THE EFFECTS OF TRIMETHOPRIM AND ERYTHROMYCIN ON 
POLYSOME METABOLISM IN ESCHERICHIA COLI

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Trimethoprim and erythromycin were shown to have different overall effects on in vivo 
polysome metabolism in Escherichia coli. In a rel A+-rel A pair of strains, trimethoprim 
treatment induces a reduction of polysome level to a variable extent, similarly to ammonoacyl-
tRNA deprivation of cells, but persisting polysomes remain dynamic structures in a state of 
continual turnover. In contrast, erythromycin stabilizes polysome level to a high value in 
either kind of strain, but maintained polysomes appear as "frozen" structures unable to 
undergo ribosome translocation.

Numerous studies have been made, at the molecular level, of the mode of action and specificity of 
trimethoprim and erythromycin, two inhibitors of bacterial protein synthesis\(^1,2\). Most results agree 
to indicate that these two drugs act at different steps of polypeptide formation. Indeed, trimethoprim 
has been shown to be involved in the initiation step by restricting the formylation of methionyl-
tRNA\(_{\text{F}}\)\(^3,4\), whereas erythromycin inhibits the elongation step by binding to the larger subunit of 
ribosomes\(^5,6\). Little is known, however, about their overall effects on the metabolism of the protein 
biosynthetic machinery in the whole cells, i.e., the polysomes.

Such effects were therefore analyzed in the present work. Firstly, the level of polysomes was 
measured in in vivo inhibitor-treated bacteria and compared to that in untreated cells. Secondly, 
the metabolic stability of polysomes was determined by studying, on the one hand, the process of 
their decay and, on the other hand, their ability to assemble at the expense of monosomes.

In addition, since it has been shown previously that polysome metabolism in procaryotes under 
protein synthesis inhibition depends on the allelic state of the rel A gene\(^7-11\), the effects of trimethoprim 
and erythromycin were examined in both a rel A\(^+\) (stringent) strain and a rel A (relaxed) strain of 
E. coli. The behaviour of polysomes under aminoacyl-tRNA deprivation of the same pair of strains 
was studied as a control throughout experiments thereafter described.

Materials and Methods

Bacterial strains and culture conditions

The previously described\(^11\) otherwise isogenic pair of E. coli strains 10B6 rel A\(^+\) and 10B6 rel A was 
used in all experiments. These strains were kindly supplied to us by Dr. S. OSAWA, Hiroshima, 
Japan. They both are arginine auxotrophs and they harbor a temperature-sensitive valyl-tRNA 
synthetase (restrictive temperature: 42°C).

Cells were grown exponentially at 30°C under forced aeration in a medium at pH 7.6 con-
taining the following components per liter: 12 g Tris; 2 g KCl; 2 g NH\(_4\)Cl; 0.5 g MgCl\(_2\)-6H\(_2\)O; 0.02 g 
Na\(_2\)SO\(_4\); 14.7 mg CaCl\(_2\)-2H\(_2\)O; 178 mg Na\(_2\)HPO\(_4\)-2H\(_2\)O; 4 g glucose; 50 mg each of the 20 L-amino 
acids; 40 mg adenine-HCl; 40 mg cytosine; 40 mg guanine-HCl; 40 mg uracil; 40 mg thymine; 5 mg 
vitamin B\(_1\).
Glucose starvation
In some experiments, bacteria were grown in the presence of a limiting amount of glucose (0.2 g/liter). Under these conditions, exponential growth was allowed to approximately $4 \times 10^8$ cells/ml. At this point, the culture quickly stopped growing due to the exhaustion of the carbon source from the medium.

Preparation of cellular lysates
The method of preparation was similar to that described by Klagsbrun and Rich. In a typical experiment, 100 ml of culture were poured over crushed ice in the presence of 100 μg/ml chloramphenicol to prevent ribosome run-off during the extraction procedure. All subsequent operations were carried out at 4°C. Cells were harvested by centrifugation for 8 minutes at 10,000 × g. The pellet was resuspended in 12 ml of a sucrose-buffer solution containing 0.5 M RNase-free sucrose, 0.016 M Tris-HCl buffer at pH 8.1, and 0.05 M KCl. Then 1.5 ml of freshly dissolved lysozyme solution (10 mg/ml in sucrose-buffer solution) and 0.3 ml of 10% (w/v) EDTA at pH 8.0 were added to produce protoplasts. The suspension was stirred for 10 minutes and 0.3 ml of 1 M MgCl₂ was added to stop lysozyme action. Protoplasts were centrifuged for 6 minutes at 12,000 × g and resuspended in 0.7 ml of freshly prepared lysing medium (0.05 M NH₄Cl, 0.01 M MgCl₂, 0.01 M Tris-HCl, pH 7.8) containing 0.5% Brij-58, 0.5% sodium deoxycholate and 5 μg/ml RNase-free DNase. After 10 minutes, the lysate was clarified by centrifugation for 10 minutes at 15,000 × g. The supernatant fraction was carefully removed and analyzed by sucrose density gradient centrifugation.

Sucrose gradient analysis
Volumes of 0.2–0.4 ml of clarified lysate were layered onto 15–40% linear sucrose gradients prepared in 0.05 M NH₄Cl, 0.01 M MgCl₂ and 0.01 M Tris-HCl, pH 7.8, buffer solution. Gradients were centrifuged in a Beckman (Palo Alto, California) SW 41 Ti rotor for 150 minutes at 39,000 rev/min, then pumped through the continuous-flow cell of a recording spectrophotometer which monitored the optical density at 260 nm.

Quantitative estimation of polysomes
The amount of ribosomal material present in polysomes was determined by measuring the relevant area under the absorbancy tracing after subtraction of the background provided by blank gradients. It was then expressed as a percentage of the total amount of ribosomal material (polysomes + monosomes + ribosomal subunits) taken as 100%.

Antibiotics and chemicals
Chloramphenicol and rifampicin were purchased from Boehringer Mannheim Gmbh. Erythromycin and trimethoprim (2,4-diamino-5[3,4,5-trimethoxybenzyl]-pyrimidine) were from Sigma Chemical Co.

RNase-free sucrose was obtained from Mann Research Labs. All other chemicals used were analytical grade from Merck Co. or Sigma Chemical Co.

Results and Discussion
In a first set of experiments, polysome level was measured in both rel A⁺ and rel A strains under various conditions of protein synthesis inhibition, and was compared to that in exponentially growing cells taken as a control. All measurements were made after a 15-minute period of inhibition. It is shown in Table 1 that, in all cases, polysome level is reduced under trimethoprim treatment (25 μg/ml). The reduction is however much more extensive in the rel A⁺ strain than in the rel A strain. This differential effect can be related to that observed when protein synthesis is blocked by the temperature inactivation at 42°C of valyl-tRNA synthetase, since then a drastic loss of polysomes occurs only in the rel A⁺ strain. It thus seems that, whatever the strain, trimethoprim-treated cells behave essentially like aminoacyl-tRNA deprived cells as far as polysome maintenance is concerned.

A quite different situation is found in cells treated with erythromycin (1 mg/ml). Indeed, in
either strain, not only polysome level is totally maintained but it is even somewhat increased over the control value. One possible explanation for this phenomenon could be that, while polypeptide elongation is blocked in the presence of the drug, a significant amount of previously free ribosomes can still attach to the messenger RNA.

No substantial variation in the proportion of persisting polysomes is observed when either kind of cells are treated simultaneously by trimethoprim and erythromycin. It thus appears that trimethoprim impairs the above-mentioned formation of additional polysomes found in the presence of erythromycin alone.

In order to check whether or not polysomes which are maintained in the absence of protein synthesis are metabolically stable structures, rifampicin, an inhibitor of RNA synthesis\(^{14}\), was added (250 \(\mu g/ml\)) to cells previously treated for 15 minutes with trimethoprim or erythromycin. Polysome level was then measured after 20 minutes of rifampicin action. It appears, in Table 1, that such treatment results in a complete breakdown of polysomes in cells pre-treated with trimethoprim. Here again, similar results are obtained in valyl-tRNA deprived cells at 42°C. In contrast, polysome level is only slightly affected in cells pre-treated with erythromycin, which indicates that, in the presence of this drug, polysomes behave like “frozen” structures due to the blocking of ribosome translocation along the messenger RNA\(^ {15}\).

Since, on the one hand, polysomes are degraded in trimethoprim-treated cells and, on the other hand, their level is however maintained to a relatively high value, one must assure that they are continuously re-synthesized. To check this point, the process of their assembly under drug treatment was therefore studied. Cultures of either strain were grown exponentially with a limiting amount of glucose then subjected to glucose starvation for 90 minutes. Under these conditions polysomes were

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Strain 10B6 rel A(^ +)</th>
<th>Strain 10E6 rel A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control at 30°C</td>
<td>46 (100)</td>
<td>56 (100)</td>
</tr>
<tr>
<td>Shift to 42°C</td>
<td>17 (37)</td>
<td>56 (100)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>19 (41)</td>
<td>35 (63)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>56 (122)</td>
<td>65 (16)</td>
</tr>
<tr>
<td>Trimethoprim + erythromycin</td>
<td>44 (96)</td>
<td>57 (102)</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0 (0)</td>
<td>&lt;3 (&lt;5)</td>
</tr>
<tr>
<td>Shift to 42°C + rifampicin</td>
<td>0 (0)</td>
<td>9 (16)</td>
</tr>
<tr>
<td>Trimethoprim + rifampicin</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Erythromycin + rifampicin</td>
<td>38 (83)</td>
<td>47 (84)</td>
</tr>
</tbody>
</table>

The percentage of polysomes relative to the control is given in brackets for each strain.

Table 2. Percentage of re-assembled polysomes in cells pre-starved for glucose.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Strain 10B6 rel A(^ +)</th>
<th>Strain 10E6 rel A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose starvation</td>
<td>&lt;3 (&lt;6)</td>
<td>&lt;3 (&lt;5)</td>
</tr>
<tr>
<td>Control at 30°C</td>
<td>52 (100)</td>
<td>62 (100)</td>
</tr>
<tr>
<td>Shift to 42°C</td>
<td>16 (31)</td>
<td>66 (106)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>23 (44)</td>
<td>57 (92)</td>
</tr>
</tbody>
</table>

The percentage of polysomes relative to the control is given in brackets for each strain.
converted into monosomes\textsuperscript{5,16}. Such polysome-free cells were then treated with trimethoprim and their polysome content was determined after 15 minutes following glucose readdition. It is shown, in Table 2, that polysomes can re-assemble partially in the rel A\textsuperscript{+} strain and almost totally in the rel A strain. In each case the extent of assembly is similar to that observed in valyl-tRNA deprived cells. One can thus conclude that polysomes which are maintained under trimethoprim treatment are actually dynamic structures which undergo continual turnover, like polysomes of amino acid starved cells\textsuperscript{7,11}. However, whether the attachment of ribosomes to messenger RNA, under these conditions, occurs only at physiological sites or at other sites as well, has still to be determined. This problem is currently under investigation in our laboratory.

Acknowledgement

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References