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REVIEW ARTICLE

Antizyme inhibitor 2: molecular, cellular and physiological aspects

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Abstract Polyamines are small organic polycations essential for cell proliferation and survival. Antizymes (AZs) are small proteins regulated by polyamines that inhibit polyamine biosynthesis and uptake in mammalian cells. In addition, antizyme functions are also regulated by antizyme inhibitors, homologue proteins of ornithine decarboxylase lacking enzymatic activity. There are two antizyme inhibitors (AZIN), known as AZIN1 and AZIN2, that bind to AZs and negate their effects on polyamine metabolism. Here, we review different molecular and cellular properties of the novel AZIN2 with particular emphasis on the role that this protein may have in brain and testis physiology. Whereas AZIN1 is ubiquitously found in mammalian tissues, AZIN2 expression appears to be restricted to brain and testis. In transfected cells, AZIN2 is mainly located in the endoplasmic reticulum–Golgi intermediate compartment and in the *cis*-Golgi network. AZIN2 is a labile protein that is degraded by the proteasome by a ubiquitin-dependent mechanism. Regarding its physiological role, spatial and temporal analyses of AZIN2 expression in the mouse testis suggest that this protein may have a role in spermiogenesis.

Keywords Antizymes · Antizyme inhibitor 2 · Polyamines · Ornithine decarboxylase like · Arginine decarboxylase · Spermiogenesis

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Abbreviations

ADC	Arginine decarboxylase
AZ	Antizyme
AZBE	Antizyme binding element
AZIN	Antizyme inhibitor
ERGIC	Endoplasmic reticulum–Golgi intermediate compartment
ODC	Ornithine decarboxylase
ODCp	Ornithine decarboxylase paralogue
ORF	Open reading frame
RT-PCR	Reverse transcription polymerase chain reaction

Introduction

The polyamines spermidine and spermine and its diamine precursor putrescine are ubiquitous small basic molecules that are essential for cell proliferation, survival and apoptosis. They modulate multiple biochemical activities including nucleic acid and protein synthesis as well as cell signaling (Igarashi and Kashiwagi 2000). Cellular and tissue polyamine contents are tightly regulated by different processes that include polyamine biosynthesis, catabolism and transport (Pegg 2009). The fact that polyamine metabolism is frequently dysregulated in cancer cells has made that process an attractive target for therapeutic intervention (Casero and Marton 2007). Polyamines themselves are critical effectors in the complex network that controls polyamine pools in mammalian cells, since they increase the levels of antizymes (AZs), a family composed by three small proteins, named AZ1, AZ2 and AZ3, which inhibit both polyamine biosynthesis and uptake (Coffino 2001; Mangold 2005). Increased polyamine levels stimulate

a translational frameshifting mechanism of AZ mRNA leading to the synthesis of a functional antizyme protein. AZ1 is a negative regulator of polyamine synthesis by inhibiting ornithine decarboxylase (ODC), a key enzyme in polyamine biosynthesis, and stimulating the degradation of ODC by the 26S proteasome (Coffino 2001). AZs also inhibit polyamine uptake by affecting a not yet well characterized mammalian transport system (Mangold 2005). In addition, AZs are regulated by ODC-related proteins termed antizyme inhibitors (AZINs), which bind to the three AZs (Mangold and Leberer 2005) and inhibit their action on both ODC activity and polyamine uptake. The first antizyme inhibitor, now known as AZIN1, which was discovered in rat liver (Fujita et al. 1982), has been extensively studied (see Kahana 2009 and Mangold 2006 for recent reviews). More recently, a novel gen paralogue of ODC, termed ODCp or ODC-like, was characterized but its biochemical function was not initially established (Pitkanen et al. 2001). Later, it was demonstrated that the mouse ODCp gene encoded a protein devoid of enzymatic activity that acted as an antizyme inhibitor protein and that was consequently named antizyme inhibitor 2 (AZIN2) (Lopez-Contreras et al. 2006). In the present review, different molecular and cellular aspects of AZIN2 as well as its possible role in testis and brain, tissues where this protein is mainly expressed, will be discussed.

Molecular and biochemical aspects of AZIN2

Gene structure

The AZIN2 gene (also known as ADC, ODCp or Azi2) is located in the chromosome 1 of the human genome (ENSG00000142920) or in the chromosome 4 of the mouse genome (ENMUSG00000028789). Forty putative orthologues have already been identified in genome databases. The structure of AZIN2 gene is similar to those of AZIN1 and ODC, containing 11 exons and 10 introns of variable length, both in humans and mice. However, as in the case of AZIN1, due to the larger size of its introns the gene span of AZIN2 is considerably larger (about fivefold) than that of the ODC gene. The first two exons of AZIN2 are noncoding; the ORF starts at exon 3 and extends to exon 11, coding for a protein of about 50 kDa. The sequences of mouse and human AZIN2 proteins exhibit 86% identity and 92% similarity. The identity between human AZIN2 and ODC is 54% and the similarity 75% (48 and 69%, respectively, in mouse). The corresponding values for AZIN2 and AZIN1 are 45 and 66% in humans and 44 and 66% in mice. The coding sequences of the three paralogues present the highest divergences in the N- and C-terminal regions, in contrast to the best conservation (86% similarity) found in the putative antizyme binding element (AZBE). Eight alternative spliced forms of AZIN2 were early detected by PCR amplification of human brain and testis cDNA libraries (Pitkanen et al. 2001). However, no additional studies on the expression of human isoforms of AZIN2 or on the possible significance of these spliced forms have been reported. Conversely, the existence of alternative splicing of AZIN2 in mice could not be demonstrated by RT-PCR analysis of RNA isolated from mouse testis (Lopez-Contreras et al., unpublished results).

Interaction with antizymes: influence of ODC activity and polyamine uptake

The molecular function of AZIN2 has been proven in several studies, using different in vitro and cellular systems that demonstrated its ability to interact with any of the three antizymes, counteracting the action that these proteins exert on the inhibition of ODC activity and stability and polyamine uptake (Kanerva et al. 2008; Lopez-Contreras et al. 2006, 2008; Snapir et al. 2008). The function of mouse ODCp as an antizyme inhibitor was first demonstrated by using co-transfection experiments and co-immunoprecipitation techniques, and consequently the name of AZIN2 was suggested (Lopez-Contreras et al. 2006, 2008). These results were later confirmed for human AZIN2 using yeast-two hybrid screening and in vitro binding assays (Kanerva et al. 2008; Snapir et al. 2008). AZIN2 interacted with the three isoenzymes (Lopez-Contreras et al. 2006) as described similarly for AZIN1 (Mangold and Leberer 2005). Although experiments using transfected cells or in vitro systems revealed that AZIN2 was as effective as AZIN1 in negating the effect of antizymes (Kanerva et al. 2008; Lopez-Contreras et al. 2006; 2008), a recent study has shown that AZIN2 protects ODC from antizyme-stimulated degradation less efficiently than AZIN1 due to its lower affinity towards AZ1 (Snapir et al. 2008).

Interestingly, it has been reported that the overexpression of AZIN2 confers growth advantage to NIH 3T3 cells (Snapir et al. 2008), similar to that found for AZIN1 (Keren-Paz et al. 2006; Kim et al. 2006), probably by increasing polyamine levels. However, the finding that a mutant form of AZIN1 that lacks the putative antizyme binding (AZBE) still retained the capacity to induce cell proliferation suggested a novel function of antizyme inhibitor in cell proliferation independent of antizymes (Kim et al. 2006). Whether the influence of AZIN2 on cell growth depends on AZBE, remains to be tested.

Finally, it must be emphasized that different experiments have clearly shown that AZIN2/ODCp is devoid of

ornithine and arginine decarboxylase activity (Coleman et al. 2004; Kanerva et al. 2008; Lopez-Contreras et al. 2006; Pitkanen et al. 2001); therefore, not sustaining the previous claim that ODCp is a mammalian arginine decarboxylase (Zhu et al. 2004), as it will be discussed later on. The lack of amino acid decarboxylating activity in AZIN2 is not surprising since, despite the high homology between ODC and AZIN2, the latter protein has substantial changes in some residues that are critical for ODC activity, apart from other differences that have been pointed out by Kidron et al. (2007).

AZIN2 protein stability

AZIN2, as its homologues AZIN1 and ODC, is a labile protein that is degraded by the proteasome. However, AZIN2 appears to be less labile than AZIN1, although both proteins, unlike ODC, are degraded by a ubiquitin-dependent mechanism (Kahana 2007; Kanerva et al. 2008; Snapir et al. 2008). Moreover, the degradation of AZIN2 by proteasome is not stimulated by antizymes, unlike in the case of ODC, instead, the binding to AZ1 or AZ3 inhibited the degradation of AZIN2 (Lopez-Contreras et al. 2009a; Snapir et al. 2008). These opposite effects of antizymes in the degradation of AZIN2 and ODC appear not be related to the differences in the C-terminal segment, since an AZIN2 chimera containing the C-terminal degradation signal is still stabilized by antizymes (Snapir et al. 2008). The fact that in double transfectants of AZIN2 and AZ3 the amount of AZ3 was markedly increased with respect to the amount found in single transfectant of AZ3, strongly suggests that the interaction of AZIN2 with AZ3 may also protect AZ3 from the degradation by the proteasome (Lopez-Contreras et al. 2009a).

Expression and subcellular localization of AZIN2

In contrast to AZIN1, which is almost ubiquitously expressed in mammalian cells, AZIN2 presents a more restricted expression. The presence of mRNA for ODCp/AZIN2 in human tissues has been studied by dot-blot hybridization experiments. By this analysis, high expression of ODCp was detected in the testis and in different areas of adult brain (Pitkanen et al. 2001). The hybridization of the same membranes with an ODC-specific probe detected low signals in the brain, in comparison with other human tissues that showed high expression levels, which initially suggested that ODCp might have a role in replacing ODC in certain cells. The expression of ODCp/AZIN2 has also been studied in mice by RT-PCR using primers specific for ODCp. These studies revealed that ODCp/AZIN2 was mainly expressed in testis and brain, in contrast to ODC, AZ1 and AZ2 that were expressed in other tissues analyzed, including kidney, liver, heart, lung, ovary, placenta and adrenal glands (Lopez-Contreras et al. 2006). Then the expression pattern of ODCp appears to be conserved in human and mice. Moreover, in situ RNA hybridization experiments have shown that AZIN2 is not evenly expressed in the different cells that constitute the brain or testes. In fact, AZIN2 mRNA is mainly found in the testicular germinal haploid cells (Lopez-Contreras et al. 2009a), whereas in the murine brain ODCp/AZIN2 appears to be mainly localized in motor and sensory nucleus, hippocampus and some cerebellar areas (Ramos-Molina 2007). However, it cannot be excluded that AZIN2 could be expressed in specific cell populations in tissues other than brain and testis. It is worth mentioning that although AZIN2 has more restricted pattern of expression than AZIN1, in the tissues where it is mostly expressed, such as brain and testis, the relative ratios of expression with regard to AZIN1 were sixfold and 23-fold, respectively (Lopez-Contreras et al. 2008).

At the cellular level, AZIN2 appears to be mainly located in the particulate compartment of both HEK 293T or COS7 transfected cells (Lopez-Contreras et al. 2006) and testicular cells (Lopez-Contreras et al. 2009a). A detailed analysis using confocal microscopy revealed that AZIN2 is mainly located in the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) and in the *cis*-Golgi network (Lopez-Contreras et al. 2009b). This location contrasts with those found for their homolog proteins ODC and AZIN1, which are mainly located in the cytosol and nuclei, respectively. Studies using several constructs of AZIN2 carrying different deletions in order to identify the region of the protein responsible for its accumulation in the ERGIC compartment indicated that the N-terminal region of AZIN2 spanning about 113 residues is essential for the ERGIC-specific subcellular location of this protein (Lopez-Contreras et al. 2009b). This observation is in agreement with the fact that this region of AZIN2 has low sequence homology with the corresponding ones in ODC and AZIN1. Moreover, when in ODC its N-terminal region was substituted by the corresponding N-terminal segment of AZIN2, the chimeric protein was mainly found in the ERGIC (Lopez-Contreras et al. 2009b). The mechanism by which AZIN2 is directed to the ERGIC network is presently unknown. The facts that in silico analyses revealed the absence of the signal peptide required for the import into the endoplasmic reticulum, and predicted a cytosolic localization of AZIN2, suggest that this protein may interact with the cytosolic side of the ERGIC, either directly or through the mediation of other partners. Interestingly, the co-expression of AZIN2 with any of the three antizymes affected AZIN2 localization, shifting the protein from the perinuclear structures of the ERGIC to the cytosol

(Lopez-Contreras et al. 2009b). Moreover, for this translocation, the existence of the antizyme binding element (AZBE) in AZIN2 was necessary, since alterations of this region prevented its translocation from the ERGIC to the cytosol mediated by antizymes. It should be pointed out that although no studies on the precise subcellular localization of AZIN2 in the male haploid cells are available, the biochemical cell fractionation of testicular extracts revealed that this protein is mainly located in membranous structures that could correspond to the ERGIC network (Lopez-Contreras et al. 2009a).

The question of whether the dual subcellular localization of AZIN2 may affect the physiological function of this protein remains to be determined. In this regard, multiple localizations of antizymes and antizyme inhibitors have been described following different experimental approaches. AZ1 has been found in the cytosol and nuclei (Gritli-Linde et al. 2001) and mitochondria (Gandre et al. 2003), whereas AZ3 appears to be located in both the cytoplasm and nucleus (Zhang et al. 2005). Translocation from one to other localization may have important cellular consequences, as exemplified by the import of AZ1 into the mitochondria that is linked to the induction of apoptosis (Liu et al. 2006). On the other hand, AZIN1 and AZ1 concentrate at centrosomes where the alteration of their relative levels causes centrosomal defects, probably by affecting ubiquitin-independent proteasomal degradation (Mangold et al. 2008).

AZIN2 and testis physiology

As commented before, AZIN2 is mostly expressed in the testis. In this regard, it must be remembered that the physiological function of polyamines in the male reproductory system is poorly understood, despite the observation that crystallization of spermine phosphate in human semen was already made by Leeuwenhoek in the XVII century. Whereas in the prostate of several mammals the biosynthesis of polyamines is characteristically high, due to its secretory function, in the rodent testis polyamines and their biosynthetic enzymes are unevenly distributed in different types of testicular cells. These cells belong to two different compartments: the seminiferous tubules formed by germ cells and supporting Sertoli cells; and the interstitium, containing various types of cells, including Leydig cells that produce testosterone. Although polyamines have been implicated in the induction of cell proliferation growth and differentiation, their role in testicular physiology has remained elusive. In adult rodent testis, the levels of ODC mRNA are relatively elevated, although in some cases no correlation with ODC activity has been established (Weiner and Dias 1992). Besides, fluctuations of testicular ODC expression appear to be finely tuned during development (Alcivar et al. 1989; Kaipia et al. 1990; Shubhada et al. 1989a, b). A detailed analysis of the expression of ODC mRNA in rat and mouse seminiferous epithelia suggested that polyamines may play an important role during late meiosis and early spermiogenesis (Kaipia et al. 1990). Spermatogenesis is the process that takes place in the seminiferous tubule of the testes, in which spermatogonia, the more primitive germ cells, proliferate and are first converted into spermatocytes that enter meiosis to yield spermatids that are finally transformed in spermatozoa. This event requires a sophisticated programme of cell differentiation, further complicated by the requirements of germ cells to traverse the seminiferous epithelium, in an orderly manner, from locations adjacent to the basement membrane into the lumen of the tubules (Lie et al. 2009). The importance of polyamine metabolism in the testis was reinforced by the generation of transgenic mice overexpressing ODC in this tissue that presented reduced fertility. In fact, the high levels of ODC activity and putrescine content in the testis of the transgenic mice were associated with marked changes in testicular morphology and impaired spermatogenesis (Halmekyto et al. 1991). Further analysis on the influence of polyamine levels on DNA synthesis, morphology and the number of cells at different stages of the cycle of seminiferous epithelium of ODC transgenic mice, revealed that putrescine stimulated DNA synthesis in spermatogonia, but reduced the number of meiotic and postmeiotic cells (Hakovirta et al. 1993). Taken together, these results suggested that polyamines may have a dual stimulatory or inhibitory action during spermatogenesis.

On the other hand, studies on the expression and role of antizymes in male reproduction were almost non-existing until the discovery of antizyme 3 (AZ3), a new paralogue of mammalian antizymes, found to be expressed specifically in the testis (Ivanov et al. 2000; Tosaka et al. 2000). It was found that AZ3 transcription was restricted to testis germ cells, starting early in spermiogenesis and finishing in the late spermatid phase (Ivanov et al. 2000). Since the AZ3 wave of expression follows the wave of high ODC expression that takes place during the early phases of spermatogenesis, it was postulated that the physiological role of AZ3 was to abolish ODC activity to avoid the detrimental effects of putrescine during spermiogenesis (Coffino 2000; Ivanov et al. 2000). The identification of the expression of AZIN2 in the testis added new insights on the requirements of a more stringent control of polyamine levels in testicular cells. The expression of AZIN2 in the mouse testis presents a very well defined pattern, both at the spatial and temporal levels. In situ RNA hybridization experiments have demonstrated that AZIN2 mRNA is present in the inner part of the seminiferous tubules, where

spermatids and spermatozoa are mainly located, suggesting that the expression of AZIN2 takes place in the haploid germinal cells (Lopez-Contreras et al. 2009a). This contention was also corroborated by studying the postnatal expression of AZIN2 during the first wave of spermatogenesis. Real-time RT-PCR analysis demonstrated that AZIN2 as AZ3 is expressed at minimal levels during the first 3 weeks after birth, the postnatal period in which the mouse testis almost does not contain haploid germ cells. In addition, the expression of AZIN2 and AZ3 started in the testis during the fourth postnatal week, in correspondence with the onset of spermiogenesis. These data, associated with the fact that immunocytochemical analysis revealed that AZIN2 protein was mainly localized in the inner part of seminiferous tubules, strongly suggest that AZIN2 may have a role in spermiogenesis (Lopez-Contreras et al. 2009a). Although different studies have shown that AZIN2 is able to interact with AZ3, affecting the action of the antizyme on ODC activity and polyamine uptake (Lopez-Contreras et al. 2008, 2009a, Snapir et al. 2009), the spatial and temporal differences observed in the expression pattern between ODC and AZ3/AZIN2 in the testis, indicate that the function of the latter pair of proteins expressed in the haploid germinal cells could be related to mechanisms different to the regulation of the key enzyme in polyamine biosynthesis. One possibility is that AZIN2 may participate in the regulation of polyamine fluxes during the differentiation of the haploid cells that takes place during spermiogenesis. In turn, changes in polyamine pools have been reported during spermiogenesis (Quemener et al. 1992) concomitantly with processes of chromatin remodeling in which histones are replaced by protamines. The fact that during the process of spermatocytes production polyamines appear to be sequestered into residual bodies (Shin et al. 2007) or in the acrosome, which is a Golgi-derived secretory granule formed during spermiogenesis (Clermont et al. 1993), besides the localization of AZIN2 in membranous structures, support a possible role of AZIN2 in the redistribution of cellular polyamines into different compartments during spermiogenesis.

In addition to regulating polyamine pools by affecting ODC activity and polyamine transport, the possibility exists that AZIN2 may affect testicular physiology by interacting with other proteins not implicated in polyamine homeostasis. In this regard, there is compelling evidence that AZ3 may interact with gametogenetin protein 1, a testicular germ-cell specific protein of unknown function expressed from late pachytene spermatocytes through round spermatids (Zhang et al. 2005). Moreover, the capacity of AZIN2 to interact with the three antizymes (Lopez-Contreras et al. 2006) opens the possibility that this protein could participate in the modulation of other targets that interact with antizymes such as the protein Smad I (Gruendler et al. 2001), cyclin D1 (Newman et al. 2004) and Aurora-A (Lim and Gopalan 2007). Finally, AZIN2 could also exert some molecular functions, independently of its interactions with AZs, as reported for AZIN1 (Kim et al. 2006).

AZIN2 in brain and the arginine decarboxylase controversy

Although AZIN2 is expressed in the brain, the specific function of AZIN2 in brain physiology has not been established. As already commented, dot-blot analysis revealed the existence of high amounts of ODCp (AZIN2) mRNA in different parts of the human adult brain, including cerebral cortex, cerebral lobes, pons, cerebellum, corpus callosum, basal nuclei, hippocampus, substantia nigra, thalamus and spinal cord, whereas in fetal brain samples lower level of expression was detected (Pitkanen et al. 2001). Although ODCp (AZIN2) is also expressed in the mouse brain (Lopez-Contreras et al. 2006), in vivo experiments on the biochemistry and physiology of this protein in animal models are scarce. Taking into consideration its molecular action as an antizyme inhibitor, it is conceivable that its physiological role should be related to the regulation of brain polyamine pools, since the presence of antizymes has been detected in rodent brain (Kilpeläinen et al. 2000; Laitinen et al. 1986). In fact, putrescine, spermidine and spermine and their metabolic enzymes are present in the central nervous system where they display specific regional distribution (Bernstein and Müller 1999). Apart from their function in brain development, polyamines are known to play different actions on neural cells through their binding to several neurotransmitter receptors (Igarashi and Kashiwagi 2000) and ion channels (Johnson 1996; Weiger and Hermann 2009; Williams 1997). Alterations in the expression and activity of different polyamine metabolic enzymes, as well as variations in polyamine levels, have been described associated to different brain insults such as cerebral ischemia (Li et al. 2007), and some mental disorders (Fiori and Turecki 2008). Moreover, present evidence suggests the existence of possible relationship between AZIN2 and agmatine, a dicationic amine that in plants and microorganisms is formed by decarboxylation of arginine. After the discovery of agmatine in rat and bovine brain (Li et al. 1994), many studies on this amine have been published, showing that agmatine may exert interesting pharmacological actions probably related to its binding to different neuroactive receptors (Grillo and Colombatto 2004; Reis and Regunathan 2000). However, whether agmatine found at relative low levels in the nervous central system has a dietary origin or is derived from arginine by the action of a putative mammalian arginine

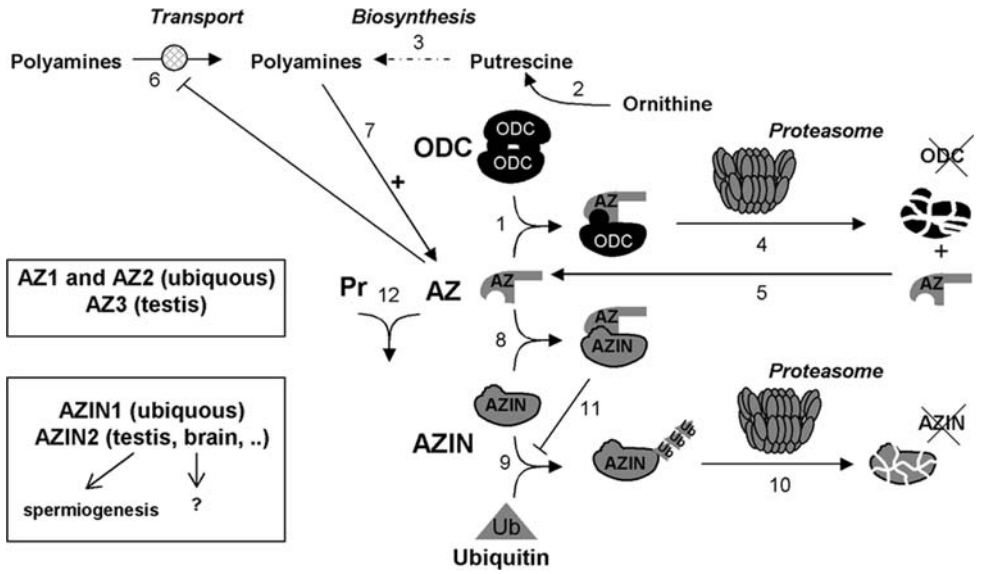

Fig. 1 Antizyme inhibitor 2 and polyamine metabolism. Antizyme (*AZ*) plays a central role in the regulation of intracellular polyamine levels. AZ binds to ornithine decarboxylase (*ODC*) (*1*), a key enzyme in polyamine biosynthesis (*2*, *3*), and promotes the degradation of ODC by the proteasome 26S by a ubiquitin independent process (*4*), in which AZ is recycled (*5*). AZ also inhibits polyamine uptake by mammalian cells (*6*), whereas increased intracellular polyamine levels stimulate AZ biosynthesis (*7*). AZ functions are regulated by antizyme inhibitor (AZIN), a protein that binds to AZ (*8*) and negates the action of this on ODC and polyamine uptake. Contrary to ODC, AZIN degradation requires ubiquitination (*9*) before interacting with the proteasome (*10*), and the binding of AZIN to AZ increases the stability of AZIN by inhibiting its ubiquitination (*11*). Moreover, AZ appears to affect different cellular processes by interacting with proteins not directly related to polyamine metabolism (*12*). In mammals there are three different antizymes (*AZ1*, *AZ2*, and *AZ3*) and two antizyme inhibitors (*AZIN1* and *AZIN2*). AZIN2 has been mainly found in brain and testis, where it appears to participate in reproductive functions

decarboxylase (ADC) is a controversial issue. Although it was suggested that ADC is present in mammalian cells (Li et al. 1994; Regunathan and Reis 2000), other studies have not supported the existence of mammalian ADC (Coleman et al. 2004; Gilad et al. 1996). More recently, a human cDNA clone that exhibited ADC activity when expressed in COS-7 cells was identified (Zhu et al. 2004), and in consequence the term ADC was included in the human data banks. Surprisingly, this sequence encoding a protein of 460 amino acids was not related to other ADC forms, but was identical to the previously cloned protein described as ODCp, lacking ornithine decarboxylase activity (Pitkanen et al. 2001). In addition, different transfection assays using either mouse or human ODCp clones clearly indicated that this protein is devoid of arginine decarboxylase activity, questioning its postulated role as mammalian ADC (Coleman et al. 2004, Kanerva et al. 2008; Lopez-Contreras et al. 2006). Furthermore, it was demonstrated that mouse ODCp really functions as an antizyme inhibitor, and it was proposed to be named as antizyme inhibitor 2 (Lopez-Contreras et al. 2006). This finding was later corroborated for the human ODCp (Kanerva et al. 2008; Snapir et al. 2008). It should also be noted that whereas ADC activity in rodent was originally located in mitochondria (Li et al. 1994; Regunathan and Reis 2000) ODCp/AZIN2 has been found in the ERGIC complex and cytosol (Lopez-Contreras et al. 2009b). Moreover, ADC activity could not be detected in the testis despite the high expression of AZIN2 (Peñafiel et al., unpublished results). Bearing in mind all these considerations, it is likely that recent experiments dealing with the expression of ADC in rat brain regions (Iyo et al. 2006; Zhu et al. 2008), using primers and antibodies targeting ODCp, what is really being studied is the distribution of AZIN2 in the brain. In fact, the regions where apparently the highest expression values of ADC were found, correlate to those described for AZIN2 (Ramos-Molina 2007). In addition, the changes observed in the levels of ADC mRNA following different treatments, including siRNA (Iyo et al. 2006), could indeed reflect variations in ODCp/AZIN2. It is also very likely that due to the stimulatory effect of AZIN2 on polyamine uptake (Lopez-Contreras et al. 2008), changes in the expression of AZIN2 may affect agmatine concentration in the brain. In summary, the role of AZIN2 in the brain could be related to the regulation of polyamine levels including agmatine in different regions, or to some other process in which antizymes could be implicated. Therefore, previous conclusions about the existence and distribution of ADC in brain should be reconsidered.

Concluding remarks and perspectives

As described here, mammalian cells possess an additional form of antizyme inhibitor named antizyme inhibitor 2 which shares some properties with antizyme inhibitor 1 and ornithine decarboxylase, but presents some specific differences. Thus, AZIN2, like AZIN1, interacts with the three antizymes, stimulating ODC activity and polyamine uptake, and therefore it may affect polyamine homeostasis and promote cell growth. AZIN2 is a short-lived protein that is degraded in a ubiquitin-dependent manner and is devoid of ornithine or arginine decarboxylase activity. It appears to be mainly located in the ERGIC compartment of cells, although its interaction with antizymes may induce the translocation to the cytosol. Unlike AZIN1, which has a ubiquitous tissular distribution, AZIN2 is expressed mostly in testis and brain (see Fig. 1). Its restricted expression in the haploid germinal cells suggests that this protein may have a role in spermiogenesis, although the mechanisms by which AZIN2 may affect the formation of spermatozoa need to be clarified. In this regard, data from a recent large-scale study in men support the view that AZIN2 may be relevant for a correct spermiogenesis, since sperm samples from teratozoospermic infertile males presented reduced expression of AZIN2 (Platts et al. 2007). On the other hand, the role of AZIN2 in the adult brain is far to be understood, although present evidence suggests that it may affect the intracellular concentration of agmatine and polyamines. Moreover, whereas in the testis AZIN2 may have the testicular-specific antizyme 3 as a partner, in brain cells this possibility is unlikely due to the lack of expression of AZ3 in neural cells.

In the future, the generation of mutant mice targeting the AZIN2 gene may provide critical information to assess the role of this protein in mouse physiology, as in the case of AZIN1 where very recent in vivo studies with mutant mice, in which the AZIN1 gene was disrupted by the gene trap technique, indicated that AZIN1 is essential for the survival of mice, exhibiting the affected mice perturbed levels of ODC, putrescine and spermidine (Tang et al. 2009). Moreover, further studies will be required to determine the role of AZIN2 in the brain and whether this protein has a role in brain development, since at least in *Xenopus laevis* this gene has been found to be expressed in a spatial and temporal manner during early embryogenesis (Cao et al. 2001). Finally, it will be of considerable interest to explore the mechanisms controlling AZIN2 expression in normal cells, as well as the possible dysregulation of AZIN2 in transformed cells, especially in those of testicular or neural origin.

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