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REVIEW

Antizyme and antizyme inhibitor, a regulatory tango

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Abstract The polyamines are small basic molecules essential for cellular proliferation and viability. An autoregulatory circuit that responds to the intracellular level of polyamines regulates their production. In the center of this circuit is a family of small proteins termed antizymes. Antizymes are themselves regulated at the translational level by the level of polyamines. Antizymes bind ornithine decarboxylase (ODC) subunits and target them to ubiquitin-independent degradation by the 26S proteasome. In addition, antizymes inhibit polyamine transport across the plasma membrane via an as yet unresolved mechanism. Antizymes may also interact with and target degradation of other growth-regulating proteins. An inactive ODC-related protein termed antizyme inhibitor regulates polyamine metabolism by negating antizyme functions. The ability of antizymes to degrade ODC, inhibit polyamine uptake and consequently suppress cellular proliferation suggests that they act as tumor suppressors, while the ability of antizyme inhibitors to negate antizyme function indicates their growth-promoting and oncogenic potential.

Keywords Antizyme · Antizyme inhibitor · Ornithine decarboxylase (ODC) · Polyamines · Cellular proliferation · Cellular transformation

Introduction

The polyamines, spermidine and spermine, and their precursor, putrescine, are positively charged aliphatic amines. Polyamines are implicated in regulating fundamental cellular processes including ion channel function, DNA folding and replication, transcription, translation, and apoptosis (for review, see [1]); most importantly, they are essential for normal as well as neoplastic cellular proliferation [2–4]. Depletion of cellular polyamines compromises cellular proliferation, which resumes upon addition of exogenous polyamines. All cells have the ability to synthesize polyamines, and most cells can also absorb polyamines from their environment by transport across the plasma membrane.

L-Ornithine decarboxylase (ODC) is the first rate-limiting enzyme in the biosynthesis pathway of polyamines. In mammalian cells, ODC provides the only route for de novo synthesis of putrescine, which is further converted to spermidine and spermine by the concerted action of downstream enzymes [5–7]. ODC is a highly regulated enzyme whose activity is induced by growth-promoting stimuli and is elevated in cells transformed by oncogenes, carcinogens, and viruses, and in a variety of malignancies [6, 8–19]. Furthermore, forced ODC overexpression is sufficient to provoke cellular transformation [20].

Being such a key regulator of cellular proliferation, it is not surprising that the intracellular ODC levels are tightly regulated at multiple control levels, especially by regulation of its stability via a polyamine mediated autoregulatory circuit. In the center of this autoregulatory circuit are small proteins termed antizymes. The most highly characterized member of this family of proteins, called antizyme-1, was originally described as a polyamine-induced ODC inhibitory activity [21]; the gene encoding a protein that yields this inhibitory activity was eventually cloned as a result of a remarkable study conducted in the laboratory of Shin-Ichi Hayashi in Japan [22]. Since the affinity of antizyme towards ODC subunits is higher than the affinity of ODC

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subunits for each other, antizyme traps transient ODC monomers to form inactive ODC-antizyme heterodimers that are recognized by the 26S proteasome, resulting in ubiquitin-independent degradation of ODC [23, 24]. Antizyme reduces cellular polyamine pools not only by stimulating ODC degradation, but also by interfering with the uptake of external polyamines [25–27].

Interestingly, an ODC-related protein termed antizyme inhibitor efficiently negates these two activities of antizyme. This protein, originally found in rat liver extract, binds antizyme with higher affinity than ODC, thus saving ODC from degradation [28]. The present review will summarize our current knowledge of the mechanisms by which antizymes and antizyme inhibitors regulate cellular polyamine homeostasis.

Synthesis of antizymes via polyamine regulated frameshifting

Antizymes are encoded by two open reading frames (ORF 1 and 2). Therefore, expression of functional antizymes requires a unique process of programmed ribosomal frameshifting [29, 30] (Fig. 1). Translation that starts either at the major translational start site (the second ATG codon) or at a minor start site (the first ATG codon) will terminate shortly thereafter at an in-frame termination codon, encoding ORF1. Since the main part of antizyme is encoded by a +1 ORF (ORF2), ribosomes must be subverted to the +1 reading frame in order to form a mature functional antizyme. Comparison of the sequence of the mature antizyme protein to the sequence of the encoding mRNA demonstrates that the actual frameshifting event occurs while the scanning ribosome encounters the last codon of ORF1 (UCC UGA UGU). In mammalian cells, the ribosome slips forward to the +1 frame encoding aspartic acid (GAU), while during translation of mammalian antizyme in yeast cells, the ribosome slips backwards to the −2 frame, establishing the same reading frame, but with an extra encoded amino acid [31, 32]. The efficiency at which this frameshifting event occurs is affected by the concentration of polyamines [29, 30, 33]. High polyamine concentration increases frameshifting efficiency, resulting in enhanced synthesis of antizyme, increased ODC degradation rate, and inhibited polyamine uptake. This dependence of frameshifting efficiency on the polyamine concentration serves as the sensing mechanism for the concentration of free intracellular polyamines.

The site of frameshifting (shifty site) and sequences around it are implicated in regulating frameshifting efficiency (for a detailed review, see [34]). Efforts are being made to determine whether and how any of these

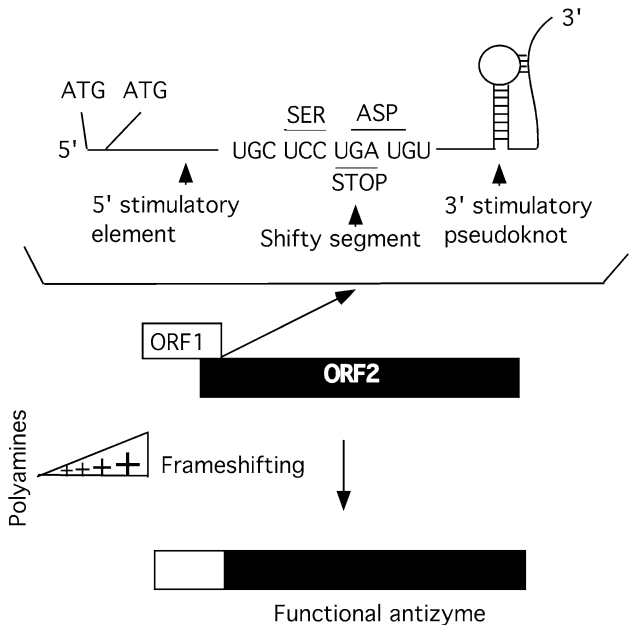

Fig. 1 Synthesis of antizyme via polyamine-stimulated frameshifting. Translation of antizyme mRNA initiates at one of two in-frame initiation codons. The scanning ribosome encounters a stop codon shortly thereafter, completing translation of the first ORF (ORF1). The sequence around this stop codon determines the ability of the ribosome to shift to a +1 frame to translate ORF2 and to generate mature functional antizyme. Increase in the free intracellular concentration of polyamines stimulates this frameshifting event. Sequences located 5′ and 3′ to the 'shifty' site also increase frameshift efficiency

sequences mediate the effect that polyamines exert on frameshifting efficiency [29, 30, 35–40]. However, it is presently unclear whether polyamines affect frameshifting through direct interaction with antizyme mRNA, or via alternative mechanisms such as interaction with the translation machinery, trans-acting factors, or by mediating interaction between such factors and the mRNA or the translation machinery. A pseudoknot located 3′ to the shifty site stimulates frameshifting. Pseudoknots were described as stimulators of −1 frameshifting, presumably by forcing the elongating ribosome to pause [41]. Pseudoknot-mediated pausing may not be required in the case of antizyme, as the scanning ribosome is forced to stop at the termination codon of ORF1. It was demonstrated that altering the stop codon at the end of ORF1 to another stop codon sequence maintains frameshifting, while frameshifting is severely inhibited when this termination codon is converted to a sense codon [29, 30].

Recently, it was demonstrated that the frameshifting event leading to the production of antizyme is stimulated by the yeast prion protein [PSI^{+}]. This prion, which corresponds to an aggregated conformation of the translational release factor eRF3, increases ribosomal pause at the stop codon of ORF1 [42]. The antiviral and antiproliferative

agent, interferon, was also demonstrated to increase antizyme production due to the interaction of interferon-induced RNase L with the release factor, eRF3 [43]. While perturbation of polyamine metabolism by interferons is compatible with their antiproliferative effect, it is not clear how such an anti-proliferative signal would enhance [PSI^+] prion "infection" and dissemination. While the above observations support the involvement of the translation termination factor in regulating programmed frameshifting, they do not necessarily support a role for the [PSI^+] prion itself in regulating frameshifting. In support of lack of such a regulatory role is the absence of the [PSI^+] prion in natural isolates of yeast [44].

Initiation of antizyme synthesis at two alternative initiation codons determines its subcellular localization

Translation of antizyme can be initiated at two in-frame initiator AUG codons, both located upstream to the site of frameshifting. Interestingly, translation initiation occurs predominantly at the second initiation codon because it is situated within a more favorable translation initiation sequence context [29, 30]. Nevertheless, the products of the two initiation events are observed both in vivo and in vitro as 24.5 and 29 kDa isoforms, respectively [29, 30, 45–47]. The segment located between the two initiation codons contains a positive amphipathic helix that is part of a mitochondrial localization signal [46, 48]. Only the less highly expressed long form is localized to mitochondria in transfected cells and imported into mitochondria in an in vitro uptake assay [48]. Nevertheless, while both forms inhibit polyamine uptake, neither affect uptake by rat liver mitochondria [25].

Interestingly, antizyme-1 also contains two independent nuclear export signals [49]. One of these signals is N-terminal, overlapping the mitochondrial localization signal, while the other resides in the central part of the protein. While the N-terminal signal is active in the context of the full-length protein, the central signal is revealed only when antizyme is N-terminally truncated, probably since it is inhibited by adjacent N-terminal sequences. In agreement with this observation, a number of studies described shuttling of antizyme between the cytoplasmic and nuclear compartments [49–51]. Based on these observations, it was suggested that antizyme is involved either in nucleocytoplasmic shuttling of ODC, and/or in the degradation of ODC in these two cellular compartments.

Multiple types of antizyme and their cellular activities

Mammalian species contain three types of antizymes, described to date [34, 38]. The prototype and the most highly investigated form is antizyme-1. Antizyme-1, which is ubiquitously expressed, was originally identified as an ODC inhibitory activity whose synthesis is stimulated by increased polyamine concentration [21, 52]. The affinity of antizyme to ODC subunits is significantly higher than the affinity ODC subunits have for each other. This, together with the weak association between the two ODC subunits resulting in their existence in equilibrium between monomers and dimers [53, 54], sets the basis for the ability of antizyme to neutralize ODC activity. Upon interaction between antizyme and a transient ODC monomeric subunit, a tight heterodimer is formed, preventing the association of ODC subunits with each other to form active ODC dimers. Although ODC inactivation is a known function of antizyme, it is actually an intermediate step in a process of targeting ODC subunits to ubiquitin-independent degradation by the 26S proteasome [24] (Fig. 2). Interaction with antizyme induces a conformational change in ODC, resulting in the exposure of a C-terminal ODC segment that serves as the proteasome recognition signal [55–58]. These antizyme-induced conformational changes enhance the interaction of ODC with the proteasome without stimulating proteasome activity. An ODC segment encompassing amino acids 117–140 is critical for binding to antizyme [59]. Trypanosoma brucei ODC, which is a stable protein, lacks the C-terminal segment [55]. Interestingly, although refractory to antizyme binding, trypanosome ODC can be converted into a rapidly degraded protein when the C-terminal segment of the mammalian enzyme is appended to its C-terminus. Although lacking the C-terminal targeting segment of the mammalian enzyme, yeast ODC is rapidly degraded in yeast cells in an antizyme-dependent manner [60, 61]. Targeting of yeast ODC to proteasomal degradation appears to be mediated by an N-terminal segment that can be replaced by the C-terminal mammalian-derived sequence [61].

Antizyme has two regions that are important for its ability to stimulate ODC degradation. The C-terminal half of the molecule is required for its interaction with ODC. However, while this interaction is sufficient to inactivate ODC, the ability to promote ODC degradation depends on the integrity of a small N-terminal segment [23, 62]. Although the specific mechanistic role of this N-terminal segment is still unknown, it was suggested that it augments the ability of the C-terminal segment of ODC to mediate proteasomal recognition [63, 64].

Antizyme acts catalytically, as following completion of its function, it is released to support another round of ODC degradation [65]; nevertheless, antizyme is a rapidly degraded protein [66, 67]. However, antizyme is not degraded together with ODC when presenting the latter to the proteasome [66]. The ODC-independent degradation of

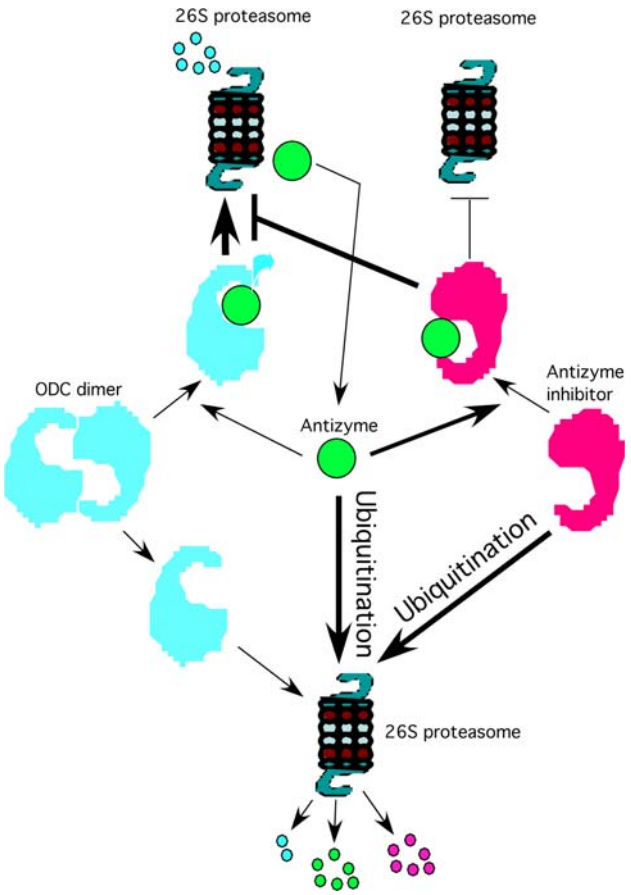

Fig. 2 Antizyme stimulates ubiquitin-independent ODC degradation, but inhibits ubiquitin-dependent antizyme inhibitor degradation. Antizyme binds both ODC and antizyme inhibitor. Binding of antizyme to a transient ODC monomer results in the exposure of the C-terminus of ODC, enabling its recognition by the 26S proteasome resulting in its ubiquitin-independent degradation. While interaction with antizyme greatly stimulates ODC degradation, ODC is also degraded in the absence of antizyme, albeit at much reduced efficiency. Antizyme, which like ODC is a rapidly degraded protein, is not degraded while presenting ODC to the proteasome, but is independently degraded in a process requiring ubiquitination. Antizyme inhibitor, which is also a short-lived protein, is targeted to degradation by ubiquitination. Its interaction with antizyme inhibits its ubiquitination and interferes with its degradation. Since the affinity of antizyme towards antizyme inhibitor is greater than its affinity for ODC, antizyme inhibitor sequesters antizyme into a stable complex, thereby reducing its ability to stimulate the targeting of ODC to degradation

antizyme is ubiquitin-dependent [66, 67]. Interestingly, the degradation of yeast antizyme that is also ubiquitin-dependent is inhibited by polyamines [67]. The antizyme recognition signals and the components of the ubiquitin system that are involved in targeting antizymes to degradation, as well as the mechanism of detachment of antizyme from ODC prior to the actual degradation are presently unknown.

In addition to regulating ODC activity and degradation, antizyme also regulates polyamine transport across the plasma membrane through an as yet undefined mechanism [26, 59, 65, 68]. In contrast to its effect in mammalian cells, the yeast antizyme has only a minor effect on polyamine uptake by yeast cells [61].

As antizyme is capable of inhibiting ODC and polyamine uptake, it is not surprising that antizyme acts as a negative regulator of cellular proliferation and of tumor development both when overexpressed in cultured cells and in transgenic mice [69–74]. In some systems, forced antizyme expression provokes apoptotic cell death [70, 71, 75, 76].

Effect of antizyme on other proteins

Although components of the polyamine metabolism seem to be the prime and natural targets of antizyme, several studies provide suggestive evidence that antizyme-1 interacts with other cellular proteins and also targets them to proteasomal degradation. Upon BMP type I receptor activation. Smad1 forms a ternary complex with antizyme-1 and the proteasome β subunit HsN3, prior to the incorporation of this subunit into the 20S proteasome. It was therefore suggested that antizyme-1, together with HsN3, may mediate targeting of Smad1 to the proteasome. BMPs further trigger the translocation of the constituents of this ternary complex into the nucleus where they mediate degradation of the CBP/p300 repressor SNIPI [77, 78]. Since there was no follow-up to these studies, the mechanism of action of antizyme-1 in this process is still unresolved. Antizyme-1 was also demonstrated to interact with and stimulate the degradation of the growth-related proteins, cyclin D1 [79], and aurora-A [80]. Since, in contrast to ODC, these two proteins are also subject to ubiquitin-dependent degradation, it is not clear whether and under what physiological conditions this antizyme-stimulated degradation operates. In addition, the relative contribution of the antizyme-mediated perturbation of cyclin D1 and aurora-A on the process of cellular proliferation remains unclear. It was also recently demonstrated that antizyme-1 and its inhibitory protein, antizyme inhibitor, localize to centrosomes, and that alteration of their relative levels affect centriole amplification, thus contributing to oncogenesis [81]. Ectopic expression of antizyme was also implicated in activation of genes involved in DNA repair resulting in increased radio-resistance of these cells [82]. However, this study did not differentiate between a direct effect of antizyme and an indirect effect through altering the intracellular polyamine pools.

Antizyme 2

The second member of the antizyme family, termed antizyme-2, has tissue distribution similar to that of antizyme-1,

but it is expressed at significantly lower levels [37]. Antizyme-2 is more conserved evolutionarily than antizyme-1 [38], suggesting it is likely to have a significant biological role. Furthermore, its minority co-existence with antizyme-1 strongly suggests that its role may be different from that of antizyme-1. While antizyme-2 inhibits ODC activity and polyamine uptake as efficiently as antizyme-1, it failed to promote ODC degradation in an in vitro reaction, but supported degradation in cells when expressed from a baculovirus-based vector [83, 84]. Since expression from baculoviral vector results in massive overexpression, it was suggested that antizyme-2 is very inefficient in supporting ODC degradation and that antizyme 2 might act as a reversible ODC storage compartment. However, while confirming the inability to support ODC degradation in vitro, a recent study demonstrated that antizyme-2 is as efficient as antizyme-1 in stimulating ODC degradation in cells [85]. In addition, this study also demonstrated that antizyme 2 is as efficient as antizyme-1 in stabilizing antizyme inhibitors.

Antizyme 3

The third member of the antizyme family, antizyme-3, is a testis-specific antizyme observed only in haploid germinal cells [86, 87]. This localization of antizyme-3 is different from that of ornithine decarboxylase mRNA, which is found mainly in the outer part of the seminiferous tubules where spermatogonia and spermatocytes are located [88]. This observation may be compatible with a recent study demonstrating that in contrast to its ability to inhibit ODC and polyamine uptake, antizyme-3 does not target ODC to degradation [85]. Consistent with this result, antizyme-3 was demonstrated to interact with the germ cell-specific protein, gametogenetin protein-1 [89]. However, the functional consequence of this interaction is not yet known.

Antizyme inhibitor regulates the regulator

As described above, antizyme is a central regulator of cellular polyamine homeostasis. Nevertheless, recent studies have demonstrated that antizyme is itself subject to regulation by an ODC-related protein termed antizyme inhibitor. Antizyme inhibitor was originally described in rat liver extract as an antizyme inhibitory activity [28]. Although it was first thought to be an ODC derivative [90, 91], cloning of the gene encoding this activity revealed that, despite its high homology to ODC, antizyme inhibitor is a distinct protein lacking ornithine decarboxylating activity [92, 93]. Antizyme inhibitor inhibits all members of the antizyme family [94].

Like ODC, antizyme inhibitor also crystallizes as a dimer [95]. However, several structural features including fewer interactions at the dimer interface, a smaller buried surface area, and lack of symmetry of the interactions between residues from the two monomers suggest that under physiological conditions antizyme inhibitor does not dimerize. Indeed, biochemical studies revealed that antizyme inhibitor remains monomeric in solution, while ODC is dimeric [95]. Since the active site of ODC is formed at the interface between the two monomers [96], the lack of physiological dimerization of antizyme inhibitor is sufficient to explain the lack of ornithine decarboxylating activity. The observation that antizyme inhibitor is unable to bind PLP provides an additional and independent explanation for the lack of enzymatic activity [95].

The existence of antizyme inhibitor as a monomer increases its availability for interaction with antizyme, partially contributing to its most important feature, namely its higher affinity for antizyme compared to that of ODC. This higher affinity provides the basis for the ability of antizyme inhibitor to rescue ODC subunits from interaction with antizyme, thus saving them from degradation. Since increased accessibility is unlikely to fully account for the higher affinity to antizyme, it must be assumed that the sequence of the relevant binding segments may also be of importance. Based on a comparison between the sequence of mouse ODC that binds antizyme and trypanosome ODC, which does not bind antizyme, the segment encompassing amino acids 117–140 was suggested as the putative antizyme-binding site of mouse ODC [97]. However, this segment probably does not constitute the entire binding site, as replacing this segment of ODC with the equivalent segment of antizyme inhibitor did not increase its affinity to antizyme (Snapir and Kahana, unpublished results). Therefore, as previously suggested, based on comparison between the structures of human and trypanosome ODC, the antizyme-binding segment may actually be larger [98].

Degradation of antizyme inhibitor

Like ODC, antizyme inhibitor is also a rapidly degraded protein. However, in contrast to ODC, it is degraded in an antizyme-independent ubiquitin-dependent manner [99]. Interaction of antizyme with ODC greatly stimulates ODC degradation. In contrast, interaction with antizyme stabilizes antizyme inhibitor by interfering with its ubiquitination [99]. It is possible that, similar to the conformational alteration that antizyme imposes on ODC resulting in exposure of the C-terminal degradation signal [57], interaction with antizyme imposes a conformational change on antizyme inhibitor, masking its, as yet unidentified, degradation signal. Since antizyme is also stabilized

when bound to antizyme inhibitor, it seems that antizyme inhibitor buffers antizyme by maintaining it in a stable complex. The behavior and destiny of this antizyme inhibitor/antizyme complex is presently unclear. It is tempting to hypothesize that increased intracellular polyamine levels may disrupt the interaction between antizyme inhibitor and antizyme, releasing antizyme to fulfill its original role.

Role of antizyme inhibitor in regulating cellular proliferation and transformation

Several lines of evidence demonstrate that antizyme inhibitor is not only capable of buffering antizyme functions but, as might be expected, it is also involved in regulating cellular growth and transformation under physiological conditions. Antizyme inhibitor mRNA is rapidly induced following growth stimulation of quiescent cells [100]. Since antizyme inhibitor mRNA is induced earlier than ODC mRNA, it was suggested that, when culture conditions improve and polyamine production is required for cell growth and proliferation, induction of antizyme inhibitor synthesis might represent a means of protecting ODC molecules from the negative effect of antizyme. DNA array analysis demonstrated increased levels of antizyme inhibitor mRNA in gastric tumors compared to adjacent healthy tissue [101]. The human antizyme inhibitor gene is located on chromosome 8q22.3, and amplification of this region is associated with several tumors [102, 103]. Ectopic overexpression of antizyme inhibitor leads to increased proliferation and to cellular transformation [104, 105]. Conversely, silencing of antizyme inhibitor using siRNA is associated with inhibition of ODC activity and with reduced cell proliferation [104–106]. More recently, it was demonstrated that antizyme inhibitor mediates the induction of ODC activity and polyamine uptake by hypoxic conditions and that this induction promotes cancer cell survival under conditions of hypoxic stress [107]. It is unclear, however, whether the growth-promoting role of antizyme inhibitor is executed only through manipulating polyamine metabolism as a recent study demonstrated that ectopic expression of wild-type antizyme inhibitor and a mutant that does not bind antizyme both stabilize cyclin D1 and promote growth [105].

Although the above studies strongly suggest that antizyme inhibitor plays an important physiological role in regulating cellular growth, more compelling evidence was recently provided demonstrating that mice harboring two disrupted alleles of antizyme inhibitor die at P0, and exhibit abnormal liver morphology that is accompanied by increased ODC degradation and perturbed biosynthesis of putrescine and spermidine [108].

As an antagonist of antizyme, antizyme inhibitor affects the cellular polyamine homeostasis both by increasing ODC activity and polyamine uptake. This is in contrast to the situation occurring in ODC overproducing cells in which polyamine uptake is actually compromised due to enhanced synthesis of antizyme. Many tumor cells display both elevated ODC activity and elevated polyamine uptake. If a direct increase in ODC activity was the sole reason for the increase in the synthesis of polyamines in these cells, polyamine uptake would be expected to be compromised. The fact that increased polyamine uptake is observed, suggests that, in these tumor cells, both antizyme inhibitor and ODC are overexpressed, or that increased antizyme inhibitor expression is the primary factor stabilizing ODC and causing increased polyamine uptake due to neutralization of antizyme. Indeed, Ras-transformed mouse fibroblasts exhibit elevated levels of ODC and antizyme inhibitor mRNAs, and elevated ODC activity and polyamine uptake [104].

Antizyme inhibitor 2

Mammalian cells contain an additional form of the antizyme inhibitor, termed ornithine decarboxylase paralogue (ODCp) or antizyme inhibitor 2, which is expressed in brain and testis [109, 110]. ODCp was demonstrated to lack ornithine decarboxylase and arginine decarboxylase activity, but is able to rescue ODC from degradation and to inhibit the antizyme-mediated inhibition of polyamine uptake; therefore, it was termed antizyme inhibitor 2 [109–113]. Like antizyme inhibitor 1, antizyme inhibitor 2 is also rapidly degraded in a ubiquitin-dependent manner [111, 113]; it stimulates ODC activity, polyamine uptake and cellular proliferation although less efficiently than antizyme inhibitor 1 [113]. Antizyme inhibitor 2 and antizyme 3 are both expressed in the haploid germinal cells, a location different from that of ODC mRNA, which localizes predominantly to the outer part of the seminiferous tubules. In addition, antizyme inhibitor 2 and antizyme 3 are expressed at minimal levels during the three first postnatal weeks, and are highly induced in the fourth week [88]. These results suggest that antizyme inhibitor 2 and antizyme 3 may have a role in spermiogenesis. However, it is presently unclear whether this role is exerted through effects on polyamine metabolism.

Concluding remarks

As demonstrated here, significant progress has been made in our understanding of the complex regulatory interactions between ODC, antizyme, and antizyme inhibitor. However,

there are still some aspects of these pathways that require additional clarification. The first relates to more detailed characterization of the physical interactions between antizyme/ODC and antizyme/antizyme inhibitor. Such analysis may reveal changes that occur in ODC and antizyme inhibitor that labilize ODC, while stabilizing antizyme inhibitor. In addition, the role of the N-terminal segment of antizyme, which does not affect binding to ODC but is required for the ability of antizyme to stimulate ODC degradation, should be further characterized [64]. Although it was demonstrated that the degradation of antizyme and antizyme inhibitor is ubiquitin-dependent [66, 99], the specific components of the ubiquitin system involved in these proteolytic processes have not yet been revealed. Upon their identification, it will be of interest to determine whether and how their activity is modulated by changes in the intracellular concentration of polyamines.

In addition to regulating intracellular polyamine homeostasis by modulating ODC stability, antizyme also regulates transfer of polyamines across the plasma membrane [26]. However, the mechanism mediating polyamine transport in eukaryotes and the way antizyme affects this process are mostly unknown and their revelation is of major interest.

Although the regulated synthesis of antizyme is tightly linked to the status of cellular polyamine levels, antizyme was also implicated in regulating the degradation of growth-regulating proteins that do not belong to the polyamine metabolic pathway [79, 80]. However, the efficiency at which antizyme targets these proteins to degradation was not compared to the efficiency with which it stimulates ODC degradation. It will also be of interest to determine whether regulation of these proteins by antizyme also mediates its cytostatic/cytotoxic effect under conditions where it does not affect polyamine metabolism.

Because of the importance of polyamines for cellular growth, polyamine metabolism has become a target for therapeutic efforts in battling cancer and other hyperproliferative diseases [114]. Inhibitors of key enzymes in the polyamine biosynthesis pathway are rather ineffective due to the ability of cells to overcome their effect by polyamine uptake. This uptake activity suggested the therapeutic use of structural analogs of polyamines that cross the plasma membrane through the polyamine uptake system. While some analogs exert their cytotoxic effect by negating the function of the physiological polyamines, others that are usually the most effective analogs also stimulate antizyme production [115]. Since the effectiveness of such toxic polyamine analogs also depends on the level of antizyme inhibitor in the treated cells, an additional challenge will be to match the optimal treatment (synthesis inhibitors vs polyamine analogs or their combination) to the composition of the molecular players in the treated cells.

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