Translational Repression in the Arginine System of
Escherichia coli*

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Abstract. Translation of bacterial mRNA, divorced from transcription, has
been obtained for enzymes of arginine synthesis; evidence has been acquired for
repression by arginine at the level of translation. mRNAs for acetylornithinase
and ornithine transcarbamylase were accumulated by arginine starvation of argR+
and argR− arginine auxotrophs derived from Escherichia coli K12. Further
transcription was inhibited with rifampicin or miracid D, and enzyme formation
was measured in the presence of either an excess of, or a restricted supply of,
arginine. For the argR+ strain 961, little mRNA was found without starvation;
for the argR− strain 977, a considerable amount of mRNA was demonstrated
even without starvation. There was relatively little translation for the argR+ strain,
but not for the argR− strain, in the presence of excess arginine, apparently
due to an accelerated degradation of mRNA in the argR+ strain under repressive
conditions.

Translational models for the regulation of the synthesis of arginine enzymes
have been considered since the development of the repression concept.1−5 By
and large, though, the idea of translational regulatory mechanisms in bacteria has
recently received relatively little attention, perhaps because of the demonstration
of transcriptional control through gene-affined repressors in such intensively
studied cases as that of the Lac system in Escherichia coli.6 Nevertheless,
translational repression has remained an attractive possibility for enzymes of
bacterial amino acid synthesis and possibly for a broader spectrum of proteins
(see ref. 7).

In the present experiments, the formation of mRNA has been separated from its
translation, and a reduction in translation under repressive conditions has been
demonstrated.

Materials and Methods. Strains: The organisms used are related to strain 619
(his−ile−met−), a derivative of E. coli K12 (ref. 8). Strain 961 (argR+) is an arginine
auxotroph derived from strain 619 (refs. 9, 10) and strain 977 (argR−, ref. 10) is a his+
recombinant of strain 961, which had received the argR− gene from the Hfr strain 3134
(ref. 8). The arginine auxotrophy of these two strains is caused by a mutation that maps
in the region of the four-gene cluster of the arginine system and is pleiotropic, affect-
ing the level of three out of the four enzymes specified by the clustered genes.10
The auxotrophs do not grow without an added arginine source, but grow at the
wild-type rate on minimal medium supplemented with arginine (and the other required
growth factors); with ornithine in the medium rather than arginine, strains 961 and 977 grow slowly, reflecting a restricted rate of arginine formation. This arginine restriction is due to the low level of argininosuccinase in the auxotrophs and, for strain 961, leads to derepressive conditions.

**Media:** Glucose–salts medium,\(^1\) supplemented with L-histidine hydrochloride, L-isoleucine, and L-methionine at 0.1 mg/ml, is used as suspension medium. L-Arginine hydrochloride or L-ornithine hydrochloride is added, as indicated.

**Cultivation:** The organisms are cultivated, at 37°C with aeration, in suspension medium with added arginine. This culture is used to inoculate a fresh portion of the same medium so as to give a turbidity corresponding to a reading of 10 in a Klett-Summer colorimeter (no. 66 filter); growth is allowed to proceed to a reading of 60. The cells are collected by centrifugation and introduced into suspension medium at 37°C, to give a colorimeter reading of 60.

**Preparation of cell extracts:** Following the experimental procedures described under **Results**, 10-ml samples of the suspensions are mixed with 2 ml of aqueous chloramphenicol (2.5 mg/ml) and immediately chilled in an ice–water bath. The cells are collected by centrifugation, suspended in 2 ml of cold 0.1 M potassium phosphate (pH 7.0) containing 1 mM glutathione, and disrupted in an MSE, 100 W, ultrasonic disintegrator with chilling. The resulting extracts are used for enzyme and protein assays.

**Assays:** For acetylornithinase (N-acetyl-L-ornithine amidohydrolase, EC 3.5.1.16), the assay and enzyme unit of Vogel and Bonner are used.\(^1\)\(^1\) For ornithine transcarbamylase (carbamoylphosphate:L-ornithine carboxamidomtransferase, EC 2.1.3.3), the assay method is that of Jones, Spector, and Lipmann,\(^1\)\(^3\) with color developed according to Prescott and Jones.\(^1\)\(^4\) One unit of activity is defined as the amount of transcarbamylase that will bring about the formation of 0.1 \(\mu\)mol of citrulline on incubation of the enzymatic reaction mixture at 37°C for 10 min. Protein is determined according to Lowry et al.\(^1\)\(^6\)

**Results.** The design of the experiments included accumulation of messenger RNA for arginine enzymes by starving the auxotrophs of arginine, inhibition of further transcription with rifampicin or miraclin D, and measurement of enzyme formation as a function of (a) arginine excess or restriction and (b) the presence of a wild type \((argR^+\)\) or mutant \((argR^-\)\) regulatory gene in the organisms. This regulatory gene governs the repressibility of all eight enzymes of the arginine biosynthetic pathway; in the organism carrying the mutant regulatory gene, all the enzymes of this path are produced at derepressed levels.

**Accumulation of arginine messages and effectiveness of rifampicin:** Fig. 1 presents evidence for the accumulation of ornithine transcarbamylase mRNA as a result of arginine starvation of the \(argR^+\) strain 961. After the addition of rifampicin, the accumulated message is detected by translation (followed for 40 min) in the presence of ornithine (which is a restrictive source of arginine, see Materials and Methods). It is seen that increasing amounts of message (corresponding to the plateau values of each curve) accumulate with increasing duration of starvation, up to 30 min, beyond which no appreciable further increases in mRNA are found. Starvation of arginine also leads to the accumulation of messages for other enzymes of the arginine system, including acetylornithinase.

The 0-min starvation curve illustrates the effectiveness of rifampicin, under the conditions used. For this curve, which reaches a plateau by 20 min of incubation, the net increase in enzyme is 15 units/mg protein. This value is small compared with the control value of 320 units/mg, obtained in the absence of rifampicin. The net value of 15 units/mg partly reflects the amount of message present in the (arginine-repressed) cells at 0-time, and presumably is largely ac-
counted for by the completion and translation of messages initiated before the addition of rifampicin. The plateau values in themselves indicate cessation of transcription. The effectiveness of rifampicin (see, for example, refs. 16–18) in the present case is further supported by experiments with EDTA-treated cells (see ref. 18): With such cells, rifampicin, at $20 \mu g/ml$, gives essentially the same results as those obtained with rifampicin, at $60 \mu g/ml$, in untreated cells. Moreover, experiments with miracid D,19 at $40 \mu g/ml$, in place of rifampicin, again give closely similar results.

**Translation of accumulated acetylornithinase message as a function of excess or restriction of arginine in argR+ and argR− strains:** The upper curve in Fig. 2A represents the formation of acetylornithinase from accumulated message, in the argR+ strain, under conditions corresponding precisely to those for ornithine transcarbamylase, as shown in Fig. 1 (top curve). A considerable accumulation of message is indicated, for translation in the presence of ornithine (under arginine restriction). In contrast, the extent of translation is greatly reduced when it proceeds in the presence of an excess of arginine. A very different picture is obtained in similar experiments with the argR− strain: As seen in Fig. 2B, translation in the presence of arginine does not result in a lower extent of translation than that obtained with ornithine. It is inferred that the repressor specified by argR is needed for the reduced translation with arginine in the argR+ strain.

**Translation of accumulated ornithine transcarbamylase message under various conditions:** In Table 1, data for ornithine transcarbamylase are given. The results obtained with arginine starvation parallel those for acetylornithinase. Without starvation, strain 961 (argR+) exhibits low extent of translation in the presence of arginine or ornithine. Strain 977 (argR−), on the other hand, shows relatively large extents of translation, whether starved or not. For the transcarbamylase, as for acetylornithinase, translation with arginine does not give lower values than that with ornithine in strain 977.

To test for the accumulation of transcarbamylase message under conditions of physiological derepression, strain 961 was treated as if for a 30-min starvation experiment, except that, during what would have been the starvation period,
l-ornithine hydrochloride (100 μg/ml) was provided; this allows some growth under derepressed conditions. After 30 min, rifampicin was added; the experiment was concluded in the usual manner. mRNA accumulated at relatively high concentration, as measured by translation in the presence of ornithine. When arginine was present, however, the extent of translation was considerably lower; this indicates persistence of the functional repressor during partial derepression.

Evidence for degradation of message under repressive conditions: The results of Fig. 2A, in relation to extent of translation, suggest that arginine either interferes with translation or promotes the disappearance of functional message or both. Data pointing to the disappearance of message were obtained in experiments with strain 961, in which message was accumulated and translated at

<table>
<thead>
<tr>
<th>Strain</th>
<th>Starvation (min)</th>
<th>Arginine source</th>
<th>Initial Specific activity</th>
<th>Final Specific activity</th>
<th>Increase</th>
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<td>808</td>
<td>949</td>
<td>141</td>
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</tbody>
</table>

* The general procedure is described in Fig. 1; starvation of arginine was as indicated, with either l-arginine or l-ornithine (as hydrochlorides, at 100 μg/ml) provided for translation. Specific activity is expressed as units of transcarbamylase per mg protein. Initial and final specific activity values were determined immediately upon addition of arginine or ornithine and at 40 min thereafter. The argR+ strain is 961; the argR- strain is 977.
25°C, first in the presence of arginine for periods from 1 to 10 min, and then (after rapid filtration to remove arginine) in the presence of ornithine for 50 min. In the 10-min experiment, in which the plateau value for translation with arginine present was reached, no further translation occurred in the presence of ornithine. Under conditions where the translation with arginine present was partial, further incubation in the presence of ornithine did lead to additional translation, but to a total extent greatly below that attained on translation with only ornithine present throughout. There was progressively less translation with ornithine present after increasing periods of translation in the presence of arginine. These results support the view that messenger degradation, obtained when an excess of arginine is present (under repressive conditions), is more rapid than that obtained with ornithine present, that is, under arginine restriction.

Discussion. As reported some time ago\textsuperscript{20}, incubation of an arginine auxotroph of \textit{Bacillus subtilis} in an arginine-free (complex) medium, followed by addition of actinomycin D at various periods of time, leads to the appearance of acetylornithine $\delta$-transaminase, provided the actinomycin is added more than 10 min after the beginning of arginine deprivation. An accumulation of translatable message during arginine starvation was thus indicated. Faanes and Rogers\textsuperscript{21} obtained evidence for the accumulation of ornithine transcarbamylase message when \textit{E. coli} auxotrophs were starved of arginine. Studies of tryptophan biosynthesis in \textit{E. coli} and in \textit{Salmonella typhimurium}, where much information is available on gene–enzyme relationships (reviewed in refs. 22–24), have yielded detailed knowledge of the accumulation of hybridizable or translatable message during tryptophan deprivation.\textsuperscript{25–28} Studies of the histidine system, likewise, have demonstrated messenger accumulation during starvation.\textsuperscript{29} In all of these cases, the mRNA accumulation appears to be relatively specific for the biosynthetic system whose end product is being restricted.

In the experiments reported here, the accumulation of mRNAs for arginine enzymes by starvation has formed the basis for an examination of the translation process (divorced from transcription) as a function of arginine supply and conditions of repression–derepression. The provision of restricted amounts of arginine, achieved by supplying ornithine to mutants with low argininosuccinase activity, has proved very useful in translation experiments, in which an excess of arginine was to be avoided. That \textit{de novo} transcription during translation has been adequately excluded is indicated by the data illustrated in Fig. 1, as well as by the results obtained with miracin D and with EDTA-treated cells.

The results in Fig. 2A show a greater extent of translation with ornithine than that with arginine. This finding, and the evidence adduced for the enhanced degradation of arginine enzyme messages in the presence of excess arginine, do not \textit{per se} implicate translation in the process of repression, although a regulatory function of arginine supply is clearly indicated. The degradation of messages in relation to tryptophan supply has been considered for the tryptophan system.\textsuperscript{30,31}

As evidence for a link between translation and the repression of the acetylornithine $\delta$-transaminase of \textit{E. coli}, it was shown that under partial inhibition by antibiotics that bind to ribosomes, such as streptomycin\textsuperscript{3} (or tetracycline, unpublished observations by R. H. Vogel and H. J. Vogel), the differential rate of
formation of the enzyme is greatly reduced for derepressed synthesis, but not for repressed synthesis. In studies of messenger translation for tryptophan enzymes of *E. coli*, Lavallé and De Hauwer\(^3\) showed that the rate of enzyme formation, under various conditions of repression–derepression, does not correlate with the concentration of the corresponding message, and concluded that repression by tryptophan occurs at the levels of both translation and transcription. For the acetylornithinase and ornithine transcarbamylase of *E. coli*, Lavallé\(^4\) found that the prompt response to addition of arginine is a lowering of the rate of enzyme synthesis below that for steady-state repression; synthesis gradually accelerates to the steady-state rate. As indicated by Lavallé, this finding cannot immediately be related to the locale at which repression occurs, in the absence of a method for the estimation of arginine messages; a translational mode of repression, however, is suggested.

The present studies clearly implicate the translation of arginine enzyme messages in the mechanism of repression by arginine, by showing that the lowered extent of translation with arginine (compared to that with ornithine), as found for the *argR*\(^+\) strain (Fig. 2A), does not occur in the *argR*\(^-\) strain (Fig. 2B). It is also significant that the arginine messages are present at relatively high levels in the *argR*\(^-\) strain, even in the absence of prior arginine starvation. Although, from general considerations and by analogy with other systems, a transcriptional as well as a translational mode of repression is plausible, the information for the arginine system available to date does not make the assumption of a transcriptional component of repression compelling. The inferred degradation of arginine messages could be triggered by the formation of a repressive complex at the ribosome.\(^4\)

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The repressive complex at the ribosome presumably includes the protein product of the argR gene, arginine, a finished arginine enzyme (see Leisinger, T., R. H. Vogel, and H. J. Vogel, Proc. Nat. Acad. Sci. USA, 64, 686, 1969), and possibly arginyl-tRNA and its synthetase (see ref. 4 and Leisinger, T., and H. J. Vogel, Biochim. Biophys. Acta, 182, 572, 1969). Conceivably, the degradation of the message could be initiated by the formation of the repressive complex leading to activation of a degradative enzyme such as ribonuclease V (Kuwano, M., C. N. Kwan, D. Apirion, and D. Schlessinger, Proc. Nat. Acad. Sci. USA, 64, 693, 1969), perhaps by a distortion of the ribosome.