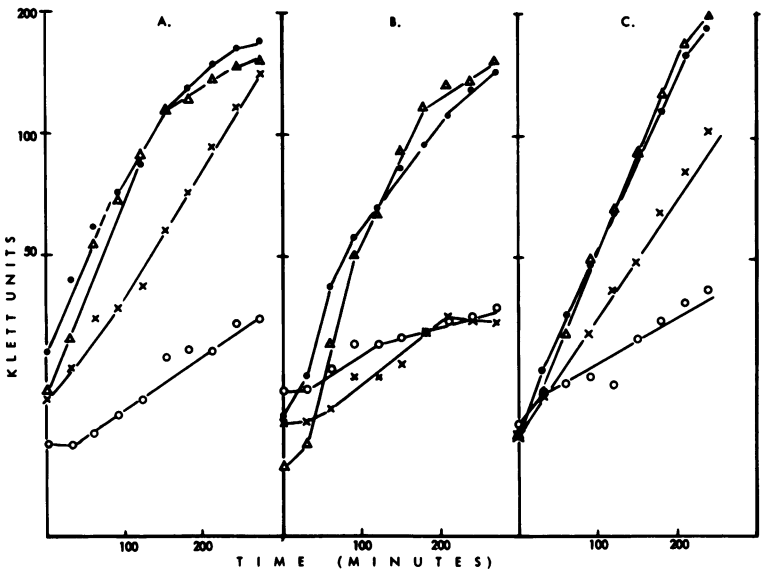

FIG. 1. *Adaptations of strain JEV73 to new nutrients: strain JEV73 previously grown on 25 μg of glycine per ml (A), 100 μg of threonine per ml (B), and 10 μg of leucine per ml (C) was subcultured into 25 μg of glycine per ml (Δ), 100 μg of threonine per ml (●), 10 μg of leucine per ml (×), and minimal medium (O). Turbidity was followed at 420 nm.*

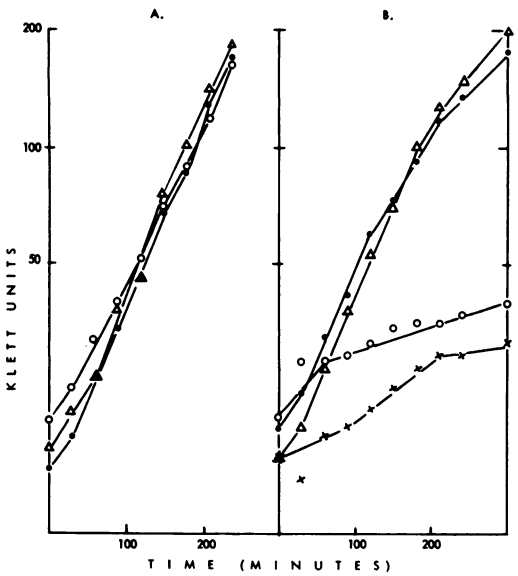

FIG. 2. *Adaptations of strain JEV73R to new nutrients. Strain JEV73R previously grown in minimal medium (A) and in 500 μg of threonine per ml (B) was subcultured in 75 μg of glycine per ml (Δ), 250 μg of threonine per ml (●), 50 μg of leucine per ml (×), and minimal medium (O). Growth was followed as indicated previously.*

(17 nmol of $HC_{14}HO$/min per mg of protein), but no activity was seen in either mutant strain (0.1 nmol of $HC_{14}HO$/min per mg of protein).

Origin of glycine in strains AT2046, JEV73, and JEV73R. The repressibility of the new pathways by threonine suggests that it originates with aspartate and forms glycine via synthesis of threonine and its cleavage, perhaps by threonine aldolase. To determine whether this pathway was being used in the various strains, they were grown with the radioactive compounds indicated below, with and without nonradioactive competitors. Nonradioactive glucose was supplied in all cases. The extent of incorporation into purines and amino acids was determined, in the former case by direct determinations of specific activity and in the latter by visual examination of radioautographs prepared from protein hydrolysates.

When strain AT2046T was grown with threonine-U-C14 (specific activity 1.4×10^4 counts/min per μmol), the purine isolated showed a specific activity of 1.42×10^4 counts/min per μmol (Table 1), indicating that four purine carbons were derived from threonine. When nonradioactive glycine was supplied along with the threonine, the purine specific activity was reduced to 0.08×10^4 counts/min per μmol. Thus, glycine prevented incorporation of threonine carbon into purine. This is consistent with the idea that, when grown with threonine, strain AT2046T derives all of its glycine and all of its C_1 from threonine.

Examination of protein hydrolysates led to the same conclusion. Carbon from threonine was found in threonine, isoleucine, and glycine and, to a lesser extent, in aspartate and glutamate. The presence of glycine in the growth

TABLE 1. *Derivation of purines in strains of E. coli K-12 grown with various radioactive precursors*[a]

Strain	Radioactive nutrilite	Sp act	C_{12} competitor	Purine sp act			No. of carbons derived from C_{14}[b]
				Adenine	Guanine	Avg	
AT2046T	[U-^{14}C]threonine	1.4	None	1.40	1.45	1.43	4
AT2046T	[U-^{14}C]threonine	1.4	Glycine	0.10	0.06	0.08	<1
JEV73	[U-^{14}C]leucine	1.2	None	0.01	0.008	0.009	<1
JEV73	[U-^{14}C]aspartate	0.8	None	0.51	0.58	0.55	2.8
JEV73R	[U-^{14}C]aspartate	0.8	None	0.30	0.33	0.32	1.4
K10	[U-^{14}C]aspartate	0.8	None	0.04	0.04	0.04	<1

[a] Specific activities: $\times 10^4$ counts/min per μmol.

[b] This is calculated as purine specific activity divided by nutrilite specific activity multiplied by the number of carbons in the nutrilite offered.

medium did not affect threonine incorporation into isoleucine, aspartate, or glutamate but greatly reduced incorporation into glycine.

Strain JEV73 could grow with leucine instead of threonine or glycine. To determine whether leucine acted as precursor of glycine or any compound usually made from glycine, strain JEV73 was grown with [U-^{14}C]leucine (specific activity 1.2 $\times$ 10^4 counts/min per μmol), and the proteins and purines were examined. Incorporation into purines was exceedingly low (0.01 $\times$ 10^4 counts/min per μmol). Leucine was the only radioactive amino acid in protein hydrolysates. Thus, leucine does not serve as a precursor of any of these compounds. This is consistent with the idea that leucine activates a new pathway rather than itself supplying cell material.

It seemed likely that the pathway activated involves conversion of aspartate to threonine. If this were true, it should be possible to show incorporation of aspartate carbon into purines (via threonine and glycine). To test this, strain JEV73 was grown with 10 μg of leucine per ml, and [U-^{14}C]aspartate (specific activity 0.83 $\times$ 10^4 counts/min per μmol) was added. The purine isolated showed a specific activity of 0.55 $\times$ 10^4 counts/min per μmol (i.e., 2.8 carbons of the purine were derived from aspartate). In protein hydrolysates, aspartate carbon was found in all compounds usually derived from aspartate (i.e., arginine, lysine, glutamate, aspartate, threonine, and isoleucine) and also in glycine. It seems then that the metabolism of aspartate by strain JEV73 is similar to that of other strains of *E. coli* (14) but includes an extra product, glycine.

Strain JEV73R is a prototroph, but still has no STHM activity. It would be expected, therefore, to derive its glycine in the same way as strain JEV73. To check this, strain JEV73R was grown with [U-^{14}C]aspartate, as before, and 10 μg of leucine per ml. In this case, incorporation into protein showed the same pattern as in strain JEV73. Incorporation into purine was substantial (0.32 $\times$ 10^4 counts/min per μmol) but lower than in strain JEV73. However, all strains studied can make aspartate de novo and, therefore, may dilute the input aspartate to varying extents in different experiments.

The actual parent strain of AT2046 not being available, the protrophic *E. coli* K-10 was taken as a control for these experiments. In this strain grown with [U-^{14}C]aspartate and leucine, the labeling pattern in proteins was identical to that in the preceding strains, except that there was no C^{14} in glycine and little in purines (0.04 $\times$ 10^4 counts/min per μmol).

The metabolic patterns thus demonstrated support the idea that strains JEV73 and JEV73R differ from prototrophic *E. coli* K-12 and from strain AT2046, as usually isolated, in their ability to convert endogenously formed threonine to glycine.

Investigation into the regulatory defect in strains JEV73 and JEV73R. Strains JEV73 and JEV73R might differ from AT2046T in their methods of regulating metabolism, such that the former strains can derive significant amounts of glycine from endogenously synthesized threonine, and the latter cannot. As will be discussed later, two sites where an alteration in feedback sensitivity might produce the phenotypes seen here are aspartokinase-homoserine dehydrogenase and threonine deaminase. Of these, only the latter was tested.

To investigate the possibility of changes in the amount of threonine deaminase synthesized, strains AT2046, JEV73 and JEV73R, as well as *E. coli* K-10, were grown with 100 μg of glycine per ml, and threonine deaminase was assayed in log-phase cells. Threonine deaminase levels were similar in all cases (Table 2).

To investigate the possibility that the enzyme itself was structurally altered, the activity of threonine deaminase as a function of inhibitor

(isoleucine, leucine) and substrate (threonine) concentration was tested in toluenized preparations of strains AT2046T, JEV73, and JEV73R. Each compound affected all strains similarly. Data are shown only for inhibition by leucine (Table 3). An alteration in the rate of threonine deaminase synthesis or in the regulation of threonine deaminase activity can therefore be excluded.

Effect of leucine on threonine cleavage in E. coli K-10. Because strains AT2046T and JEV73 differ in their ability to grow with leucine, one would suppose that the mutation resulting in strain JEV73 should be involved some way in leucine metabolism. However, it could also be that leucine has some metabolic effect in all strains of *E. coli* and that an unrelated mutation in strain JEV73 makes the leucine effect sufficient to allow growth in strain JEV73 although it is not sufficient for strain AT2046T.

Leucine is indeed known to have several effects in *E. coli* (3, 10). One which seems to be related to the present work is its ability to shift the pattern of threonine metabolism in *E. coli*. Thus, *E. coli* K-10 was grown with [U-^{14}C]aspartate or [U-^{14}C]threonine, each with (100 μg/ml) or without leucine. In the presence of both leucine and threonine, considerable amounts of threonine carbon were found in purine (Table 4). Such conversion was not seen with exogenous threonine only, or with aspartate, either with or without leucine. The same pattern was seen in protein hydrolysates, glycine being heavily labeled only in the culture grown with both threonine and leucine. Thus, leucine stimulates the conversion of threonine, but not of aspartate, to glycine. This phenomenon is of interest on its own and is being pursued. However, it is relevant here in that if a mutation in strain JEV73 led to increased amounts of internal threonine it would be reasonable to expect from the preceding that the presence of leucine in the medium might produce increased conversion of threonine to glycine.

TABLE 2. *Threonine deaminase levels in strains of E. coli K-12*

Strain	Threonine deaminase[a]
K10	0.55
AT2046T	0.61
JEV73	0.63
JEV73R	0.55

[a] Micromoles of keto acid/30 min per milligram of protein.

TABLE 3. *Effect of leucine on threonine deaminase activity in strains of E. coli K-12*

Leucine concn (10^{-3} M)	% Activity		
	AT2046	JEV73	JEV73R
0	100	100	100
0.4	97	97	106
0.8	99	93	86
1.2	63	68	74
1.6	36	46	34
2.0	23	26	24
3.0	9	0.5	13
4.3	1	6	5

Source of glycine in strain HM100. JEV73R is a prototroph that grows without STHM, deriving all its glycine from aspartate via threonine. JEV73 does the same, though only in the presence of leucine. A third mutant in which the source of glycine (and serine) has not been clear is strain HM100 growing on formate and glucose (7). This strain is a serine/glycine auxotroph that cannot grow in the absence of serine or glycine unless formate is present and that does not incorporate formate carbon into either serine or glycine.

The present findings suggested the possibility that in strain HM100 the pathway from threonine was activated not by leucine, but by formate. To test this, strain HM100 was grown with [U-^{14}C]aspartate and formate. No incorporation of C^{14} into purines was detected. The source of serine and glycine in this strain therefore remains uncertain.

DISCUSSION

Only one quantitatively important pathway of glycine biosynthesis has been described in *E. coli* growing in unsupplemented medium, this proceeding from serine via STHM. Since an STHM-deficient strain requires glycine for growth, it has been considered that this is the only pathway for glycine biosynthesis in *E. coli* (12).

In this paper, the establishment of a second pathway by a series of mutational events is described, as is an unexpected relationship between leucine and glycine metabolism.

The starting strain for these studies is a subclone, AT2046T, of a previously described strain, *E. coli* AT2046. AT2046T is unable to synthesize STHM and requires glycine or threonine for growth. From AT2046T was isolated a mutant strain, JEV73, which can use leucine as well as glycine or threonine. From that strain, a further derivative was isolated: the prototroph strain JEV73R. The fact that strain JEV73R

TABLE 4. *Derivation of purines in E. coli K-10 grown with various radioactive precursors*[a]

Radioactive nutrilite	Sp act	C_{12} competitor	Purine sp act			No. of carbons derived from C_{14}
			Adenine	Guanine	Avg	
[U-^{14}C]aspartate	0.8		0.04	0.02	0.03	<0.1
[U-^{14}C]aspartate	0.8	Leucine	0.05	0.07	0.06	~0.1
[U-^{14}C]threonine	1.4		0.09	0.02	0.05	~0.1
[U-^{14}C]threonine	1.4	Leucine	0.6	0.4	0.5	1.5

[a] Specific activities: $\times 10^4$ counts/min per μmol.

grows in unsupplemented medium, i.e., can make its own glycine but contains no STHM, demonstrates that the mutations have activated a new pathway of glycine biosynthesis.

The pathway thus activated involves the conversion of aspartate carbon through threonine to glycine. Strains JEV73 and JEV73R both incorporate carbon from aspartate into glycine. This is not seen to any significant extent in a representative prototroph (*E. coli* K-10) studied here, nor was it seen in earlier studies (14).

That this pathway is, in fact, the pathway providing glycine is also indicated by the fact that growth with threonine renders strain JEV73R unable to grow in unsupplemented medium. Thus, growth with threonine represses the synthesis of enzymes needed for growth in minimal medium, and it seems likely that these are enzymes involved in conversion of aspartate to threonine. A similar phenomenon is described for strain JEV73.

What metabolic changes could result in the phenotypes described here, i.e., the leucine-dependent endogenous synthesis of glycine from aspartate in strain JEV73 and the leucine-independent synthesis of glycine in strain JEV73R?

Strain AT2046T differs from strain AT2046 in its ability to use exogenous threonine for growth. A similar derivation of threonine-utilizing strains from a serine/glycine auxotroph has been described by Van Lenten and Simmonds (18), who correlated the ability to use threonine with the rate of conversion of threonine to glycine by resting cells.

Even the strain that used exogenous threonine, however, could not use endogenous threonine as the sole source of glycine. It did show a lower requirement for glycine than did its parent strain, and this was ascribed to an ability to make some glycine via endogenously synthesized threonine (18). Similarly, Pizer and Potochny (13) considered that endogenous threonine could not support rapid growth of a serine/glycine auxotroph because *E. coli* normally maintains its endogenous threonine pool at too low a level to permit diversion of endogenous threonine to glycine. The conclusion that the threonine pool is kept low in *E. coli* was also reached by Roberts et al. (14). Pizer and Potochny indeed suggested that, if strains had different levels of regulation of threonine biosynthesis, they might be able to derive varying proportions of their serine and glycine from threonine.

Now the fact that strain AT2046T grows on exogenous threonine and converts it to glycine, as indicated by tracer studies, demonstrates clearly that it can make the enzyme(s) necessary to convert threonine to glycine. Strain JEV73, derived directly from it, therefore must also be able to make the enzyme(s). If then the mutation that resulted in JEV73 was a regulatory mutation that set the endogenous threonine pool at a higher level, the conditions for the use of endogenous threonine as a source of glycine would be met. That is, the cell would have both sufficient threonine and the enzymes needed to cleave it. That this is indeed happening, albeit only in the presence of leucine, is indicated by the conversion of aspartate to glycine in strain JEV73, though not in a typical prototroph.

What sort of alteration might increase the threonine pool? One possibility, suggested to us by T. Newman and M. Levinthal, Purdue University, could be an alteration in threonine deaminase. This however, seems to be excluded. The enzyme of the mutant strains is synthesized at the same level and shows the same sensitivity to isoleucine and leucine, as well as the same affinity for threonine, as does the enzyme of the parent AT2046.

A second possibility would be a site in the pathway from aspartate to threonine. Several points of regulation by threonine have been described within this pathway (11). Thus, the activation of aspartate is carried out by three distinct aspartokinases, one of which is controlled both by repression and inhibition by threonine. The later conversion of aspartic

semialdehyde to homoserine is carried out by two homoserine dehydrogenases, one of which is similarly subject to threonine repression and inhibition. The first step entirely specific to threonine is homoserine kinase, a single enzyme, subject to repression but not inhibition by threonine.

Suppose that the feedback inhibition of the threonine-sensitive aspartokinase-homoserine dehydrogenase were altered. This should increase the homoserine pool, which should in turn increase the threonine pool, since homoserine kinase is relatively insensitive to threonine. Under these conditions, the cell could maintain relatively higher pool levels of threonine by endogenous synthesis. However, cells pregrown with threonine would have decreased levels of homoserine kinase, it being sensitive to repression, and then when transferred to leucine would be unable to synthesize sufficient threonine to convert it to glycine. This hypothesis seems plausible, but has not been tested.

The endogenous synthesis of glycine in strain JEV73 depends on the presence of exogenous leucine. This leucine appears to have a catalytic or regulatory function. Very low amounts of leucine suffice, and no conversion of [^{14}C]leucine to any product that might be expected to be lacking (glycine, C_1, purines, or threonine) could be detected. Thus, leucine appears to permit the functioning of some reaction needed by strain JEV73. We have shown that leucine increases the conversion of [^{14}C]threonine to glycine but not that of [^{14}C]aspartate to glycine in a prototrophic strain. It would seem then that leucine has an effect on the portion of the pathway between threonine and glycine and not on that between aspartate and threonine.

This difference between the effect on threonine and on aspartate, as well as the effect of leucine in sparing glycine, could be explained if leucine induces threonine aldolase or some similar enzyme. In this case, strain JEV73 would have an increased threonine pool due to alterations in the regulation of threonine biosynthesis, but would need the presence of exogenous leucine to induce threonine conversion to glycine. This latter conversion would be constitutive in strain JEV73R.

The establishment of a second pathway for glycine biosynthesis then results from an alteration in the regulation of threonine biosynthesis and a coincidental effect of leucine on threonine conversion to glycine. Why should leucine have such an effect?

Leucine has, in fact, a large number of metabolic effects. It is known as an inducer of threonine and serine deaminases (3, 10). It has been considered to induce glycine cleavage to C_1 (E. B. Newman, J. Fraser, R. Potter, and V. Kapoor, manuscript in preparation). It has been shown here to induce threonine cleavage to glycine. It would seem then that leucine acts as a signal for increased catabolism of at least some amino acids (threonine, serine, and glycine) and perhaps of others as yet untested.

Leucine, however, also has anabolic effects. It is well known as a strong positive effector of glutamic dehydrogenase (19). Indeed, it has recently been shown in rat liver mitochondria that leucine greatly increases the assimilation of ammonia into glutamate and decreases its incorporation into urea (6).

We suggest, therefore, that an increase in leucine concentration is a signal for the cell to increase the catabolism of some amino acids which happen to be in excess and to increase the assimilation of the ammonia thus produced. That is, leucine could be considered to be a signal for nitrogen scavenging.

This might be related to the metabolic position of leucine at the end of a long many-branched metabolic pathway, a final product which is not a precursor of any other metabolic substrate. An increase in intracellular leucine might occur when leucine is synthesized faster than it is used. But since leucine is being used only as a source of protein, such an increase should occur only when the rate of utilization of leucine for protein synthesis is lower than the rate of leucine synthesis. If the slowdown in protein synthesis reflects a decrease in nitrogen available for amino acid synthesis, the role of leucine might be to scavenge nitrogen from amino acids that are present and use that nitrogen to produce those that are absent. This restoration of amino acid balance would allow further protein synthesis, perhaps then adjusting the cell to new environmental conditions. It may be relevant then that serine deaminase is induced by a shortage of inorganic nitrogen, as well as by leucine, and by glycine (3).

We would then view the establishment of a second pathway of glycine biosynthesis as described here as being due to the interplay of two phenomena—a general effect of leucine increasing threonine conversion to glycine as one of many effects of leucine on metabolism, and a specific alteration in the regulation of threonine biosynthesis.

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