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Directions for Polyamine Research

The history of the polyamines predates that of the nucleic acids—first being referred to in Leeuwenhoek's description of spermatozoa presented to the Royal Society in London. He described crystals in seminal fluid, later found to be spermine phosphate, but the appropriate recognition of the importance of these compounds in cellular and molecular processes is still wanting. It is clearly recognized that all cells stimulated to reproduce show early rises in biosynthetic enzymes involved in polyamine biosynthesis and subsequent increases in polyamine levels. When such accumulation is prevented, through use either of inhibitors of polyamine biosynthesis or of cells which have been mutated with regard to the biosynthetic enzymes, cellular functions are disrupted. However, the critical role that the polyamines play in these cellular functions is not well appreciated. This is at least partly due to the fact that studying these ubiquitous, highly charged molecules poses enormous difficulties, and that as such, specific functional correlates are not easily ascertained.

Isolation and identification of the enzymes involved in polyamine biosynthesis has proceeded with great success, and the extensive regulation involved in polyamine biosynthesis is now appreciated. All genes for the key enzymes have been cloned and have been expressed in *Escherichia coli*. The crystallographic structure of these enzymes should be available in the near future. Specific nucleic acid sequences and structures that respond to polyamines in terms of regulation are being identified, and the possibility that these sequences can be incorporated into other genetic materials and thus confer polyamine regulation upon their genetic products is an intriguing concept. Nevertheless, as we get closer to understanding the molecular biology of these enzymes and their exquisite regulation, we must focus once again on the end-product of this biosynthetic machinery, the polyamines themselves, and upon their functions within cells.

Polyamines are unique not only because of their extensive regulation, but also because the charge on the polyamine is distributed *in space*, separated by specific atomic distances, in contrast to the point charges provided by magnesium, calcium, etc. As with other cations, potential and real functions are numerous. Interactions with nucleic acids, proteins, membranes, and other intracellular organelles are well described, despite the fact that the exact nature of these interactions is only now being elucidated. The ability of the polyamines to induce conformational changes in nucleic acids, the subject of a future *Prospect* (Feuerstein et al.) in the Journal, may be related to genetic control-mechanisms. The physiological compound most able to induce B to Z transition in nucleic acids is spermine, a compound available within mammalian cells at high concentration. It appears that polyamines may both play a role in stabilizing membrane structures and also actually facilitate the formation of specific structures. Polyamine functions are presently being addressed using molecular biologic approaches, specific inhibitors of polyamine biosynthesis (now available for virtually every enzyme in the biosynthetic pathway), and more recently with the use of polyamine analogues. These analogues may interact at specific sites in competition with naturally occurring polyamines, but may have altered function at those sites.

Important areas for consideration include the following:

—A major methodologic impediment to defining polyamine function is our inability to ascertain subcellular distribution and compartmentation of the polyamines. A *Prospect* by Rowland Davis published in the December issue of the Journal relates to studies of polyamine distribution in nonmammalian cells. Our ability to extend these studies to mammalian cells is essential.

—Specific cellular transport mechanisms exist for the cellular entry and efflux of polyamines. The function, regulation, and possible use of these transport systems for the delivery of chemotherapeutic agents are undergoing active investigation. These studies will be facilitated by

the cloning and expression of the transport proteins and their comparison to other transporters.
—The use of mutants in the polyamine field has been most valuable, particularly with regard to bacteria and yeast, and an extension of these studies into mammalian cells should help address many areas of interest.
—The biosynthesis of the polyamines responds to a variety of intracellular signals, including those generated by growth factors, hormones, tumor promoters and oncogenes. Polyamine biosynthesis is regulated at many levels, and the mechanisms by which signal transduction pathways exert their influence are presently an area of intense interest. In addition, a biological role of the polyamines themselves may be to influence specific molecular steps of particular signal transduction pathways.
—The subject of a *Prospect* in this Journal by P. Coffino and A. Poznanski is the possibility that oxidation products of the polyamines are regulatory. Although this hypothesis is in need of significant expansion and data, it is an intriguing notion worthy of consideration.

The knowledge so far gained in the basic science investigations of these compounds has been applied to clinically relevant issues. Monitoring polyamines in a variety of physiological fluids and in red cells relates biologically and perhaps clinically to the presence of tumors and other disease states. The observation that tumors as rapidly growing entities contain high levels of polyamines is the basis for such diagnostic studies. The fact that inhibition of polyamine biosynthesis can reduce cellular growth has formed the focus for the therapeutic approach. For parasitic diseases and cancer, polyamine biosynthesis inhibitors and polyamine analogues have provided new and promising therapeutic tools. The ability to go freely back and forth between the basic science laboratory and the clinical setting has been another intriguing element in the polyamine field. In another *Prospect* by C. C. Wang published in this Journal, molecular biological techniques were used to develop an understanding of the difference in sensitivity to an inhibitor of ornithine decarboxylase, difluoromethylornithine, between trypanosomes and mammalian cells. The trypanosome is exquisitely sensitive to this enzyme inhibitor, whereas the mammalian cell is less so. As described in the *Prospect*, an understanding of the construction of the ornithine decarboxylase molecule provides at least a portion of the answer.

The future for further basic and clinical studies related to the polyamines is bright. Translation of data from the basic science laboratory into the clinical sphere has not only been accomplished, but also is likely to be further expanded. Many scientists use polyamines freely, knowingly and unknowingly, in their experimental protocols, whether it be to crystallize nucleic acids, improve endonuclease reactions, or use reticulocyte lysate systems that are rich in polyamines. The time has come to understand their functions.

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