Metabolic Regulation of Aminoacyl-tRNA Synthetase Biosynthesis in Bakers' Yeast

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The specific activities of 15 aminoacyl-tRNA synthetases in Saccharomyces cerevisiae were measured after growth under a variety of conditions that produced a range of cell-doubling times. The specific activity of each synthetase increased as cell-doubling time decreased. Control experiments eliminate the possibility that these results are due to preferential recovery of synthetases, or to the presence of activators in the faster growing cultures or inhibitors in the slower growing ones. These observations run counter to the expectation that synthetases in bacteria and yeast are negatively regulated by free amino acids, or, more likely, by aminoacyl-tRNA. In fact, as the growth medium was enriched, generation times decreased, and synthetase and aminoacyl-tRNA levels increased. It is suggested that cytoplasmic aminoacyl-tRNA synthetases may be more or less coordinately controlled such that their response to growth follows the pattern observed for ribosome production and RNA synthesis. This suggests the possibility of coordinated response of genes for components of the protein synthetic apparatus.

Regulation of aminoacyl-tRNA synthetases is of particular interest because of their essential role in the protein synthetic apparatus. Hence, the control of synthesis of these enzymes must have wide ramifications for the synthesis of other proteins and a host of cellular growth processes. Most research on the regulation of enzymes, including aminoacyl-tRNA synthetases has been formulated in the light of the Jacob-Monod Model (1).

Many reports on the regulation of aminoacyl-tRNA synthetase levels have dealt with control of their synthesis in bacteria and focused on the response of synthetase activity to amino acid restriction (2-9). In many cases, some degree of synthetase derepression has been observed when the supply of a required amino acid is restricted. Such findings have been interpreted as indicating that the amino acid or some derivative thereof is involved in the regulation of synthetase production. Some investigations of the control of synthetase biosynthesis have implicated aminoacyl-tRNA as a co-repressor and suggested that an inverse relationship exists between the level of an aminoacyl-tRNA and the rate of synthetase production (5, 6, 10). However, some evidence exists that there is no change in synthetase production during amino acid restriction (3, 8, 11).

Some of the apparent conflict arises out of technical difficulties associated with the experiments. It has been reported that in some cases, an auxotroph restricted for the necessary amino acid produces a transient derepression of the cognate synthetase; this may have been missed by some investigators. Also, restriction has in some cases resulted in an apparent decrease in synthetase levels when in fact, biosynthesis of the appropriate synthetase had increased but so had turnover of that same molecule (12).

The present studies of the control of synthetase production in yeast were undertaken in an effort to make more efficient the isolation of large quantities of synthetase in pure form for enzymatic and structural studies. It soon became apparent that synthetase levels in yeast correlate inversely with the doubling time of the cells. While this work was in progress, Parker et al. (13) reported a correlation between the growth rate of Escherichia coli and Salmonella typhimurium and the levels of two synthetases. They observed a linear relation between the level of valyl- and arginyl-tRNA synthetases in these organisms and the first order rate constant for doubling of the cells, and they termed this pattern metabolic regulation. Our subsequent studies indicate that for the 15 synthetases tested in Saccharomyces cerevisiae, common bakers' yeast, such metabolic regulation occurs, in an apparently coherent manner.

MATERIALS AND METHODS

Reagents—Glass beads (200-μm diameter) (Minnesota Mining and Manufacturing Co.) were cleansed by soaking in concentrated HNO₃ followed by multiple washings with distilled H₂O and oven drying. Yeast extract and peptone were from Baltimore Biological Laboratory. N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid from Calbiochem, radioactive amino acids from New England Nuclear, and all enzymes from Worthington Biochemicals.

Buffers—Buffer A contains 0.05 M phosphate (K⁺), 2 × 10⁻⁴ M EDTA, and 10% (v/v) glycerol, at pH 7.5.

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Cell Lines—Saccharomyces cerevisiae haploid strains Z175d and MG421 were kindly provided by M. Grenson (Université Libre de Bruxelles, Belgium). The latter strain is a leucine auxotroph (leu⁻) isolated from the former, wild type, strain.

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Media and Methods of Culture—All media with the exception of M contained 1% (w/v) yeast extract and 2% (w/v) peptone. In addition, YPD contained 2% (w/v) d-glucose, TPG, 2% (v/v) glycerol, YPS 8% (w/v) sucrose, and YP succ, 5% (w/v) succinic acid. M is the minimal medium described by Kowalski and Fresco (14), but modified to

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A + 2A, the cell-doubling time, \( t_{\text{doubling}} = \frac{\ln 2}{k} \). Hence, k values are collected on a Millipore filter. Radioactivity on the dried filter was excess 5% cold trichloroacetic acid, and the resulting precipitate was upon the reduction of NADP as determined spectrophotometrically mixture for 10 min at 37°C. The reaction was stopped by addition of protein concentration, was incubated with 100 \( \mu \)l of the final reaction depending on the amount required to give a linear response with import, since only the relative activities are of interest in this study.

A control, containing all components except glucose 6-phosphate served as the blank for each assay.

Protein Determination — Protein concentration in extracts was determined by means of the spectrophotometric method of Ehrenmann et al. (15).

Synthetase Assays — Aminoacyl-tRNA synthetase activities were determined from the rate of aminoaoylation of yeast tRNA with the use of \(^{14}C\)- or \(^{15}H\)-labeled amino acids. For assay of each synthetase, a stock solution was prepared containing 1 to \( 10^{-4} \) \( \mu \)M \(^{14}C\)-aminoacid with a specific activity of 30 mCi/mmole (except methionine, which was 11 mCi/mmole) of \(^{15}H\)-aminoacid with a specific activity of 100 mCi/mmole. Each stock solution was then mixed with an equal volume of a solution 80 mM in N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid buffer at pH 7.5, 16 mM in ATP, 16 mM in MgCl\(_2\), 120 mM in KCl, and also containing 20 \( A_{260} \) units/ml of unfracti- nated budding yeast tRNA. Although the Mg/ATP ratio used is not equal to that of all synthetase systems, it was considered of no import, since only the relative activities are of interest in this study. Usually between 1 and 10 \( \mu \)g of protein from a crude extract, depending on the amount required to give a linear response with protein concentration, was incubated with 100 \( \mu \)l of the final reaction mixture for 10 min at 37°C. The reaction was stopped by addition of excess 5% cold trichloroacetic acid, and the resulting precipitate was collected on a Millipore filter. Radioactivity on the dried filter was determined in the toluene system in a scintillation counter.

Glucose-6-Phosphate Dehydrogenase Assay — The assay is based upon the use of NADP as a coenzyme and determined spectrophotometrically at 340 nm (16). In each assay 1.35 ml of 0.055 M Tris buffer, pH 7.8, plus 0.0033 M MgCl\(_2\), 0.05 ml of 6 mM NADP, and 0.2 ml of 0.1 M glucose 6-phosphate were combined in a cuvette. Reaction was started by the addition of between 20 and 100 \( \mu \)g of crude extract protein. Specific activities are expressed as \( A_{260} \) units/min/\( \mu \)g of protein. A control, containing all components except ethanol, served as the blank for each assay.

Alcohol Dehydrogenase Assay — The method relies on the change in absorbance at 340 nm due to the reduction of NAD (17). For each assay 0.8 ml of 0.025 M phosphate buffer, pH 8.5, plus 0.05 ml of 60% ethanol, and 0.2 ml of 2.5 \( \times \) 10\(^{-4} \) M NAD were mixed in a cuvette. Reaction was started by addition of between 2 and 10 \( \mu \)g of crude extract protein. Specific activities are expressed as \( A_{260} \) units/min/\( \mu \)g of protein. A control, containing all components except ethanol, served as the blank for each assay.
others and were examined for only a few aminoacylation activities. Fig. 1 shows for each synthetase the relative specific activity in a series of cultures (expressed as some value relative to that of the fastest growing culture, which was set at 100 for each synthetase) plotted against the first order rate constant, \( k \), for cell doubling in those cultures. It can be seen that the specific activity of each synthetase increased as the generation time of the culture decreased.

It is noteworthy that the experiments with a leucine auxotroph followed the general trend. When this auxotroph was grown under conditions of leucine restriction, i.e. in the presence of leucine and a high concentration of valine, a very low \( k \) value, \( k = 0.097 \text{ h}^{-1} \), correlated with a very low leucyl synthetase specific activity. A stimulation of this synthetase, as might be expected from derepression, did not occur. Moreover, when this mutant was grown without leucine restriction, both large \( k \) values and higher synthetase levels were observed.

### Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Medium</th>
<th>Culture volume</th>
<th>Flask volume</th>
<th>( k )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG421</td>
<td>M + 10^{-5} M leu + 10^{-4} M val</td>
<td>100</td>
<td>300</td>
<td>0.097</td>
</tr>
<tr>
<td>Δ1278b</td>
<td>YPG</td>
<td>100</td>
<td>300</td>
<td>0.195</td>
</tr>
<tr>
<td>M4821</td>
<td>YPD</td>
<td>100</td>
<td>300</td>
<td>0.294</td>
</tr>
<tr>
<td>MG421</td>
<td>M + 10^{-3} M leu</td>
<td>100</td>
<td>300</td>
<td>0.308</td>
</tr>
<tr>
<td>Δ1278b</td>
<td>M</td>
<td>100</td>
<td>300</td>
<td>0.336</td>
</tr>
<tr>
<td>Δ1278b</td>
<td>YPD</td>
<td>100</td>
<td>300</td>
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<tr>
<td>Δ1278b</td>
<td>YPD</td>
<td>200</td>
<td>1000</td>
<td>0.462</td>
</tr>
<tr>
<td>Δ1278b</td>
<td>YPS</td>
<td>100</td>
<td>300</td>
<td>0.226</td>
</tr>
<tr>
<td>Δ1278b</td>
<td>YPG</td>
<td>100</td>
<td>300</td>
<td>0.212</td>
</tr>
<tr>
<td>Δ1278b</td>
<td>YPSuc</td>
<td>100</td>
<td>300</td>
<td>0.198</td>
</tr>
</tbody>
</table>

To test whether the general trend for variation of specific activity of synthetases with growth rate is due to differential recovery of protein during the preparation of crude extracts, the ratio of total soluble protein in each crude extract to wet weight of cells was examined. This ratio was found to be essentially constant for all cultures regardless of growth rate. Moreover, as can be seen in Fig. 2, the levels of alcohol dehydrogenase and glucose-6-phosphate dehydrogenase do not vary greatly with generation time. All these results indicate that the extraction procedure is not responsible for the observed variation of specific activities with growth rate.

The possibility that there are synthetase inhibitors in the slow growing cells or activators in the fast growing cells (or both), was also examined. When portions of crude extracts from slow growing and fast growing cells were mixed, the activity was found to be additive. Taken together, these results indicate a real variation of aminoacyl-tRNA synthetase level with growth rate that we believe reflects synthetase...
been reported (24-26). These conflicting observations place the cultures of mutant MG421, and open
tinction (3, 8, 11, 22, 23), and a few instances of restriction of non-
tRNA was not identical with that used for the other four

tRNA's and aminoacylated-tRNA's.  

Dependence of tRNA and Aminoacyl-tRNA Levels on Growth Rates—An examination was made of the levels of
total tRNA^{16}u and aminoacylated and deacylated tRNA^{16}u in cells with widely different generation times. In all three cases
examined, as expected (21), the levels of total tRNA^{16}u and leucyl-tRNA^{16}u were lower in the slower growing cells (Table II). In each case, one culture of slow growing cells and one of
fast growing cells were examined in parallel. Since the last
two cultures in Table II were prepared and examined on
different occasions, the enzyme used to measure the levels of
tRNA was not identical with that used for the other four
cultures; however, this is of no consequence since it is only the
general trend that is important. It is clear from these data that the faster growing cells always had a higher concentration of
tRNA^{16}u and aminoacylated-tRNA^{16}u.

**DISCUSSION**

In most investigations of the control of synthetase produc-
tion, the levels of synthetase were examined after amino acid
restriction (2-11). Derepression in response to amino acid re-
striction implicates the amino acid or some derivative thereof
as the functioning corepressor. Such results have led to the
suggestion that in bacteria at least 10 synthetases respond to
amino acid-mediated repression (12, 13) and dismissed for the valyl and
arginyll synthetases of *Escherichia coli* and *Salmonella typhi-
murium*, because one enzyme is located near the origin and the other near the terminus of DNA replication in these
organisms. Although there is little information concerning the
mode of DNA replication in relation to the growth rate of yeast
(97), the likelihood that multiple synthetase gene copies are the basis for the metabolic regulation of synthetases observed seems remote.

In view of the various control experiments performed, it is
most unlikely that the observed correlation of synthetase level
with growth rate could be due to preferential recovery of these
enzymes from the faster growing cultures. Furthermore, cer-
tain enzymes that are probably constitutively synthesized (28-
30) and are not involved in protein synthesis are present at the
same levels in all extracts regardless of the culture’s growth
rate, and, therefore, are not subject to metabolic regulation.

The possibility that mitochondrial synthetases gave rise or
contributed to the higher activities found in fast growing cells
when compared to slower growing cultures is also not tenable. Glucose was used as carbon source in six cultures (Table I),
and a seventh employed sucrose, and both these sugars are
fermentable carbon sources that result in poor mitochondrial
development (31-33). Moreover, the number of mitochondria
in haploid strains of yeast is usually between 7 and 17 (34), and
mitochondrial synthetases are generally specific for mitochon-
drial tRNAs (22, 35). Hence, any contribution to synthetase
activity by mitochondrial enzymes must be negligible.
The work presented in this paper demonstrates that the levels of at least 15 aminoacyl-tRNA synthetases inSaccharomyces cerevisiae increase as the growth rate increases, and that these higher levels are most likely due to an increase in the actual number of synthetase molecules, as well as the proportion of the total protein that they represent. It would appear, therefore, that the increase in synthetase levels is due to increased transcription and translation of the synthetase genes. Although the alternative possibility that these levels reflect an inverse relationship between cell growth rate and preferential degradation of synthetases has not been excluded, it seems most unlikely. Indeed, for two synthetases in S. typhimurium, this possibility has been eliminated (13).

The factors which affect the rate of cell growth are presently ill defined. Certainly, the control of the various processes associated with growth must be complex, and they must be linked in some unknown way to the mechanism responsible for the metabolic regulation of aminoacyl-tRNA synthetases we have shown here. All 15 synthetases showed the same qualitative response to growth rate, though the threshold for cell growth rate necessary to elicit a response in synthetase level seemed to vary. Nevertheless, what is striking and new in this study is the evidence that the level of many and possibly all cytoplasmic aminoacyl-tRNA synthetases must be more or less coordinately controlled. This is not inconsistent with the finding that different synthetases are organized into aggregates in eukaryotes (36, 37). This response of synthetases to growth also seems to follow the pattern that has been observed for ribosome production and RNA synthesis (21). This suggests the possibility of coordinated response of genes for components of the protein synthetic apparatus, perhaps by some compound(s) with a role in protein synthesis (such as guanosine tetraphosphate (38, 39), or a protein factor with an essential role). Alternatively, there is the possibility that metabolic regulation is merely the result of the increased accessibility of relevant portions of the genome to transcription during DNA replication. In any event, our results suggest that reduced levels of aminoacyl-tRNA are not principally responsible for the increased levels of synthetases.

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