Temperature-Dependent Variation in the Extent of Methylation of Ribosomal Proteins L7 and L12 in *Escherichia coli*

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The amount of $\epsilon$-$N$-monomethyllysine in ribosomal proteins L7 and L12 in *Escherichia coli* is dependent upon the cell growth temperature. At 37°C or above, very small amounts were detected. Dramatic increase in the content of $\epsilon$-$N$-monomethyllysine in these proteins was observed when the growth temperature was lowered.

In 1972, Terhorst et al. (9) reported that both ribosomal proteins L7 and L12 from *Escherichia coli* MRE600 contain approximately 0.5 molecule of $\epsilon$-$N$-monomethyllysine (MML) per molecule of protein. Since then, we have carried out extensive studies on the methylation of ribosomal proteins from different *E. coli* strains (2) and were unable to confirm this. Only very small amounts of MML ($\leq$0.05 molecule per molecule of protein) were detected in either protein (2). The biological significance of methylation is unclear at present. Since proteins L7 and L12 are involved in a number of factor-dependent hydrolyses of GTP during protein biosynthesis (1, 3, 4), it has been suggested that they may constitute a receptor site on the ribosome where the soluble factor-GTP complexes are bound (6, 8). In an effort to investigate the function of methylation of ribosomal proteins, it is of importance to clarify the presence of MML in proteins L7 and L12.

In all our previous studies, cells were grown at 37°C in a minimal medium supplemented with [methyl-$^{14}$C]methionine and harvested at late log phase (2). Proteins L7 and L12 were separated by two-dimensional polyacrylamide gel electrophoresis, extracted, and hydrolyzed. High-voltage paper electrophoresis showed only very small amounts of MML ($<$0.1 molecule per molecule of protein) detected at 37°C (Fig. 1). Surprisingly, when the growth temperature was lowered, the amounts of MML increased dramatically (Fig. 1). A summary of the amounts of MML in proteins L7 and L12 from cells grown at different temperatures is presented in Table 1. When the growth temperature is 27°C, proteins L7 and L12 contain approximately 0.6 molecule of MML per molecule of protein. As the growth temperature is increased, there is a concomitant decrease in the amounts of MML in both proteins L7 and L12. MML was also identified by extracting the MML regions from the paper electropherogram and subjecting them to descending paper chromatography in a buffer system containing pyridine-acetone-3 M NH$_4$Cl (10:6:5, vol/vol). Under these conditions only MML was detected (data not shown).

Several possibilities could account for the presence of large amounts of MML in proteins L7 and L12 from cells grown at low temperatures. First, since the rate of protein synthesis is slower at low temperatures (e.g., 27°C), the ribosomes that are synthesized at lower temperatures may have a different configuration as compared to those that are synthesized at a higher temperature (e.g., 37°C). The methylation of proteins L7 and L12 may be required so that these ribosomes can function at low temperatures. Alternatively, the slower rate of ribosome assembly at lower temperature may allow proteins L7 and L12 to be exposed for a longer time and result in methylation by a protein methylase. It is also possible that at higher temperatures the protein methylase is not functioning properly and results in the production of less MML in proteins L7 and L12. Conversely, a methyl-removing enzyme may be active at higher temperatures and promote the removal of the methyl groups from MML of proteins L7 and L12. Thus the amounts of MML in each protein could be regulated by the enzymes. Experiments are currently underway to differentiate the above possibilities. It will also be of interest to investigate whether similar variation in the amount of MML in proteins L7 and L12 also exists during the cell growth cycles under different nutritional conditions.

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and Wittmann

NOTES

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1200

100

Fig. 1. Temperature-dependent variation in the extent of methylation of protein L7. E. coli JC355 cells were grown at various temperatures in a minimal medium (2) containing 14 μg of all 20 amino acids per ml except methionine. The concentration of [methyl-14C]methionine was 5 μg/ml. Cells were harvested at late log phase by chilling quickly to 0 to 4°C by shaking for approximately 5 s in a dry ice-acetone bath. A 30-ml volume of carrier cells which had been similarly processed was added to the above labeled cells, and the mixture was centrifuged at 12,000 rpm for 5 min. The cells were stored at -70°C. To isolate the ribosomes, cells were suspended in 4 ml of a buffer containing 0.01 M tri(hydroxymethyl)aminomethane-hydrochloride (pH 7.8), 0.01 M MgCl2, 0.05 M KCl, and 3 μg of deoxyribonuclease per ml, and were disrupted with a French press at 0 to 4°C. The extracts were then centrifuged at 16,000 rpm for 30 min to remove the cell debris and unbroken cells. The supernatants were then centrifuged at 40,000 rpm for 2 h to pellet the ribosome. After suspending the pellets in the above buffer without deoxyribonuclease, 70 absorbance units at 260 nm of ribosomes was added, and ribosomal proteins were prepared by the rapid addition of 2 volumes of glacial acetic acid by the procedure of Hardy et al. (5). The supernatant protein solutions were lyophilized to dryness and dissolved in 0.15 ml of the sample gel solution used for the first-dimension run of the two-dimensional polyacrylamide gel electrophoresis procedure of Kalschmidt and Wittmann (7). After electrophoresis and staining of the gel, protein L7 was cut out of the gels, extracted, and hydrolyzed as described previously (2). MML was separated from methionine and its oxidized products by electrophoresis at 1,200 V for 40 min in 0.05 M sodium borate buffer (pH 9.3). After electrophoresis, 1-cm strips were counted in a scintillation counter using a toluene-based scintillation fluid (Formula 949, New England Nuclear). Zero indicates the origin of sample application.

Table 1. Variation of MML in proteins L7 and L12 from E. coli JC-355

<table>
<thead>
<tr>
<th>Growth temp (°C)</th>
<th>Molecules of MML/molecule of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>L7</td>
</tr>
<tr>
<td>30</td>
<td>L12</td>
</tr>
<tr>
<td>32.5</td>
<td>L7</td>
</tr>
<tr>
<td>37</td>
<td>L12</td>
</tr>
<tr>
<td>40</td>
<td>L7</td>
</tr>
</tbody>
</table>

a The amount of MML in each protein was determined as described in the legend of Fig. 1. Since protein L12 comigrated with protein S6, in the above calculations we assumed equal amounts of each protein were present.

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LITERATURE CITED