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A New Perspective on Ornithine Decarboxylase Regulation: Prevention of Polyamine Toxicity is the Overriding Theme

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Abstract The polyamines are essential cellular components for growth. Control of a key regulated enzyme of polyamine biosynthesis, ornithine decarboxylase (ODC), as a function of growth, is an area of intense interest. A unique regulatory property of ODC is the short half-life of the protein, which has been suggested to be an important factor in rapid activation of polyamine biosynthesis after cells are mitogenically stimulated. In this paper, it is argued that the biological significance of the short half-life of ODC is unrelated to the rate of its induction to a new steady state by growth factors, which is in fact limited by the relatively long half-life of the ODC mRNA. Instead, I suggest that the rapid turnover of ODC protein becomes of significance when cells cease growth and expeditious downregulation of the enzyme is important in preventing polyamine overproduction, which would result in cytotoxicity in the arrested cells. Although mitogenic activation of ODC expression has been studied extensively, there is very little known about the mechanisms controlling downregulation of polyamine biosynthesis during the arrest of animal cell growth. These considerations suggest that this would be a fertile area of future inquiry.

Key words: polyamine biosynthesis, ornithine decarboxylase, down regulation, animal cell growth

The polyamines (putrescine, spermidine, and spermine) are highly charged, low molecular weight compounds that are found ubiquitously in living cells at concentrations orders of magnitude higher than most metabolites [1–5]. Because of their positive charge, the polyamines bind tightly to nucleic acids and other negatively charged cellular constituents and, thus, only minor fractions of the total intracellular pools are metabolically active [6]. These amines are required for optimal growth of both prokaryotic and eukaryotic cells [3–5] and it is generally assumed that they exert their biological functions by virtue of their positive charge.

Ornithine decarboxylase (ODC) catalyzes a key regulated step in polyamine biosynthesis, the conversion of ornithine to putrescine, and its cellular activity is modulated by a variety of cellular stimuli [7]. The pioneering studies of Diane Russell and her colleagues revealed that ODC activity was elevated after stimulation of a variety of growth-regulated biological systems. For example, cellular ODC activity is compared, as a function of time, to the accumulation of intracellular polyamines in mitogen-activated T-lymphocytes in Figure 1. Enzyme activity began to accumulate prior to 5 h after cell activation and reached peak activity at about the time of entry into S phase. As expected, polyamine levels lagged somewhat behind the increase in ODC activity.

Kahana and Nathans demonstrated that ODC mRNA was elevated in resting fibroblasts in response to growth stimuli and that this induction did not require prior protein synthesis [9]. Other genes showing a similar primary response to growth stimulation encode an interesting group of regulatory proteins that is composed, in part, of a number of proto-oncogene products [10,11]. With many of these primary response genes, the half-lives of both their protein and mRNA products are quite short, being on the order of minutes. Since the rate of approach of a cellular macromolecule to a new steady state level depends only on its half-life, the cellular products of these genes are capable of rapid excursions in response to altered rates of transcription [see reference 12 for a general review of the influence of half-life on the rate of accumulation of a macromolecule]. An interesting situation exists in the case of ODC. In this case, the

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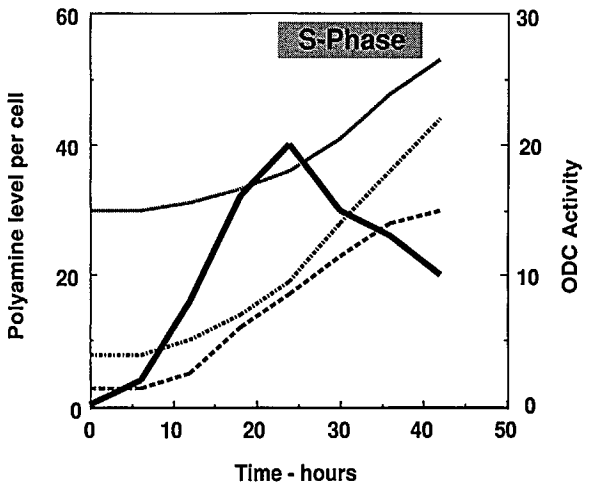

Fig. 1. Comparison of the levels of ODC and polyamines after activation of T-lymphocytes with mitogen. T-lymphocytes were activated with concanavalin A at zero time and ODC (——), putrescine (----), spermidine (-·--), and spermine (·····) were followed as a function of time. The polyamine and ODC data were redrawn from references 14 and 36, respectively.

protein turns over quite rapidly, with a half-life on the order of 10 to 20 min, while the half-life of ODC mRNA is rather long, varying from 2 to 5 h depending on the cell type [13]. This means that the rates of change in steady state level of ODC protein brought about by changes in transcription rate are limited by the stability of the mRNA and the significance of the short half-life of the protein is not immediately apparent.

Individuals working in the polyamine field traditionally interpreted the short half-life of ODC as allowing a rapid increase in polyamine biosynthesis. Furthermore, it was suggested that elevated polyamine levels were required in the initial emergence of cells from quiescence in response to trophic stimuli. In the extreme, this view led to the unsubstantiated hypothesis that polyamines were regulatory molecules that participated in the control of cell growth, and in particular ribosomal RNA production [reviewed in reference 8]. In the context of this conjecture, however, it is curious that the accumulation of the polyamines themselves has been found to be rather delayed in all systems studied. For example, in T-lymphocytes (Fig. 1), where this has been examined with precision, increases in total cellular polyamine levels clearly lag behind accumulation of rRNA [14] and are considerably slower than the early regulatory events, including changes in proto-oncogene expression. Therefore, based solely on the delayed kinetics of polyamine accumulation, which is probably due in part to the low turnover of the intracellular pools of these compounds, one must conclude that the growth processes, in which elevated polyamines may participate, should occur rather late after mitogenic activation. Thus, there is strong reason to question this traditional view that the most distinctive regulatory attribute of ODC, its short protein half-life, arises from the necessity to control production of essential regulatory molecules, which in turn act early in the process of mitogenic activation. In contrast, I will argue in this paper that the regulatory properties of ODC are of singular importance at times of growth arrest. It will be suggested that post-transcriptional mechanisms of regulating ODC are particularly significant in preventing overproduction of polyamines as cells enter into a quiescent state.

ROLE OF POLYAMINES IN CELL GROWTH

Since the discovery of the ubiquitous occurrence of polyamines at high intracellular concentrations, there has been considerable interest in their biological functions. Studies using genetic and pharmacological tools have clearly demonstrated the essentiality of these compounds for normal growth processes in both prokaryotic and eukaryotic cells [reviewed in reference 5]. In considering molecular sites of polyamine action in animal cells, the kinetics of polyamine accumulation (see above) suggest a role for these compounds late in the process of mitogenic activation, perhaps in DNA replication or cell division. In mutants of *E. coli*, which lack the ability to produce normal cellular levels of putrescine and spermidine, the movement of DNA replication forks is defective [15]. In mitogen-activated T-lymphocytes (Fig. 1), blocking polyamine accumulation with inhibitors that act at two different sites in the biosynthetic pathway revealed minor, if any, effects on the synthesis of RNA or protein prior to 20 h after activation and, of even more significance, the cells *initiated* DNA replication (S phase) at the same time as those with a normal complement of polyamines [16–18]. In contrast to the normal entry of the cells into S phase, DNA replication per se was inhibited in nuclei isolated from the polyamine-deficient cells [19], reflecting a lengthened S phase in the intact cells [16–18]. It has still not been resolved whether this behavior results from a direct role of the polyamines in the replication process or whether these compounds might be involved in the expression of S phase-specific

enzymes, thereby indirectly influencing DNA synthesis [20]. However, regardless of the detailed functions of the polyamines in cell growth, it is clear that they act rather late in the mitogenic program. Thus, it seems unlikely that the biological significance of the short half-life of ODC protein resides in a necessity to rapidly turn on the synthesis of polyamines.

POLYAMINES ARE CYTOTOXIC AT INAPPROPRIATE CONCENTRATIONS

The toxic influence of spermine on microbes has been recognized for many years [2,3]. At least one mechanism for this toxicity seems clear; exposure of *E. coli* to high concentrations of the polyamines inhibits protein synthesis [21]. It seems likely that this toxic effect is due to replacement of magnesium ions at critical sites in the ribosomes with polyamines, leading to a loss of native structure of the two ribosomal subunits [22,23] and their constituent RNAs [24]. In *E. coli,* both polyamine biosynthesis and metabolism are postured to prevent the accumulation of toxic intracellular concentrations of these cations. Both of the biosynthetic pathways leading to putrescine are under endproduct control [25,26], which should prevent overproduction. Perhaps of equal importance, when *E. coli* is grown to stationary phase, all of the intracellular spermidine is converted to the glutathionyl derivative and, when these cells are exposed to high endogenous spermidine or spermine, large quantities of the acetylated derivatives are excreted into the culture medium [reviewed in reference 3]. Thus, metabolism and excretion of the polyamines compensate in part for intracellular accumulation under these physiological conditions.

In the case of animal cells, studies of polyamine toxicity are confounded by the presence of polyamine oxidase in bovine serum present in culture media [27]. The action of this enzyme on spermidine and spermine gives rise to acrolein [28], which is highly toxic itself and masks any toxic influence due directly to the polyamines. Thus, the issue of cytotoxicity of high intracellular levels of polyamines in animal cells is unresolved. However, there are multiple mechanisms in eukaryotic cells, which prevent intracellular accumulation of polyamines to high levels. Polyamines are degraded in animal cells by acetylation followed by oxidation [29]. It has not been established whether excess polyamines or their acetyl derivatives can be excreted from animal cells, as with bacteria. However, in *Neurospora crassa,* excretion is clearly a highly significant mechanism for ridding the cells of excess polyamines [30]. An extremely important mode of preventing overproduction is the negative regulation of ODC by polyamines, employing what appears to be a complex pattern of post-transcriptional mechanisms. One component of feedback regulation of ODC level may be at the level of translational initiation [reviewed in reference 31], although this interpretation has recently been brought into question [32]. An additional mechanism of negative regulation of ODC by polyamines seems to be through modulation of the stability of the protein [33]. Whether this latter mode of control is mediated via the inhibitory protein, ODC antizyme [34], has not been established. Regardless of the detailed mechanisms involved, it is clear that eukaryotic cells have several means to prevent the accumulation of intracellular levels of polyamines that might be detrimental. This suggests that in animal cells, as well as in bacteria, inappropriate concentrations of the polyamines are to be avoided.

THE FLIP SIDE OF ODC CONTROL: DOWNREGULATION IS OF PARAMOUNT IMPORTANCE

It is of interest to contrast the behavior of ODC with other genes that show a primary response to growth factors, such as c-*myc,* the *jun* family, the *fos* family, and *egr*1/*zif*268 [10,11]. These other genes code for transcription factors, which act physiologically by regulating expression of other genes that function further along in the processes of mitogenic activation and cell cycle progression. These other immediate early genes are "poised for action"; in those cases where it has been examined, the half-lives of both the mRNAs and the protein products are on the order of a few minutes, leading to extremely rapid induction kinetics. This is presumably because these gene products are required to act early in order for the mitogenic process to move forward. In contrast, peak induction of ODC mRNA is later than most of the other primary response genes [35]. Since ODC transcription is activated as early as that of c-*myc* [13], the delay in the peak response of ODC mRNA seems not to be at the transcriptional level, and must be due, at least in part, to the long half-life of the ODC message. This more gradual accumulation of ODC mRNA, relative

to the products of other primary response genes, seems consistent with the evidence summarized above indicating that the products of ODC activity, the polyamines, are not required in mitogen-activated cells until nearly a day after the initial activating events.

If ODC mRNA accumulates relatively slowly among the early message species, and if elevated levels of the polyamines are needed only long after the initial act of cell stimulation, why should ODC protein have one of the shortest half-lives known? It seems that the initial interpretation that this short half-life allows rapid elevation of activity is not correct, since the rate of ODC induction to a new steady state is limited by the stability of its mRNA. If not required for induction, the instability of ODC protein could be important for downregulation of enzyme activity. As argued above, cells have evolved elaborate schemes to prevent overproduction of the potentially cytotoxic polyamines. An extension of this logic suggests that if the extremely short half-life of ODC protein is not important for the kinetics of its induction during mitogenic activation, it could be required for rapidly extinguishing active enzyme when growth ceases. Because of the long half-life of this mRNA, an abrupt downregulation of ODC activity upon growth arrest could only result from post-transcriptional regulatory processes such as those discussed above. These processes may be brought into play either as a direct result of removing the mitogenic stimulus (or exposure of the cells to a negative growth stimulus) or as a result of feedback inhibition as polyamines begin to accumulate in the arresting cells. These possible mechanisms of downregulation of polyamine biosynthesis in growth-arrested cells have not been examined to my knowledge. From the arguments presented in this paper, it seems that this aspect of ODC regulation is at least as important from a biological standpoint as its induction and should be studied in detail.

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