Regulation of polyamine biosynthesis by antizyme
and some recent developments relating the induction of
polyamine biosynthesis to cell growth

Review

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This review considers the role of antizyme, of amino
acids and of protein synthesis in the regulation of
polyamine biosynthesis.

The ornithine decarboxylase of eukaryotic cells and
of Escherichia coli can be non-competitively inhibited
by proteins, termed antizymes, which are induced by di-
and poly- amines. Some antizymes have been purified
to homogeneity and have been shown to be structurally
unique to the cell of origin. Yet, the E. coli
antizyme and the rat liver antizyme cross react and
inhibit each other's biosynthetic decarboxylases. These
results indicate that aspects of the control of poly-
amine biosynthesis have been highly conserved through-
out evolution.

Evidence for the physiological role of the antizyme
in mammalian cells rests upon its identification in
normal uninduced cells, upon the inverse relationship
that exists between antizyme and ornithine decarboxy-
lase as well as upon the existence of the complex of
ornithine decarboxylase and antizyme in vivo. Furthmore, the antizyme has been shown to be highly
specific; its $K_{eq}$ for ornithine decarboxylase is $1.4 \times 10^{11}$ M$^{-1}$. In addition, mammalian cells contain an
anti-antizyme, a protein that specifically binds to the
antizyme of an ornithine decarboxylase-antizyme
complex and liberates free ornithine decarboxylase from
the complex. In E. coli, in which polyamine bio-
synthesis is mediated both by ornithine decarboxylase
and by arginine decarboxylase, three proteins (one
acidic and two basic) have been purified, each of which
inhibits both these enzymes. They do not inhibit the
biodegradative ornithine and arginine decarboxylases nor
lysine decarboxylase. The two basic inhibitors have
been shown to correspond to the ribosomal proteins
$S_{28}/L_{26}$ and $L_{34}$, respectively. The relationship of the
acidic antizyme to other known E. coli proteins
remains to be determined.
In mammalian cells, ornithine decarboxylase can be induced by a broad spectrum of compounds. These range from hormones and growth factors to natural amino acids such as asparagine and to non-metabolizable amino acid analogues such as α-amino-isobutyric acid. The amino acids that induce ornithine decarboxylase as well as those that promote polyamine uptake utilize the sodium dependent A and N transport systems. Consequently, they act in concert and increase intracellular polyamine levels by both mechanisms. The induction of ornithine decarboxylase by growth factors, such as NGF, EGF, and PDGF as well as by insulin requires the presence of these same amino acids and does not occur in their absence. However, the inducing amino acid need not be incorporated into protein nor covalently modified.

It is suggested that the induction of ornithine decarboxylase activity and of polyamine biosynthesis occurs when the prerequisites for protein synthesis, cell division and growth are present. These include the availability of amino acids transported by Systems A and N and the attainment of critical intracellular concentrations of these amino acids.

Introduction

The regulation of ornithine decarboxylase (L-ornithine carboxylase, EC 4.1.1.17) and of polyamine biosynthesis has been reviewed (1-6). We shall discuss the regulation of polyamine biosynthesis by antizyme and the relationship of amino acids and protein synthesis to polyamine biosynthesis.

The polyamines are among the few remaining metabolites about whose function we have very little solid information, even though they exist in cells in millimolar concentrations and their biosynthesis appears to be intimately related to cell division and growth.

Ornithine decarboxylase converts ornithine to putrescine and is widely distributed in eukaryotes and prokaryotes (7,8). Putrescine was first shown to be an essential growth factor in *Hemophilus parainfluenzae* by Herbst and Snell (9). Subsequently, Ham showed putrescine to be necessary for the optimal growth of several mammalian cell lines (10) while Bottenstein and Sato (11) and Pohjanpelto (12) identified putrescine as the main factor responsible for the acceleration of growth of human fibroblasts.

Spermidine and spermine are formed by the addition of one and two aminopropyl groups, respectively, to the terminal amino groups of putrescine. In *Escherichia coli*, putrescine can also be formed through arginine decarboxylase (L-arginine carboxylase, EC 4.1.1.19), which decarboxylates arginine to form agmatine. The latter is then hydrolyzed to putrescine by agmatine ureohydrolase (agmatine amidohydrolase, EC 3.5.3.11) (13,14) (Fig. 1).

Mammalian Cells

Beck et al. noted that administration of puromycin to rats induces liver ornithine decarboxylase (15). It was suggested that puromycin
inhibited the synthesis of a protein inhibitor of ornithine decarboxylase (15). Fong et al. further pursued these studies in cell cultures and found that H-35 hepatoma cells exposed to putrescine produce a non-competitive inhibitor of ornithine decarboxylase that binds to ornithine decarboxylase in a salt-dissociable linkage (16). Since this non-competitive protein inhibitor of ornithine decarboxylase was induced in response to the product of the reaction, it represented a novel mechanism of control of enzymatic activity, and we named it the antizyme of ornithine decarboxylase (17). Initially controversial, because of the difficulty in purifying the antizyme and because of the high concentrations of polyamines that were used at the time to induce the appearance of the antizyme, these observations have been confirmed (18-35).

Antizyme to ornithine decarboxylase has been induced in a variety of eukaryotic cells including fibroblasts, nerve and hepatic cells (19), as well as HeLa (26) and hepatoma cells (23,24) and in thyroid cells (28) and in plant cells (32,33) as well as in tissue secretions such as milk (36).

The antizyme to ornithine decarboxylase was shown to exist also in normal uninduced cells (18). In such cells it was not found in the free form but in a bound form in the nuclei or in the post-ribosomal nucleoprotein particles (the latter can be isolated by extensive
High salt concentrations (0.3 M KCl) are required to extract the total antizyme of the post-ribosomal particles. The extracted material is of low specific activity. High specific activity antizyme can be extracted from these subcellular components by micromolar concentrations of putrescine and spermidine (18). It was suggested that the polyamines may interact in a specific manner with the antizyme, resulting in a dissociation of the antizyme from the ribonucleoprotein particles.

In addition to putrescine, the naturally occurring polyamines spermidine and spermine also induce the antizyme to ornithine decarboxylase. Aliphatic diamines ranging from two to ten carbons in chain length have also been shown to induce the antizyme to ornithine decarboxylase (19,23,25,28,35,37). Consequently, it would appear that the inhibition of polyamine biosynthesis is a general cellular response when cells are exposed to aliphatic amines.

The physiological role of the antizyme to ornithine decarboxylase was investigated by Heller and Canellakis with starved cell cultures that have minimal levels of ornithine decarboxylase (19,34). In such cell cultures, free antizyme could be induced with concentrations of putrescine as low as 10^{-6} to 10^{-7} M, because such cells contain minimal amounts of intracellllular ornithine decarboxylase to complex the antizyme that is induced. Consequently, they are very sensitive indicators of any free antizyme that may be produced. By alternately inducing ornithine decarboxylase (with asparagine) or its antizyme (with putrescine), and determining the activity of their respective free forms, it was shown that if the cells had been pre-induced for one activity, the appearance of the opposing activity was delayed. To induce measurable amounts of active ornithine decarboxylase in cells that had been pre-induced for antizyme it was necessary either to increase the concentration of asparagine or to extend the experiment for longer periods of time. Similarly, in cells that had been pre-induced for ornithine decarboxylase, high concentrations of putrescine were required to induce free antizyme; alternatively, the cells had to be incubated for extended periods at lower concentrations of putrescine. In agreement with the in vitro evidence that antizyme acted as a non-competitive inhibitor of ornithine decarboxylase, at no time could the two activities be detected concurrently. These results provided evidence for the inverse relationship between ornithine decarboxylase and its antizyme.

More recently, the presence and the turnover of the ornithine decarboxylase-antizyme complex has been determined in vivo through the use of difluoromethyl ornithine (DFMO) (38), an ornithine decarboxylase activated irreversible inhibitor of the enzyme. Hayashi and his collaborators (39) found that the DFMO-inactivated ornithine decarboxylase exchanges with the native ornithine decarboxylase that exists in an inactive complex with antizyme. This exchange results in the liberation of the native ornithine decarboxylase which can be assayed enzymatically and permits quantitation of the inactive complex of ornithine decarboxylase and antizyme and of the rate of its disappearance. A similar assay has been used by Brosnan, who has shown the existence of such ornithine decarboxylase-antizyme complexes in milk (40) and independently by Mitchell, who has studied their kinetics of appearance in H-35 cells (41).
The physiological role of such a complex has to be considered. In the above experiments, Hayashi and Mitchell demonstrated that the ornithine decarboxylase-antizyme complex disappears rapidly following its formation in vivo. Since the disappearance of ornithine decarboxylase could only be incompletely accounted for by the appearance of the ornithine decarboxylase-antizyme complex, it is thought that the formation of these complexes provides a more rapid means of degradation of ornithine decarboxylase (39,41).

However, these results do not exclude an alternate interpretation. We had earlier proposed that inactive complexes of ornithine decarboxylase and antizyme become associated with subcellular components to create reservoirs of an inactive or of a cryptic form of ornithine decarboxylase from which active ornithine decarboxylase can be released rapidly upon demand (1,30). Arguments against such an interpretation would be that the total amount of cellular ornithine decarboxylase can be accounted for by the coincidence between the titratable active ornithine decarboxylase and the protein bound $^3$H-DFMO (35). However, most such studies have been made with partially purified cell extracts or 100 000 g supernatant fractions of cell extracts (35). Consequently, any forms of ornithine decarboxylase that are associated with subcellular fractions would not be measured. This limitation of these studies has been noted (35).

The following evidence can be marshalled in favor of the alternate hypothesis. There exists in rat liver a protein that could be responsible for the activation of free ornithine decarboxylase from its inactive complexes with antizyme. This is the anti-antizyme discovered by Hayashi and his collaborators (31,42,43). This protein binds strongly to the antizyme present in an enzymatically inactive complex of ornithine decarboxylase-antizyme complex, dissociates antizyme from its ornithine decarboxylase-antizyme complex and liberates active ornithine decarboxylase (31,43). It should also be noted that upon exposure of cells to $^3$H-DFMO, radioactivity is also associated with subcellular components (44,45). It has been assumed that this radioactivity is due to the presence of active ornithine decarboxylase; however, Kyriakidis et al. found that $^3$H-DFMO reacts not only with active ornithine decarboxylase, but also with the inactive enzyme present in the ornithine decarboxylase-antizyme complex (46). Consequently, following treatment of cells with $^3$H-DFMO, an ornithine decarboxylase-antizyme complex bound to subcellular components would be indistinguishable from active ornithine decarboxylase by autoradiography. Therefore, the possibility that the particle-bound radioactivity is due to ornithine decarboxylase-antizyme complexes should be seriously considered.

As a non-competitive inhibitor of ornithine decarboxylase, antizyme has been used to measure amounts of ornithine decarboxylase and compare them with ornithine decarboxylase activity (33,46). The quantitation of ornithine decarboxylase in cell extracts has also been performed with $^3$H-DFMO (47-49) and with antibodies to ornithine decarboxylase (35,49-55). Under conditions of long-term induction (46-55) the increases in ornithine decarboxylase activity in mammalian cells were always accompanied by corresponding increases in the amount of ornithine decarboxylase protein.

It is becoming apparent that when ornithine decarboxylase is
induced in tissues, the increase in the ornithine decarboxylase mRNA is much less than the increase in ornithine decarboxylase activity (56-59). This may be explained by an increased stability of the mRNA or by the possible existence of more than one species of ornithine decarboxylase. However, it has also been found by Kameji et al. (59) that upon induction of ornithine decarboxylase with thioacetamide, the total amount of free immunotitratable ornithine decarboxylase protein plus the mRNA associated immunotitratable newly synthesized proteins are also less than the amount necessary to account for the increase in enzyme activity. One factor that may help explain these discrepancies is the finding of Kitani and Fujisawa that the activity of the partially purified mammalian enzyme can be enhanced by phospholipids (60). It may also explain the rapid and transient activation that occurs within two minutes of exposure of mouse kidney slices to androgen, that was noted by Koenig et al. (61,62), since trophic stimuli are known to activate phospholipid metabolism (63).

The rat liver antizyme of ornithine decarboxylase has been recently purified 600,000-fold to homogeneity by Kitani and Fujisawa (64). This feat was only made possible following the comparably extensive purification of rat liver ornithine decarboxylase by Hayashi and his collaborators (65) and by Kitani and Fujisawa (66). The use of affinity columns of highly purified ornithine decarboxylase was a major tool in the purification procedure, resulting in a 5500-fold purification of rat liver antizyme in a single passage. The rat liver antizyme, mol.wt. = 22,000, and pl = 6.8, exhibits a high affinity for rat liver ornithine decarboxylase, $K_{eq} = 1.4 \times 10^{11} \text{ M}^{-1}$. The maximal activity of antizyme that can be induced in rat liver by the administration of putrescine is approximately equivalent to the maximal amount of ornithine decarboxylase that can be induced following administration of dexamethasone (29,64).

The question of the existence of alternate forms of antizyme and of ornithine decarboxylase has been raised (1,30) and remains to be further elucidated. Antizyme preparations do not completely inactivate ornithine decarboxylase activity (46) and there is evidence that there are components in tissue extracts that modify the interaction between ornithine decarboxylase and antizyme (21). Chromatographic separation of two forms of antizyme from chicken liver has been achieved by Grillo et al. (67). Similarly, there is a great deal of cumulative evidence for the existence of more than one form of ornithine decarboxylase (68-71), and recently Berger et al. (57) have identified two distinct mRNAs in mouse kidney coding for ornithine decarboxylase.

The role of amino acids in the regulation of polyamine biosynthesis

Chen and Canellakis found that ornithine decarboxylase could be induced in a salts-glucose solution (Earle's Balanced Salts Solution) and have used this solution to determine the critical requirements for polyamine biosynthesis (52). They found that, contrary to what had been found in more complex incubation media, cAMP would not induce ornithine decarboxylase unless a minimal concentration of asparagine,
which in itself was inadequate to induce ornithine decarboxylase, was present (52). It became apparent that the effect of cAMP was indirect and was mediated through asparagine.

Using the salts-glucose media, Costa et al. found asparagine to be a requirement for the induction of ornithine decarboxylase by luteinizing hormone in CHO cells (54), while Vicesp-Madore et al. (55) showed the induction of ornithine decarboxylase to be Na⁺ dependent and that in addition to asparagine, which was the best inducer, certain non-metabolizable amino acids such as α-amino-isobutyric acid, N-methyl asparagine and others were also effective inducers of ornithine decarboxylase. These results established that the metabolism of the inducing amino acid and its covalent modification was not necessary for the induction of ornithine decarboxylase (55).

Rinehart and Chen showed that the natural amino acids, as well as non-metabolizable analogues, that utilize the System A transport system stimulate the uptake of polyamines (72).

It has now become evident that the sodium dependent System A and N amino acid transport systems play a central role in polyamine biosynthesis. Amino acids that are transported by these systems not only stimulate the uptake of polyamines but are also required for the induction of ornithine decarboxylase activity (73). However, it was also found that in order that these amino acids induce ornithine decarboxylase, it is necessary that they achieve a critical intracellular concentration. Furthermore, much as cAMP would not induce ornithine decarboxylase in the absence of asparagine, it was now found that growth factors such as platelet derived growth factor, nerve growth factor, epidermal growth factor and insulin, added to a variety of responsive cells, will not induce ornithine decarboxylase unless a System A or N inducing amino acid, such as asparagine, is present (74). Significantly, asparagine could again be replaced by an unnatural amino acid analogue such as α-amino-isobutyric acid.

It was concluded that growth factors do not induce ornithine decarboxylase activity directly, but their effect is mediated through System A and N amino acids and does not require the covalent modification of these amino acids. It is evident that the induction of ornithine decarboxylase activity by cAMP and by cAMP-mediated growth factors (NGF, EGF and PDGF) as well as by insulin is not a self-contained reaction but is dependent upon the presence of an inducing amino acid. Since α-amino-isobutyric acid is known not to be metabolized intracellularly (75,76), the inducing amino acids may be acting as gratuitous inducers, in the manner of isopropyl β-D-thiogalactose-pyranoside (IPTG) during the induction of β-galactosidase in E. coli (77).

Consequently, these two coordinated effects of the System A and N amino acids upon the uptake of polyamines and upon the induction of ornithine decarboxylase, reinforce each other and both contribute to an increased intracellular polyamine concentration.

E. coli

Kyriakidis et al. (20) isolated from E. coli a non-competitive inhibitor protein of the E. coli ornithine decarboxylase. This protein was purified to homogeneity, and found to be an acidic protein, pl = 3.8, and to have an apparent mol.wt. = 49 500. Its intracellular
concentration increased when *E. coli* were grown in the presence of increasing concentrations of putrescine and spermidine and it inhibited the mammalian ornithine decarboxylase. Because of the overlap in these various properties with the mammalian antizyme, this inhibitory protein of *E. coli* was also named antizyme (20,78,79).

Two basic proteins, with pI greater than 9.5, have since been purified from *E. coli*, with biological properties very similar to those found for the acidic antizyme; these were named, provisionally, antizymes 1 and 2 (Az-1 and Az-2) (78).

The two basic *E. coli* proteins were shown to inhibit both the biosynthetic ornithine decarboxylase and the biosynthetic arginine decarboxylase (Fig. 1); they did not inhibit the corresponding biodegradative ornithine, arginine and lysine decarboxylases of *E. coli* nor did they inhibit S-adenosyl methionine decarboxylase (79). Unlike the biosynthetic decarboxylases, the biodegradative decarboxylases are not constitutive, but are induced when *E. coli* are grown in low pH media; their function appears to be one of raising the intracellular pH (80-82).

To define more closely the specificity of inhibition of decarboxylases by the various antizymes, a lysine decarboxylase that requires pyridoxal phosphate and does not decarboxylate ornithine or arginine was purified about 200-fold from *E. coli*. It differs from the well-studied biodegradative lysine decarboxylase of *E. coli* which has a pH optimum of 5.5 (83), in having a high pH optimum, about 9.0 (C. Panagiotidis, unpublished results). It appears to be a biosynthetic enzyme similar to the biosynthetic ornithine decarboxylase and arginine decarboxylases (82,84) and is reminiscent of the lysine decarboxylase that was referred to by Leifer and Maas (85). However, cadaverine, the product of lysine decarboxylase activity, is known not to replace putrescine for growth (86). This new lysine decarboxylase was also found not to be inhibited by these two basic antizyme proteins (C. Panagiotidis, unpublished results).

Both the acidic antizyme of *E. coli* and the rat liver antizyme were also tested for their ability to inhibit these various decarboxylases and the results are presented in Table 1. It is evident that all these proteins that were first identified as non-competitive inhibitors of ornithine decarboxylase inhibit only the polyamine biosynthetic decarboxylases. Both the acidic and the basic *E. coli* antizymes inhibit the structurally dissimilar rat liver ornithine decarboxylase. In a complementary fashion, the rat liver antizyme inhibits the biosynthetic ornithine and arginine decarboxylases of *E. coli*; in fact, it appears to be more effective against the *E. coli* ornithine decarboxylase than the rat liver enzyme.

Huang et al. (87) found that the inhibition of *E. coli* ornithine decarboxylase caused by the acidic antizyme and by the basic *E. coli* antizymes is relieved to different degrees by DNA and by synthetic nucleic acid polymers indicating preferences for certain nucleic acid sequences (87). The inhibition of rat liver ornithine decarboxylase by the *E. coli* antizymes shown in Table 1 is relieved by nucleic acids (S. C. Huang, unpublished results). It should be noted, however, that the inhibitions exerted by rat liver antizyme upon these various decarboxylases is not prevented by nucleic acids (W. Fong, unpublished results).
Table 1. Comparison of activity of various antizyme preparations against various decarboxylases

In each case, 2-3 units of the decarboxylase were titrated with the antizyme fraction indicated. The units of antizyme required to lower the decarboxylase activity by 20% was determined, as estimated from the linear portion of the curve.

<table>
<thead>
<tr>
<th>Antizymes</th>
<th>E. coli ODC</th>
<th>E. coli ADC</th>
<th>Rat liver ODC</th>
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<tr>
<td>E. coli</td>
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<tr>
<td>S20/L26</td>
<td>20*</td>
<td>70</td>
<td>188</td>
</tr>
<tr>
<td>L34</td>
<td>20*</td>
<td>64</td>
<td>172</td>
</tr>
<tr>
<td>Acidic Az</td>
<td>20*</td>
<td>133</td>
<td>182</td>
</tr>
<tr>
<td>Rat liver</td>
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<tr>
<td>Antizyme</td>
<td>11</td>
<td>48</td>
<td>20*</td>
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</tbody>
</table>

ODC = ornithine decarboxylase; ADC = arginine decarboxylases; * Inhibitor units in these cases were defined as being equivalent to the 20 decarboxylase units (see above). All other inhibitor values were normalized to this value.

It was found that a variety of synthetic nucleic acid polymers, including ribo- and deoxyribo- polymers, will also relieve the inhibition of ornithine decarboxylase by the E. coli antizymes (87); additional experimental data in support of this statement are presented in Figs. 2 and 3. These results and the specificities indicated are not unlike those obtained for various DNA and other nucleic acid binding proteins which, in addition to their indicated specificity for specific DNA sequences, also exhibit varying degrees of specificity for binding to a variety of synthetic nucleic acid polymers (88-91).

The ability of DNA and other nucleic acids to relieve the inhibition of ornithine decarboxylase by the acidic antizyme (pI = 3.8), and the relative specificity shown in the binding of these proteins to various nucleic acid sequences, suggests that this reaction may prove to be more specific for certain nucleotide sequences and may not only represent a non-specific interaction between basic proteins and acidic nucleic acid polymers. At this time additional experiments are under way in our laboratory to understand the exact mode of this interaction and its underlying specificity.

Panagiotidis and Canellakis (92) determined the partial amino acid sequences of Az-1 and of Az-2 and found them to be identical with corresponding amino acid sequences of the $S_{20}$/$L_{20}$ (93) and $L_{34}$ r-proteins (94) respectively, within the limits of the methods used. The identity of Az-1 and of Az-2 with the respective r-proteins was further verified by isolating ribosomes from E. coli MA255 by standard procedures (95). The basic ribosomal proteins were then
Fig. 2. Reversal of the inhibition of ornithine decarboxylase by antizyme 1, through the addition of deoxynucleotide homopolymers. *E. coli* ornithine decarboxylase (2.0 units) was inhibited with 1.8 units of *E. coli* antizyme 1 and the resultant complex was titrated with deoxynucleotide homopolymers.

Extracted (96). Partial purification of these basic ribosomal proteins through a CM-Biogel A column, followed by electrophoresis of this fraction in an acid-urea acrylamide gel, showed two main components which travelled to the same positions as did Az-1 and Az-2. Furthermore, their inhibition of ornithine decarboxylase activity was found to be relieved by added DNA. These experiments established that Az-1 is in fact the $S_{20}/L_{25}$ r-protein while Az-2 is the $L_{34}$ r-protein. The $S_{20}/L_{25}$ is a basic inter-ribosomal protein that is shared by the small and large ribosomal subunits (93), while the $L_{34}$ r-protein is a basic protein associated exclusively with the large ribosomal subunit (94).

Holtta et al. had found that the activity of the biosynthetic *E. coli* ornithine decarboxylase is greatly increased by nucleotides, especially GTP (97), while it is inhibited by ppGpp (98). These investigators considered the requirement of GTP for protein synthesis and the accumulation of ppGpp during starvation of *E. coli* that are under stringent control, and suggested that changes in polyamine pools may be responsible for the stringent effect in *E. coli* (98). The accumulation of ppGpp leads to the cessation of stable RNA synthesis (99,100) and appears to be related to the fidelity of protein synthesis (101,102).
Fig. 3. Reversal of the inhibition of *E. coli* ornithine decarboxylase by antizyme I, through the addition of ribonucleotide homopolymers. Experimental details as in the legend to Figure 2, except that the titration was performed with ribonucleotide homopolymers. rA, rC, rG and rU refer to poly A, poly G, poly C and poly U respectively.

Sakai and Cohen examined the suggestion of Holtta et al. in a K⁺ requiring *E. coli* mutant (103) maintained at various growth rates. They found a lack of correlation between the putrescine levels, the levels of GTP or of ppGpp and the rates of RNA synthesis. However, the control mechanism may be more complex than originally assumed, and it may be necessary to reconsider the Holtta et al. suggestion (98). It may be necessary to take into account the inhibition of ornithine decarboxylase by the $S_{20}/L_{25}$ and the $L_{34}$ r-proteins because, during starvation of bacteria that are under stringent control, the synthesis of ornithine decarboxylase and the uptake of polyamines are also inhibited (104). Furthermore, recent evidence suggests that there may exist a closer interrelationship between polyamines and protein synthesis. In addition to the well-known stimulation of the rate of protein synthesis in vitro by polyamines, Cooper et al. have found that hypusine, a putrescine derivative, is covalently linked to the protein elongation factor Ef-1 (105). In addition, Mitsui et al. (106) have shown that, in a polyamine requiring strain of *E. coli*, the addition of polyamines is necessary for the optimal synthesis of a discrete 62K protein. One function of this 62K protein is to stimulate the synthesis of MS2 phage RNA replicase in the presence of spermidine (106).
Discussion

The *E. coli* ornithine decarboxylase is known to be inhibited by a variety of basic and acidic proteins. These include polylysine (87), histones and heparin (S.C. Huang, unpublished experiments). The rat liver ornithine decarboxylase has been shown to be inhibited by the acidic heparin (60). However, it should be noted that the relatively neutral (pI = 6.8) rat liver antizyme inhibits both the *E. coli* biosynthetic ornithine and arginine decarboxylases and does not inhibit the corresponding *E. coli* biodegradative decarboxylases or lysine decarboxylase. On the other hand, the acidic *E. coli* antizyme and the basic S_{20}/L_{25} and L_{34} r-proteins inhibit the biosynthetic *E. coli* ornithine and arginine decarboxylases as well as the rat liver ornithine decarboxylase but not the corresponding biodegradative *E. coli* enzymes nor the *E. coli* lysine decarboxylase. There appears therefore to be an underlying specificity of interaction among these biosynthetic polyamine enzymes with certain proteins which is masked by an apparent non-specificity in their interaction with various basic and acidic polymers.

Another underlying similarity in the behavior of the *E. coli* and the rat liver polyamine biosynthetic enzymes is that their inhibition by these various inhibitory proteins can be relieved by other polymers. On the one hand, the inhibition of both the rat liver ornithine decarboxylase and of the *E. coli* ornithine and arginine decarboxylases by the *E. coli* inhibitory proteins is reversed by nucleic acids. On the other hand the inhibition of the rat liver ornithine decarboxylase by rat liver antizyme is overcome only by a protein and not by nucleic acids.

It is as yet unclear whether the acidic antizyme and the S_{20}/L_{25} and the L_{34} r-proteins are the only acidic and basic *E. coli* proteins that inhibit the polyamine biosynthetic decarboxylases. Our method of isolation of these proteins includes acid extraction of the 10,000 g supernatant fraction of *E. coli* lysates (78). During this procedure the only effective inhibitors of ornithine decarboxylase that are extracted are the acidic antizyme and the basic S_{20}/L_{25} and L_{34} r-proteins. It is therefore possible that these enzymes are inhibited by other acidic or basic proteins of *E. coli* which our method does not extract.

The interaction between the biosynthetic polyamine enzymes and the ribosomal proteins suggests that it may reflect upon the rates of protein synthesis and/or upon the rates of polyamine synthesis in *E. coli*. The interrelationship between polyamine synthesizing enzymes and protein synthesis is further accentuated by the finding that an increase of the intracellular concentration of certain amino acids is in itself adequate for the induction of polyamine biosynthesis in eukaryotic cells. Furthermore, growth factors and hormones will not induce polyamine biosynthesis in these cells unless the inducing amino acids are present and unless the intracellular concentration is above a minimal concentration range. These data suggest that the presence of critical concentrations of amino acids is necessary for the initiation of polyamine-dependent growth processes.

Should other such inhibitory proteins to polyamine synthesizing enzymes exist, it will be most interesting to define their physiological
function and to determine whether their function is dependent upon the various polyamine synthesizing enzymes. It remains to be determined whether this interplay between polyamine synthesizing enzyme, nucleic acid associated proteins, nucleic acids and amino acids has a physiological function, such as permitting the modulation of polyamine biosynthesis at localized regions of RNA and DNA and modifying nucleic acid dependent reactions, or whether we are noting reactions with limited physiological significance.

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