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Minireview

Polyamines as regulators of cell activation

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The polyamines putrescine, spermidine and spermine naturally occurring in all living beings play an important though poorly understood role in various cellular activities [1]. On the basis of their chemical structures (at physiological pH these polyamines are protonated and possess two, three and four positive charges, respectively), it has been suggested that they may contribute to the neutralization of the negative charge on the DNA backbone. The charge distribution in the spermine molecule, which is the final product of polyamine biosynthesis found mainly in the cell nucleus, makes it bind strongly to two phosphate groups in each strand of the DNA helix. Thus spermine can stabilize the helix by binding its two strands together. These changes can have important consequences for DNA-protein interaction in the cell. The polyamines also stabilize other double-helical structures, such as stems and loops in RNA. These interactions may be the basis for their stimulatory effects on DNA, RNA and protein synthesis [2]. Changes in the activity of such fundamental processes would be expected to have profound effects on cellular physiology, but it is very difficult to demonstrate clearly the biological relevance of these effects.

In vertebrates, biosynthesis of polyamines begins with ornithine being converted by ornithine decarboxylase (ODC)[1] to putrescine. To produce other polyamines an aminopropyl group must be added. *S*-Adenosylmethionine decarboxylase (SAMDC) is responsible for making available aminopropyl groups, the addition of which sequentially converts putrescine to spermidine and then to spermine. Both decarboxylases are key, rate-limiting enzymes in polyamine biosynthesis. Activities of both of these enzymes, and hence polyamine levels can undergo enormous changes, increasing and/or decreasing very rapidly in response to diverse stimuli [3, 4]. Upon growth stimulation, ODC activity increases several hundred-fold. Both decarboxylases show extraordinary lability and are among the most rapidly degraded proteins in mammalian cells [2, 5, 6]. The expression of ODC is regulated through negative feedback by polyamines at translational and post-translational levels [7, 8]. There is evidence for the existence of macromolecular inhibitor of ODC that may regulate the activity of the enzyme under some circumstances [9].

What do polyamines do? Why are the enzymes that carry out their biosynthesis controlled in such complex ways and capable of such rapid changes?

Polyamines are essential for life. Cells can be depleted of polyamines by either genetic or pharmacological means, and a sufficient de-

[1]Abbreviations used: DFMO, difluoromethylornithine; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF-1, insulin like growth factor 1; LPS, lipopolysaccharide; MGBG, methylglyoxal-bis(guanylhydrazone); NMDA, (*N*-methyl-D-aspartate); ODC, ornithine decarboxylase; PDGF, platelet derived growth factor; SAMDC, *S*-adenosylmethionine decarboxylase

gree of depletion is toxic or even lethal to organisms ranging from bacteria to mammals [1, 6]. The importance of polyamines for cell growth is clearly manifested by P22 cells, the mutant progeny of a Chinese hamster ovary cells, devoid of ornithine decarboxylase activity and consequently unable to produce their own polyamines. In effect, the cells cannot grow in the absence of exogenously added polyamines [10]. The application of specific inhibitors of polyamine biosynthesis e.g. difluoromethylornithine (DFMO), an inhibitor of ODC or methylglyoxal-bis(guanylhydrazone) (MGBG), an inhibitor of SAMDC profoundly alters cell proliferation [11 - 14].

During the last decade there has been a huge number of articles in the area of polyamine research, and a great many of them pointed to an association of polyamine biosynthesis with cell proliferation and differentiation. The pattern of changes in cellular polyamine content and activities of polyamine-synthesizing enzymes were found to be temporally related to proliferative as well as developmental processes (for review see [3, 4, 15]). However, the expression of ODC can also be induced in terminally differentiated cells, such as neurons and macrophages, during their functional activation, which raises the possibility that polyamines can participate in the regulation of long-lasting changes in these cells [4]. This article summarizes some data concerning participation of polyamines in the regulation of cell function, and attempts to answer the question what is the physiological role of polyamines.

Polyamines in the regulation of the cell cycle

When cells are stimulated to grow and divide, the synthesis of polyamines is rapidly induced. The intracellular levels of polyamines as well as the activities of enzymes involved in their synthesis are highly regulated in response to growth factors such as PDGF, EGF and FGF [16 - 19], hormones [20] and tumor promoters [21, 22] in human and rodent fibroblasts. Mitogenic lectins such as concanavalin A and phytohemagglutinin stimulate ODC and polyamine formation in mouse and human lymphocytes [23 - 26]. In different types of cells several peaks of ODC activity and polyamine accumulation have been reported following cell cycle stimulation. The first peak, occurring early after stimulation, has been observed only in cells stimulated to proliferate from quiescence and has not been demonstrated in continuously dividing cells. The second peak, detectable in all types of proliferating cells, occurs at the mid to late G_1-S phase and is clearly correlated with the time of DNA replication [27, 28]. Indeed, it has been suggested that polyamines are critical for the S phase of the cell cycle [3, 25].

However, recent studies point to polyamine involvement in activation of the cell cycle by the so called competence growth factors. Competence growth factors (e.g. PDGF – platelet derived growth factor) initiate some biochemical changes and prime cells to be responsive to progression factors (e.g. EGF – epidermal growth factor or IGF-1 – insulin like growth factor 1) responsible for S phase progression. In mouse 3T3 fibroblasts PDGF (competence factor) is the stronger inducer of ODC gene expression, while EGF has a much lesser potency [16]. In contrast, in human WI38 fibroblasts, EGF (acting in this case as competence factor) causes strong ODC mRNA accumulation [17].

The expression of genes encoding polyamine biosynthesizing enzymes increases early after stimulation of quiescent fibroblasts but is unchanged during progression throughout the cell cycle [29]. Studies of the effects of polyamine depletion in serum stimulated fibroblasts also support the role of polyamines during G_0-S transition when the initiation of the cell cycle takes place [30]. All these data suggest that the role of polyamines is not restricted to the S phase; however, the exact nature of the ODC-dependent part of the cell cycle has not been clarified.

In order to determine polyamine-dependent steps in the cell cycle, we have studied the effects of polyamine depletion elicited by DFMO and MGBG on the proliferation of T lymphocytes as well as on the expression of some growth-regulated genes. We found that the ability of mitogen-stimulated mouse T lymphocytes to enter DNA synthesis was markedly inhibited by MGBG in a dose-dependent manner. This effect was particularly strong in the presence of fetal bovine serum containing a high level of activities of polyamine oxidases (polyamine degrading enzymes) [23]. Our studies of the effects of polyamine depletion on gene expression revealed marked inhibition of the expression of genes encoding cytoskeletal proteins. This inhibitory effect was visible dur-

ing the early phase of G_0-S transition, suggesting polyamine involvement in the regulation of early phase of the cell cycle [31].

To clarify which part of the cell cycle is polyamine-dependent, we have studied the effects of exogenous polyamines on cell proliferation. Unfortunately, the addition of polyamines to cells cultured *in vitro* causes a technical difficulty due to the cytotoxic effect of polyamines in the presence of fetal bovine serum, a standard component of the medium [32]. We established conditions for culturing of lymphocytes in the presence of horse serum which contains very low levels of polyamine oxidizing enzymes. Then we could demonstrate that exogenously added polyamines, putrescine and spermine, are able to induce lymphocyte proliferation. The kinetics of the polyamine-induced cell cycle was similar to that observed with the mitogen concanavalin A [23].

Moreover, putrescine and spermine, at concentrations which were effective in induction of the cell cycle, produced a significant increase of the expression of genes encoding the main cytoskeletal proteins: β-actin, vimentin and α-tubulin. Both the kinetics and the extent of the increase of gene expression following polyamine treatment resembled the stimulation produced by the mitogen. It is important to note that the expression of those genes is induced early during the G_0-S transition and may serve as a convenient landmark of that part of the cell cycle. Our results suggest that polyamines are capable of inducing the cell cycle by acting as mitogens and this effect can be mediated by their influence on gene expression [23].

Our results showing the regulatory role of polyamines in the cell cycle are in good agreement with the recent data that ODC belongs to a family of protooncogenes. It has been reported that the expression of ODC becomes constitutively activated during cell transformation induced by carcinogens, oncogenic viruses, or oncogenes [33 - 35]. Recently, Auvinen *et al.* [36] showed that the increased expression of ODC (50 - 100 times over the endogenous level) induced morphological transformation of fibroblasts and anchorage-independent growth. Blocking of ODC by treatment with antisense oligodeoxynucleotides or specific inhibitors prevents cell transformation. Probably the increase in expression of ODC and in its transforming activity is associated with changes in tyrosine phosphorylation of a protein of 130 kDa with unknown function. These data indicate that ODC is both necessary and sufficient for transformation, and support the notion of a regulatory role of polyamines in induction of the cell cycle.

Recently, it has been shown that the c-*myc* protooncogene is a potent activator of the ODC gene [37]. The c-*myc* protooncogene is a key regulator of competence phase of the cell cycle. Introduction of the c-*myc* gene under the control of inducible or constitutive promoters into fibroblasts or lymphocytes abrogates the dependence of those cells on growth factors [4]. Microinjection of recombinant c-*myc* renders fibroblasts competent [38]. Although the precise function of c-*myc* in regulation of the cell cycle is unknown, it has recently been demonstrated that Myc protein acts as transcription factor regulating expression of other genes. Some studies suggest that ODC is one of the physiologically relevant transcriptional targets of Myc protein. It has been demonstrated that c-*myc* can activate ODC transcription by binding to the *myc* binding sequence found in the promoter region of the ODC gene [37]. These results point again to polyamine involvement in regulatory pathways leading to induction of the cell cycle.

Polyamines and activation of terminally differentiated cells

The role of polyamines does not seem to be restricted only to proliferation. Many stimuli which provoke the functional activation of terminally differentiated cells may lead to a rapid induction of ODC and polyamine accumulation. Many stressful procedures like hypertonic infusions, chemical or mechanical treatment caused an increase of ODC activity in rat liver. A very high increase in ODC activity and gene expression occurs in organs undergoing hypertrophy: in androgen-induced hypertrophic growth of kidney and in cardiac hypertrophy brought about by thyroxin treatment (for review see [4]).

Involvement of polyamine biosynthesis activation in the early phase of the central nervous system response to a lesion has been demonstarted in various models of mechanical and ischemic injury [39, 40]. In the brain, increases in ODC activity can be seen following: mechan-

ical, thermal or toxic injury; global and focal ischemia; blood-brain barrier opening by mannitol or freezing; lesion or electroshock-induced seizures [41]. A number of experiments have suggested that the polyamines play a critical role in the entry of calcium through receptor-operated or voltage-sensitive calcium channels. If polyamine synthesis is blocked by DFMO, the various stimuli are no longer capable of inducing calcium entry. Recently, a link has been demonstrated between the polyamines and the NMDA (*N*-methyl-D-aspartate) receptor, which possesses a modulatory site sensitive to spermine and spermidine. Since the compounds which antagonize the effects of the polyamines on the NMDA receptor are potent neuroprotective agents *in vitro* and in animal models of NMDA receptor mediated neurotoxicity, it has been suggested that polyamines may contribute to neuronal death [41]. The effects of the polyamines in the nervous system are not restricted to those described above, and are far from being fully understood.

Polyamine involvement in functional activation of human macrophages

There are data available indicating the role of polyamines in monocyte/macrophage activation within the immune system. Macrophages play an important role in defending the body against bacterial and fungal infections and belong to tumoricidal effector cells [42]. In response to different stimuli such as interferon γ, tumor necrosis factor and bacterial lipopolysaccharide (LPS), they are activated to produce reactive oxygen metabolites [43, 44]. A respiratory burst, a process that generates reactive oxygen metabolites used for killing of microorganisms and tumor cells, is essential for macrophages.

It has been shown that ODC activity was induced by LPS and different immunoadjuvants in macrophage-like cell lines [45, 46]. Kierszenbaum *et al.* [47] reported the reduced capacity of macrophages to perform their phagocytic function following inhibition of ODC by DFMO. Additionally, Prosser & Wahl [48] observed the inhibition of collagenase production in LPS-treated macrophages following DFMO treatment. These data suggested some functional significance of polyamines for macrophage stimulation. However, at that time little was known regarding the role of polyamines in the function of cells within the immune system.

Working in collaboration with Prof. Angelo Messina's group from the University of Catania (Italy), we found that the stimulation of human and mouse macrophages with various stimuli such as lipopolysaccharide (LPS), interferon γ and tumor necrosis factor leads to ODC mRNA accumulation in a few hours after the addition of the drugs [49, 50]. We also demonstrated the importance of polyamines for the functional activation of human monocytes/macrophages. Using the antisense oligodeoxynucleotides targeted against ODC mRNA we could directly correlate down-regulation of ODC expression and the biological response of macrophages [50]. We tested the production of oxygen radicals, which is one of the major biological functions of activated macrophages involved in cytotoxicity. Moreover, we found that the polyamine depletion produced by the application of the inhibitors of polyamine biosynthesis also blocked the production of oxygen radicals [50, 51]. These inhibitory effects cannot be due to the cytotoxic effects of either drug because cells were viable and responded quite well to control stimulation. The inhibitory effect of MGBG was reversible by spermine [51].

Our findings clearly demonstrated that the ODC pathway and polyamine synthesis is an important intracellular component in the sequence of events that leads to functional activation of macrophages. Moreover, these results added an important point to the discussion about the possible use of inhibitors of polyamine metabolism in tumor chemotherapy. Despite some promising results in experimental models, clinical trials of DFMO and MGBG were not successful. The antiproliferative effects of inhibitors on tumor cells grown in culture usually cannot be matched *in vivo*. Our results raise a doubt whether treatment with polyamine synthesis inhibitors would be beneficial because of the adverse effects on the potentially important tumoricidal effector cell activity. Our results demonstrating that DFMO and MGBG block the cytokine-activated respiratory burst in macrophages and the mitogen-induced proliferation of T lymphocytes exemplify the impairment of an important function of the immune system as a consequence of polyamine depletion.

Are polyamines the regulators of cell activation?

All data presented above suggest that the conventional view of the nature and role of polyamines should be altered or augmented as follows: polyamines are potent cellular regulators which play a regulatory role in different biological phenomena, some of which are seemingly unrelated. ODC induction followed by polyamine accumulation is associated with the transition of cells from quiescence into the cell cycle, the induction of terminal differentiation in cells and the increased activity of mature, nondividing cells such as neurons and macrophages. These phenomena have been referred to collectively as *cell activation*; we introduced this term a few years ago to describe a ubiquitous phenomenon preceding and leading to the establishment of a new phenotype [4].

Cell activation is a process by which cells adapt their phenotype in response to environmental signals. All of the aforementioned cases of cell activation share a similar pathway: the external signals (growth factors, cytokines, neurotransmitters) activate through their specific receptors the formation of second messengers. Generation of second messengers results in activation of some genes whose protein products act as third messengers spreading an activating signal into the cell nucleus. Third messengers, by influencing expression of other genes, act as transcription modulators mediating establishment of a new phenotype.

We summarized previously [4] the data about the expression of c-*fos*, c-*myc* and ODC genes in different biological phenomena and formulated the idea that these genes create a link between the transduction of extracellular signals and long-term genomic responses (Fig.1).

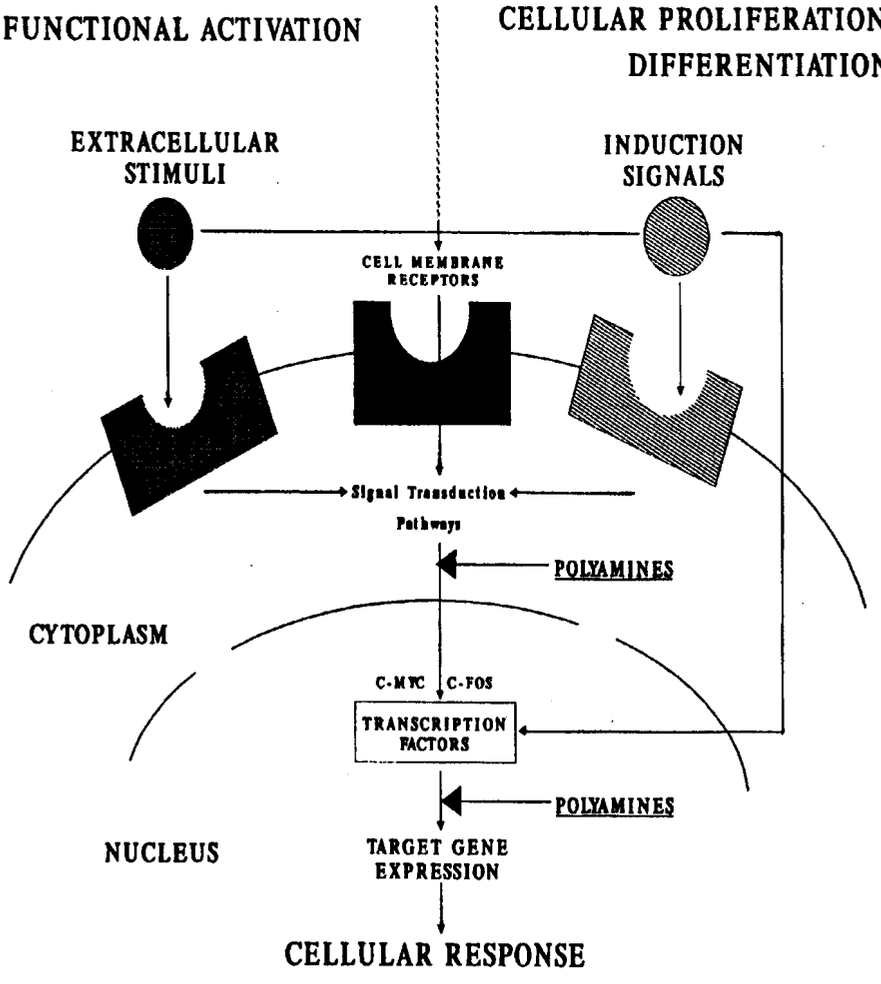

Fig. 1. A scheme that relates cell stimulation by first messengers, transduction pathways and transcription factors to final cellular response.
The possible role of the polyamines as intracellular messengers in this stimulus-coupling pathway is postulated.

We proposed that the gene encoding ODC belongs to activation-related genes such as some protooncogenes (e.g. c-*fos*, c-*myc*). At that time this hypothesis was a speculation, particularly with respect to ODC and polyamines. Our findings that polyamines play a regulatory role in the induction of the cell cycle of T lymphocytes and participate in the functional activation of macrophages support the hypothesis mentioned above. Additionally we provided evidence that polyamines can directly regulate the cell cycle and act as transcriptional regulators influencing the expression of cell growth-dependent genes. The recent finding that the gene encoding ornithine decarboxylase is a protooncogene essential for regulation of cell growth and transformation suggests again that polyamines are potent regulators of cell function. In this light it is evident that rapid and complex changes in polyamine biosynthesis are necessary to keep their regulatory potential under fine control. This minireview on the role of polyamines cannot answer some of the fundamental questions related to their exact mechanism of action. However, this paper does provide an answer to the questions: what the polyamines do and why is their biosynthesis controlled in such complex ways.

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