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Evidence That the Majority of Leucine Transfer Ribonucleic Acid Is Not Involved in Repression in *Salmonella typhimurium*

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Leucine transfer ribonucleic acid (tRNA) was almost fully charged, and the isoleucine-valine and leucine enzymes remained derepressed when trifluoroleucine was added to a leucine auxotroph. High levels of charged leucine tRNA and derepression were also found in a leucyl-tRNA synthetase mutant.

Previous reports have shown that many aminoacyl-transfer ribonucleic acid (tRNA) synthetases are involved in repression of their corresponding amino acid biosynthetic enzymes (8). In the case of histidine and valine, it appears that amino acid attachment to tRNA is required for regulation (4, 9). It has recently been reported that leucine must be activated by leucyl-tRNA synthetase to act in repression of the isoleucine-valine and leucine enzymes in *Salmonella typhimurium* (1). The present study was initiated to examine the role of leucine tRNA in repression.

A possible correlation between repression and charged leucine tRNA was tested. A leucine auxotroph of *S. typhimurium leu-130* was grown in minimal medium (2) with excess isoleucine and valine and varying concentrations of leucine. The cells were harvested at the same point in exponential growth and divided into two portions for RNA extraction and for determinations of the level of the isoleucine-valine and leucine enzymes. The amount of charged leucine tRNA was determined by periodate oxidation as described previously (11). A direct correlation was found between the level of charging and repression (Fig. 1). When leucine tRNA was fully charged, the isoleucine-valine and leucine enzymes were repressed. Conversely, these enzymes were highly derepressed when leucine tRNA was minimally charged. Partial repression was found at intermediate levels of charged leucine tRNA.

Additional experiments were performed as these just described, except that various concentrations of 5',5',5'-trifluoro-DL-leucine (fluoroleucine) were added to the growth medium. The addition of low amounts of the analogue increased leucine tRNA charging to 85%. However, the isoleucine-valine and leucine enzymes remained high (Table 1). When the level of fluoroleucine was increased to 50 μg/ml or higher, charged leucine tRNA increased only slightly. Under these conditions, as reported previously (6), the isoleucine-valine enzymes but not the leucine enzymes were repressed.

An additional indication that most of leucine tRNA may not be essential for repression was obtained with a leucyl-tRNA synthetase mutant of *S. typhimurium* MF708. This mutant was isolated as resistant to fluoroleucine and has the same growth rate in minimal medium as the wild type (M. Freundlich, *unpublished data*). The level of charged leucine tRNA was about the same when strain MF708 or wild type was grown in minimal medium (Table 2). However, the isoleucine-valine and leucine enzymes were derepressed 3- to 10-fold in the mutant. The addition of isoleucine decreased charged leucine tRNA by about 12% in both strains and increased enzyme levels in the mutant to almost fully derepressed amounts.

These results indicate that, if leucine tRNA is involved in repression, only a small part of it is necessary for regulation. An alternative explanation is possible if leucyl-tRNA synthetase is part of the repression mechanism, i.e., as a protein repressor (1). Thus, derepression in strain MF708 would result not from the inability of this mutant to charge a minor leucine tRNA species but from an alteration in the synthetase preventing binding to an operator. The ability of small amounts of fluoroleucine to charge leucine tRNA but not cause repression could be a quantitative reflection of a fluoroleucine-adenylate synthetase complex inadequate for repression but

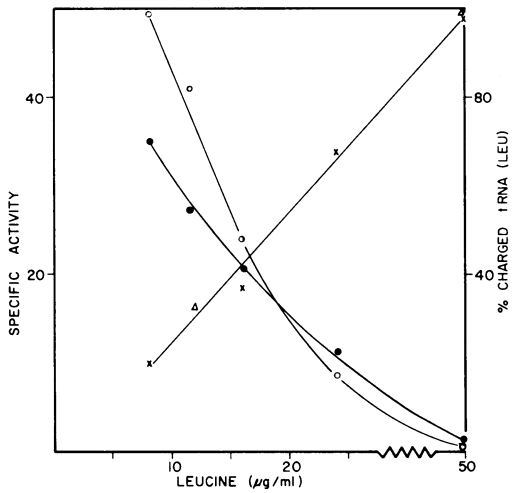

FIG. 1. *Correlation between repression and level of charged leucine tRNA in Salmonella typhimurium leu 130. The cells were grown in minimal medium as described previously (11) with* L-*isoleucine (50 μg/ml),* L-*valine (100 μg/ml), and varying concentrations of* L-*leucine. The cells were harvested at the same point in exponential growth and divided into two portions for RNA extraction and for enzyme determinations. Acetohydroxy acid synthetase (10) and β-isopropylmalate synthetase (5) were determined by methods previously described. Specific activity is expressed as micromoles of product formed per hour per milligram of protein. The extraction and determination of percentage of charged leucine tRNA was as described previously (11), except that the concentration of sodium periodate was 4 × 10⁻³ M. In some experiments (Δ), trichloroacetic acid was added to a final concentration of 5% to stop growth. The amount of charged leucine tRNA then was determined by a procedure suggested by F. C. Neidhardt as described by Folk and Berg (3). Other symbols: acetohydroxy acid synthetase (○), β-isopropylmalate dehydrogenase (●), percentage of charged leucine tRNA (×).*

TABLE 1. *Effect of fluoroleucine on repression and charged leucine tRNA in S. typhimurium leu-130*[a]

FL added to medium[b] (μg/ml)	Leucine tRNA charged[c] (%)	Specific activity		
		Threonine deaminase[d]	Aceto-hydroxy acid synthetase	β-IPM dehydrogenase
0	35	58.4	60.2	30.6
18	80	60.8	77.2	31.0
25	82	57.4	73.5	40.6
75	85	8.6	3.4	42.8

[a] Abbreviations: FL, fluoroleucine; β-IPM dehydrogenase, β-isopropylmalate dehydrogenase.

[b] Minimal medium plus L-isoleucine (50 μg/ml), L-valine (100 μg/ml), and L-leucine (10 μg/ml).

[c] The extraction and determination of percent charged leucine tRNA was as described previously (11), except that the concentration of sodium periodate was 4 × 10⁻³ M.

[d] Threonine deaminase was measured by methods described previously (7). Other experimental conditions were the same as described in Fig. 1. Threonine deaminase and acetohydroxy acid synthetase are isoleucine-valine biosynthetic enzymes. β-IPM dehydrogenase is specific for leucine biosynthesis.

sufficient for aminoacyl-tRNA formation. Although we cannot choose between these alternatives, the data are consistent with the hypothesis that one or two minor leucine tRNA species are required for repression of the isoleucine-valine and leucine enzymes in *S. typhimurium* (6).

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TABLE 2. *Level of charged leucine tRNA and derepression in strain MF708 and wild type*[a]

Additions to minimal medium (μg/ml)	Strain	Leucine tRNA charged (%)	Specific activity		
			Threonine deaminase	Acetohydroxy acid synthetase	β-IPM dehydrogenase[b]
None	MF708	77	50.2	32.0	28.6
None	Wild type	82	12.8	3.1	3.2
Isoleucine 100	MF708	63	84.2	72.8	46.8
Isoleucine 100	Wild type	72	13.6	4.2	5.4

[a] The extraction and determination of percentage of charged leucine tRNA were as described previously (11), except that the concentration of sodium periodate was 4 × 10⁻³ M. For other experimental conditions, see Fig. 1.

[b] β-Isopropylmalate dehydrogenase.

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