Polyamine homoeostasis

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Abstract

The polyamines are essential for a variety of functions in the mammalian cell. Although their specific effects have not been fully elucidated, it is clear that the cellular polyamines have to be kept within certain levels for normal cell function. Polyamine homoeostasis in mammalian cells is achieved by a complex network of regulatory mechanisms affecting synthesis and degradation, as well as membrane transport of polyamines. The two key enzymes in the polyamine biosynthetic pathway, ODC (ornithine decarboxylase) and AdoMetDC (S-adenosylmethionine decarboxylase), are strongly regulated by feedback mechanisms at several levels, including transcriptional, translational and post-translational. Some of these mechanisms have been shown to be truly unique and include upstream reading frames and ribosomal frameshifting, as well as ubiquitin-independent proteasomal degradation. SSAT (spermidine/spermine N\textsuperscript{1}-acetyltransferase), which is a crucial enzyme for degradation and efflux of polyamines, is also highly regulated by polyamines. A cellular excess of polyamines rapidly induces SSAT, resulting in increased degradation/efflux of the polyamines. The polyamines appear to induce both transcription and translation of the SSAT mRNA. However, the major part of the polyamine-induced increase in SSAT is caused by a marked stabilization of the enzyme against degradation by the 26S proteasome. In addition, active transport of extracellular polyamines into the cell contributes to cellular polyamine homoeostasis. Depletion of cellular polyamines rapidly induces an increased uptake of exogenous polyamines, whereas an excess of polyamines

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down-regulates the polyamine transporter(s). However, the protein(s) involved in polyamine transport and the exact mechanisms by which the polyamines regulate the transporter(s) are not yet known.

Introduction

A number of anabolic processes in the mammalian cells are highly dependent on adequate levels of polyamines [1,2]. A decrease in cellular polyamine concentrations often results in a reduction of nucleic acid and protein syntheses, eventually giving rise to cell growth inhibition [3]. Induction of cell growth is usually associated with an increase in cellular synthesis and levels of polyamines; however, too high concentrations of the polyamines may be toxic to the cells, inducing cell death or apoptosis. Thus cellular polyamine concentrations are usually maintained within rather narrow limits. This is achieved by a careful balance of synthesis, degradation and uptake of the polyamines [2,4]. These processes are all highly regulated and the polyamines exert a strong feedback control by affecting all of these steps in the polyamine metabolic pathway. In the present chapter the control of polyamine homoeostasis in mammalian cells will be described in more detail.

Polyamine synthesis

The polyamine biosynthetic pathway includes four different enzymes, i.e. ODC (ornithine decarboxylase), AdoMetDC [AdoMet (S-adenosylmethionine) decarboxylase], spermidine synthase and spermine synthase (Figure 1). The rate-limiting step in biosynthesis of polyamines appears to be either the decarboxylation of ornithine or the decarboxylation of AdoMet. These metabolic steps, catalysed by ODC and AdoMetDC, are also the key regulatory steps in the polyamine biosynthetic pathway. Both ODC and AdoMetDC have extremely high turnover rates in the cell [5–7], whereas spermidine synthase and spermine synthase are considerably more stable [8]. Owing to the fast turnover of ODC and AdoMetDC, the cellular levels of the enzyme proteins and thus the corresponding enzyme activities are rapidly altered when the synthesis and/or degradation of the enzymes are changed. Both enzymes are subject to a strong feedback control by the polyamines (Figure 1). ODC and AdoMetDC are rapidly up-regulated when cells become depleted of their polyamine content. On the other hand, both enzymes are down-regulated when cells are exposed to an excess of polyamines.

ODC

ODC is one of the enzymes in the mammalian cell having the shortest biological half-life [5,6]. Usually the half-life of ODC varies between 15 and 60 min, but can be as short as a few minutes in some cases. The turnover of ODC has been shown to be highly affected by the cellular polyamine levels [5,6,9]. Provision of an excess of polyamines to the cells will rapidly result
in a faster degradation of the enzyme, reducing the half-life even further. On the other hand, a stabilization of ODC occurs when cells are depleted of their polyamines. All changes in the ODC degradation rate will rapidly result in a change in the level of ODC protein, and thus in the enzyme activity, due to the fast turnover of the enzyme. As a result of this, there is an inverse relationship between cellular polyamine content and ODC activity, which tends to stabilize polyamine levels in the cell. Significant progress has been made in the understanding of the mechanisms involved in the rapid degradation of ODC, as well as in how polyamines affect the turnover of the enzyme, revealing several unique processes. ODC is, like most other short-lived and regulatory proteins, degraded by the proteolytic complex called the 26S proteasome [5]. Usually, targeting proteins for degradation by the 26S proteasome involves covalent binding of multiple ubiquitin molecules to the protein. However, ODC is one of the very few proteins that are degraded by this proteolytic system without prior ubiquitination. In fact, ODC was the first example of a non-ubiquitinated protein being degraded by the 26S proteasome [5]. Instead, targeting of ODC for degradation by the 26S proteasome occurs by a binding of a specific protein, named Az (antizyme), to the enzyme (Figure 2) [5,6,9]. Az was discovered more than 30 years ago by Canellakis and colleagues [10]. They demonstrated that exposure of cells to high levels of putrescine resulted

Figure 1. Feedback regulation of polyamine synthesis
The regulatory steps in polyamine biosynthesis are catalysed by ODC and AdoMetDC. Both of these enzymes have short half-lives and are regulated at various levels by the polyamines. Putrescine has a stimulatory effect on AdoMetDC processing and enzyme activity. dcAdoMet, decarboxylated AdoMet; MTA, methylthioadenosine.
in the induction of a unique short-lived protein, which strongly inhibited ODC activity. They also proposed the name Az for this group of inhibitory proteins. However, it was not until later that it was demonstrated that, besides inhibiting the ODC activity, Az induced the degradation of the ODC protein by the 26S proteasome [4–6,9]. ODC is functionally a dimer and the two active sites, which are formed at the dimer interface, contain amino acids from both subunits [4]. The binding of Az occurs at the monomer level and thus promotes the dissociation and inactivation of the dimer (Figure 2) [6].

The synthesis of Az is stimulated by polyamines. Thus an excess of polyamines down-regulates ODC by affecting the production of Az, which inhibits the enzyme as well as stimulates its degradation [5]. On the other hand,
a depletion of cellular polyamine content results in a stabilization of ODC protein due to a decrease in Az production. Studies of the mechanism by which the polyamines affect the synthesis of Az revealed a truly unique regulatory mechanism. The cloning and sequencing of Az mRNA demonstrated that the message contains two major, partially overlapping, ORFs (open reading frames) and that the expression of the full-length Az required a ribosomal +1 frameshift (Figure 3) [5,6]. It was also shown that polyamines stimulated the synthesis of Az by promoting the +1 frameshifting [11]. Programmed ribosomal frameshifting is an extremely rare event and is mainly known from various viruses. So far, synthesis of Az is the only known example of a regulated frameshifting occurring in mammalian cells.

Truncations and mutations in the mammalian ODC have revealed that the C-terminal part of the enzyme is essential for the rapid degradation of the protein [12]. Interestingly, ODC from the parasite (*Trypanosoma brucei*) causing African sleeping sickness lacks this part of the protein and is a metabolically stable protein [13]. However, if *T. brucei* ODC is recombined with the C-terminal part of mammalian ODC the parasitic enzyme is transformed into a short half-life protein when expressed in mammalian cells [13]. The C-terminal part of mammalian ODC has been shown to be essential for the recognition by the proteasome and binding of Az to ODC is believed to affect the structure of the monomer in such a way that the C-terminal part is exposed, targeting the protein for degradation by the 26S proteasome [6].

The amount of Az available for ODC binding is not only regulated at the translational level. In addition, Az activity is regulated by a specific protein

![Figure 3. Ribosomal frameshifting in translation of Az mRNA](image-url)
named AzI (Az inhibitor) (Figure 2) [4,5,9]. AzI is highly homologous with ODC without being enzymatically active (lacks essential amino acids). However, like ODC, AzI binds Az. The binding affinity between AzI and Az is stronger than that between ODC and Az. Thus, in the presence of AzI, less Az is available for binding to ODC, which results in a decreased degradation of the enzyme. Even though AzI most probably fulfils an important function in polyamine homoeostasis, not much is known about its regulation. To make everything even more complicated, three different forms of Az (Az1–Az3) and two different forms of AzI (AzI-1 and AzI-2) have been discovered [6,9]. However, it was recently demonstrated that AzI mRNA contains a small uORF (upstream ORF), which mediates polyamine-induced inhibition of the translation of the downstream main ORF [14]. Thus, in the presence of polyamines, AzI should be down-regulated, giving rise to a reduction of ODC due to degradation by the 26S proteasome. The importance of AzI was also recently demonstrated using knockout mice with a disrupted Oaz1 (mouse AzI-1) gene [15]. Homozygous mutant mice died at the newborn stage with abnormal liver morphology. Further analysis of embryos of the homozygous mice indicated that the deletion of the Oaz1 gene induced a degradation of ODC and a reduction of polyamine biosynthesis.

Both Az and AzI have a rapid turnover in the cell and are degraded by the 26S proteasome in an ubiquitin-dependent process [9]. Even though Az binding to ODC induced degradation of ODC by the proteasome, degradation of Az was unaffected by the interaction with ODC. On the other hand, binding of Az to AzI stabilized AzI against degradation by the proteasome.

In addition, to induce changes in ODC turnover rate, the polyamines appear to affect ODC synthesis. However, this feedback control of ODC synthesis is not correlated with any changes in ODC mRNA levels, indicating an effect on translation rather than on transcription (or stability) of the ODC mRNA [16]. Like many of the other mRNAs encoding growth-related proteins, mammalian ODC mRNA contains a long GC-rich 5′ UTR (untranslated region), which strongly inhibits the translation of the subsequent ORF. The expression of ODC has been shown to be stimulated by the initiation factor eIF-4E (eukaryotic initiation factor 4E). This initiation factor contains an RNA helicase activity, which is important for the melting of secondary structures of the 5′ UTR. Moreover, the 5′ UTR of ODC mRNA has been suggested to contain an IRES (internal ribosomal entry site), making cap-independent translation possible [17]. However, so far no evidence exists that the 5′ UTR of ODC mRNA is important for the polyamine-mediated control of ODC synthesis.

AdoMetDC

Synthesis of spermidine and spermine is achieved by the addition of an aminopropyl group to putrescine and spermidine respectively. These aminopropyl groups are derived from decarboxylated AdoMet, which is
produced by AdoMetDC. Mammalian AdoMetDC belongs to the group of very few enzymes that uses pyruvate, instead of pyridoxal 5'-phosphate, as a cofactor [18]. The pyruvate is generated by an autocatalytical cleavage of the AdoMetDC protein after synthesis [19]. Mammalian AdoMetDC is produced as a pro-enzyme which is rapidly cleaved into two differently sized subunits, which both are part of the active heterotetrameric form of the enzyme [18]. The cleavage occurs at a serine residue (in the larger subunit), which then is converted into pyruvate. The processing of the AdoMetDC pro-enzyme has been shown to be stimulated by putrescine, which also has a direct stimulatory effect on the catalytic activity of the enzyme [18].

Mammalian AdoMetDC is also highly regulated and its turnover is very fast. Like mammalian ODC, AdoMetDC is degraded by the 26S proteasome [7]. However, unlike the degradation of ODC by the 26S proteasome, the degradation of AdoMetDC is dependent on ubiquitination of the protein. Interestingly, the degradation rate appears to be very dependent on the availability of the substrate AdoMet [7]. In the presence of low substrate levels, the degradation of AdoMetDC is decreased, whereas high substrate levels are believed to induce a more rapid degradation. The binding of AdoMet in the active site occasionally gives rise to transamination of an amino group from the substrate to the bound pyruvate, converting it into alanine and thus irreversibly inactivating the enzyme [7]. Although this occurs at a very low frequency, it may be substantial in the presence of high concentrations of the substrate. The transaminated form of AdoMetDC appears to be more rapidly degraded than the active form of AdoMetDC, explaining the substrate-mediated difference in degradation rate [7]. In addition, the turnover of AdoMetDC is affected by the cellular supply of spermidine and spermine in such a way that decreased polyamine levels give rise to a stabilization of the enzyme against degradation [18]. However, the mechanism by which the polyamines affect the degradation of AdoMetDC is still unknown.

The polyamines spermidine and spermine negatively regulate the synthesis of AdoMetDC [18]. Both polyamines are effective, but spermine appears to be a more potent regulator than spermidine. Part of this control appears to be achieved at the transcriptional level, since the AdoMetDC mRNA level is changed according to the polyamine status of the cells. Depletion of cellular spermidine and/or spermine induces an increase, whereas an excess of these polyamines results in a decrease in the AdoMetDC mRNA level. In addition to affecting the AdoMetDC mRNA steady-state level, spermidine and spermine induce changes in the efficiency by which the AdoMetDC mRNA is translated [18]. The efficiency is increased in situations of low polyamine levels and vice versa. This effect by the polyamines appears to be dependent on a short uORF of the AdoMetDC mRNA [20]. The uORF codes for the peptide MAGDIS and the production of this peptide seems to be essential for the polyamine-mediated effect on the translation of the AdoMetDC reading frame. Mutating the initiation codon of the uORF, as well as changing the
nucleotide sequence coding for the C-terminus of the peptide, abolishes the polyamine-mediated regulation of AdoMetDC mRNA translation [20]. It has been shown that, during translation of the uORF, ribosomes pause close to the termination codon. Changing sequences coding for the C-terminus of the MAGDIS peptide reduces the pausing of the ribosomes making translation of the downstream reading frame more efficient. The time the ribosomes are stalled is dependent on the polyamines. The polyamines prolong the pausing period, which reduces downstream translation of AdoMetDC mRNA [20].

**Polyamine degradation**

Spermidine and spermine may be degraded to putrescine and spermidine respectively, in a two-step process usually referred to as ‘the polyamine interconversion pathway’ (Figure 4) [21]. The first step in this pathway is an acetylation of the N1-nitrogen of the polyamine producing N1-acetylsperrmidine or N1-acetylsperrmine, catalysed by the enzyme SSAT (spermidine/spermine N1-acetyltransferase). The acetylation is followed by an oxidation catalysed by a FAD-dependent peroxisomal polyamine oxidase. The preferred substrates of this polyamine oxidase are the N1-acetylated derivatives of spermidine and spermine. Spermidine and spermine are poor substrates of this enzyme unless they are acetylated. The enzyme is nowadays referred to

![Figure 4. Feedback regulation of polyamine degradation](image)

Spermidine and spermine may be converted into putrescine and spermidine respectively, in a two-step process, catalysed by SSAT and APAO. The rate-limiting step is catalysed by SSAT, which has a fast turnover and is induced by the polyamines. Spermine can also be converted into spermidine in a single step catalysed by SMO. SMO expression is induced by polyamine analogues, and most likely also by spermine.
as the APAO (N\textsuperscript{1}-acetylpolyamine oxidase) to distinguish it from the recently discovered SMO (spermine oxidase), which preferentially uses spermine as a substrate (see below). The results of the oxidation of N\textsuperscript{1}-acetylspermidine and N\textsuperscript{1}-acetylspermine by APAO are putrescine and spermidine respectively, as well as 3-acetamidopropanal and hydrogen peroxide [21]. Putrescine may be further oxidized by diamine oxidase, whereas spermidine may undergo another round in the interconversion pathway. The acetylated derivatives of spermidine and spermine may also be excreted from the cells. The exact mechanisms by which the acetylated polyamines are excreted are still not clear. However, recently SLC3A2, which is part of a heterodimeric cationic amino acid transporter, was identified as being involved in the export of especially putrescine, but also of acetylated spermidine [22]. The SLC3A2 protein was shown to be co-localized with SSAT on the plasma membrane, and both proteins were co-immunoprecipitated using antibodies against either SLC3A2 or SSAT, suggesting a functional interaction [22]. Interestingly, SLC3A2-dependent export of putrescine appeared to be associated with uptake of arginine, indicating an exchange reaction. Arginine, being a substrate for ornithine production, is essential for polyamine biosynthesis. However, whether this efflux system plays a physiological role in cellular polyamine homoeostasis or not remains to be established.

SSAT catalyses the rate-limiting step in the polyamine interconversion pathway [23]. The activity of APAO normally greatly exceeds that of the acetylase. Like ODC and AdoMetDC, SSAT has a very fast turnover with a half-life as short as 15 min, whereas APAO is a stable enzyme [23]. Thus SSAT may respond quickly to changes in the synthesis or degradation of the enzyme. The rapid turnover of SSAT is mediated by the 26S proteasome and is dependent on ubiquitination of the protein [24]. SSAT is strongly induced by a variety of stimuli, including various toxins and hormones. However, the enzyme is also induced by polyamines (Figure 4) and some of their analogues, such as N\textsuperscript{1},N\textsuperscript{12}-bis(ethyl)spermine and N\textsuperscript{1},N\textsuperscript{12}-bis(ethyl)norspermine [24]. Thus, similar to ODC and AdoMetDC, SSAT plays an important role in polyamine homoeostasis. A minor part of the increase in SSAT activity observed after a polyamine load is explained by an increase in the level of SSAT mRNA, indicating a transcriptional effect. A putative polyamine-responsive element has been identified in the promoter of the SSAT gene [25]. Part of the increase in SSAT expression may be due to an increase in the efficiency by which the SSAT mRNA is translated. However, the major part of the polyamine-induced increase in SSAT appears to be caused by a marked stabilization of the enzyme against degradation. SSAT degradation has been shown to be strongly inhibited by the binding of polyamines or N\textsuperscript{1},N\textsuperscript{12}-bis(ethyl)spermine to the enzyme [24]. The rapid turnover of SSAT, like that of ODC, is dependent on the C-terminal region of the protein. Truncation or mutations in this region completely abolish the rapid degradation of SSAT [24]. It is conceivable that the binding of polyamines or their analogues to SSAT induce a conformational
change in the enzyme protein that prevents the exposure of the C-terminal region to the proteasome.

In addition to being a substrate for the polyamine interconversion pathway, spermine may be degraded to spermidine, 3-aminopropanal and hydrogen peroxide in a single step catalysed by the enzyme SMO (Figure 4) [26,27]. Like APAO, SMO is a FAD-dependent oxidase but, unlike the former enzyme, SMO has a high specificity for spermine as a substrate. In great contrast with APAO, SMO is highly induced by several of the polyamine analogues, indicating a role in polyamine homoeostasis [26]. However, the regulation of SMO appears to be mainly at the level of mRNA transcription/stability, rather than translational/post-translational [28].

Polyamine uptake

In addition to regulating polyamine levels by synthesis, degradation and efflux, cells are equipped with an active transport system for the uptake of polyamines [29]. Large amounts of polyamines are found in the food we eat. Bacteria in the intestinal system produce and excrete considerable quantities of polyamines. It is conceivable that a large fraction of these exogenous polyamines are absorbed from the intestines and later taken up and used by cells in the body. However, to what extent cells rely upon endogenous compared with exogenous polyamines is not yet clear. Nevertheless, in situations when the polyamine biosynthetic machinery is insufficient, cells would certainly be more dependent on extracellularly derived polyamines. Results from experiments using cells deficient in polyamine transport indicate that the transport system may also be involved in the salvage of polyamines normally excreted from cells [30].

The exact mechanisms and the proteins involved in polyamine transport are still not identified. Whether there are individual transport systems for the various polyamines or only a single transporter, capable of transporting all of the polyamines, is not clear. Results obtained indicate that uptake of polyamines by mammalian cells, at least partly, occurs by a mechanism involving cell-surface heparin sulfate proteoglycans and endocytosis [31]. The polysaccharides of the proteoglycans are negatively charged and may interact with the positively charged polyamines with affinities even stronger than the interaction between DNA and polyamines. Moreover, it was recently demonstrated that polyamine uptake in human colon cancer cells follows a dynamin-dependent and clathrin-independent endocytic route, which is negatively regulated by caveolin-1 [32]. However, in spite of large efforts, a mammalian transporter responsible for polyamine uptake has not yet been cloned. Nevertheless, a cell-surface protein, capable of transporting both putrescine and spermidine, has been cloned from the protozoan pathogen *Leishmania major* [33].

The polyamine transporter is an important part of the polyamine homoeostatic system in mammalian cells [29]. The activity of the polyamine transporter is partly regulated by cellular polyamine levels. Cellular depletion of polyamines results in a marked increase in the cellular uptake of exogenous
polyamines. On the other hand, the polyamine transporter is down-regulated when cells are exposed to an excess of polyamines. This feedback regulation is dependent on protein synthesis and involves a protein with a very fast turnover [29]. Interestingly, it has been shown that Az, which is induced by an excess of polyamines (and has a fast turnover), besides regulating the degradation of ODC, also appears to negatively regulate the cellular polyamine transporter [29]. Cells in which Az is expressed to high levels exhibit a marked reduction in polyamine uptake. All three different forms of Az (Az1–Az3) have been shown to effectively down-regulate polyamine transport. However, the mechanism by which Az affects polyamine uptake is so far unknown.

It is conceivable that the therapeutic effect of inhibitors of ODC or AdoMetDC against proliferative disorders, such as cancer, may partly be neutralized by an increased cellular uptake of exogenous polyamines [34]. Any decrease in cellular polyamine levels will rapidly induce an elevated uptake of polyamines from the extracellular environment. Results from various model systems strongly indicate that the therapeutic effect of the ODC inhibitors could be dramatically improved if the uptake of exogenous polyamines can be blocked [34,35].

Conclusions

Cellular polyamine homoeostasis is achieved by effective feedback mechanisms at a multitude of levels, including synthesis, degradation and transport (Figure 5). At present, we understand some of these mechanisms in part, but most of the molecular details are still unknown. The polyamine metabolic pathway is a potential target for therapeutic agents against a variety of diseases,

Figure 5. Regulation of polyamine homoeostasis
Polyamine homoeostasis is achieved by a balanced control of polyamine synthesis, degradation and uptake.
including cancer. However, the usefulness of these agents may depend on the understanding and control of the molecular mechanisms involved in the cellular control of polyamine homoeostasis. Moreover, such understanding may constitute the basis for future development of new and more effective drugs interfering with cellular polyamine homoeostasis.

**Summary**

- **Polyamine homoeostasis in mammalian cells is achieved by a complex network of regulatory mechanisms affecting synthesis, degradation and uptake of the polyamines.**
- **All key enzymes in polyamine biosynthesis and degradation have extremely short half-lives and are subject to strong feedback regulation by the polyamines at various levels.**
- **Some of the mechanisms involved in polyamine homoeostasis are truly unique, including, for example, upstream reading frames and ribosomal frameshifting, as well as ubiquitin-independent proteasomal degradation.**
- **The polyamine metabolic pathway is a potential target for therapeutic agents against a variety of diseases, and the understanding of the molecular mechanisms involved in cellular polyamine homoeostasis may form the basis for development of new and more effective drugs.**

**References**


