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Management of Polyamine Pools and the Regulation of Ornithine Decarboxylase

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The management of polyamine synthesis and polyamine pools differs fundamentally from that of most other small molecular-weight endproducts. The polyamines are vital to growth and important cellular functions, but they are toxic in excess. I argue here that their multivalent cationic character, leading to binding to cell constituents, precludes fluent feedback inhibition of synthesis. This has led to the development of elaborate alternative regulatory mechanisms controlling ornithine decarboxylase, the key initial enzyme of the pathway. Poorly regulated polyamine synthesis and the toxicity of polyamines impose upon cells a need to control uptake and to dispose of excess polyamines. Recent data on polyamine transport suggest unorthodox mechanisms of accomplishing these functions.

Key words: polyamine synthesis, polyamine transport, ornithine decarboxylase control

PROPERTIES AND CELLULAR DISTRIBUTION OF POLYAMINES

The polyamines (putrescine, spermidine, and spermine) are multivalent, aliphatic cations having two, three, or four positive charges at physiological pH (Fig. 1). Accordingly, they bind readily to ribosomes, DNA, RNA, and membranes. Polyamine-auxotrophic mutants demonstrate the indispensability of polyamines, even if their cellular roles remain uncertain. However, the elaborate control of one of the first enzymes of the pathway, ornithine decarboxylase, suggests the importance of managing polyamine levels properly. The activity of this enzyme, one of the first to rise in cells entering a rapidly growing state, is regulated by a balance of mechanisms including transcription, translation, and rapid turnover of the enzyme protein. My thesis here is that unusual schemes of managing polyamine pools have developed around the tendency of polyamines to bind to cellular anions. In particular, the elaborate regulation of ornithine decarboxylase reflects the difficulty of using polyamines as effectors of feedback inhibition, and fine control of pools often requires polyamine excretion.

Biologists have argued about the functions of polyamines for years. An older generation of biochemists developed an abiding impatience with work on polyamines because no one could prove their indispensability in any individual process. Part of the

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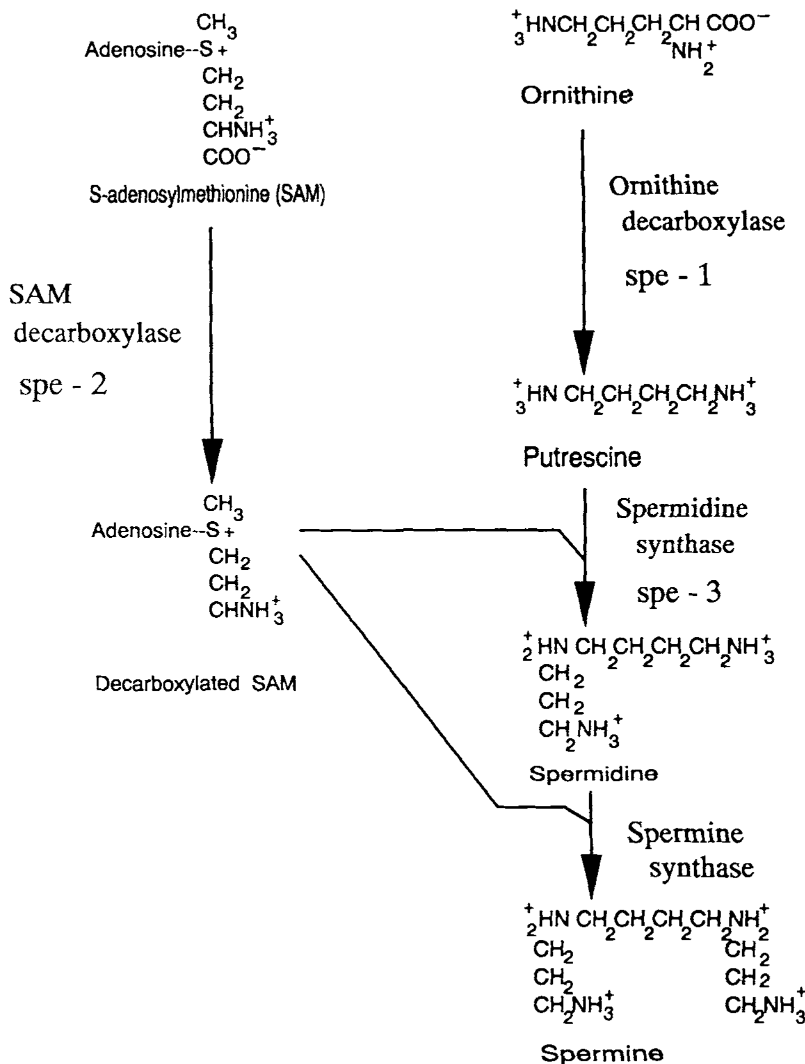

Fig. 1. Pathway of polyamine biosynthesis in animals and fungi. (Methylthioadenosine, the byproduct of the spermidine and spermine synthase reactions, is not shown.) The pool sizes of the polyamines in exponential cultures of *Neurospora crassa*, per mg, dry weight, are approximately 0.8 nmol putrescine, 18 nmol spermidine, and 0.4 nmol spermine. *spe-1*, *-2*, and *-3*, genetic loci of *Neurospora* that govern the major enzymes of the pathway, are shown.

problem resides in the fact that while polyamines are the best available cations for certain functions, omission or substitution is often tolerable in vivo and in vitro. For instance, bacteriophage T4 virions contain a large amount of putrescine, but polyamine-starved hosts are able to package DNA in phage heads without putrescine [1]. Polyamines associate with ribosomes and tRNA [2,3], but bacterial cells continue to grow slowly, albeit with less fidelity of translation, with little or none of the normal polyamines [4]. However, the existence of polyamine auxotrophs shows that eucary-

otes have an absolute requirement for polyamines. In such cells, processes affected by polyamine starvation, such as protein and nucleic acid synthesis, are complex, and have not revealed clearly the specific roles of the polyamines [5]. We are left to think of polyamines as facilitators of many processes, similar to the roles of Mg^{2+}, K^{+}, and other common ions. Whatever their roles, polyamines are essential for cells making their way in a competitive natural world.

No rigorous demonstration that polyamines were largely bound in vivo was available until recently. Our own tracer work in *Neurospora* [6] was among the first to show unequivocally that at least 80–90% of the spermidine pool (representing about 95% of the polyamine of *Neurospora*) was not on the main line of synthesis. In the experiment, radioactive ornithine was presented to cells and followed in its metabolism to spermine. With determinations of specific radioactivity, we asked whether the label was diluted by the resident spermidine pool as it was converted from putrescine to spermine. It was, but very little. The demonstration that little of the cellular polyamine is involved in metabolism is consistent with the later demonstration that only a small fraction of the polyamines is involved in regulation of the pathway [7].

Other types of experiment also show that polyamines are bound in the cell. Igarashi has shown that polyamine transport in bacteria is unidirectional, but that transport into bacterial membrane vesicles is not. The difference is due to the binding of polyamines to the contents of the intact cells [8]. Our studies on *Neurospora,* in which polyamines enter the cell in growth conditions by a diffusional process, show cellular materials must be saturated with polyamines before equilibration takes place across the plasma membrane. The same work showed that the normal cellular polyamine content is determined by the binding sites available [9]. In agreement with this, permeabilized bacterial and fungal cells retain polyamines, bound to cellular constituents [8–10].

Where in the cell are the polyamines? Many have speculated on this question, and few have definitive answers. Inferences drawn from in vitro studies [e.g., 11,12] have led most of us to believe polyamines bind to nucleic acids, ribosomes, and membranes. More recent NMR studies confirm the association with ribosomes and tRNA [2,13]. Non-aqueous extraction techniques, applied to mammalian liver cells, show that 16% of spermidine and spermine is in the nucleus [14]. Our own studies showed that 25% of the spermidine of *Neurospora* cells lay within vacuoles, complexed with polyphosphate [6,15]. However, we could not localize the cytosolic portion of the sequestered spermidine, which was double the vacuolar content.

Workers with polyamine auxotrophs showed that although normal cells had substantial pools of polyamines, the mutants could grow on minuscule amounts of polyamines. The long period needed to deplete endogenous pools to growth-limiting levels made it difficult to isolate such mutants. This makes the point emphasized some time ago by Canellakis [16] that most of the polyamines in the cell are dispensable, and therefore their location in cells cannot guide us to their essential functions.

Taken together, the findings that growth is not impaired until a very small minimum of polyamines is reached, that only small fractions of the polyamines are involved in metabolism, and that most polyamines are bound to cell constituents demonstrate that the bulk of polyamines in cells are a metabolic red herring. The "sticky" nature of these compounds, however, may have peculiar consequences in the management of polyamine synthesis and disposal.

MANAGEMENT OF NORMAL OR LIMITING AMOUNTS OF POLYAMINES

Cells generally fine-tune the steady-state amount of a biosynthetic endproduct by feedback inhibition, an allosteric response of an early pathway enzyme to the endproduct. One of the peculiarities of polyamine biosynthesis is that feedback inhibition of ornithine decarboxylase, the most highly regulated enzyme, has never developed in any organism. Feedback inhibition must work in a defined range of parameters, particularly the strength of inhibition [17]. If inhibition is too weak, the steady-state pool is unnecessarily large and the response to excess is damped; if it is too strong, unstable and increasingly strong oscillations in synthetic rate occur upon perturbation of pools. The polyamines are unusual in the dependence of their chemical activity on binding to cell constituents. Given normal fluctuations in binding sites, competitors for the binding sites, cell water, or ionic strength, the level of polyamines in the unbound state may be quite erratic, especially in rapidly dividing cells. This may be why feedback inhibition of ODC never evolved. The instantaneous concentration of free polyamines is a poor reflection of the polyamines available to the cell, and adjustments of polyamine synthetic rate in response to the concentration of free polyamine would be inappropriate.

The lack of feedback inhibition of ODC may have promoted a large array of mechanisms controlling the amount of active ODC protein [18]. These mechanisms have in almost all cases included rapid enzyme turnover, often polyamine induced, as a part of the regulatory process. Turnover is balanced by rapid access to increased enzyme: by polyamine-regulated translation of preexisting ODC mRNA; by recruitment of enzyme from an inactive enzyme-antizyme complex; and by alteration of enzyme from inactive to active form. These control mechanisms have a time-constant much greater than feedback inhibition, and are thus damped against rapid fluctuations of free polyamine concentration in cells. Moreover, a slower response is tolerable because the polyamines, bound with varying affinities to cellular anions, can easily be recruited for use if the free polyamine concentration falls, even over a long time. The control of the pathway by a dynamic balance of synthesis and degradation of ODC, and of binding and release of polyamines, has given the study of polyamines its most distinctive character.

Our work in *Neurospora* illustrates how growth is affected by the onset of polyamine starvation. A mutant lacking arginase grows well in a minimal medium. When arginine is added, however, ornithine synthesis is feedback inhibited. Without arginase, no ornithine can form, and the polyamine pathway is deprived of a substrate. In about 30 min, the available (vacuolar) ornithine is gone, and the polyamine pool begins to fall. Growth continues almost normally until the pool reaches 10% of its normal level, at which point growth slows down to about one-half normal [19]. Growth then continues indefinitely [20], owing to the synthesis of cadaverine and aminopropylcadaverine (analogs of putrescine and spermidine) from lysine [21]. Cadaverine is made by the highly derepressed ornithine decarboxylase in the absence of ornithine.

Without an analog such as aminopropylcadaverine, *Neurospora* has an absolute requirement for spermidine, even if putrescine is plentiful [22]. Mutants (*spe-1*) lacking ODC fail to grow at all in minimal medium (unlike the arginaseless mutant, they cannot form cadaverine). If they begin growing, they stop when the residual spermidine pool, carried over by the inoculum, falls below 2 nmol per mg, dry weight.

However, *spe-2* mutants, lacking S-adenosylmethionine decarboxylase (Fig. 1), behave quite differently. Unlike *spe-1* mutants, they accumulate copious amounts of putrescine and grow well until their spermidine pool falls to about 0.3 nmol per mg, dry weight. The difference between the two mutants demonstrates that putrescine fulfills functions of spermidine up to a point, but that some functions have an absolute requirement for spermidine. Possibly these functions could be identified by the use of this mutant strain.

The conclusion from the study of mutants—in all organisms studied—is that cellular polyamines can be recruited for some time from the bound state for growth if their synthesis is interrupted.

MANAGEMENT OF EXCESS POLYAMINES

Eucaryotic cells rarely encounter excess polyamines. Cells control polyamine synthesis via ornithine decarboxylase, and in *Neurospora* at least, spermidine synthesis is limited by spermidine synthase even if its substrates are plentiful. Moreover, polyamines do not often flood the environment. Toxicity of polyamines is exerted in some cases by aldehydes produced during polyamine degradation. Nevertheless, because of the lack of feedback inhibition and the existence of polyamine uptake systems, cells may occasionally accumulate growth-inhibiting levels of the polyamines themselves [23] (Davis, unpublished). Cells deal with excess cellular polyamine not only by reducing synthesis, but also by polyamine turnover, conjugation, compartmentation, excretion, and control of uptake.

1. Polyamine Turnover, Conjugation, and Compartmentation

Mammalian cells have an elaborate polyamine interconversion system, which adjusts the ratios of polyamines, and which degrades excess polyamines. A number of plants and microbes also have this capability, and many microbes catabolize polyamines as carbon and nitrogen sources. Many of these pathways involve acetylation, and acetylpolyamines are not only degraded, but excreted [24]. Other conjugates, such as the glutathionyl-spermidine of *E. coli* and trypanosomes [25], and the hydroxycinnamic acid amide derivatives of plants [26], remove polyamines from the pool, and thus might mitigate adverse effects the unconjugated polyamines might have. The conjugated polyamines, however, doubtless have more significance as reserves or as secondary products than as detoxified forms of polyamines [26].

Polyamines may also be removed from the cytosol by sequestering them in cellular organelles. The discretionary capacity of most cell types may be limited, but *Neurospora,* and perhaps many fungi and plants, can sequester polyamines within vacuoles. We have described the physiological behavior of *Neurospora* vacuoles in relation to basic amino acids previously [15,27,28], and vacuoles respond to excess polyamines in the same way. Excess polyamines are concentrated in the vacuole, where they displace other monovalent cations such as arginine, ornithine, and lysine. Accordingly, vacuoles of the spermidine-starved *spe-3* mutant, lacking spermidine synthase, are loaded with putrescine. A mutant (*puu-1*) we have isolated, suffering uncontrolled putrescine uptake (see below), sequesters putrescine in vacuoles up to their capacity before the mutant becomes intoxicated by putrescine flooding the cytosol (Davis and Ristow, unpublished).

2. Polyamine Excretion and Uptake

Frequently cancer cells release excessive polyamines into biological fluids. Some normal tissues also excrete polyamines; the human prostate excretes copious spermine into the seminal fluid. This cannot be a vital feature of seminal fluid, because mouse seminal fluid, and that of many other mammalian species, has virtually no polyamines [29]. In most cases, excretion is probably a simple diffusional process, after intracellular levels of free polyamines become elevated.

Neurospora does not catabolize polyamines, and excretion of polyamines may be an essential capability [30]. In stationary phase, *Neurospora* continues making spermidine, and slowly excretes most of it [9]. In ornithine-starved cells derepressed for ODC, addition of ornithine leads to a sudden burst of putrescine synthesis, much of which appears in the medium. Similarly, putrescine-accumulating *spe-3* mutants (see above) slowly excrete putrescine during their limited growth in minimal medium [9].

Perhaps the most unusual adaptation of cells to excess polyamines is the control of uptake of environmental polyamine. The accumulation of most ionic solutes that have been studied in fungi is limited by rapid counterflow (often diffusional "leakage") or by transinhibition of further uptake by a high concentration of solute already in the cell.

Wild-type *Neurospora* normally does not actively concentrate polyamines from normal growth media, but does so only from low-ionic-strength buffers, especially if cells are washed with both EGTA and other broad-spectrum chelators. The recessive *puu-1* mutant, by contrast, actively concentrates large amounts of polyamine if it is present in the medium because the activity is abnormally resistant to Ca^{2+} in the growth medium. Correlated with this is a deficiency in amino acid uptake. We do not know the basis of this pleiotropic phenotype, but it is clearly maladaptive: the mutant is greatly inhibited by polyamine concentrations to which the wild type is indifferent. Similarly, the cyanobacterium *Anabena,* when exposed to putrescine at high pH, develops toxic levels of intracellular putrescine by ion trapping. The lethal effect of putrescine is exerted here on the ribosomes, to which putrescine becomes conjugated [31].

Whatever the nature of these polyamine transport systems, they require restraint, if, as in the cases above, the organism cannot excrete or degrade polyamines rapidly enough. We have speculated that the polyamine uptake system of *Neurospora* is not really a polyamine uptake system at all [30]. The affinities for putrescine and spermidine are low (600 and 150 μM, respectively), suggesting that the system might be devoted to the uptake of other cations entirely. If it serves a broad-specificity transport function (for vital trace metals, for instance), restraint of the entry of toxic levels of its substrates must be built in. So far, we have failed to obtain mutants lacking the system, despite an easy selection method. It may indeed be that the uptake system has an unrecognized, indispensable role.

The diffusional transport of polyamines, seen in many organisms, poses an additional, intriguing question. It is unlikely that these multivalent cations pass through a lipid bilayer, and the mechanism of this transport remains to be discovered. It is in fact uncertain whether an unusual ion-pairing mechanism, such as a phospholipid-amine interaction, together with the aliphatic character of the carbon skeleton, could permit polyamines to get through a membrane. It is more likely that the diffusional entry is a nonspecific transit via proteins embedded in the membrane, with such low affinity that

it does not appear to be a saturable process. An intriguing possibility for the widespread occurrence of diffusional entry is that cells must in some way dispose of polyamines; if this is not an energy-requiring process, it is likely to be bidirectional.

In conclusion, the study of polyamines has slowly made us aware of how the chemical nature of these compounds have forced cells to handle them in unusual ways. It is likely that much information collected in the past can be evaluated more successfully in light of our newer understanding.

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REFERENCES

1. Hafner EW, Tabor CW, Tabor H: J Biol Chem 254:12419–12426, 1979.
2. Frydman B, Frydman RB, de los Santos C, Garrido DA, Goldemberg SH, Algranati ID: Biochim Biophys Acta 805:337–344, 1984.
3. Cohen SS, Morgan S, Streibel E: Proc Natl Acad Sci USA 64:669–676, 1969.
4. Tabor H, Hafner EW, Tabor CW: J Bacteriol 144:952–956, 1980.
5. Marton LJ, Morris DR: In McCann PP, Pegg AE, Sjoerdsma A (eds): "Inhibition of Polyamine Metabolism." New York: Academic Press, 1987, pp 79–105.
6. Paulus TJ, Cramer CL, Davis RH: J Biol Chem 255:8608–8612, 1983.
7. Davis RH, Krasner GN, DiGangi JJ, Ristow JL: Proc Natl Acad Sci USA 82:4105–4109, 1985.
8. Kashiwagi K, Kobayashi H, Igarashi K: J Bacteriol 165:972–977, 1986.
9. Davis RH, Ristow JL: Arch Biochem Biophys 271:315–321, 1989.
10. Tabor CW, Tabor H: J Biol Chem 241:3714–3723, 1966.
11. Igarashi K, Sakamoto I, Goto N, Kashiwagi K, Honma R, Hirose S: Arch Biochem Biophys 219:438–443, 1982.
12. Kakegawa T, Sato E, Hirose S, Igarashi K: Arch Biochem Biophys 251:413–420, 1986.
13. Frydman B, de los Santos C, Frydman RB: J Cell Biochem [Suppl] *14C*:90, 1990.
14. Sarhan S, Seiler N: Biol Chem Hoppe-Seyler 370:1279–1284, 1989.
15. Cramer CL, Davis RH: J Biol Chem 259:5152–5157, 1984.
16. Canellakis ES, Viceps-Madore D, Kyriakidis DA, Heller JS: Curr Top Cell Regul 15:155–202, 1979.
17. Savageau M: J Mol Evol 5:199–222, 1975.
18. Hayashi S-I (ed): "Ornithine Decarboxylase: Biology, Enzymology, and Molecular Genetics," International Encyclopedia of Pharmacology and Therapeutics, Sec 129. New York: Pergamon Press, 1989.
19. Paulus TJ, Davis RH: J Bacteriol 145:14–20, 1981.
20. Davis RH, Lawless MB, Port LA: J Bacteriol 102:299–305, 1970.
21. Paulus TJ, Kiyono P, Davis RH: J Bacteriol 152:291–297, 1982.
22. Pitkin J, Davis RH: Arch Biochem Biophys 278:386–391, 1990.
23. Ramakrishna S, Guarino L, Cohen SS: J Bacteriol 134:744–750, 1978.
24. Pegg AE: Cancer Res 48:759–774, 1988.
25. Tabor H: Microbiol Revs 49:81–99, 1985.
26. Flores HE, Protacio CM, Signs MW: In Poulton JE, Romeo JT, Conn EE (eds): "Plant Nitrogen Metabolism," Recent Advances in Phytochemistry, Vol 23, New York: Plenum Press, 1989.
27. Cramer CL, Vaughn LE, Davis RH: J Bacteriol 142:945–952, 1980.
28. Vaughn LE, Davis RH: Mol Cell Biol 1:797–806, 1981.
29. Williams-Ashman HG, Canellakis ZN: Perspect Biol Med 22:421–453, 1979.
30. Davis RH, Ristow JL: Arch Biochem Biophys 267:479–489, 1988.
31. Guarino LA, Cohen SS: Proc Natl Acad Sci USA 76:3660–3664, 1979.