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The Leucine Regulon of *Escherichia coli* K-12: a Mutation in *rblA* Alters Expression of L-Leucine-Dependent Metabolic Operons

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We have isolated and characterized a highly pleiotropic *Escherichia coli* mutant affected in the activity of a number of enzymes involved in different metabolic pathways, all of which are regulated by leucine. Selected for its ability to grow with L-serine as sole carbon source, the *rbl-1*::Tn*10* mutant had high levels of L-serine deaminase activity (due to increased transcription of the structural gene) and of another amino acid-degrading enzyme, L-threonine dehydrogenase, and decreased transcription of the operons *serA* and *ilvIH*, coding for biosynthetic enzymes. The *rbl* mutation suppressed the slow growth of a *metK* mutant, deficient in *S*-adenosylmethionine synthetase. Furthermore, *metK* mutants spontaneously accumulated faster-growing *rbl*-like derivatives, and a commonly used *metK* strain, RG62, carries such a mutation. The *rbl* gene is located near 20 min on the *E. coli* genetic map. All phenotypes of the *rbl* mutant could be observed in *rbl*$^+$ strains cultivated in the presence of L-leucine, and exogenous L-leucine had little further effect on the *rbl* strains. We propose that the *rbl* gene product is the regulator of a global response to leucine.

Escherichia coli and other enteric bacteria possess a number of programmed responses to particular types of environmental changes, by which whole sets of genes are turned on or off according to the immediate needs of the cell (reviewed in reference 29). Each of these global responses (13) is governed by a specific regulator. The regulators work by various mechanisms. These include specific sigma factors, as in the responses to heat shock (*rpoH*; 16, 30) and nitrogen starvation (*ntrA*; 18). Other positive transcriptional activators regulate the activities of enzymes involved in anaerobic respiration (*fnr*; 43), carbohydrate catabolism (*crp*; 20), repair of oxidative damage (*oxyR*; 8), capsular polysaccharide synthesis (*rosA*; 14), and the response to phosphate starvation (*phoB*; 50). A transcriptional repressor controls the synthesis of the DNA repair enzymes belonging to the SOS response (*lexA*; 23).

A number of these global responses are mediated by two-component regulatory systems, in which a membrane sensor protein, in response to an environmental signal, phosphorylates a cytoplasmic effector protein, which in turn regulates the transcription of certain genes (42). In many cases the specific effector to which the regulator responds is not known, and in some cases the regulator itself has not yet been identified. In practice, a genetically defined regulator has been a convincing argument for the existence of specific global responses and has provided an essential tool for determining the member genes.

We have previously suggested that the branched-chain amino acid L-leucine may be the effector of a global response in *E. coli*, since the transcription of a number of genes is increased or decreased when L-leucine is present in the growth medium (11, 32). In this work, we describe an *E. coli* mutant in which the expression of several leucine-regulated genes from different metabolic pathways is no longer affected by exogenous L-leucine. The mutant behaves as though L-leucine were constantly present in the medium. We call the gene *rbl*, for regulation by leucine, and suggest that it codes for the regulator of a new global response.

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MATERIALS AND METHODS

Cultures. The strains used, all derivatives of *E. coli* K-12, are described in Table 1. The minimal medium used and the methods for monitoring growth have been described previously (35). Because strain CU1008 and all of its derivatives carry a deletion in *ilvA*, isoleucine and valine, 50 μg/ml each, were added to all media.

Map locations. Map locations were determined first by conjugation and then by transductions, using strains with conveniently placed Tn*10* insertions as devised by Singer et al. (44).

Other genetic techniques. Transductions and transformations were performed as described elsewhere (24, 28).

Enzyme assays. L-Serine deaminase (L-SD) was assayed as previously described in toluene-treated whole cells (35). One unit of L-SD was taken to be the amount of enzyme which catalyzed the formation of 1 μmol of pyruvate in 35 min.

Cystathionine-β-lyase was assayed in whole cells by the method of Hunter et al. (19), using toluene instead of lysozyme, as suggested to us by R. C. Greene.

β-Galactosidase was assayed in whole cells according to the method of Miller and expressed in his units (28).

Proline oxidase was assayed by a slight modification of the method of Dendinger and Brill (10), using whole cells grown in glycerol minimal medium with proline (1 mg/ml).

Tryptophanase was assayed by the method of Botsford and DeMoss (5), using whole cells grown in glycerol minimal medium in which the nitrogen source was replaced with L-tryptophan (500 μg/ml).

Analog sensitivity tests were performed by placing various concentrations of γ-glutamyl methyl ester (GGME) on filter disks (21) and also by streaking strains to be tested on glucose minimal medium plates containing 2 mM GGME.

Isolation of mutants. Strain MEW1 was infected with λ::Tn*10* Δ*att* c*I857*(ts) obtained from J. Wood (51), and tetracycline-resistant colonies were obtained according to her method (51) and screened for those able to grow with serine as carbon source. The *rbl* mutation from one isolate was transduced to strain MEW1, selecting for tetracycline

TABLE 1. Bacterial strains

Strain	Genotype or relevant characteristics	Source
CU1008	*E. coli* K-12 *ilvA*	L. S. Williams
MEW1	Δ*lac* derivative of strain CU1008	35
KEC9	CU1008 *ssd*	34
DRN-1	*serA*::Mu dl	38
RG	Wild-type *E. coli* K-12, parent of RG62	R. Matthews (25)
RG62	RG *metK* spontaneous ethionine-resistant derivative of RG now described as *metK rbl-62*	R. Matthews (25)
CV975	*ilvIH*::*lacZ*	J. M. Calvo
CV1008	CV975 *ihb*::Tn*10*	J. M. Calvo
MEW22	*sdaA*::λ *plac*mu9 Kan^r	47
MEW28	*sdaA*::Cm^r	Su Hong Sheng
MEW26	MEW1 *rbl*::Tn*10*	This work
MEW30	MEW1 *metK62*	This work
MEW31	MEW1 *metK62 rbl*::Tn*10*	This work
MEW32	MEW31 *sdaA*::Cm^r from MEW28	This work
MEW33	MEW1 *rbl-62*	This work
MEW34	MEW1 *metK62 rbl-62*	This work
MEW35	MEW22 *ssd* by transduction of *ssd* from KEC9 into MEW22 *metB*	This work
MEW36	MEW22 *rbl*::Tn*10*	This work
MEW37	DRN-1 *ssd*	This work
MEW38	DRN-1 *rbl*::Tn*10*	This work
MEW39	DRN-1 *rbl-62*	This work
MEW40	MEW38 *sdaA*::Cm^r	This work
MEW41	DRN-1 *ihb*	This work
MEW42	CV975 *rbl*::Tn*10*	This work
MEW43	MEW1 *ihb*::Tn*10*	This work
MEW44	MEW22 *ihb*::Tn*10*	This work

resistance and verifying use of serine as carbon source. This strain was called MEW26.

Principles of strain constructions. Alleles derived from strain RG62 are indicated as *-62*. Strains were constructed by transductions as follows.

rbl from strain MEW26 was transferred by selecting for tetracycline resistance and verifying that the transductants grew with L-serine as carbon source, except for derivatives of MEW22, in which this verification could not be made.

To transfer *rbl-62*, we first transduced Tn*10* from Singer strain 18478 (44) into RG62, selecting for tetracycline resistance, and then used a tetracycline-resistant transductant which grew with L-serine as donor of *rbl-62*.

metK62 from strain RG62 was transduced into strain DRN-1 *serA*, selecting for serine-independent growth. These transductions were done in the presence of L-leucine, which facilitates growth of *metK* mutants (see text). Strains were verified by GGME resistance. Strain DRN-1 *serA* was constructed by insertion of Mu dl prophage into *serA* (38). It has since acquired a second Mu prophage which creates no auxotrophy and synthesizes no β-galactosidase.

ssd was transferred from strain KEC9 in two steps. First the recipient was made *metB* by selecting tetracycline resistance from Singer strain 5052 *btuB*::Tn*10 metB*. A methionine-requiring recipient was transduced to methionine independence, and other characteristics of the strain were verified as required.

ihb::Tn*10* was transferred from strain CV1008 by antibiotic selection.

sdaA::Cm^r was transferred by selecting chloramphenicol resistance and verifying that the strains could not grow with a combination of serine, glycine, and leucine as carbon source.

RESULTS

Isolation of an *rbl*::Tn*10* mutant. Wild-type *E. coli* K-12 is unable to grow on L-serine as sole carbon source unless L-leucine and/or glycine are also present in the medium. We have previously described a mutant of *E. coli* affected at the *ssd* (*ecfB*) locus and able to grow on L-serine alone. This pleiotropic mutation caused overproduction of the enzyme L-SD, which converts L-serine to pyruvate; it also affected the energy metabolism and antibiotic resistance of the cells (34). Other mutations have also been shown to confer the ability to grow on L-serine (6).

To determine whether it was possible to isolate L-serine utilizers by gene knockout mutations, we selected for growth on L-serine from a pool of MEW1 cells carrying Tn*10* insertions at random locations on the chromosome. We transduced the Tn*10* insertion from one such isolate back into strain MEW1, selecting for tetracycline resistance, and verified that the transductants could grow on L-serine. This procedure showed that the insertion mutation was sufficient to permit growth on L-serine; we called it *rbl-1*::Tn*10*, for regulation by leucine. One *rbl-1*::Tn*10* transductant, strain MEW26, was kept for further study.

To locate this mutation, we used a streptomycin-resistant derivative of MEW26 and the Hfr kit of Singer et al. (44). We selected recombinants resistant to both kanamycin (carried by the Hfr) and streptomycin and screened for loss of the ability to grow on L-serine and loss of tetracycline resistance. The wild-type allele of the insertion in strain MEW26 was most frequently transferred with inserts at 12 min, using strain 12203 (95%), and at 17 min, using strain 12206 (67%). We located *rbl-1*::Tn*10* more precisely by P1-mediated transduction (28). The mutation was 15 to 20% cotransducible with an insert near 20 min (72 of 412 transductants scored with strain 18528) (44) and 40 to 50% cotransducible (67 of 147 transductants scored) with *serC* at 20.1 min. Thus, the *rbl* gene is located near 20 min. This map location is clearly different from that of previously described mutations permitting growth on L-serine: *ssd* at 88 min (34), *gos-3* and *gos-5* at 90 min (6), and *metJ* at 89 min (6).

The *rbl* mutant MEW26 grew slightly more slowly than its parent strain MEW1 in glucose minimal medium, with a doubling time of 80 min compared with 58 min for strain MEW1. The presence of L-leucine in the medium had essentially no effect on the growth rate of the wild-type strain but increased that of the *rbl* mutant to about 65 min, suggesting that L-leucine synthesis is limiting in the mutant.

Increased L-SD activity in the *rbl* mutant. The previously described *ssd* mutant KEC9, selected for its ability to grow on L-serine as carbon source, was shown to overproduce the enzyme L-SD (34). The *rbl-1*::Tn*10* strain MEW26, grown in glucose minimal medium, also made more L-SD than the parent strain MEW1, although less than the *ssd* mutant (Table 2). This presumably accounts for the ability of the mutant to grow on L-serine, since an increase in L-SD per se has been shown to permit growth (47). In this respect, the *rbl* mutant differs from the *gos-3*, *gos-5*, and *metJ* strains, which do not overproduce L-SD (6).

Formation of active L-SD requires posttranslational activation (31). However, the increased activity observed in the *ssd* and *rbl* mutants reflected an increased rate of transcription of the *sdaA* gene, which has been shown to be the structural gene of L-SD (H. S. Su, personal communication).

TABLE 2. L-SD and TDH activity of *rbl* mutants and related strains of *E. coli* K-12[a]

Strain	Relevant genotype	L-SD activity[b]	TDH activity[c]
MEW1	Wild type	10	5.1
KEC9	*ssd*	214	ND[d]
MEW26	*rbl*	96	40
MEW43	*ihb*	93	42

[a] Assays were done on mid-exponential-phase cultures grown in glucose minimal medium at 37°C. Values are averages of two or three different experiments.
[b] Expressed in milliunits per 100 Klett units of cells as described previously (35).
[c] Expressed as nanomoles of aminoacetone formed by 0.3 ml of a 500-Klett-unit suspension of cells in 20 min as described previously (32).
[d] ND, Not determined.

This effect of *ssd* and *rbl* on *sdaA* transcription was shown by means of an *sdaA*::*lacZ* protein fusion, constructed by insertion of λ p*lac*mu9, putting the structural gene for β-galactosidase under control of the *sdaA* promoter (47). β-Galactosidase activity was increased sevenfold in the *rbl-1*::Tn*10* *sdaA*::*lacZ* strain MEW36 and ninefold in the *ssd sdaA*::*lacZ* strain MEW35 (Table 3, experiments 1 to 3).

In wild-type strains, L-SD activity is known to be induced by the presence of L-leucine in the medium. The fourfold induction factor is about half that due to the *rbl* mutation. These effects were not additive; in the *rbl* mutant, L-leucine stimulated L-SD activity only 1.2-fold further (Table 4). L-Leucine exerted similar effects on the *sdaA*::*lacZ* fusion: 4-fold induction in the *rbl*$^+$ strain, compared with only 1.2-fold further induction in the *rbl* mutant (Table 4).

Increased TDH activity in the *rbl* mutant. If the *rbl* mutation affects regulation by L-leucine, other enzymes regulated by this amino acid might also have altered activity in the *rbl* mutant. A second enzyme whose activity is stimulated by the presence of L-leucine in the growth medium is L-threonine dehydrogenase (TDH; 32), which catalyzes the first step in a pathway of glycine synthesis from threonine (11, 32, 39, 40). Since the *rbl* mutation seemed to mimic the effect of exogenous L-leucine on L-SD activity, we examined its effect on TDH activity. The *rbl* mutant had sevenfold-higher TDH activity than the wild-type strain grown in glucose minimal medium (Table 2). The presence of L-leucine in the medium stimulated the wild-type level threefold but had essentially no effect on the TDH level in the *rbl* mutant (Table 4).

Decreased *serA* transcription in the *rbl* mutant. The enzyme phosphoglycerate dehydrogenase, encoded by the *serA* gene and responsible for the first step specific to L-serine synthesis, also exhibits leucine-sensitive regulation of activity. However, unlike L-SD and TDH, it is expressed at lower levels in the presence of exogenous L-leucine (27). To examine the effect of the *rbl* mutation on *serA* transcription, we introduced the *rbl-1*::Tn*10* allele into a *serA*::*lacZ* fusion strain. β-Galactosidase activity was decreased fivefold in the *rbl* mutant (Table 3, experiments 5 and 7). The presence of L-leucine in the medium decreased the wild-type level twofold but had essentially no effect in the *rbl* mutant (Table 4).

The *ssd* mutation had no effect on *serA* expression (Table 3, experiments 5 and 6). This finding suggests that the *ssd* and *rbl* gene products belong to different, although possibly overlapping, regulatory systems.

Decreased *ilvIH* transcription in the *rbl* mutant. The *ilvIH* operon encodes the two subunits of acetolactate synthase III, involved in branched-chain amino acid synthesis (45).

TABLE 3. Comparison of strains carrying *rbl*, *ihb*, and *ssd* mutations

Expt	Strain	Relevant genotype	Activity[a]			
			L-SD activity[b]	β-Galactosidase from promoters of[c]:		
				serA[d]	*sdaA*[e]	*ilvIH*[f]
1	MEW22	*sdaA*::*lacZ*			50	
2	MEW35	*sdaA*::*lacZ ssd*			450	
3	MEW36	*sdaA*::*lacZ rbl*			350	
4	MEW44	*sdaA*::*lacZ ihb*			375	
5	DRN-1	*serA*::*lacZ*		2,350		
6	MEW37	*serA*::*lacZ ssd*		2,250		
7	MEW38	*serA*::*lacZ rbl*	83	375		
8	MEW39	*serA*::*lacZ rbl62*		375		
9	MEW41	*serA*::*lacZ ihb*	84	375		
10	MEW40	*serA*::*lacZ rbl* *sdaA*::Cm^r		425		
11	CV975	*ilvIH*::*lacZ*	13			275
12	CV1008	CV975 *ihb*	38			10
13	MEW42	CV975 *rbl*	51			10

[a] Values are averages of two or three different experiments and are given to the nearest 25 U except those less than 50, which are given to the nearest 5 U.
[b] Expressed as in Table 2.
[c] Assayed in exponential-phase cells grown in glucose minimal medium (with additions as needed) and reported in Miller units (28).
[d] Expressed in units from a *serA*::*lacZ* fusion.
[e] Expressed as units from an *sdaA*::*lacZ* fusion.
[f] Expressed as units from an *ilvIH*::*lacZ* fusion.

Transcription of the *ilvIH* operon, like that of the *serA* gene, is decreased in the presence of exogenous L-leucine (46).

To study the effect of the *rbl* mutation on *ilvIH* transcription, we introduced the *rbl-1*::Tn*10* mutation into strain CV975, an *ilvIH*::*lacZ* fusion strain kindly provided by J. M. Calvo. The *rbl* mutation caused a 27-fold drop in the level of *ilvIH* transcription (Table 3, experiments 11 to 13).

Tryptophanase and proline oxidase are not affected in the *rbl* mutant. Since both L-SD and TDH are amino acid-degrading enzymes, we examined the possibility that the *rbl* mutation induced synthesis of other enzymes involved in amino acid catabolism. We assayed L-tryptophanase and L-proline oxidase activities in wild-type (MEW1) and *rbl* (MEW26) strains. The *rbl* mutation had no effect on the level of either enzyme (data not shown), excluding the hypothesis that the *rbl* gene is a general regulator of amino acid-degrading enzymes.

It has previously been shown that L-proline oxidase activity is not affected by the presence of L-leucine in the growth medium (48). We also found that L-tryptophanase activity is unaffected by L-leucine (data not shown). These observations are thus consistent with the idea that the *rbl* gene product is the specific mediator of leucine effects.

The *rbl* and *ihb* genes are probably identical. J. M. Calvo and his collaborators have described a protein that binds to the regulatory region of the *E. coli ilvIH* operon (41) and mediates its repression by L-leucine. They have also isolated a mutant, *ihb*::Tn*10*, in which this protein is absent (personal communication). They have shown that the *ihb* mutation is located near 20 min and causes decreased expression of the *ilvIH* operon. J. M. Calvo suggested to us (personal communication) that our instances of leucine regulation might be modulated by the same protein. In this case, the *rbl* gene might be identical to *ihb*, the structural gene for the *ilvIH* binding protein.

To test this, we examined the effect of the *ihb*::Tn*10* mutation from strain CV1008 on L-SD and TDH activity and

TABLE 4. Regulation by leucine in the *rbl* mutant

Expt	Activity assayed	Strain	Relevant genotype	Activity in minimal medium (A)	Activity in minimal medium with leucine (B)	Effect of leucine, B/A × 100
1	L-SD[a]	MEW1	*rbl*$^+$	11	46	418
2		MEW26	MEW1 *rbl*	67	73	118
3	TDH[a]	MEW1	*rbl*$^+$	5	18	305
4		MEW26	MEW1 *rbl*	36	43	112
5	β-Galactosidase from *sdaA*[b]	MEW22	*sdaA*::*lacZ rbl*$^+$	56	225	400
6		MEW36	MEW22 *rbl*	425	530	125
7	β-Galactosidase from *serA*[b]	DRN-1	*serA*::*lacZ rbl*$^+$	2,350	1,360	58
8		MEW38	DRN-1 *rbl*	450	500	113

[a] Expressed as in Table 2.
[b] Expressed as in Table 3.

on *sdaA*::*lacZ*, *serA*::*lacZ*, and *ilvIH*::*lacZ* expression. The results show that the two mutations, *ihb* and *rbl*, had quantitatively similar effects in all cases: L-SD activity, TDH activity, and *sdaA* expression were increased, and *serA* and *ilvIH* expression were decreased (Table 2; Table 3, experiments 1, 4, 5, and 9). Furthermore, the *ihb* strain CV1008 was able to grow with L-serine as sole carbon source, whereas the parent strain CV975 could not.

The identity of phenotypic effects and genetic location is consistent with the idea that the *ihb* and *rbl* mutations affect the same gene and that the product of this gene regulates the activity of L-SD and TDH and the expression of the *sdaA*, *serA*, and *ilvIH* genes.

Effects of *rbl* mutations in a *metK* strain. Strain RG62, a *metK* mutant deficient in *S*-adenosylmethionine (SAM) synthetase activity, was isolated by Greene et al. as an ethionine-resistant mutant (15). It had less than 3% residual SAM synthetase activity and high levels of methionine biosynthetic enzymes and was resistant to GGME. More recently, this strain was reported to have an unusual ability to degrade exogenously supplied L-serine (26), suggesting that it might have high L-SD levels. R. Matthews kindly provided us with her isolate of strain RG62. When grown in glucose minimal medium, it did indeed have high L-SD activity, 71 mU/100 Klett units versus 30 for the *metK*$^+$ parent strain. Furthermore, strain RG62 was able to grow with L-serine as sole carbon source, whereas the parent could not.

We had observed that another *metK* strain did not overproduce L-SD and could not grow with L-serine (data not shown). This suggested that these properties of RG62 might be due not to the *metK* mutation but rather to an unsuspected *rbl*-like mutation present in the strain. To test this possibility, we first transduced the *metK* mutation from RG62 to our strain background, by cotransduction with *serA*$^+$ (recipient strain DRN-1). Of the transductants, 10 to 20% were resistant to GGME, consistent with the established genetic map distance between *serA* and *metK* (4).

We characterized one such *metK62* transductant, MEW30, in more detail. In addition to GGME resistance, it had high β-cystathionase activity (product of the *metC* gene), as expected from a *metK* strain (data not shown). However, unlike RG62, it had low L-SD activity (Table 5, experiment 5) and was unable to grow with L-serine as sole carbon source. Furthermore, MEW30 grew exceedingly slowly in glucose minimal medium. The fastest growth rate we measured (120-min doubling time) was only half that of the parent strain, but the growth rate was variable and usually even slower. RG62, on the other hand, had a doubling time of 80 min, only slightly longer than that of the wild-type. The growth rate of MEW30 was considerably increased by addition of L-leucine to the medium (doubling time of 85 min); this may be related to the observation that exogenous L-leucine increased the residual SAM synthetase activity three- to fourfold in the RG62 mutant (15).

Since our *metK62* transductants did not acquire the ability of RG62 to grow with L-serine, we tested for the presence of an *rbl*-like mutation, using the same system as for the mapping of *rbl*. Strain RG62 carried such a mutation at the same map position, 50 to 70% cotransducible with an insertion at 20 min. We were also able to move the mutation into strain MEW1 by cotransduction with a Tn*10* insertion near 20 min (created during the mapping studies). These transductants had high L-SD activity but were not resistant to GGME (Table 5, experiment 4), and they had low cystathionase activity (data not shown). When this mutation was transduced into the *serA*::*lacZ* fusion strain DRN-1, it caused a sixfold drop in *serA* expression (Table 3, experiment 8), similar to results for the *serA*::*lacZ rbl*::Tn*10* strain MEW38. We conclude that RG62 harbors a second mutation, an *rbl* allele, and suggest that it be called *rbl-62*.

The fact that exogenous L-leucine increased the growth rate of our *metK62* transductant MEW30 suggested that the relatively fast growth rate of RG62 might be due to its *rbl-62* mutation. To test this possibility, we transduced the *rbl-1*::Tn*10* mutation from MEW26 and the *ihb*::Tn*10* mutation from CV1008 into a newly constructed, slow-growing *metK62* strain, selecting for tetracycline resistance on LB plates. In glucose minimal medium, both the *metK62 rbl-1*::Tn*10* and the *metK62 ihb*::Tn*10* double mutants grew at the same rate as RG62 (80-min doubling time) and much faster than the *metK* parent strain. Their growth rate was about the same as that of the *metK62* strain growing in the presence of L-leucine.

TABLE 5. L-SD activity and GGME resistance of *rbl* and *metK* mutants and related strains of *E. coli* K-12

Expt	Strain	Relevant genotype	L-SD activity[a]	GGME resistance[b]
1	MEW1	Parent	10[c]	−
2	RG62	*metK62 rbl-62*	71	+
3	MEW26	*rbl*::Tn*10*	96[c]	−
4	MEW33	*rbl-62*	66	−
5	MEW30	*metK62*	11	+
6	MEW31	*metK62 rbl*::Tn*10*	65	+

[a] Expressed as in Table 2.
[b] As judged by growth on glucose minimal medium containing 2 mM GGME. +, Resistance; −, sensitivity.
[c] Value taken from Table 2.

TABLE 6. Selection of cells growing on serine during growth of a *metK* mutant[a]

Step	Culture	No. of cells producing colonies on minimal medium with given carbon source		% Serine utilizers, B/A × 100
		Glucose (A)	L-serine (B)	
a	Inoculum	1.4×10^8	5×10^2	4×10^{-4}
b	Culture 1	6.4×10^7	1.5×10^4	2.5×10^{-2}
c	Culture 2	8×10^8	2.4×10^6	0.3
d	Culture 3	1.6×10^9	1.6×10^7	1.0
e	Culture 4	7.5×10^8	1.4×10^8	19

[a] A recently transduced isolate of strain MEW30 was plated on glucose and serine minimal plates (step a), inoculated into liquid glucose minimal medium at 37°C, grown overnight, and plated on the same two media (step b). It was then subcultured into fresh glucose minimal medium (culture 2) at 0.2 ml/20 ml of culture, and that culture was incubated overnight, plated (step c), and subcultured. The experiment was continued in the same way for cultures 3 and 4. Each subculture allowed approximately eight generations of growth. Columns A and B show the number of cells in 1 ml of each culture at the time of subculture, as judged by colony counts on glucose and serine minimal media, respectively. Glucose was provided at 0.2%. The results given are those of one experiment; a second gave similar results.

The *metK* mutant accumulates derivatives able to grow on L-serine. Strain RG62, like most *metK* strains described, was isolated by resistance to a methionine analog, in this case ethionine (15). It grew a little more slowly than the wild type but was not severely handicapped, especially as used in later studies (25, 26). Since the same *metK62* allele transduced into another strain still conferred slow growth, it seemed likely that the secondary *rbl-62* mutation had arisen during culture of the original *metK* isolate and that the double mutant had been selected by virtue of its faster growth rate.

To determine whether we could reproduce this type of event, we isolated a single colony of strain DRN-1, newly transduced to *metK62* (Ser^+), and grew it in glucose minimal medium, periodically testing the number of cells able to grow with L-serine as sole carbon source. By the fourth subculture (about 30 generations), 19% of the cells could grow on L-serine (Table 6). No such accumulation of L-serine utilizers was observed during growth of the parent strain MEW1. We conclude that an *rbl*-like mutation(s), permitting growth on L-serine, accumulated during cultivation of a *metK* strain in glucose minimal medium.

Although many rapidly growing *metK* derivatives could grow on L-serine, others could not. There is clearly more than one way to restore rapid growth to *metK* strains.

Effects of the *rbl* mutation are not due to high L-SD levels. The *rbl-1*::Tn*10* mutant, selected for increased L-serine degradation, has a highly pleiotropic phenotype. We analyzed several of the phenotypes in strains lacking L-SD activity. A *metK62 rbl-1*::Tn*10 sdaA*::Cm^r triple mutant grew as rapidly as the *metK62 rbl-1*::Tn*10* parent (and much faster than the *metK* single mutant), indicating that the *rbl* mutation could compensate for the slow growth of the *metK* mutant even in the total absence of L-SD activity. Similarly, in the *serA*::*lacZ rbl-1*::Tn*10 sdaA*::Cm^r strain MEW40, *serA* transcription was decreased (Table 3). We conclude that these phenotypes are not secondary effects resulting from L-SD overproduction in *rbl* strains.

DISCUSSION

We have isolated and characterized an *E. coli* mutant selected for its ability to grow with L-serine as sole carbon source and affected in the activities of enzymes involved in several different metabolic pathways. In each case, the enzymes are similarly affected in wild-type strains by the presence of L-leucine in the growth medium, and indeed the phenotype of the mutant is similar to that of wild-type strains cultivated with L-leucine. These observations led us to name the gene *rbl* (for regulation by leucine) and to suggest that it codes for the regulatory element of a new global response, the leucine regulon. The mutant studied, *rbl-1*::Tn*10*, is due to a Tn*10* insertion, presumably resulting in the total absence of any product; this in turn suggests that the effect of exogenous L-leucine is to inactivate the *rbl* gene product.

The *rbl* mutation increased the activities of L-SD and TDH sevenfold (Table 2). The presence of L-leucine during growth stimulated the wild-type activities five- and threefold, respectively, but had essentially no effect on the mutant levels (Table 5). We showed that the effect of *rbl* on L-SD was transcriptional, using an *sdaA*::*lacZ* fusion, which places β-galactosidase synthesis under control of the promoter of *sdaA*, the L-SD structural gene (H. S. Su, personal communication). Similarly, the *rbl* mutation strongly decreased transcription of the *serA* and *ilvIH* operons, coding for phosphoglycerate dehydrogenase and acetolactate synthase III, respectively (Table 3). Both of these operons are repressed in the wild-type strain by growth with L-leucine (27, 45, 46).

Synthesis of the leucine biosynthetic enzymes is known to be decreased in the presence of L-leucine. If this regulation is mediated by the *rbl* gene product, then the *rbl* mutant may have such low levels of these enzymes that L-leucine is limiting for growth and the cells are partially starved. This could explain the apparent paradox that although the *rbl* mutant behaves as though it were constantly in the presence of excess leucine, the addition of L-leucine to the growth medium actually increases the growth rate.

L-Tryptophanase and L-proline oxidase activities were unaffected by the presence of the *rbl* mutation or by the presence of L-leucine in the medium. We previously suggested (12, 32) that L-leucine might trigger increased expression of degradative functions in rich medium, with a concomitant decrease in biosynthetic functions. The scheme is clearly less general than this, since not all catabolic functions are affected by the *rbl* mutation or by L-leucine.

We located the *rbl* gene near 20 min on the *E. coli* genetic map. In the same region is found the *ihb* gene, identified by its effects on expression of the *ilvIH* operon (41). J. M. Calvo suggested to us (personal communication) that the *ihb* gene might code for a general mediator of leucine effects, and we have shown that the two mutations, *rbl* and *ihb*, have quantitatively similar effects on L-SD and TDH activity and on *sdaA*, *serA*, and *ilvIH* transcription (Tables 2 to 4). These observations suggest that *ihb* and *rbl* refer to the same gene.

The *ihb* gene codes for a protein that binds to the regulatory region of the *ilvIH* operon in the absence of leucine but not in its presence (41). Ricca et al. (41) suggested that this protein might be a transcriptional activator of the *ilvIH* operon. If the *rbl*/*ihb* gene product is directly responsible for all effects described in this work, we would extent this hypothesis and propose that in the absence of L-leucine, the Rbl/Ihb protein stimulates the expression of the *ilvIH* and *serA* operons and represses that of *sdaA* (L-SD) and the gene coding for TDH. In this respect, the Rbl/Ihb protein would be reminiscent of another global regulatory protein, the cyclic AMP receptor protein, which, in the presence of cyclic AMP, activates the transcription of some operons and represses that of others (12).

Several other genes in the 20-min region have been identified as regulators of L-leucine-sensitive operons and thus may be related to or identical to *rbl/ihb*. These include *livR* (1, 37) and *oppI* (2, 3). The *livR* gene codes for a regulator of two high-affinity L-leucine transport systems, both of which are repressed in the presence of L-leucine (1, 37) and derepressed in the *livR* mutant. The *oppI* gene product regulates the *oppABCD* operon, which encodes an oligopeptide permease in *E. coli* (2, 3). Leucine has been shown to stimulate transcription of this operon (2).

A surprising aspect of this work is the demonstration that the *metK* mutations, reducing SAM synthetase activity, have more profound effects on cell metabolism than previously expected and that these effects are greatly mitigated by inactivation of the *rbl* gene product. Indeed, we suggest that most commonly studied *metK* strains are in fact double mutants, often *metK rbl*.

The *metK* strain studied here, RG62, is the prototype *metK* strain, isolated by virtue of its ethionine resistance and shown to have less than 3% wild-type SAM synthetase activity when cultivated in the absence of L-leucine (15). It was recently reported that strain RG62 is able to degrade L-serine rapidly, and this was attributed to the *metK* mutation (26). We found that strain RG62 indeed had unusual serine degradation properties: it grew rapidly with L-serine as carbon source and had high levels of L-SD. However, this was not due to the *metK* mutation; these properties did not follow when we transduced the *metK* mutation from RG62 into another strain.

The *metK* transductants, unlike strain RG62, grew very slowly unless provided with L-leucine or suppressed by either the *rbl* or *ihb* mutation. In fact, we showed that RG62 harbors a mutation, *rbl-62*, which resembles *rbl-1* in its location near 20 min, causes L-SD overproduction, decreases *serA* expression, and permits growth on L-serine. Furthermore, during growth in glucose, our slow-growing *metK* transductant rapidly accumulated fast-growing derivatives, many of which had acquired the ability to grow on L-serine (Table 6). We presume that during the purification of the ethionine-resistant RG62, it accumulated an *rbl* mutation, allowing it to grow much faster than the original *metK* strain. It is clear then that RG62, previously characterized as a *metK* mutant, is in fact a *metK rbl* double mutant.

Nonetheless, we have shown previously that other strains derepressed for methionine biosynthesis, including *metJ* mutants, are able to grow slowly with L-serine as carbon source (6). This ability depended on overproduction of the *metC* gene product, L-cystathionase, and was not accompanied by an increase in L-SD (6).

Other phenotypes ascribed to the *metK* mutation may also have to be reconsidered. Matthews and Neidhardt reported that strain RG62 required serine and isoleucine for growth at 44°C (26). L-SD is normally increased at high temperature (35). If this effect is additive with the stimulation due to the *rbl* mutation, then L-serine degradation could be quite severe in RG62 at 44°C. This, coupled with decreased *serA* expression, could explain the serine requirement. The isoleucine requirement could result from the known sensitivity of *E. coli* to L-serine, which is relieved by isoleucine (9, 49).

Strain RG62 has also been reported to display constitutive synthesis of the heat shock protein lysyl-tRNA synthetase II, product of the *lysU* gene, and this property was not corrected by the presence of a cloned *metK*$^+$ gene (25). This finding suggests that the constitutivity may result from the *rbl* mutation in RG62 rather than the *metK* allele. Possibly supporting this idea is the observation that lysyl-tRNA synthetase II is induced by glycyl-L-leucine (17).

Another intriguing mutant is the temperature-sensitive *htrA*::Tn*10* described by Lipinska et al. (22) which, at temperatures above 42°C, could grow in minimal medium only if provided with either L-serine or L-leucine. We are investigating possible interactions of the *rbl* locus in this phenotype.

The extremely slow growth rate of *metK* mutants may be a result of a low pool of SAM leading to problems with methylation reactions. The *rbl* mutation might alleviate these problems by providing a new source of methyl groups or by bypassing the need for some critical methylation reaction. The high level of TDH in the *rbl* mutant suggests that it may derive much of its glycine from threonine (7, 11, 32, 39, 40). If so, it must make less glycine from serine. In this case, the *rbl* mutant may have major alterations in its one-carbon metabolism. Whereas the cell normally produces much of its C_1 units in the conversion of serine to glycine, this strain, with its decreased conversion of serine to glycine, must rely on some other route (33, 36).

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ADDENDUM IN PROOF

J. Calvo and co-workers have isolated mutants that have properties similar to those described for the *rbl* mutants discussed here. We have agreed that the locus mapping near *aroA* and *serC* that affects expression of *sdaA*, *serA*, and *ilvH* and activity of threonine dehydrogenase is assigned the locus designation *lrp* (leucine-responsive regulatory protein).

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