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Polyamines in Microorganisms

CELIA WHITE TABOR* AND HERBERT TABOR

Laboratory of Biochemical Pharmacology, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Bethesda, Maryland 20205

INTRODUCTION

Polyamines are polycationic compounds that are present in all biological materials and have been implicated in a wide variety of biological reactions, including synthesis of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein. The most common polyamines are putrescine (1,4-diaminobutane) and spermidine; spermine is also present in many, but not all, biological materials. Although it is clear that the polyamines are essential for normal growth and have important physiological roles, we still cannot state with any certainty their specific molecular functions in vivo.

The purpose of this review is to summarize those studies that have been carried out on polyamines in microorganisms. Note should be made of the historical importance of studies in microorganisms to the polyamine field. Even though the polyamines were first discovered in animal tissues, the biosynthetic pathway and many of the biological effects of polyamines were shown in microorganisms long before these systems were defined in animals. Studies with both procaryotic and eucaryotic cells have confirmed the universality of both the presence of these amines and most of the steps in their biosynthesis. Studies on these amines in microorganisms have been particularly useful because of the suitability of these systems for genetic, metabolic, and enzymatic experiments. In particular, the recent isolation of mutant bacterial and yeast strains that lack polyamines affords particularly convenient systems for studying the role of polyamines in vivo.

Since a very large number of papers have been published on polyamines in microorganisms, animals, and plants, all cannot be reviewed or cited in a review of this size. Even the number of studies on microorganisms is too great, and therefore this review will be restricted to those few systems for which the physiology, biochemistry, and genetics have been studied in some depth. In particular, we will concentrate on the studies done on *Escherichia coli*, *Saccharomyces cerevisiae*, *Neurospora crassa*, *Aspergillus nidulans*, and *Physarum polycephalum*, even though this limitation omits many interesting papers.

Various aspects of polyamine research have been covered in a number of symposia and reviews, and references to some of these have been included in the bibliography (11, 12, 25, 26, 30, 31, 68, 84, 133, 139, 159, 168, 177, 190, 198–200, 210, 213, 220, 234, 236). Most of the reviews concentrate on polyamines in higher organisms, but two are specifically concerned with viruses (31) and fungi (190). The earlier history of polyamine research will not be covered in this article, but has been reviewed in references 62, 210, and 220.

POLYAMINES IN *ESCHERICHIA COLI*

Biosynthesis of Putrescine, Agmatine, and Cadaverine

Putrescine is synthesized either by ornithine decarboxylase or by the combined action of arginine decarboxylase and agmatine ureohydrolase (Fig. 1). Two types of ornithine decarboxylase occur in *E. coli*: a biodegradative (induced) and a biosynthetic (constitutive) enzyme.

Biodegradative (induced) ornithine decarboxylase. Gale showed that a number of amino acid decarboxylases, including ornithine decarboxylase, are markedly induced by growth under semianaerobic conditions at low pH in rich media containing excess amino acids (reviewed in references 47 and 133). The biodegradative ornithine decarboxylase was purified to homogeneity from *E. coli* U44 grown in such a medium containing excess ornithine (6, 7, 132). Under these conditions ornithine decarboxylase represents 7% of the protein present in a crude extract.

In contrast to other induced amino acid decarboxylases, which have acid pH optima, the pH optimum for this enzyme is 6.9. The purified enzyme is specific for ornithine and requires pyridoxal phosphate for activity. The enzyme contains phosphopyridoxyllysine linked to histidine. At pH 7.0, the enzyme is a dimer with a relative molecular weight (M_r) of 160,000; at lower pH values, aggregation occurs.

It is noteworthy that, as opposed to the biosynthetic form of the enzyme, the biodegradative form of ornithine decarboxylase cannot be found in most strains of *E. coli*, including *E. coli* K-12 (7).

Biosynthetic (constitutive) ornithine decarboxylase. Morris and Pardee found that the induced form of ornithine decarboxylase that had been described by Gale was not the form present in *E. coli* grown at neutral pH, but that another type of ornithine decarboxylase with different characteristics was present (133, 138, 140, 141). This biosynthetic (or constitutive) form of ornithine decarboxylase appears to be present in all strains tested. The amount of biosynthetic ornithine decarboxylase present is considerably less than the amount of the biodegradative form of the enzyme found under the special cultural conditions described above (i.e., 0.023% compared with 7% of total protein).

Biosynthetic ornithine decarboxylase has been purified to near homogeneity from *E. coli* UW44 grown under aerobic conditions in relatively purified medium without ornithine (7, 132). The purified enzyme is specific for ornithine, with <0.1% decarboxylase activity towards arginine or lysine. Pyridoxal phosphate is required for activity. The enzyme is a dimer with a subunit M_r of 82,000. The pH optimum is 8.1, compared with 6.9 for the biodegradative enzyme. Immunological studies show no cross-reactivity between the biodegradative and biosynthetic forms of ornithine decarboxylase.

The enzyme is inhibited by both putrescine and spermidine (7, 211) and is activated by guanosine triphosphate (GTP) and other nucleotides (7, 77). In contrast to almost all other ornithine decarboxylase preparations from other sources, *E. coli* ornithine decarboxylase is not inhibited by the mechanism-based inhibitor α-difluoromethylornithine (88). It is, however, inhibited by both monofluoromethylornithine (14) and monofluoromethylputrescine (90).

Mutants lacking ornithine decarboxylase. Mutants have been described that are deficient in biosynthetic ornithine

* Corresponding author.

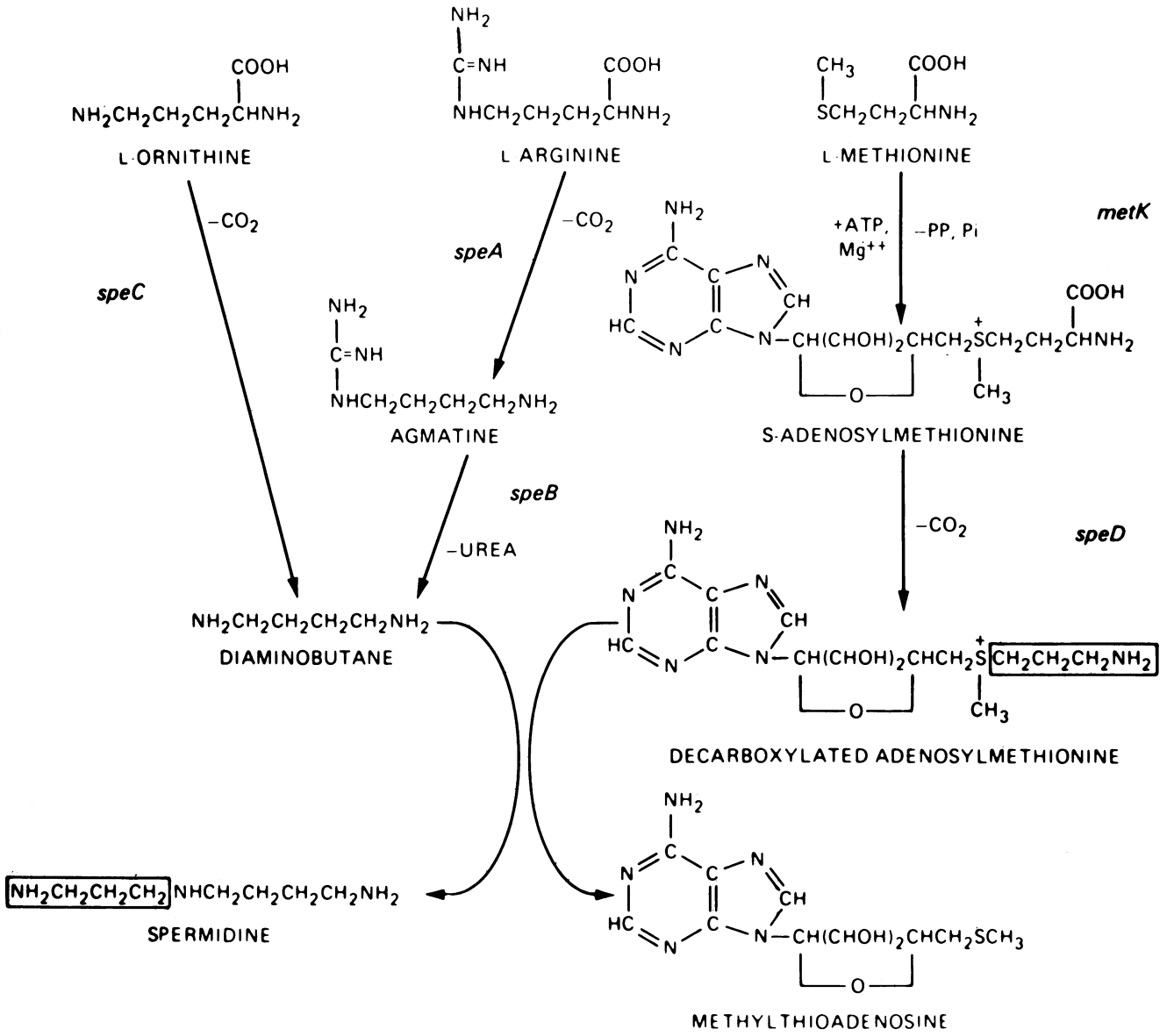

FIG. 1. Pathway for the biosynthesis of putrescine (1,4-diaminobutane) and spermidine in *E. coli*.

decarboxylase (39, 64, 203). Deletion mutants (64) which completely lack this enzyme have been obtained by mutagenesis by a method in which bacteriophage Mu is inserted into an appropriate high frequency of recombination (Hfr) donor (206, 208). These mutations (*speC* mutants) have been located at 63.4 min on the *E. coli* chromosome, and the gene order for this region has been established as *metC glc speC metK speB speA serA* (24, 39, 64). These studies, and other studies on CO_2-producing reactions in the polyamine biosynthetic pathway, were facilitated by the development of a mass screening technique in which, after mutagenesis, individual clones are placed in the separate wells of a 96-well microtiter dish, and the $^{14}CO_2$ released from a ^{14}COOH-labeled substrate is measured after precipitation on BaOH-impregnated filter paper (203, 218). Mutants have not been described for the induced ornithine decarboxylase, and the location of its gene is not known. High-copy-number plasmids (derivatives of pBR322) have been constructed that overproduce the biosynthetic form of ornithine decarboxylase (24, 204).

Biodegradative arginine decarboxylase. Arginine decarboxylase has also been shown to exist in two forms. A biodegradative (inducible) form is present in large amounts when *E. coli* B is grown in rich medium containing arginine, especially if the medium is acidic and conditions are semianaerobic. On the other hand, when the bacteria are grown in purified media at neutral pH, only the biosynthetic form (see next section) is found.

The biodegradative enzyme was initially studied by Gale (47, 132, 133) and has been purified to homogeneity and crystallized (15, 18–20). Under the conditions for induction, arginine decarboxylase accounts for about 3% of the bacterial protein.

Arginine is the preferred substrate for the enzyme, but other guanidine derivatives with an α-amino group, such as L-canavanine, are also substrates. L-Lysine is not attacked,

but L-ornithine is decarboxylated at a slow rate. Arginine decarboxylase requires pyridoxal phosphate for activity. It contains 10 binding sites for pyridoxal phosphate per enzyme molecule ($M_r \cong 820,000$). The pyridoxyllysine formed is located next to a histidine group, as is true for several other amino acid decarboxylases (18).

Arginine decarboxylase occurs in two stable aggregation states, a dimer (M_r 160,000) and a decamer (M_r 820,000). The decamer appears to contain two heterologously bonded pentamers, which dissociate to five isologously bonded dimers at low concentrations of monovalent ions or at a pH >6.5. Reassociation can occur and is favored by divalent and monovalent cations, substrate analogs, or pH <6. Kinetic studies of reassociation at the pH optimum (pH 5.2) indicate that the decamer represents the active form of the enzyme at this pH (148).

No genetic information is available on the gene coding for the biodegradative form of arginine decarboxylase.

Biosynthetic arginine decarboxylase. The biosynthetic enzyme has been purified from *E. coli* UW44 grown with vigorous aeration at neutral pH in a semipurified medium. The crude extract contains only 0.07% of its protein as biosynthetic arginine decarboxylase. The enzyme was purified to homogeneity and crystallized (238).

The pH optimum is 8.4. The enzyme has an absolute requirement for both pyridoxal phosphate and Mg^{2+}. Two moles of pyridoxal phosphate are bound per mole of enzyme. The enzyme decarboxylates only arginine and has no activity towards lysine or ornithine. In the presence of Mg^{2+} and pyridoxal phosphate, the enzyme exists as a tetramer of M_r 296,000, but can exist in monomeric or dimeric forms, depending on the environment (238, 239). The enzyme is inhibited by putrescine and spermidine and is irreversibly inactivated by α-difluoromethylarginine (89).

Wu and Morris (238) found that the monomeric form of the enzyme, as isolated, is a mixture of M_r 71,000 and M_r 75,500. That this mixture is not an artifact of isolation was shown recently by Boyle et al. (24), who found both forms when arginine decarboxylase was synthesized in minicell preparations containing a multicopy plasmid with the *speA* gene. S. M. Boyle (personal communication) has also shown by pulse-chase experiments that the 74,000-M_r form is a precursor of the 70,000-M_r form and that the latter is found in the periplasmic space. These data would explain the earlier findings (212) that exogenous arginine is more easily converted to putrescine than is arginine formed endogenously.

Mutants lacking biosynthetic arginine decarboxylase. Mutants have been reported that are deficient in the biosynthetic form of arginine decarboxylase (64, 105, 135, 136, 203); deletion mutants that completely lack the enzyme have also been obtained (64, 208). The gene for biosynthetic arginine decarboxylase (*speA*) has been located at 62.8 min on the *E. coli* chromosome and is very close to the gene for agmatine ureohydrolase (*speB* (24, 105). It may be of interest that several of the genes involved in polyamine biosynthesis are clustered in this area of the chromosome; there is no evidence, however, for the existence of an operon (64).

The gene for biosynthetic arginine decarboxylase has been placed in a high-copy-number plasmid (pBR322), and strains containing this plasmid overproduce the enzyme (24, 204).

Agmatine ureohydrolase. The conversion of agmatine to putrescine and urea has been demonstrated both in intact *E. coli* cells and in cell extracts (131, 141). Agmatine ureohydrolase represents the only pathway for urea biosynthesis in *E. coli* (137). No urease is present in *E. coli*, and the urea accumulates in the medium.

Mutants lacking agmatine ureohydrolase have been reported (64, 74, 135, 203, 208). The gene (*speB*) coding for this enzyme is located at 62.8 min on the *E. coli* chromosome (24, 64). High-copy-number plasmids containing the *speB* gene have been constructed (24, 204). Agmatine ureohydrolase has recently been purified to homogeneity from this overproducing strain (S. M. Boyle, personal communication).

Lysine decarboxylase. Even though cadaverine or its aminopropyl derivative is not present in *E. coli* grown in purified media, these amines are present when growth takes place in crude media or if the biosynthesis of putrescine is blocked by mutation (43, 49, 64).

Lysine decarboxylase has been purified to homogeneity from *E. coli* B grown on media containing lysine at pH 5.5 under semianaerobic conditions (i.e., inducing conditions) (17, 178, 179). About 2% of the protein in the extract is lysine decarboxylase. The pH optimum of the enzyme is 5.7. The enzyme is specific for L-lysine, *S*-aminoethyl-L-cysteine, and δ-hydroxy-L-lysine. It can exist either as a dimer (M_r 154,000 to 156,000) or as a decamer. Lysine decarboxylase requires pyridoxal phosphate for activity.

Two genes have been described that are involved in lysine decarboxylase activity (207). One gene, *cadR*, is located at 46 min on the *E. coli* map and appears to be a regulatory gene for lysine decarboxylase; mutants carrying mutations in this gene overproduce lysine decarboxylase even when grown on purified media under noninducing conditions. *cadR* mutants are also resistant to S-aminoethylcysteine and may be the same as the *lysP* mutant described by Popkin and Maas (164).

Another gene, *cadA*, maps at 92 min on the *E. coli* map; mutants carrying mutations in this gene have essentially no lysine decarboxylase activity (207). Mutants with low lysine decarboxylase activity have also been obtained by Goldemberg (49).

Some evidence has been presented for a second, much less active constitutive lysine decarboxylase (49, 231) that is inhibited by putrescine and spermidine. However, the data are still too incomplete to permit any definitive conclusions on the presence of this second enzyme.

Biosynthesis of Spermidine

Methionine adenosyltransferase (*S*-adenosylmethionine synthetase). *S*-Adenosylmethionine synthetase was first found in liver by Cantoni in 1953 and was subsequently demonstrated in *E. coli* and elsewhere. As in the case of the liver enzyme, the *E. coli* enzyme carries out the reaction methionine + adenosine triphosphate (ATP) → *S*-adenosylmethionine + inorganic phosphate (P_i) + inorganic pyrophosphate (PPi). The *E. coli* enzyme has been purified to homogeneity and crystallized from a *metJ* (derepressed) strain containing an overproducing plasmid (111, 112). The purified enzyme also has an inorganic tripolyphosphatase activity, stimulated by *S*-adenosylmethionine, similar to that found for the liver and yeast enzymes. The mechanism of the reaction has been studied with the use of this homogeneous preparation (109, 111) and has been shown to involve an enzyme-bound tripolyphosphate intermediate. For other references to these and other studies on the substrate and inhibitor specificities of the enzyme, see the bibliography in our recent review on methionine adenosyltransferase (199).

Mutants deficient in methionine adenosyltransferase are easy to obtain since they are resistant to ethionine toxicity (58, 59, 63). No completely deficient mutants have been

obtained, nor has it been possible to obtain any deletions in the *metK* gene. The *metK* gene maps at 63 min on the *E. coli* chromosome (63, 81, 105). High-copy-number plasmids containing the *metK*$^+$ gene have been constructed; these strains overproduce the enzyme and are very useful for preparative purposes (24, 111, 112, 204). The *metK* gene has recently been sequenced (110a).

S-Adenosylmethionine decarboxylase. *S*-Adenosylmethionine decarboxylase was first described in *E. coli* extracts in 1958 (209) and has been purified to homogeneity (113, 114, 233; reviewed in reference 199). The native enzyme has an M_r of 108,000 and is composed of six identical subunits. Each subunit has one covalently attached pyruvoyl group which is essential for activity. The enzyme requires divalent cations.

The enzyme from *E. coli* differs from the eucaryotic organism enzymes in that the latter do not require divalent cations and are usually stimulated by putrescine. Both procaryotic and eucaryotic enzymes contain the essential pyruvoyl group. In contrast to most other decarboxylases, *S*-adenosylmethionine decarboxylase from all sources shows no requirement for pyridoxal phosphate. Evidence has been presented for a Schiff base reaction between *S*-adenosylmethionine and the pyruvoyl group of the enzyme (113). The decarboxylation proceeds with retention of the steric configuration (3).

The presence of a covalently linked pyruvoyl group in an enzyme is rather unusual and was first shown by Riley and Snell (172) for histidine decarboxylase from *Lactobacillus* sp. strain 30a. The only other enzymes containing this moiety are proline reductase from *Clostridium sticklandii*, phosphatidylserine decarboxylase from *E. coli*, and L-aspartate-α-decarboxylase from *E. coli* (reviewed by Recsei and Snell [170]).

S-Adenosylmethionine decarboxylase is reversibly inhibited by methylglyoxal-bis(guanylhydrazone) and ethylglyoxal-bis(guanylhydrazone); the K_i of these inhibitors is much higher for the *E. coli* enzyme than for the enzyme from prostate or yeast. Other inhibitors include decarboxylated *S*-adenosylmethionine,5′-(dimethylsulfonio)-5′-deoxyadenosine, and *S*-adenosyl-3-methylthiopropylamine (85, 113, 161, 234).

Point mutations and deletion mutations have been described in the gene (*speD*) for *S*-adenosylmethionine decarboxylase, resulting in decrease or complete absence of the enzyme (64, 202, 203, 208, 218). The gene has been mapped at 2.7 min on the *E. coli* chromosome. High-copy-number plasmids have been constructed containing the *speD*$^+$ gene, and strains containing these plasmids have been used for the purification of the enzyme (113, 114, 204).

Spermidine synthase (putrescine aminopropyltransferase). Spermidine is synthesized by the following reaction: putrescine + decarboxylated adenosylmethionine → spermidine + thiomethyladenosine. This enzyme has been purified to homogeneity. It has an M_r of 73,000 and contains two subunits. Cadaverine and spermidine can also serve as substrates, but the rate is slower than with putrescine (22). There is disagreement on the kinetics and stereochemistry of the reaction (54, 163, 241). The more recent studies (54, 163), which used nuclear magnetic resonance (NMR) measurements with stereospecifically labeled deuterated adenosylmethionine, indicated that inversion of the configuration occurred at the reacting methylene group, supporting an S_N2 displacement reaction.

Spermidine synthase is strongly inhibited by *S*-adenosyl-1,8-diamino-3-thiooctane and by dicyclohexylamine (160).

Polyamine Concentrations in *E. coli*

Gram-negative bacteria such as *E. coli* usually have high concentrations of polyamines (72). For example, when *E. coli* B or K-12 is grown to mid-logarthmic phase in minimal media at neutral pH, the cells contain approximately 13.1 μmol of putrescine and 4.7 μmol of spermidine per g (wet weight) of cells. The total intracellular concentration of putrescine is thus approximately 0.02 M and that of spermidine is about 0.006 M (196, 211, 219). These are rather high concentrations of cations, and the values are comparable to those for the intracellular concentration of Mg^{2+} (104). Under these growth conditions, no putrescine or spermidine is present in the medium, and no other diamines or polyamines have been detected in either the cells or the medium. As opposed to yeast and animal tissues, no spermine is present in *E. coli*.

Influence of culture conditions. The concentration of polyamines is markedly affected by the culture conditions. Consequently, it is difficult to evaluate any concentrations published in the literature unless enough details are given concerning the medium and other growth conditions. Both the total amount of polyamines and the putrescine-spermidine ratio are affected. Some of the factors that are important are the pH of the medium, the amount of aeration, the presence of such amino acids as arginine and lysine, the age of the culture, and the growth rate (209, 211). Thus, the amine content of cells harvested in the stationary phase after growth in crude media is higher than the values given above for cells grown in minimal media and harvested in logarithmic phase. Similarly, cultures that accumulate ornithine or arginine because of genetic or metabolic blocks increase their amine levels by decarboxylation of these amino acids (reviewed in reference 213). There have also been a number of studies on the influence of potassium ion concentration and osmolarity on the polyamine control of *E. coli* (143, 144, 175).

Putrescine and spermidine are decreased or absent in mutants that are defective in putrescine biosynthesis when these strains are grown in amine-free media. Such cells have small amounts of cadaverine (1,5-diaminopentane) and its aminopropyl derivative, even though these amines are not normally present in *E. coli* (43, 64).

Transport of polyamines into *E. coli*. The polyamine content of the bacteria is also affected by the amount of polyamines present in the medium, since active transport systems are present for putrescine and spermidine (196). Spermine is also taken up by *E. coli*, even though it is not normally present. The rate of spermine uptake is 10 times greater at pH 8 than at pH 7.

A transport system for putrescine and spermidine has also been described by Höltje (75), who found that these amines are taken up by the same inducible sytem that transports streptomycin into the cell. The relation of this transport system to the ones described above is not clear.

In the above transport systems, the polyamines appear to be transported against a concentration gradient; i.e., the total intracellular concentration of amines is greater than the concentration in the medium. However, it is not possible to evaluate whether a real concentration gradient occurs. We do not know the concentration of free amines within the cell, since it is likely that a large fraction of these basic compounds form complexes with intracellular polyacids.

Intracellular localization of polyamines. So far it has not been possible to devise experiments to demonstrate the localization of the polyamines in specific intracellular struc-

tures. When the cells are disrupted for assay, the basic polyamines form complexes with the intracellular polyacids. Thus, even though isolated organelles, such as ribosomes, can be shown to contain polyamines, we do not know whether this observation reflects the in vivo distribution of polyamines before lysis (193).

Minicells which have no DNA still contain a high concentration of polyamines (117), indicating that all of the polyamines are not normally in the nucleus.

Regulation of Biosynthetic Enzymes

Although many factors have been shown to affect polyamine biosynthesis in *E. coli*, the regulatory mechanisms by which many of these effects occur are still largely not understood. A large number of interacting pathways are involved, and hence overall regulation is necessarily complex. For example, there are two parallel pathways for the formation of putrescine, a pathway for the formation of decarboxylated *S*-adenosylmethionine, and a pathway for the biosynthesis of ornithine and its conversion to arginine. In addition, some of the substrates in the overall reactions, such as arginine, methionine, and *S*-adenosylmethionine, are involved in many other intracellular reactions and are also regulated by the end products of these reactions.

Control by intracellular concentrations of ornithine and arginine. The most striking difference between polyamine biosynthesis in *E. coli* and that in yeast and animal tissues is the presence in *E. coli* of both ornithine decarboxylase and arginine decarboxylase (133, 138, 141). In contrast to *E. coli*, yeast and animal tissues do not have arginine decarboxylase and synthesize putrescine only by the ornithine decarboxylase pathway. It is of interest that many plants have arginine decarboxylase and synthesize putrescine from arginine via agmatine (200).

The relative degree of use of each pathway by *E. coli* was carefully quantitated by Morris and Koffron (138) under various growth conditions. They showed that the relative flux through the ornithine or arginine decarboxylase pathway is controlled by the intracellular concentration of ornithine; intracellular arginine concentrations are usually rather constant and thus do not normally affect the flux significantly. Under normal growth conditions in minimal medium, more putrescine is synthesized by the ornithine decarboxylase pathway than by the arginine decarboxylase pathway. However, the ornithine decarboxylase pathway is used much less if the intracellular concentration of ornithine is lowered; such a fall in ornithine levels occurs, for example, when the biosynthesis of ornithine is inhibited and repressed by the addition of arginine to the growth medium. It should be noted that, in contrast to many other microorganisms, arginase is not present in *E. coli*, and thus arginine is not converted to ornithine.

The absence of arginase in *E. coli* may be responsible for the evolutionary survival of the two distinct and parallel pathways for putrescine biosynthesis. The arginine decarboxylase pathway ensures the biosynthesis of putrescine and spermidine under a variety of growth conditions.

It is striking, however, that even if the intracellular concentrations of both ornithine and arginine are markedly decreased, total polyamine biosynthesis is still substantial. For example, if an ornithine auxotroph of *E. coli* is grown in a chemostat with limiting amounts of ornithine or arginine in the perfusion fluid, the total polyamine level is still 16 to 50% of normal. Even under these conditions, where growth is limited by the low concentration of ornithine or arginine, 9 to 18% of the added amino acid is converted to putrescine and spermidine (211).

Control by intracellular concentrations of polyamines. In addition to control by the concentration of ornithine and arginine, the amount and activity of the ornithine and arginine decarboxylases are also controlled by the amount of polyamines present in the cell. Thus, when putrescine or spermidine was added to the chemostat experiments mentioned above, the activity of the ornithine and arginine decarboxylases decreased both in vivo and in vitro, indicating both repression and inhibition (211). However, the studies were not extensive, and no attempt was made to determine by immunological techniques whether there was an actual decrease in the amount of immunologically reactive enzyme.

Control by guanine nucleotides and by cyclic AMP. Ornithine decarboxylase is stimulated by GTP and inhibited by ppGpp (7, 76). Some relation of polyamines to the stringent response has been suggested (50, 76, 83). In one study, upon removal of a required amino acid, polyamine accumulation stopped in a stringent strain, but not in a relaxed strain (76). In another study (83), polyamines were shown to stimulate ppGpp synthesis in a polyamine-requiring mutant. Some evidence for a negative control by cyclic adenosine monophosphate of ornithine decarboxylase, arginine decarboxylase, and agmatine ureohydrolase at the transcriptional level in *E. coli* in vivo has also been reported (23, 181, 237). In general, however, more extensive experiments are needed before the in vivo significance of these observations can be established.

Control by antizyme. An intriguing, but still unclear, type of regulation is the finding of Canellakis and his associates (26, 69, 70, 100–102) that ornithine decarboxylase can be inhibited by three intracellular proteins that they called antizymes. These proteins form noncovalent enzymatically inactive complexes with ornithine decarboxylase; the binding and the inhibition are reversible. Similar inhibition has been observed with arginine decarboxylase. Two basic proteins and one acidic protein with antizyme activity have been found in *E. coli* and have been purified to homogeneity. Antizyme proteins have also been purified from other sources (26, 46, 67, 95). The inhibitory action of the antizymes can be reversed by an anti-antizyme (46, 67, 102) or by nucleic acids (80); this anti-antizyme activity may account for the activator found in *E. coli* (101). Although the role and mechanism of the antizyme action are unclear, the increase in antizymes when cells are grown in the presence of polyamines may indicate some physiological role for these proteins.

Two basic antizyme proteins of *E. coli* have recently been identified as the ribosomal proteins S20/L26 and L34 (151).

Metabolism

Formation of glutathionylspermidine. In cells growing logarithmically in minimal media there is no obvious metabolism of spermidine (192). However, when the cells reach stationary phase and the culture becomes slightly acid and somewhat anaerobic, all of the spermidine is found as glutathionylspermidine (γ-glutamylcysteinylglycylspermidine). Isotopic experiments indicated that the spermidine of glutathionylspermidine is in equilibrium with free intracellular spermidine. Glutathionylspermidine represents about 50% of the total intracellular glutathionine. Upon dilution of the culture into fresh medium, glutathionylspermidine is rapidly hydrolyzed to spermidine (214).

Enzymatic activities have been found in *E. coli* extracts for both the synthesis and breakdown of glutathionylspermidine, but no detailed studies have been carried out on the enzymes involved (197). Glutathionylspermidine has been isolated only from *E. coli* and has not yet been detected in other sources. The physiological role for this intriguing compound is completely unclear. (See Addendum in Proof.)

Acetylation of putrescine and spermidine. Contrary to earlier reports, only traces of acetylputrescine or acetylspermidine are found in cells growing logarithmically in minimal medium (192). Significant acetylation of the amines has been found in two situations. (i) Storage of a culture in the cold is associated with the formation of monoacetylputrescine and monoacetylspermidine, and (ii) addition of high concentrations of spermidine or spermine to the growth medium results in the formation of considerable monoacetylspermidine or monoacetylspermine (192). Acetylated polyamines have also been found under conditions in which large amounts of polyamines are formed endogenously (169).

Recently, *E. coli* has been shown to have a spermidine N-1 acetyltransferase which is still active at 4°C and may account for the acetylation noted in the cold in vivo (115).

Effects of Polyamines In Vivo

E. coli offers a particularly useful system for studying the physiological role of polyamines because mutants are available which do not contain any amines. It is desirable to study the effects of polyamine deprivation and supplementation in vivo, since it is very difficult to know which of the many effects described in vitro are important physiologically and which merely reflect the rapid formation of complexes and precipitates when polyamines are mixed with polyacids, such as DNA.

Despite the availability of mutants that contain no polyamines, we still cannot make any definitive statements on the physiological function of the polyamines. The experiments described in the next paragraph definitively indicate that polyamines are involved in some aspect of protein biosynthesis. However, it is important to emphasize that there is no reason to assume that polyamines affect only a single system.

Effects on protein biosynthesis in vivo. Surprisingly, mutant strains that do not contain any polyamines can still grow indefinitely, although the rate is only about 30% of the rate observed after polyamine addition (64, 207). However, when certain *rpsL* (*strA*) mutations are introduced into these strains, the requirement for polyamines becomes absolute; i.e., there is no growth in the absence of added polyamines (217). Since *rpsL* mutations are known to affect the S12 ribosomal protein (147), the most likely explanation for this effect is that the conformation or stability of the protein-synthesizing ribosomal complex is adversely affected by an abnormal S12 ribosomal protein, especially when no polyamines are present. This defect would be reversed, at least in part, by addition of polyamines. Alternatively, one might speculate that in these strains some ambiguity is necessary for optimal growth; *rpsL* mutations would decrease this ambiguity and polyamines would act by restoring some ambiguity in the translation process, i.e., comparable to the effect of streptomycin in the conditional-lethal mutants of Gorini (55, 147). An unexplained relationship between polyamine deprivation and streptomycin action has also been reported by Goldemberg and Algranati (52), who showed that the inhibition of protein synthesis by streptomycin is decreased in polyamine-starved bacteria.

From strains that have an absolute requirement for polyamines for growth, mutants can be isolated that no longer have this absolute requirement. Even though these new mutants can grow in the absence of polyamines, they are not true revertants; they still do not make polyamines and are still *rpsL*. The genetic locus for this phenotypic change has not been determined accurately but is in the ribosomal area (216). These suppressor strains are reminiscent of the *ram* mutation described by Gorini et al. (55).

More direct evidence for the involvement of polyamines in protein biosynthesis in vivo was obtained by studying the growth of bacteriophage T7 in *E. coli* mutants that contain no polyamines. Wild-type bacteriophage T7 grows well in polyamine-deficient hosts even if no polyamines are added to the medium. On the other hand, some amber mutants of this phage do not grow in polyamine-deficient hosts even if the host has an amber suppressor gene. However, if polyamines are added to the medium, these amber mutants give near-normal bursts. This requirement for polyamines for optimal translation of amber codons in vivo was confirmed by showing that infecting polyamine-depleted *E. coli* cells with bacteriophage T7 carrying an amber mutation in gene 1 results in an increased accumulation of the amber fragment of the gene 1 protein (215).

Another approach to the question of the in vivo involvement of polyamines in protein synthesis is that of Algranati et al. (1, 2, 53). They compared extracts from polyamine-depleted and polyamine-supplemented mutants with respect to ribosomal pattern, protein biosynthesis, and streptomycin-ribosome interaction. They have reported that such extracts have defective 30S ribosomal subunits, changed ribosomal profile, and decreased protein synthesis associated with decreased initiation. Igarashi et al. (82) attributed the defect in the 30S ribosome to a decrease in the S1 protein.

Recently, Mitsui et al. (129, 130) studied the proteins synthesized shortly after putrescine addition to polyamine-deficient *E. coli* strains. They found that polyamine administration resulted in a very rapid increase in the PI protein (M_r 62,000) and a slower increase in the ββ′ subunits of RNA polymerase. The PI protein was purified to homogeneity and found to have a stimulatory effect in in vitro protein-synthesizing systems.

The above results on the effects of polyamines on protein synthesis in vivo are consistent with earlier observations that, when polyamines are restored to polyamine-deficient mutants, protein synthesis recovers before DNA or RNA synthesis (240). On the other hand, Morris and Hansen (134) reported that upon polyamine starvation of a polyamine-deficient mutant, the decrease in both the rate of polypeptide elongation (β-galactosidase) and messenger RNA (mRNA) synthesis is proportional to the decrease in cellular growth rate.

Effects on DNA replication in vivo. Geiger and Morris (48) studied DNA replication in their polyamine-deficient mutants and concluded that polyamines affect the rate of movement of the DNA replication fork rather than the initiation of DNA synthesis.

Effects on osmotic fragility of spheroplasts and protoplasts. A possible involvement of polyamines on membrane or cell wall stability is suggested by their ability to decrease the lysis of spheroplasts and protoplasts when diluted into hypotonic media (61, 106, 186, 191). Polyamines have also been shown to stabilize halophilic organisms and a variety of other fragile bacteria (reviewed in references 210 and 213). There is no definitive evidence yet that these findings reflect a normal physiological function of the polyamines. In this

connection, however, it may be of interest to note the presence of cadaverine covalently linked to a peptidoglycan of another species of microorganism (*Selenomonas ruminantium*) (91).

Polyamines in bacteriophages. The various studies on the presence of polyamines in bacteriophages and viruses have been reviewed previously (11, 31). The earliest definitive study on the presence of polyamines in bacteriophages was the finding of Ames et al. (4, 5) that T-even bacteriophages contain putrescine and spermidine. These amines represent one-third of the cations needed to neutralize the phage DNA. Since T-even bacteriophages are impermeable, these analyses represent the most convincing data for a DNA-polyamine interaction in vivo. Despite the presence of the polyamines as integral components of bacteriophage T4, the polyamines do not appear to be essential since bacteriophage T4 grows well and has a normal burst size in amine-deficient mutants (64). An earlier report (43) that bacteriophage T4 did not grow in another polyamine-deficient organism may be explained by an increase in the lag period, associated with the lower growth rate of the polyamine-deficient host.

It is more difficult to interpret data on the polyamine content of the permeable T-odd bacteriophages since these bacteriophages may have taken up the polyamines after the cells were lysed or may have lost their amines to the medium during purification. It is clear, however, that the T-odd bacteriophages, such as wild-type bacteriophage T7, do grow well in polyamine-deficient hosts (64). The importance of divalent cations or polyamines to the stability of these T-odd phages, however, is shown by earlier experiments in which bacteriophage T5 was inactivated by treatment with sodium citrate or ethylenediaminetetraacetate (EDTA); addition of polyamines (or higher concentrations of divalent cations) prevents this inactivation (205).

The importance of intracellular cations to bacteriophage reproduction is also suggested by recent experiments (97) showing that cellular leakage of K^+, Mg^{2+}, putrescine, and spermidine occurs after T7 infection of *E. coli* K12-M and suggesting that this leakage might be the cause of arrested phage morphogenesis. Similar cellular leakage was found after infection of *E. coli* K-12 (λ) with bacteriophage T4 rII; the decreased phage yield observed in this system could be prevented by addition of Mg^{2+} or polyamines (44).

In contrast to the findings with bacteriophages T4 and T7, bacteriophage λ does not grow in polyamine-deficient hosts, either after induction of a lysogen or after a lytic infection. The defect in the λ infections seems to be relatively early in the λ life cycle, since no killing of the host was observed. Other experiments, however, implicated a polyamine requirement for later steps in λ replication as well, since polyamines were shown to be required for optimal packaging of phage heads or for optimal joining of phage heads and phage tails (16, 65, 87). In vitro studies also showed that spermidine is required for the integration of λ DNA into chromosomal DNA and stimulates the excision of λ prophage (56, 145).

Effects of spermidine analogs in vivo. Morris and his associates (86, 103) have studied the effect of a series of spermidine analogs with the structure $NH_2(CH_2)_nNH(CH_2)_3NH_2$ on reversing the decreased growth rate and decreased rate of synthesis of protein and mRNA observed in a mutant deficient in putrescine biosynthesis. *sym*-Norspermidine ($n = 3$) was equivalent to spermidine; compounds with $n = 5$ or $n = 6$ were partially effective, but compounds with $n = 7$ or $n = 8$ were essentially inactive. Similar results were obtained when these analogs were used to stimulate the movement of the DNA replication fork in polyamine-deficient cells (48). These authors pointed out that their results indicated some specificity in the interaction of spermidine at its site of action, rather than a nonspecific acid-base interaction.

In contrast to these results, these homologs showed comparable activity to spermidine in stabilizing *E. coli* protoplasts against osmotic lysis (186).

Toxicity of spermine. High concentrations of spermine (>500 μg/ml) are bactericidal for *E. coli*. The toxicity of spermine is much greater at pH 8 than at pH 7, presumably as a result of increased transport at the higher pH. Protein synthesis appears to be required for the toxic effect since the toxicity is inhibited by chloramphenicol (119, 174, 195).

Much higher toxicity is observed when spermidine or spermine is added together with a beef serum amine oxidase preparation (195). The toxic agent is presumably the aldehyde resulting from the oxidation of the terminal (or primary) amino group(s) (201).

Effects of Polyamines In Vitro

A very large number of experiments have been reported on the effects of polyamines in a variety of enzymatic systems isolated from *E. coli*. These studies are all of interest in showing the potential actions of polyamines. As indicated above, however, it is very difficult to decide whether an observed effect of polyamines in vitro is physiologically significant or merely the result of a nonspecific interaction of the polyamines with acidic groups on the enzyme, substrate, or product of the reaction.

There are too many papers on the in vitro effects of polyamines to permit their citation in this review, especially since they have been covered in several recent reviews and symposia (12, 25, 68, 139, 168, 180, 198, 200, 235). It also seems artificial to present in vitro studies according to the source of the extracts or enzymes utilized. It is obvious that the results of in vitro studies with systems derived from nonmicrobiological sources are applicable to comparable studies in systems derived from *E. coli* or other microorganisms and vice versa. Furthermore, some of the most significant in vitro studies, such as on the interaction of polyamines and polyacids (such as nucleic acids), are carried out at a chemical level; the source of the reagents is usually of secondary significance. For these reasons, we shall list only some of the in vitro studies here and refer the reader to the other reviews for more extensive discussions and references.

Of particular relevance to the in vivo studies on protein biosynthesis discussed in a previous section are the effects of polyamines on ribosome dissociation and subunit stability and on in vitro translation systems, especially with regard to increasing the fidelity of in vitro translation. Polyamines are usually added as part of the optimal incubation mixture recommended for in vitro protein translation systems.

Polyamines have been shown to improve discrimination during the initial binding of aminoacyl transfer RNA (tRNA) to the ribosome as well as in the subsequent proofreading steps. Other studies have been concerned with the stimulation of aminoacyl-tRNA synthetase by spermidine, especially in the absence of Mg^{2+}, and the effect of polyamines on tRNA conformation.

Other studies include studies on polyamine-DNA interactions by X-ray, linear dichroism, NMR, and electron micros-

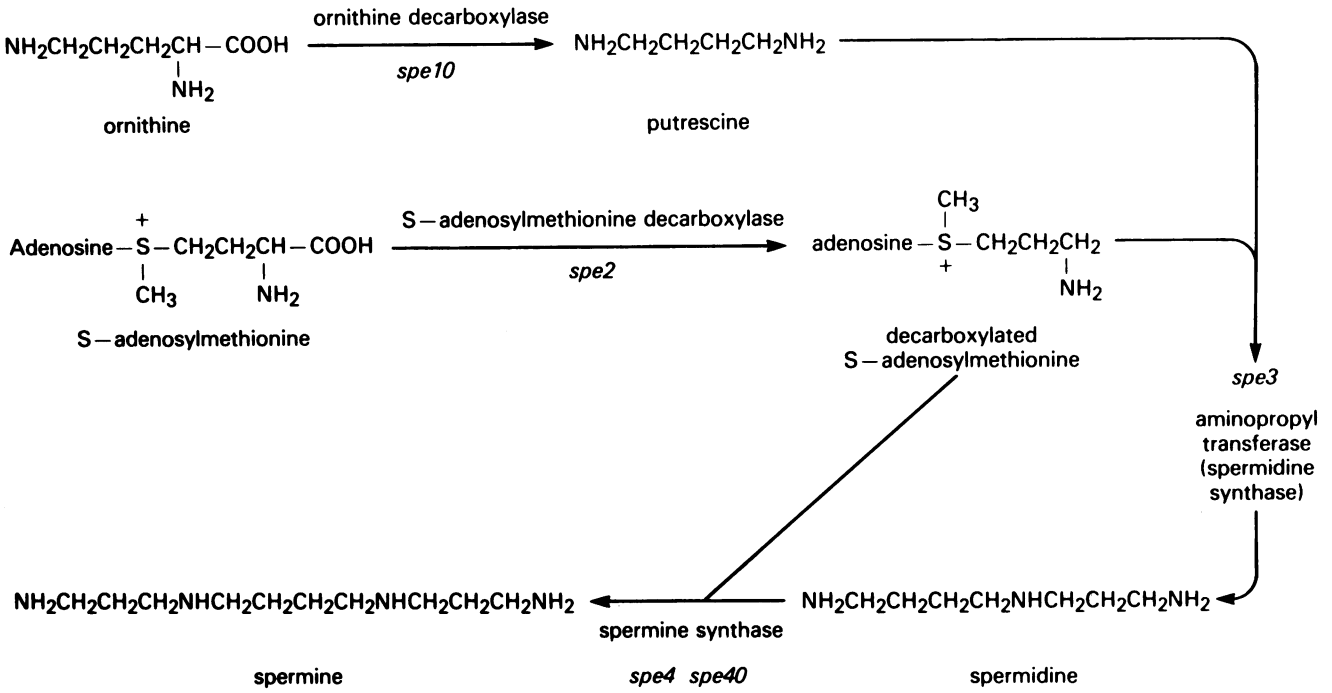

FIG. 2. Polyamine biosynthetic pathway in *S. cerevisiae*.

copy; requirement of spermidine for catenation of supercoiled DNA by gyrase; and B-DNA to Z-DNA conversion. A wide variety of effects of polyamines have been reported on such enzymes as aminoacyl-tRNA synthetases, T4 DNA ligase, and many others.

In most experiments, but not all, the effects observed after polyamine addition in vitro can be duplicated, at least in part, by the addition of larger amounts of Mg^{2+}. In some systems, the reverse is also true; namely, polyamines can decrease (but not abolish) the requirement for Mg^{2+}. Although this interrelationship of Mg^{2+} and polyamines in vitro makes it difficult to interpret the specificity of the polyamine effects observed in these experiments, such observations do not necessarily negate the physiological significance of the polyamine effects. As shown in the previous section, polyamines definitely have important physiological effects in vivo, and further work needs to be done to clarify which of the in vitro observations are applicable in vivo.

POLYAMINES IN *SACCHAROMYCES CEREVISIAE*

Concentration of Amines

Putrescine, spermidine, and spermine are present in *S. cerevisiae* (33, 165, 232). The concentrations vary with the strain and the culture conditions; one strain growing in logarithmic phase in minimal medium had 0.45 μmol of putrescine, 2 μmol of spermidine, and 0.3 μmol of spermine per g (wet weight) of cells (33). No derivatives of these amines have been reported.

Biosynthesis of Putrescine

S. cerevisiae has the same biosynthetic pathway for the amines (Fig. 2) as that described in animal cells. This has been demonstrated by both biochemical and genetic studies. In *S. cerevisiae*, the decarboxylation of ornithine appears to be the only mechanism for the biosynthesis of putrescine (Fig. 2) (35, 232). This was shown by the complete absence of putrescine both in mutant strains unable to synthesize ornithine (232) and in mutant strains lacking ornithine decarboxylase (35, 79, 232).

Purification of ornithine decarboxylase. Ornithine decarboxylase has been purified 1,500-fold to homogeneity from a derepressed strain (223, 224). As isolated, the purified enzyme is a monomer of M_r 68,000. However, the protein appears to have an M_r of 86,000 in vivo, since the 86,000-M_r form is obtained if the extracts are prepared rapidly in the presence of a proteolytic inhibitor (phenylmethylsulfonylfluoride) (225). Although the 86,000-M_r form has been partially purified (224), little work has been done on this form of the enzyme. The enzyme requires pyridoxal phosphate and is stabilized by thiols. Putrescine is a competitive inhibitor, whereas the mechanism-based inhibitor α-difluoromethylornithine irreversibly inactivates the enzyme in vitro (166, 225). Ornithine decarboxylase has also been purified 2,100-fold to homogeneity from *Saccharomyces uvarum*. As isolated, this enzyme has an M_r of 71,000 (166).

Mutants lacking ornithine decarboxylase activity. Mutants lacking ornithine decarboxylase have been isolated in three different laboratories, and the mutations have been designated *spe1* or *spe10* (35, 79, 232). Recent work has indicated that the *spe1* and *spe10* genes are the same; the *spe1* and *spe10* mutations do not complement each other (S. K. Taneja, H. Tabor, and C. W. Tabor, unpublished data). The ornithine decarboxylase gene has been cloned and can be expressed in both *S. cerevisiae* and *E. coli* (45). The specific map position is not known.

spe1 and *spe10* mutants have no putrescine and very little or no spermidine or spermine after several subcultures in amine-free media. One of these strains has an absolute requirement for putrescine, spermidine, or spermine for

growth; the others have very long doubling times (30 to 60 h in the absence of added amines) (35, 232). In addition to these growth defects, the deprived mutants cannot sporulate and cannot maintain the double-stranded RNA killer plasmids (37, 226). One mutant also showed a defect in the transport of [^{14}C]choline and the incorporation of [^{14}C]choline into phosphatidylcholine (79).

Secondary mutations arise in *spe10* mutant strain cultures after prolonged incubation in the absence of amines (35); these strains are then able to grow in the amine-deficient medium. The new mutants are not the result of reversions of the original *spe10* mutations, but of new mutations in the genes coding for spermidine aminopropyltransferase, the last step in the biosynthesis of spermine. Two classes of these mutations have been identified. One group (*spe10-SPE40* mutants) grows at a nearly normal rate and has high levels of ornithine decarboxylase and substantial intracellular polyamines. In contrast, in the second group (*spe10-spe4* mutants), ornithine decarboxylase activity is still too low to be detectable; these strains grow faster than the *spe10* parent, however, although the growth rate is still very slow.

The mechanism of the reversal of the *spe10* mutation by *spe4* or *SPE40* mutations is completely unknown. Since the cloning experiments mentioned above show that *spe1* (*spe10*) codes for the structural gene for ornithine decarboxylase, it is unclear why defects in this gene can be reversed by mutations in the *spe4* or *SPE40* gene.

Regulation of ornithine decarboxylase. The regulation of *S. cerevisiae* ornithine decarboxylase is not well understood. Although mutants have been obtained which have a markedly increased level of ornithine decarboxylase activity (35), they have not been adequately characterized; multiple genetic sites seem to be involved (unpublished data). Ornithine decarboxylase activity is much higher in rapidly growing cultures at low cell densities than in dense cultures (94).

The half-life of the *S. cerevisiae* ornithine decarboxylase, determined after protein synthesis has been stopped with cycloheximide or trichodermin, is 6 h (223). This is not a very short half-life compared with the 10- to 20-min half-life found in other eucaryotic cells.

Effects of polyamines on ornithine decarboxylase. Addition of spermidine and spermine to the growth medium leads to a sharp, rapid fall in ornithine decarboxylase activity (half-life of 2 h; almost complete loss of activity in 4 h) (223). This loss in activity is not associated with any significant loss of the immunologically reactive enzyme protein and requires active protein synthesis. These findings indicate a posttranslational modification of the enzyme. The nature of this modification, however, is still unknown, but it does not seem to be due to a transamidation with putrescine or spermidine (225), which had been postulated (176), or a change in the K_m for pyridoxal phosphate, as occurs in *Physarum polycephalum* (see below). No evidence for an antizyme has been found yet in *S. cerevisiae*.

Mutants that do not synthesize spermidine or spermine, such as *spe2* and *spe3* mutants, have high ornithine decarboxylase activities and are useful as the starting material for the purification of the enzyme (223, 224). These findings, as well as the relationship of the *spe10* and *SPE40* mutations discussed above, indicate a regulatory system involving control of ornithine decarboxylase activity by spermidine and spermine, with the possible additional involvement of the *spe3* or *spe4* gene product. Putrescine is a competitive inhibitor of ornithine decarboxylase in vitro. Spermidine and spermine have only a slight inhibitory effect on the activity in vitro.

Biosynthesis of Spermidine and Spermine

Methionine adenosyltransferase (*S*-adenosylmethionine synthetase). The aminopropyl moiety of spermidine and spermine is derived from *S*-adenosylmethionine. *S*-Adenosylmethionine biosynthesis has been extensively studied in *S. cerevisiae* since the initial work of Mudd and Cantoni in 1958 (142) and has been reviewed very recently (199). Two forms of methionine adenosyltransferase have been separated and purified. These represent two independent proteins encoded by two separate genes (27). As with the analogous enzymes from other sources, each form of the enzyme carries out two reactions: (i) the synthesis of *S*-adenosylmethionine from ATP and methionine and (ii) the ordered splitting of P_i and PP_i from a putative enzyme-bound inorganic tripolyphosphate (28).

***S*-Adenosylmethionine decarboxylase.** *S*-Adenosylmethionine decarboxylase has been purified 12,000-fold to homogeneity (32, 36); the native enzyme is composed of two subunits, of 41,000 M_r each. The activity is lost after reduction with sodium borohydride or treatment with carbonyl-binding reagents. The enzyme has 1 mol of covalently linked pyruvate per mol of subunit; this pyruvate is required for activity. Pyridoxal phosphate is not present in the enzyme (32, 165). The enzyme resembles other adenosylmethionine decarboxylases from other eucaryotic organisms in that it is activated by putrescine but not by Mg^{2+} and is inhibited reversibly by methylglyoxal-bis(guanylhydrazone) and by decarboxylated *S*-adenosylmethionine.

Mutants lacking *S*-adenosylmethionine decarboxylase. Mutants deficient in *S*-adenosylmethionine decarboxylase activity (*spe2*) have been isolated after mutagenesis of *S. cerevisiae* (33, 232). The mutated gene, which is tightly linked to *arg1* (33), has been definitively located on chromosome XV by Hilger and Mortimer (73). In some of these mutants, the enzyme activity is not detectable, and no spermidine or spermine is synthesized. In other mutants, only a partial loss of the enzymatic activity is found, and decreased amounts of spermidine and spermine are present (33). The enzyme from one of the latter mutants is temperature sensitive, suggesting that the affected gene codes for the structural protein. Most of the strains with a marked deficiency in *S*-adenosylmethionine decarboxylase overproduce both ornithine decarboxylase and putrescine (33–35).

The effects of spermidine and spermine deprivation in these strains are: (i) marked slowing of the growth rate; (ii) loss of ability of diploids homozygous for the *spe2* gene to sporulate; and (iii) loss of double-stranded RNA killer plasmids K_1 and K_2 (33, 39, 226). However, since such strains have a high putrescine content, they are able to maintain the L-A-E double-stranded plasmid which is lost in strains lacking all three amines, i.e., lacking ornithine decarboxylase (226).

Mutants lacking putrescine aminopropyltransferase and spermidine aminopropyltransferase. *S. cerevisiae* has two separate enzymes, putrescine aminopropyltransferase (spermidine synthase) and spermidine aminopropyltransferase (spermine synthase), coded for by independent genes, which carry out the biosynthesis of spermidine and spermine, respectively (34, 35). The enzymes have not been purified.

Mutants lacking putrescine aminopropyltransferase (*spe3* mutants) have been isolated from a mutagenized culture but have not been studied extensively (34). These *spe3* strains lack both spermidine and spermine. The *spe3* mutation has not been mapped. The phenotypes of the *spe3* mutants

include those listed above for *spe2* mutants, which also lack spermidine and spermine. In addition, adenosylmethionine and decarboxylated adenosylmethionine concentrations are elevated in *spe3* mutants, and the adenosylmethionine decarboxylase activity is very low (5% of the wild-type level) (34).

Strains lacking spermidine aminopropyltransferase and therefore lacking spermine have been isolated among *spe10* mutants as bypassers of the amine requirement for growth (35); i.e., these double mutants can grow in minimal medium lacking polyamines (see section on ornithine decarboxylase above). Two classes of mutants have been identified. (i) One class of mutations (*spe4*) is recessive and may result from a mutation of the structural gene for spermidine aminopropyltransferase. These strains have no detectable spermidine aminopropyltransferase either in vivo or in vitro. These mutants do not have detectable ornithine decarboxylase, and no putrescine, spermidine, or spermine is found in the cells. The growth rate of the *spe10-spe4* strains is very slow but is faster than that of the parent *spe10* strain. *SPE10*$^+$-*spe4* strains have also been constructed and grow at a normal rate; these strains contain no spermidine aminopropyltransferase and no spermine. (ii) The second class of mutants contain a mutation, *SPE40*, which is dominant and is not linked to the *spe4* gene. These *SPE40* strains grow at a nearly normal rate and have high ornithine decarboxylase activity and moderate levels of putrescine and spermidine, but no detectable spermidine aminopropyltransferase or spermine. The *SPE40* mutation is tightly linked to the *spe10* mutation, and it is unknown whether the two mutations are on the same gene. The nature of the interaction between the *spe4* and *SPE40* mutations and *spe1* (*spe10*) is completely unclear.

Effects of Polyamines In Vitro on tRNA of *S. cerevisiae*

Polyamines have been found to affect the tRNA of *S. cerevisiae* in vitro in two ways. First, the accuracy and extent of tRNA processing in vitro is increased by spermidine (158). The effect appears to be a stimulation by polyamines of a specific membrane-associated endonuclease required for the processing. Second, many studies on the effects of polyamines on the structure and activity of tRNA have been carried out with several tRNAs isolated from *S. cerevisiae*. For reviews of this area, see references 31, 180, 198, and 200.

POLYAMINES IN *PHYSARUM POLYCEPHALUM*

Studies of *P. polycephalum* are of interest because the life cycle of this organism may permit eventual correlation of the polyamine profile with different stages of development. Another advantage of this organism is that synchronized cultures may be used.

Polyamine concentrations. Growing microplasmodia of *P. polycephalum* contain high concentrations of both putrescine (89 μmol/g of protein) and spermidine (38 μmol/g of protein); very low levels of spermine have also been detected (126). When the microplasmodia differentiate into dormant spherules, the putrescine content is reduced to about one-third the original level, but the spermidine content is unchanged.

Ornithine decarboxylase and adenosylmethionine decarboxylase. Cytoplasmic ornithine decarboxylase has been purified to homogeneity by two laboratories with different results. In one study (13), the homogeneous protein (obtained after 3,500-fold purification) was a dimer, with an M_r of 80,000; the subunit size was 43,000 M_r. In another study, the enzyme (after a 5,000-fold purification) was a monomer with an M_r of 52,000 (121); two forms of the enzyme (A and B forms) with the same molecular weight have been obtained. (See below for discussion of these two forms.) In addition to these preparations of cytosolic ornithine decarboxylase, another form of ornithine decarboxylase has been purified from nucleoli of *P. polycephalum* (9). This preparation differed from the cytosolic preparations in its molecular weight (M_r 70,000) and amino acid composition.

Adenosylmethionine decarboxylase has been found in crude extracts of *P. polycephalum* but has not been purified. This enzyme, in contrast to the *E. coli* enzyme, is inhibited by Mg^{2+}; it also differs from the *S. cerevisiae* and mammalian enzymes in that putrescine has no effect on its activity. The enzyme is not stimulated by pyridoxal phosphate, but it is not known whether covalently bound pyruvate is present (126).

Variations in the activity of both of these enzymes during the cell cycle and during germination of the spherules have been described (126). Both enzymes have a very rapid turnover rate; the half-lives, assayed as enzymatic activity, are 14 and 21 min for ornithine decarboxylase and adenosylmethionine decarboxylase, respectively.

Regulation of polyamine biosynthesis in *P. polycephalum*. The regulation ornithine decarboxylase in *P. polycephalum* has been studied extensively by Mitchell and his associates (120–128). The very short half-life of the enzyme is comparable to the values obtained for ornithine decarboxylase in higher eucaryotes. This short half-life permits a very rapid and sensitive response by the cell to an increased or decreased need for polyamines. However, no immunological studies have been carried out to test whether the short half-life is due to protein modification or to protein turnover.

As mentioned above, two forms of the enzyme are found that differ in their affinity for pyridoxal phosphate and in their inhibition by difluoromethylornithine. (i) The more active form (A form) has a low K_m for pyridoxal phosphate. (ii) The less active form (B form) has a very high K_m for pyridoxal phosphate and therefore has almost no activity when measured in the presence of low concentrations of pyridoxal phosphate. In rapidly growing cells, about equal amounts of each form are present. The B form is favored by growing the cells in the presence of spermidine or spermine, by inhibiting protein synthesis, and by high osmolarity of the growth medium (120, 123–125, 127).

The A form can be converted to the B form in vitro by the addition of stoichiometric amounts of a partially purified protein (protein-converting factor) (120–122). This conversion is usually measured by the relative activities found in the presence of high and low concentrations of pyridoxal phosphate. The A-to-B conversion in vitro requires the presence of spermidine or spermine, but the amines are not incorporated into the enzyme. This conversion is inhibited by ATP and by other nucleotides, as well as by high ionic strength. This modification of the A form to the B form results from a posttranslational covalent modification of the enzyme and is very different from the inactivation of ornithine decarboxylase by antizyme which was discussed in the *E. coli* section. The mechanism of the A-form-to-B-form conversion, however, is still completely unknown.

Another postulated type of posttranslational modification is the phosphorylation of nucleolar ornithine decarboxylase reported by Kuehn and co-workers (9, 40). These authors reported that purified nucleolar ornithine decarboxylase is closely associated with a polyamine-activated protein kinase

which, in the presence of ATP, phosphorylates and inactivates the ornithine decarboxylase in vitro. However, the significance of these observations is not clear, since the nucleolar particles contain only a small percentage of the total ornithine decarboxylase of the organism, and the extent of purification of the nucleolar enzyme was not defined.

POLYAMINES IN *NEUROSPORA CRASSA*

The most important studies on polyamines in *N. crassa* are concerned with the compartmentation of the amines in vacuoles and the characterization of mutants deficient in the biosynthesis of putrescine. *N. crassa* contains putrescine, spermidine, small amounts of spermine, and diaminopropane (42, 66, 229). By studying the flux of labeled amines, Davis and his associates (154–156) have studied the regulation of polyamine biosynthesis, turnover, and intracellular distribution in *N. crassa*. They have shown that much of both the putrescine and spermidine present is sequestered in vacuoles and is not freely available for biosynthetic conversion to spermidine and spermine, respectively. These data were confirmed by isolation of the vacuoles (38). Ornithine, which is the only source of endogenous putrescine, is also almost completely localized in the vacuoles and is not freely exchangeable with cytosolic ornithine (21, 92). This intracellular localization of both ornithine and the amines plays an important role in the control of polyamine biosynthesis. *N. crassa* is the only microorganism for which intracellular sequestration of polyamines in vacuoles has been shown in vivo.

In *N. crassa*, putrescine is synthesized only by ornithine decarboxylase, a cytosolic enzyme (230). Two types of mutants have been described that are not able to synthesize putrescine. (i) One type (*aga*) lacks arginase and thus cannot convert arginine to ornithine. Added arginine represses ornithine biosynthesis completely, and therefore no amines can be made. In the presence of arginine, *aga* strains grow very slowly unless ornithine or polyamines are added to the medium (41). These cultures contain no putrescine, spermidine, or spermine, but do contain cadaverine and aminopropylcadaverine. Indirect evidence has been presented that cadaverine is formed by a derepressed ornithine decarboxylase with a weak lysine decarboxylase activity and that the slow growth is supported by these small amounts of cadaverine (42, 157). (ii) A second type of mutant (*spe-1*) lacks ornithine decarboxylase and cannot grow without exogenous polyamines (116, 157). These studies indicate that amines are essential for the growth of *N. crassa*.

The first demonstration that the aminopropyl moiety of spermidine is derived from methionine was done with *N. crassa* (57). No further studies have been carried out on the specific steps involved in the biosynthesis of spermidine and spermine in *N. crassa*.

POLYAMINES IN *ASPERGILLUS NIDULANS*

The first mutant with an absolute requirement for putrescine for growth was the *A. nidulans* auxotroph isolated by Sneath in 1955 (183). (Earlier, in 1948, Herbst and Snell [71] had shown that a natural isolate of *Haemophilus parainfluenzae* required putrescine for growth. See reference 198 for references to other early papers on the growth-stimulating effects of polyamines in various microorganisms.) The mutation (*puA*) was later shown to map in linkage group II and to result in a deficiency in ornithine decarboxylase (29, 78, 187). The growth defect could be reversed by the addition of putrescine or relatively high concentrations of spermidine or spermine. The requirement for high concentrations of the latter amines was later shown to result from poor transport of these amines (184, 185). Putrescine auxotrophy also occurs with double mutants that are both blocked in ornithine biosynthesis and lack arginase (8).

Independent transport systems were shown to exist for putrescine and spermidine. More recently a recessive mutant has been described (*spsA1*) that has a more effective uptake system for spermidine and spermine (184); the mechanism of the change in the transport system has not been clearly defined. The *spsA1* mutation also increases the toxicity of spermidine and spermine. The *spsA1* gene is located between *galE* and *cnxH* on group III.

A. nidulans contains substantial concentrations of spermidine and spermine and a lower concentration of putrescine. The specific enzymes involved in the biosynthetic pathway have not been purified. However, it has been shown (189) that both ornithine decarboxylase and adenosylmethionine decarboxylase increase 50- to 100-fold in germinating conidia; both enzyme activities fall approximately 20 h later. The concentration of intracellular amines also increases, but to a lesser extent.

Ornithine decarboxylase turns over rapidly, with a half-life of 35 min. The half-life is increased to 5 h when the cells are grown in the presence of the putrescine analog 1,4-diaminobutanone; 1,4-diaminobutanone has been shown to protect ornithine decarboxylase from proteolysis in vitro (188). Ornithine decarboxylase is inhibited competitively by putrescine. No arginine decarboxylase has been found in *A. nidulans*.

There have been a number of studies (reviewed byStevens and Winther in reference 190) attempting to correlate polyamine levels in the *puA* mutant with DNA, RNA, and protein biosynthesis, but the results have been inconclusive. In general, in these studies, as well as comparable studies in other systems, the results are difficult to interpret because polyamine pool sizes do not respond immediately to polyamine deprivation or to polyamine supplementation and temporal relationships do not necessarily indicate a direct cause-and-effect relationship.

UNUSUAL AMINES AND AMINE DERIVATIVES IN MICROORGANISMS

It is striking that in almost all microorganisms, the major, or only, polyamines are putrescine, spermidine, and spermine. There are a number of interesting exceptions, and some of these are described below.

Thermophilic organisms. A variety of novel polyamines have been described in *Thermus thermophilus* and have been reviewed by Oshima (149, 150). The structures are:

norspermidine, $NH_2(CH_2)_3NH(CH_2)_3NH_2$
sym-homospermidine, $NH_2(CH_2)_4NH(CH_2)_4NH_2$
thermine, $NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
thermospermine, $NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$
caldopentamine,
$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
homocaldopentamine,
$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$.

Most of these amines have also been found in other thermophilic organisms; a few have also been reported in mesophilic bacteria and a few higher organisms. The polyamine composition of the extreme thermophiles depends on the growth temperature; cells at high temperatures contain more tetraamines and pentaamines than do cells at lower temperatures.

Oshima (150) also showed that no activity is found in an in vitro protein-synthesizing system from *T. thermophilus* if the incubation temperature is between 65 and 75°C unless spermine is present. Mg^{2+} and diamines are ineffective.

Essentially no work has been done on the biosynthesis of these interesting compounds, although Paulin et al. (153) reported that *Clostridium thermohydrosulfuricum* has a propylamine transferase activity that can use 1,3-diaminopropane as a substrate in the synthesis of thermine. The biosynthetic pathway for 1,3-diaminopropane in these organisms is not known. The only known biosynthetic pathway for 1,3-diaminopropane is the oxidation of spermidine by a *Serratia* sp. spermidine dehydrogenase (10, 194).

Polyamine-containing antibiotics. A number of antibiotics produced by bacteria and fungi contain spermine, spermidine, or a closely related polyamine derivative in covalent linkage. Umezawa (227) recently reviewed his studies on some of these polyamine-containing compounds, especially those that have antitumor activity. Some compounds in this general group are bleomycins, spergualins, tallysomycins, cinodine, and the edeines (60, 99, 227, 228).

Polyamine-containing siderophores. Agrobactin and parabactin, two siderophores (microbial ferric ion transport compounds), are catechol derivatives of a threonylspermidine tertiary amide (146, 162). These compounds are found in the medium of several microorganisms.

2-Hydroxyputrescine (1,4-diaminobutane-2-ol). 2-Hydroxyputrescine has been isolated from *Pseudomonas* sp. strain Kim (98, 221, 222), which contains no spermidine, and from *Pseudomonas acidovorans* (93), which contains 50 mM putrescine, 3 to 5 mM spermidine, and 45 mM hydroxyputrescine. In *Pseudomonas* sp. strain Kim, hydroxyputrescine is more effective than putrescine in stabilizing the ribosomes (173).

α-Putrescinylthymine. Bacteriophage ΦW-14, isolated from *P. acidovorans*, contains a covalently bound putrescine in its DNA. A hypermodified base, α-putrescinylthymine, is the only known example of a covalently bound polyamine in DNA. This new base replaces 50% of the thymine in the phage. The modification occurs at the polynucleotide level, with label from ornithine appearing in the hypermodified base in vivo; in extracts, labeled putrescine is incorporated into the DNA. Studies with amber mutants indicate that a portion of the α-putrescinylthymine is essential for the production of viable phage, probably because of its effect on DNA packaging in the phage head (96, 107, 108, 118, 182).

POLYAMINES AND DIFLUOROMETHYLORNITHINE

Special mention should be made of the many studies done with difluoromethylornithine, since the introduction of this compound has markedly influenced the course of polyamine research in recent years. Difluoromethylornithine and its monofluoro analog are mechanism based ("suicide"), irreversible inhibitors of ornithine decarboxylase. They are very nontoxic, and because of their mechanism-based action, they are very specific for ornithine decarboxylase.

Difluoromethylornithine has been used extensively in a large number of studies designed to determine the effect of loss of ornithine decarboxylase in vivo in animals, tissue cultures, and microbiological materials. The use of this inhibitor is particularly important for organisms for which no ornithine decarboxylase mutants are available. However, despite the dramatic inhibition of the enzyme in vitro, in vivo inhibition is not complete, and the treated cells or bacteria usually still contain some residual polyamines, especially spermine. In addition, resistant cell lines develop.

There have been numerous studies on the practical application of difluoromethylornithine therapy in the chemotherapy of neoplasias and of some infectious diseases and as an abortifacient. However, despite the marked inhibition of ornithine decarboxylase and decreased polyamine levels, the overall therapeutic value has so far been disappointing even when this drug was combined with other inhibitors of polyamine biosynthesis or other therapies. Work in this area is still going on actively, and some promising results have been obtained in the treatment of trypanosomiasis and coccidiosis.

Because of the many papers in this area, we are not including specific references here. The reader is referred to the recent reviews of Williamson (236) and of Sjoerdsma and Schechter (182a).

ADDENDUM IN PROOF

Recently, a related glutathione-spermidine conjugate has been identified in trypanosomatids and shown to be a cofactor for the glutathione reductase of the organism (A. H. Fairlamb, P. Blackburn, P. Ulrich, B. T. Chait, and A. Cerami, 1985, Science, in press; A. H. Fairlamb and A. Cerami, Mol. Biochem. Parasitol. **14**:187–198, 1985). The structure is N_1,N_8-*bis*(L-gamma-glutamyl-L-hemicystinylglycyl)spermidine.

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