Polyamines: New Cues in Cellular Signal Transduction
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Ca\textsuperscript{2+} influx and fill SR Ca\textsuperscript{2+} stores. In relation to abnormal pacemaker function, drugs or disease processes that elevate SR Ca\textsuperscript{2+} may act via low-voltage-activated SR Ca\textsuperscript{2+} release to abnormally enhance atrial pacemaker automaticity. Abnormal pacemaker activity may therefore arise from Ca\textsuperscript{2+}-mediated mechanisms that need not invoke delayed afterdepolarizations and triggered activity, which require spontaneous diastolic Ca\textsuperscript{2+} release from an SR overloaded with Ca\textsuperscript{2+}. Because latent atrial pacemakers are more dependent on low-voltage-activated SR Ca\textsuperscript{2+} release than primary pacemakers, they may be more susceptible to Ca\textsuperscript{2+}-mediated dysrhythmic activities. Moreover, diastolic SR Ca\textsuperscript{2+} release may play a more important role in generating activity in pacemaker cells lacking \( I_f \). An understanding of the different mechanisms underlying atrial pacemaker activity may provide insight into the etiology and ultimate prevention of certain types of atrial arrhythmias.

References

4. DiFrancesco D, Ferroni A, Mazzanti M, and Tromba C. Properties of the naturally occurring polyamines putrescine, spermidine, and spermine are involved in signal transduction. This has been demonstrated by using inhibitors for polyamine biosynthesis (such as \( \alpha \)-difluoromethylornithine) or adding polyamines to cultured cells. Different polyamines, preferentially activated protein kinases (tyrosine kinases and MAP kinases), stimulated the expression of nuclear protooncogenes (myc, jun, and fos).

The distance between the cellular outer membrane and the nucleus seems to be tiny, only 20 \( \mu \text{m} \). Yet that minute distance encompasses a major mystery: how do the cells of higher organisms respond to the many growth signals that they receive from the environment? The answer is crucial to understanding such long-standing questions in cell biology as what causes cancer and how cellular growth is initiated or arrested. It has been well established that growth factors (mitogens) bind to specific receptors located on the cellular membrane. The mitogen-receptor complexes then trigger a cascade of events, including the activation of Ras by converting it from the GDP bound form to the GTP form. The activation of protein kinases is considered to be the next step in signal transduction, and we now recognize its critical role in the regulation of cell growth.
and development. The extracellular signal-regulated kinases (ERKs), also referred to as mitogen-activated protein kinases (MAPKs), play a number of important roles in signal transduction in eukaryotic cells (see Fig. 1). They are phosphorylated by MAPK/ERK kinases (MEKs), which are, in turn, activated by Raf. ERKs, which play important roles in signal transduction, are then phosphorylated by MEK. From there, signals are transduced to the nucleus, where histone H3 (His) is phosphorylated and thereby changes the structure of chromatin. Nuclear oncogenes, which may serve as transcription factors, are also activated by phosphorylation. CREB is a cAMP binding site located on the ornithine decarboxylase (ODC) gene. Another oncogene, Src, is also induced by mitogens, leading to the activation of another pathway, PKA and PKC, protein kinases B and C; SOS, son-of-sevenless protein; PI3K, phosphatidylinositol 3-kinase; FRAP, FKBP-rapamycin-associated protein; FKB, FK506 binding protein, CREB, cAMP-responsive element binding protein.

**Polyamines and proliferation processes**

The naturally occurring polyamines (the diamine putrescine, the triamine spermidine, and the tetra-amine spermine) are ubiquitous polycations (Fig. 2). They are present in all prokaryotic and eukaryotic cells thus far studied. They stabilize nucleic acids and stimulate their replication (6). Polyamines are therefore essential for growth processes, and they have also been associated with carcinogenesis. Their assay in biological fluids has been used for cancer diagnosis and for monitoring anticancer treatment (10). The intracellular concentration of polyamines can be regulated by 1) biosynthesis, 2) uptake, 3) oxidation, and 4) acetylation.

**Biosynthesis.** The rate-limiting step in the biosynthesis of polyamines is the conversion of ornithine into putrescine (Fig. 2). This step is catalyzed by ornithine decarboxylase (ODC; E.C.4.1.1.17), which has also been defined as an oncogene (1). ODC has an extremely short half-life (15–25 min in eukaryotic cells), and its synthesis is induced by hormones (10). Inhibition of ODC by specific inhibitors such as α-difluoromethylornithine (DFMO) results in inhibition of malignant growth, abortion, and prevention of parasitic growth (6).

**Uptake.** In addition to biosynthesis, cellular polyamine concentrations can be modulated by uptake. The transport of polyamines by yeast and bacterial cells has been studied extensively, and transporters have been isolated and cloned (6). The rate of polyamine uptake by bacteria is energy dependent and is a function of external pH and exogenous amine concentrations. The transporters can be either membrane-associated proteins or contain transmembrane-spanning segments, some of which bind ATP (6).
Oxidation. Oxidation and/or excretion are the main processes leading to the reduction in cellular polyamine levels. Polyamines and diamines can be oxidized by specific polyamine or diamine oxidases. Some of the oxidation products exhibit biological activity. Thus \(L\)-aminobutyric acid, which plays an important role in neural function, can be formed from putrescine.

Acetylation. Acetylation is another metabolic pathway that leads to a decrease in active polyamine levels (6). This reaction is catalyzed by specific acetyltransferases and causes the reduction in the net positive charge density of the polyamines. Acetylpolyamines and diamines are not tightly bound to cellular negatively charged molecules (such as nucleic acids, phosphoproteins, or phospholipids) and thus may destabilize nucleic acids. Polyamine excretion is also enhanced by acetylation.

Despite the wide distribution of polyamines in nature, their exact biological functions have not yet been elucidated. Many biologists were puzzled by two questions: 1) is there a need for three different naturally occurring polyamines and 2) does each of them fulfill a specific function?

In this short review, we will try to explain some specific functions of the different polyamines. It will be shown that spermidine enhances the phosphorylation of threonine and tyrosine residues in ERK1 and ERK2 and that both the diamine putrescine and triamine spermidine stimulate the phosphorylation of proteins by tyrosine kinases. The activation of kinases by polyamines triggers the expression of nuclear oncogenes.

It is evident from Fig. 2 that putrescine can be converted into spermidine or spermine. This occurs when cells are saturated with putrescine. In our studies, cells were starved before the addition of the polyamines, polyamines did not reach saturation, and therefore the interconversion was minimal.

Polyamines and protein kinases

The Ras/MAPK cascade is the best-defined pathway involved in cell proliferation. In this pathway, a central role is played by proteins p42 and p44. Various recent studies suggested that polyamines are involved in the expression and activation of MAPKs. Thus ODC-overproducing transfectants showed enhanced MAPK (7) and tyrosine kinase (2) activities. In those studies, no differential effect of one of the polyamines has been reported. We have recently demonstrated (4) that spermidine preferentially stimulated the phosphorylation of p42 and p44 (Fig. 3). The MAPK pathway is essential for growth. PD-98059 [2-(2-amino-3′-methoxyphenyl)-oxanaphthalen-4-one] selectively inhibits the MAPK-activating enzymes (MEKs). This inhibitor inhibits ERK phosphorylation and ODC activity (7).

Polyamines and the expression of nuclear oncogenes

Growth-associated genes, such as c-fos and c-myc proto-oncogenes, are activated during cellular proliferative processes. This activation is due to signals transmitted from the cellular membrane to its nucleus. Following mitogenic stimulation, a simultaneous activation of polyamine biosynthesis and the transcription of the c-fos protooncogene has been observed. Similarly, malignant transformation leads to an
increase in polyamine biosynthesis, deregulation of ODC, and the amplification of protooncogenes. It has been demonstrated that polyamines stimulate the transcription of c-myc and c-fos (11), but the preferential role of each of the polyamines on protooncogene expression has not been explored. The involvement of polyamines in the expression of nuclear protooncogenes has previously been confirmed by the finding that DFMO blocked the expression of c-fos and c-myc in cultured cells (11). We have recently reported that spermidine at micromolar concentrations stimulated the transcription and translation of c-myc (Fig. 3) in cultured rat kidney epithelial cells (12). On the other hand, putrescine was more active (Fig. 4) in preferentially stimulating the expression of c-fos and c-jun in those cells (12). These findings suggest that each of the polyamines has a preferential effect in controlling nuclear protooncogene expression.

Polyamines and signal transduction

It can be seen in Fig. 1 that signals are transduced from the cellular membrane to its nucleus by activating kinases, including tyrosine kinases. Subsequently ras, MEKs, and ERKs are activated. During the last steps of the signal transduction process, nuclear protooncogenes and transcription factors are expressed. It is remarkable that many of these steps are regulated by polyamines and that DFMO, which inhibits polyamine biosynthesis, prevents the transfer of information from the membrane to the nucleus. All of this strongly suggests that polyamines play a role in signal transduction.

An ODC-oncogene loop

Protooncogene products, expressed downstream of the MAPK cascade, include the transcription factors activator protein 1 (AP-1) or c-Myc. AP-1 is either a homodimer of Jun or a heterodimer of Jun and Fos, which bind to a common DNA binding site located in introns 3, 5, and 11 of the ODC gene (13). The Myc protein, on the other hand, is a transcription factor that regulates the expression of genes by binding to the specific DNA sequence CACGTG (5). Among the target genes for Myc regulation is ODC (5). Both Myc and AP-1 are substrates of phosphorylation by MAPK. If polyamines, indeed, regulate the formation of Myc and AP-1, then a reciprocal effect of these nuclear oncogenes and polyamine synthesis could be conceived. Such a polyamine-oncogene loop has been proposed by Flamingi (7).

The expression of ODC can also be regulated by cAMP (3). Spermidine may regulate the activity of protein kinase A (8). The ODC gene has a cAMP binding site. Therefore, another cAMP loop may be envisaged.

Conclusions

It now appears that the naturally occurring polyamines not only stabilize cellular nucleic acids and/or membranes but also play a pivotal role in activating protein kinases and transcription factors. These findings may explain the role of polyamines in cellular proliferation and differentiation processes. Moreover, carcinogenesis could be related to the accumulation of polyamines in cancer cells. The expression of ODC, which catalyzes the formation of putrescine from ornithine, can be regulated by factors that are controlled by polyamines such as Myc, Jun, Fos, and cAMP, thus forming several reciprocal loops. All of these findings stress the importance of naturally occurring polyamines in controlling essential physiological events and may explain the distribution of more than a single polyamine in nature.

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References