Characterization of *lip* expression in *Salmonella typhimurium*: analysis of *lip::lac* operon fusions

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Strains of *Salmonella typhimurium* which have an auxotrophic requirement for lipoic acid were isolated by mutagenesis with the transposable element *Mu dJ*. The chromosomal location of these insertion mutations was determined to be at 14 map units by bacteriophage P22-mediated cotransduction. The *lip* gene is transcribed in the clockwise direction relative to the *S. typhimurium* genetic map. Strains with *lip::lac* operon fusions were used to characterize the transcriptional activity of the *lip* promoter. Transcription of the *lip* gene is not regulated by catabolite repression or lipoic acid concentration. The data indicate that the *lip* gene product is expressed constitutively at a low level.

Introduction

Lipoic acid is an 8-carbon unbranched fatty acid (octanoic acid) that contains a terminal 6,8-disulphide (dithiolane) functional group. It is enzymically activated through formation of an amide linkage to the ϵ -amino group of a specific lysine residue on the apoenzyme. The terminal disulphide group participates in the acyl transfer reactions of the 2-oxo acid dehydrogenase multienzyme complexes, which metabolize pyruvate and 2-oxoglutarate (Reed & Yeaman, 1987). It also performs a similar acyl transfer function in the cleavage of glycine to ammonia, carbon dioxide and methylenetetrahydrofolate (Kochi & Kikuchi, 1976).

The detailed enzymic mechanism resulting in the synthesis of lipoic acid is unknown, but several key steps in the biosynthetic sequence have been demonstrated. Octanoic acid serves as the immediate precursor for the 8-carbon chain (White, 1980). Sulphur is directly inserted at the C-6 and C-8 positions with inversion of configuration at the prochiral centre. Cysteine appears to serve as the source of sulphur, although it has not been determined whether the two sulphur atoms are inserted simultaneously or sequentially (Dupre *et al.*, 1980).

Previous studies with enteric bacteria have identified a genetic locus (*lip*) that encodes a lipoic acid biosynthetic function (Herbert & Guest, 1968). Mutations at this locus result in an auxotrophic requirement for lipoic acid. The *lip* locus is situated at 14 map units on the *Salmonella typhimurium* chromosome and is cotransducible with the *leuS* and *nadD* genes (Hughes *et al.*,

1983). No direct enzyme assay for the *lip* gene product has yet been developed. Thus, the isolation and characterization of *lip::Mu dJ* (Kan, *lac*) operon fusions and their use to evaluate the transcriptional activity of the *lip* promoter is described in this study.

Methods

Bacterial and bacteriophage strains. The bacterial strains used in this study and their sources are listed in Table 1. All strains are derivatives of *S. typhimurium* LT2. Bacteriophage P22 HT 105/1 *int-201* was used for routine transductional crosses. *Mu dJ* [*Mu dJ11734* (Kan, *lac*)] is a conditionally transposition-defective derivative of *Mu d1* (Casadaban & Cohen, 1979) which forms operon fusions (Castilho *et al.*, 1984).

Culture media. NCE (no carbon E) minimal medium base (Davis *et al.*, 1980), with the addition of D-glucose (14 mM), was used routinely as the defined medium. Alternative carbon sources included acetate (25 mM), succinate (25 mM), pyruvate (25 mM), glycerol (14 mM), maltose (14 mM), lactose (14 mM) and galactose (14 mM). Alanine, cysteine, glycine, serine and uracil were added as needed to defined medium at a final concentration of 0.1–0.5 mM (Davis *et al.*, 1980). Luria-Bertani (LB) broth (Miller, 1972) and NCE medium base with the addition of Casamino acids (Difco, 0.8%) and 5 μ M-lipoic acid served as the complex media. Agar (Difco) was added at a final concentration of 1.5% (w/v) to solidify complex media. Noble Agar (Difco, 1.5%) was added to solidify minimal medium. Antibiotics were added at the following final concentrations to defined and complex culture media respectively: sodium ampicillin 15 mg l⁻¹ and 30 mg l⁻¹, tetracycline hydrochloride 10 mg l⁻¹ and 20 mg l⁻¹, kanamycin sulphate 125 mg l⁻¹ and 50 mg l⁻¹, and streptomycin 1000 mg l⁻¹ and 200 mg l⁻¹. Inorganic chemicals were supplied by J. T. Baker Chemicals and Fisher Scientific. Organic chemicals and biochemicals were supplied by Aldrich, Boehringer Mannheim and Sigma. Restriction endonucleases were supplied by Gibco BRL and Promega.

Table 1. *Bacterial strains*

Strain	Genotype†	Source‡
TR2647	<i>pyrC7 rpsL1/F'</i> _{ts} 114 <i>lac</i> ⁺	J. Roth
TR3933	<i>lip-2 hisD8531</i>	J. Roth
TT6748	<i>pncB185::Tn10</i>	J. Roth
TT7247	<i>zbe-1023::Tn10</i>	J. Roth
TT7252	<i>zbe-1028::Tn10</i>	J. Roth
TT10288	<i>hisD9953::Mu dJ</i>	J. Roth
RT791	<i>hisA9944::Mu d1</i>	J. Roth
RT1140	<i>pro-1931::Tn10d-Tet</i>	
RT1141	<i>zbd-6165::Mu dJ</i>	
RT1142	<i>lip-42::Mu dJ</i>	
RT1142	<i>lip-47::Mu dJ</i>	
RT1146	Δ <i>proAB47/F'</i> _{ts} 128 <i>pro</i> ⁺ <i>lac</i> ⁺	J. Roth
RT1148	<i>DUP2704 [(zbd-6168)*Tn10* (zbe-1028)] lip-46::Mu dJ</i>	
RT1175	<i>cya::Tn10</i>	J. Escalante
RT1182	<i>rpsL1</i>	
RT1184	<i>lip-41::Mu dJ</i>	
RT1185	<i>lip-45::Mu dJ</i>	
RT1186	<i>lip-46::Mu dJ</i>	
RT1187	<i>lip-49::Mu dJ</i>	
RT1208	<i>lip-46::Mu dJ zbe-1023::Tn10</i>	
RT1209	<i>zbd-6169::Tn10d-Tet</i>	
RT1210	<i>lip-50::Mu dJ</i>	
RT1215	<i>lip-47::Mu dJ cya::Tn10</i>	
RT1235	<i>lip-47::Mu dJ cya::Tn10 crp*</i>	
RT1236	<i>lip-47::Mu dJ crp::Tn10</i>	
RT1241	<i>lip-50::Mu dJ pncB185::Tn10</i>	
RT1242	<i>lip-50::Mu dJ pro-1931::Tn10d-Tet</i>	
RT1243	<i>lip-50::Mu dJ pncB185::Tn10/F'</i> _{ts} 114 <i>lac</i> ⁺	
RT1244	<i>lip-50::Mu dJ pro-1931::Tn10d-Tet/F'</i> _{ts} 114 <i>lac</i> ⁺	

† Genetic nomenclature for the chromosomal duplication in strain RT1148 follows the recommendations of Schmid & Roth (1983) and Hughes & Roth (1985). The *crp** mutation makes expression of catabolite-repressed genes independent of cAMP.

‡ Strains for which no source is shown were produced during this study. J. Roth, University of Utah, Salt Lake City; J. Escalante, University of Wisconsin, Madison.

Transductional methods. P22 lysates were prepared on donor strains of *S. typhimurium* as described by Davis *et al.* (1980). Donor bacteriophage and recipient cells were mixed at a multiplicity of infection of 0.01 and spread onto selective medium. Phage-free transductants were isolated by streaking non-selectively on modified green indicator medium (Jeter, 1990). Phage-free clones were checked for P22 sensitivity by cross-streaking with the clear-plaque mutant phage P22 H5.

Mutant isolation. A P22 lysate was grown on strain TT10288 (*hisD9953::Mu dJ hisA9944::Mu d1*) and used to create random *Mu dJ* chromosomal insertions in an LT2 recipient (Hughes & Roth, 1988). Kanamycin-resistant mutants were selected on LB agar supplemented with lipoic acid and kanamycin and were subsequently replica-plated onto NCE Casamino acids agar plates with and without lipoic acid. Colonies which required exogenous lipoic acid for growth were chosen for additional characterization. Mutant strains containing *lip::lac* operon fusions were detected by testing for β -galactosidase activity on LB agar containing 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-Gal) (Silhavy & Beckwith, 1985). *Lac*⁺ fusion strains formed blue colonies.

Cotransductional mapping. *Lip*⁻ strains were transduced to tetracycline resistance with P22 lysates grown on donor strains TT7247

(*zbe-1023::Tn10*) and TT7252 (*zbe-1028::Tn10*). These strains contain *Tn10* transposon insertions that are cotransducible with the *lip* locus (Hughes *et al.*, 1983). Tetracycline-resistant colonies were replica-plated to LB agar with tetracycline and kanamycin and scored for growth (i.e. retention of the *lip::Mu dJ* insertion). Cotransduction frequencies were used to estimate the physical distances between the genetic markers (Wu, 1966).

Formation of duplications. Merodiploid strains containing duplications of the *lip* region were constructed using *Tn10* and *Tn10d-Tet* insertions near the *lip* gene as regions for homologous recombination (Maloy & Roth, 1983). Strain RT1142 (*lip-47::Mu dJ*) was transduced to tetracycline resistance with a P22 lysate grown on pooled cells containing random chromosomal insertions of *Tn10d-Tet* (Way *et al.*, 1984). Transductants were scored for loss of kanamycin resistance and a *Lip*⁺ phenotype (replacement of *lip::Mu dJ* with *lip*⁺) by replica plating. These strains contain *Tn10d-Tet* insertions near the *lip*⁺ allele. Several of these strains were transduced to kanamycin resistance with a lysate grown on RT1208 (*lip-46::Mu dJ zbe-1023::Tn10*). Kanamycin-resistant transductants were scored for retention of the *Lip*⁺ phenotype. Strains that contain both *lip-46::Mu dJ* and *lip*⁺ alleles were presumed to be chromosomal duplications created by homologous recombination between the *Tn10* and the *Tn10d-Tet* insertions. These putative duplication strains were streaked for purification on NCE glucose minimal medium containing tetracycline and kanamycin. Overnight cultures of each strain were grown in LB broth, diluted by a factor of 1×10^8 and spread onto LB agar (no selective pressure). One hundred isolated colonies from each culture were tested for segregation of the *lip*⁺ and *lip*⁻ alleles.

Conjugational methods. Strain TR2647 (*pyrC7 rpsL1/F'*_{ts} 114 *lac*⁺) was inoculated into NCE lactose broth with a uracil supplement and incubated overnight at 30 °C. Strains RT1241 (*lip-50::Mu dJ pncB185::Tn10*) and RT1242 (*lip-50::Mu dJ pro-1931::Tn10*) were grown in NCE glucose medium supplemented with proline and lipoic acid. The *F'*_{ts} *lac* plasmid was transferred by spreading equal volumes of donor and recipient cultures onto NCE lactose agar supplemented with proline and lipoic acid and incubating at 30 °C. Purified *Lac*⁺ colonies from each mating were streaked onto the same medium and incubated at 42 °C to select colonies containing a stable *Hfr* integrate (Chumley *et al.*, 1979). *Hfr* derivatives of RT1241 and RT1242 served as donors in conjugative matings with RT1182 (*rpsL1*). Equal volumes of donor and recipient cells were spread onto NCE glucose medium containing proline, lipoic acid, tetracycline and streptomycin. The Tet^r Str^r colonies from each mating were tested by replica plating for simultaneous inheritance of either an auxotrophic requirement for proline (*ProAB*⁻ phenotype) or resistance to 6-aminonicotinic acid (*PncB*⁻ phenotype).

β -Galactosidase assays. β -Galactosidase enzyme activities were measured by the procedure described by Miller (1972). Specific activities are expressed as nmol *o*-nitrophenol (ONP) formed min⁻¹ per OD₆₅₀ unit. Wild-type *S. typhimurium* LT2 (*Lac*⁻) served as the negative control for all enzyme assays. β -Galactosidase enzyme activities from RT1146 (Δ *proAB47/F'*_{ts} 128 *pro*⁺ *lac*⁺) induced with isopropyl β -D-thiogalactopyranoside (IPTG) served as the positive control. All reported values are the means of five replicate assays. β -Galactosidase activities were compared by analysis of variance to test the hypothesis that they were all equal ($\alpha = 0.05$). Homogeneity of variance was determined by Box's small-sample *F* approximation. Student-Newman-Keuls (SNK) *post-hoc* multiple comparison test was also performed ($\alpha = 0.05$) where indicated.

Lipoic acid deprivation. An overnight culture of strain RT1142 was grown in NCE succinate medium supplemented with 5 μ M-lipoic acid. The culture was washed twice in NCE buffer and resuspended in NCE succinate medium without lipoic acid to an OD₆₀₀ of 0.20. The cultures

were incubated at 37 °C with aeration for an additional 8 h. β -Galactosidase activities were measured in samples taken from these cultures at 2 h intervals.

Results

Isolation and characterization of Lip⁻ mutants

Seven strains which contained chromosomal Mu *dJ* insertions that conferred a Lip⁻ phenotype were isolated independently. These mutants were initially identified by their lack of growth on NCE Casamino acids medium without lipoic acid supplementation. The ability to grow on defined solid medium with alternative carbon sources was also tested. Growth rates of some cultures were determined by measuring the increase in OD₆₀₀ in liquid medium. Exogenous lipoic acid was required under all culture conditions tested, except for anaerobic growth on glucose alone and aerobic growth on glucose supplemented with either lysine and methionine or acetate and succinate, which is in agreement with previously published results (Herbert & Guest, 1968).

Genetic mapping

The *lip* locus is situated at 14 map units on the *S. typhimurium* chromosome (Sanderson & Roth, 1988). Transductional crosses with other genetic markers in the region indicated that all seven of the Mu *dJ* insertions that conferred a Lip⁻ phenotype were located at the *lip* locus. All *lip* alleles tested in this study were 95–100% cotransducible with *zbe-1023::Tn10* and 8–14% cotransducible with *zbe-1028::Tn10*, which is similar to the results of a previous study (Hughes *et al.*, 1983).

Chromosomal direction of transcription

Orientation of the *lip* promoter was established by creating Hfr insertions of the temperature-sensitive plasmid F_{ts}114 *lac*⁺ in strains RT1241 (*lip-50::MudJ pncB185::Tn10*) and RT1242 (*lip-50::MudJ pro-1931::Tn10d-Tet*) and determining the direction of chromosomal transfer in a conjugal mating. The *lac* genes carried by the Mu *dJ* transposon served as a region of homology for recombination between the plasmid and the bacterial chromosome. The orientation of the plasmid-encoded *lac* genes and its relation to the direction of transfer have been described (Maloy & Roth, 1983). The *lip-50::Mu dJ* insertion in the recipient strains confers a Lac⁻ phenotype. RT1241 and RT1242 also contain single insertions of *Tn10* or *Tn10d-Tet* on

either side of the *lip* gene. The direction of transfer was determined by selecting Tet^r transconjugants from each mating and scoring for simultaneous inheritance of the derivative phenotype. Two hundred and seventeen transconjugant colonies inherited *Tn10d-Tet* located in the *proAB* genes (at 7 map units), whereas one colony inherited *Tn10* located in the *pncB* gene (at 20 map units). These data indicate that the *lip* gene is transcribed in the clockwise direction with respect to the *S. typhimurium* genetic map.

Transcriptional activity

Transcription of the *aceEF* and *sucAB* genes, which encode the E1 and E2 subunits of the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes, is repressed by glucose (Spencer & Guest, 1987). This repression is mediated by cAMP and the cAMP receptor protein. Strains containing *lip::lac* fusions were grown in NCE Casamino acids medium supplemented with glucose, cAMP or glycerol, and β -galactosidase levels were measured to determine whether *lip* transcription was regulated by catabolite repression. Enzyme activity in NCE Casamino acids medium without any of these supplements was used as the baseline value. Transcription of *lip* was not affected by the addition of glucose, glycerol or cAMP to the medium (Table 2). Strains containing additional mutations in the *crp* and *cya* genes (both null mutations and *crp*^{*}, which makes expression of catabolite-repressed genes independent of cAMP) were also constructed and tested. β -Galactosidase activities in these strains indicated that these mutations had no effect on *lip* transcription. These data support the conclusion that the *lip* gene does not respond to catabolite repression and is not regulated by cAMP and the cAMP receptor protein.

Transcription of the *aceEF* and *sucAB* genes is also sensitive to high levels of acetate and succinate (Spencer & Guest, 1987). β -Galactosidase activities in *lip::lac* fusion strains were measured in minimal media containing acetate and succinate as sole carbon sources. These compounds had no effect on *lip* transcription (Table 2).

Lipoic acid functions as a cofactor in the conversion of glycine to serine and a one-carbon unit (glycine cleavage reaction) in *Escherichia coli* (Plamann *et al.*, 1983). The effects of glycine and serine supplementation on *lip* transcription were tested. It has been postulated that cysteine serves as the sulphur donor in the synthesis of lipoic acid from octanoic acid. Thus, cysteine supplementation was also evaluated. None of these amino acids had a specific effect on *lip* transcription (Table 2). However, a nonspecific increase in β -galactosidase activity was observed in some cases (see Discussion).

Table 2. β -Galactosidase specific activities of *lip::lac* operon fusions in various culture media

Carbon source in NCE minimal growth medium†	β -Galactosidase specific activity‡ in strain:					
	RT1142 <i>lip::lac</i>	RT1148 <i>lip::lac lip⁺</i>	RT1215 <i>lip::lac cya</i>	RT1236 <i>lip::lac crp</i>	RT1235 <i>lip::lac cya crp*</i>	LT2
<i>Aerobic culture conditions</i>						
Casamino acids (CAA)	54.1 $\pm$ 3.9 ^a	37.0 $\pm$ 1.7 ^b	64.2	41.9	62.1	0.6
CAA + glucose	60.8 $\pm$ 4.4 ^a	27.0 $\pm$ 1.1 ^b	68.5	39.8	62.6	0.0
CAA + glucose + cAMP	54.9 $\pm$ 1.9 ^a	42.3 $\pm$ 3.9 ^b	65.2	ND	ND	0.1
CAA + glycerol	48.8 $\pm$ 1.5 ^a	38.0 $\pm$ 1.1 ^b	63.1	39.8	44.7	0.0
Glucose	30.5 $\pm$ 2.9 ^b	32.0 $\pm$ 6.9 ^b				
Acetate	28.4 $\pm$ 4.3 ^b	21.5 $\pm$ 4.6 ^b				
Succinate	22.7 $\pm$ 3.5 ^b	28.4 $\pm$ 2.5 ^b				
Pyruvate	23.9 $\pm$ 2.6 ^b	24.9 $\pm$ 0.2 ^b				
Glycerol	28.5 $\pm$ 1.4 ^b	28.4 $\pm$ 5.0 ^b				
Glucose + acetate + succinate (no lipoic acid)	23.9 $\pm$ 3.1 ^b	23.4 $\pm$ 0.4 ^b				
Glucose + lysine + methionine (no lipoic acid)	22.9 $\pm$ 3.1 ^b	23.7 $\pm$ 3.4 ^b				
Glucose + serine	44.2 $\pm$ 7.4 ^a	34.0 $\pm$ 3.7 ^b				
Glucose + glycine	54.7 $\pm$ 2.7 ^a	31.9 $\pm$ 3.8 ^b				
Glucose + cysteine	49.0 $\pm$ 2.7 ^a	29.1 $\pm$ 4.6 ^b				
Amino acid pool A	29.5 $\pm$ 3.9 ^b	36.9 $\pm$ 3.9 ^b				
Amino acid pool B	28.6 $\pm$ 3.9 ^b	32.2 $\pm$ 3.9 ^b				
<i>Anaerobic culture conditions</i>						
Glucose (no lipoic acid)	37.8 $\pm$ 3.6 ^b	25.5 $\pm$ 1.5 ^b				
Glucose	38.3 $\pm$ 8.0 ^b	24.7 $\pm$ 4.6 ^b				
CAA (no lipoic acid)	54.9 $\pm$ 1.8 ^a	32.4 $\pm$ 3.2 ^b				
CAA	55.7 $\pm$ 0.7 ^a	37.1 $\pm$ 4.0 ^b				

† All media contained lipoic acid (5 μ M) unless indicated otherwise. Composition of amino acid pool A: methionine, proline, alanine, cysteine, valine, serine, phenylalanine and arginine. Composition of amino acid pool B: histidine, asparagine, glutamic acid, tryptophan, isoleucine, aspartate, leucine, lysine and threonine. Final amino acid concentrations in the culture medium were as described in Davis *et al.* (1980).

‡ β -Galactosidase specific enzyme activities are presented as the mean values of five replicate trials. (ND, Activity not determined.) Standard error and statistical classification are included for strains RT1142 and RT1148. Superscripted *a* and superscripted *b* β -galactosidase activities differed at $P = 0.05$ by Student–Newman–Keuls analysis of ordered means. RT1142 has β -galactosidase values which fall into both the high (*a*) and low (*b*) activity groups, whereas all values from the duplication strain RT1148 only fall into the low activity group.

S. typhimurium does not require lipoic acid for anaerobic growth. However, transcriptional activity of the *lip* gene under anaerobic culture conditions was similar to the aerobic activity in the same medium (Table 2).

The effect of lipoic acid deprivation on *lip* transcription was determined by measuring β -galactosidase activity in strain RT1142 after a shift to lipoic-acid-free medium. Lipoic acid starvation did not induce *lip* transcriptional activity. On the contrary, a decrease in transcriptional activity was observed, which probably reflects a general lowering of metabolism in non-dividing cells. Addition of lipoic acid to the starved cultures resulted in renewed cell division and a corresponding increase in transcriptional activity (Fig. 1).

The effect of excess lipoic acid on *lip* transcription was evaluated by measuring β -galactosidase activity in a merodiploid strain (RT1148) containing both a *lip::lac*

Fig. 1. Effects of lipoic acid depletion on *lip* transcription. □, Δ OD₆₀₀; ●, β -galactosidase activity.

fusion and a *lip⁺* allele within a chromosomal duplication of the *lip* region. The *lip⁺* allele eliminated the requirement for an exogenous lipoic acid supplement by providing a functional gene product. Supplementation with lipoic acid had no effect on *lip* transcription. No

change was observed over a 500-fold increase in exogenous lipoic acid concentration. However, the presence of the wild-type *lip* allele in the merodiploid strain was correlated with a lower level of β -galactosidase activity than in the *lip::lac* haploid strain (RT1142) on certain growth media (see Discussion).

Analysis of variance and *post-hoc* evaluation by Student–Newman–Keuls multiple comparison test indicated that all β -galactosidase activities could be divided statistically into two groups. The members of each group are indicated in Table 2.

Discussion

All known *lip* mutations, including the new Mu *dJ* insertions identified in this study, occur at a single genetic locus (14 map units) on the *S. typhimurium* chromosome. This region is presumed to encode the lipoic acid biosynthetic enzyme that catalyses the incorporation of sulphur into octanoic acid. The location of the gene encoding the lipoic acid-activating enzyme, which attaches lipoic acid to the E2 subunits of the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase complexes, remains unknown.

Several models for the transcriptional regulation of the *lip* gene were tested by measuring β -galactosidase levels in *lip::lac* fusion strains under a variety of culture conditions. The experimental data indicated that transcription initiation from the *lip* promoter does not require cAMP or cAMP receptor protein and is not sensitive to glucose concentration. Thus, the *lip* gene is not regulated by catabolite repression. Growth on acetate, succinate or pyruvate (rather than glucose) as sole carbon and energy sources also had no differential effect. Anaerobic growth conditions did not cause a decrease in *lip* expression. β -Galactosidase enzyme activities in a merodiploid (duplication) strain indicated that excess exogenous lipoic acid does not repress transcription. Thus, end-product repression is not indicated as a mechanism for *lip* regulation. Recently, Ali & Guest (1990) found that overexpression of the E2 subunit of the pyruvate dehydrogenase complex leads to accumulation of the unlipoylated form of the protein. This observation, which suggests that the synthesis of lipoic acid is not induced in response to an increased cellular demand for it, also supports the results of this study.

Statistical analysis of β -galactosidase activities under all growth conditions tested in this study suggests that the *lip* promoter is expressed constitutively at a low rate but that there are two distinct levels of expression (Fig. 2). These levels appear to be related in some way to nutrient availability, but our data do not suggest the exact nature of this relationship. Defined culture media containing a

Fig. 2. Division of β -galactosidase activities into statistical groups. β -Galactosidase specific activity in strain RT1142 (+) and strain RT1148 (O) is plotted. Horizontal bars represent mean activity within each group. Groups differed with $P = 0.05$. Numerical data are presented in Table 2.

single carbon source and lipoic acid produced lower activities. Supplementation of these same media with amino acids which can serve as a source of carbon skeletons increased transcription from the *lip* promoter approximately twofold (Table 2). Growth rates were slower in defined media with a single source of carbon (generation times from 41 to 95 min) than in supplemented media (26 to 35 min). Yet this enhancement of *lip* transcription does not appear to be a simple function of growth rate alone. The growth rates of the haploid strain (RT1142) and the merodiploid strain (RT1148) were identical in each medium tested. Nevertheless, the transcriptional activity in RT1142 increased in supplemented media while the transcriptional activity in RT1148 did not. The chromosomal duplication provides both a functional gene product *in trans* to the *lip::lac* fusion and a second copy of the *lip* promoter. Either or both of these elements might be modulating the level of *lip* transcription, but if so, the molecular mechanism for this interaction remains to be determined.

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