

Review

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The role of bacterial antizyme: From an inhibitory protein to AtoC transcriptional regulator

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Abstract

This review considers the role of bacterial antizyme in the regulation of polyamine biosynthesis and gives new perspectives on the involvement of antizyme in other significant cellular mechanisms. Antizyme is a protein molecule induced by the end product of the enzymic reaction that it inhibits, in a non-competitive manner. The bacterial ornithine decarboxylase is regulated by nucleotides, phosphorylation and antizyme. The inhibition of ornithine decarboxylase by antizyme can be relieved to different degrees by DNA or by a variety of synthetic nucleic acid polymers, attributed to a specific interaction between nucleic acid and antizyme. Recently, this interplay between bacterial antizyme and nucleic acid was determined by discerning an additional function to antizyme that proved to be the *atoC* gene product, encoding the response regulator of the bacterial two-component system AtoS-AtoC. The gene located just upstream of *atoC* encodes the sensor kinase, named AtoS, that modulates AtoC activity. AtoC regulates expression of *atoDAEB* operon which is involved in short-chain fatty acid metabolism. Antizyme is thus referred to as AtoC, functioning both as a post-translational and transcriptional regulator. Also, the AtoS-AtoC signal transduction system in *E. coli* has a positive regulatory role on poly-(R)-3-hydroxybutyrate biosynthesis. The properties and gene structural similarities of antizymes from different organisms were compared. It was revealed that conserved domains are present mostly in the C-domain of all antizymes. BLAST analysis of the *E. coli* antizyme protein (AtoC) showed similarities around 69–58% among proteobacteria, α -proteobacteria, enterobacteria and the thermophilic bacterium *Thermus thermophilus*. A working hypothesis is proposed for the metabolic role of antizyme (AtoC) describing the significant biological implications of this protein molecule. Whether antizymes exist to other enzymes in different tissues, meeting the criteria discussed in the text remains to be elucidated.

Review

In 1978 Seymour Cohen, the father of the field of polyamines posed the question "what do the polyamines do?" in his excellent article [1]. Since then, thousands of papers appeared in the literature concerning the metabolic role of polyamines. We will not attempt to discuss all the recent progress in the field, which has been well

documented in book form [2-8] as well as in a number of excellent reviews [9-15]. Instead, we shall only consider the role of antizyme (Az) in the regulation of ornithine decarboxylase (L-ornithine carboxylase, EC 4.1.1.17, ODC), its involvement in the bacterial two-component signal transduction system AtoS-AtoC [16], as well as its implication in other significant cellular functions.

The role of Az in various organisms

Az was discovered by Canellakis and co-workers in rat liver and several cell lines. It is a 26.5 kDa protein, induced by polyamines, the product of ODC action. Az forms complex with ODC, inhibiting that way the enzyme activity in a stoichiometric manner, non-competitively [17,18]. The inactive ODC-Az complex can be dissociated by high salt concentrations. The induction of Az by exogenously added polyamines is inhibited by cycloheximide or puromycin, but not by actinomycin D indicating that polyamines induce antizyme by stimulating the translation of its mRNA [13]. This explains an old paradoxical finding that ODC activity was stimulated in rat liver when puromycin was administered to animals [19].

Since then, three different Azs have been identified in eukaryotic cells, possessing different roles at the cellular levels: a) Az1 inhibits ODC and then directs its degradation by the 26 S proteasome. ODC when bound to Az1 is efficiently degraded by the proteasome and Az1 is usually recycled to act again [13,20]. This type of degradation of ODC usually occurs in an ATP-dependent, but ubiquitin-independent manner. Polyamines can trigger a +1 translational frameshift on Az mRNA, allowing the complete Az1 protein to be expressed [13,21]. ODC can be released from Az1 by another protein called "anti-antizyme", which liberates ODC in the presence of growth stimuli having higher affinity for Az1 than for ODC [22]. b) Az2 shares similar properties with Az1, including the regulatory frameshifting. It does not stimulate, however, degradation of ODC under certain conditions, but alters polyamine homeostasis, by down-regulating polyamine uptake independently of the effects on ODC, thus playing a negative role in the regulation of polyamine transport [23,24]. c) Az3 is expressed to a limited extent in testis germ cells, at a particular stage of spermatogenesis [25,26]. The pattern of Az3 expression suggests that it acts by sharply limiting polyamine accumulation in cells that have finished DNA synthesis and meiotic reduction and are about to be remodeled into mature spermatozoa [25].

Searches on genomic databases have revealed that the Azs comprise a widespread family of conserved homologues [27]. In humans, five non-allelic Az homologues have been detected. Two copies are presented in zebra fish *Danio rerio*. Az has also been cloned from fruit flies (*Drosophila melanogaster* and *Drosophila virillis*) and is found in a number of invertebrate species (*Bombyx mori*, *Caenorhabditis elegans*, *Onchocerca volvulus*, *Haemonchus contortus* and *Pristionchus pacificus*). Moreover, Az activity has been detected in a wide variety of organisms, from plants to eubacteria [8,9,14,15,23-32].

The question is why a cell or an organism needs more than one type of Az with distinct functions? Their distinct

functions may be due to their special time of expression, compartmentalization or different place and way of degradation of ODC.

Regulation of polyamine biosynthesis in bacteria

Regulation of ODC allosterically by nucleotides

Two ODCs, the biosynthetic and the biodegradative have been characterized in *E. coli* [33,34]. The biodegradative ODC is induced by low pH and by the presence of ornithine in the growth medium. The activity of the biosynthetic ODC is modulated by a number of positive and negative effectors [11]. The positive effectors include nucleotides, GTP being the more effective [34], while ppGpp acts as a negative effector [35]. The requirement for GTP in protein synthesis and the accumulation of ppGpp during starvation of *E. coli* that are under stringent control, suggest that changes in polyamine pools may be responsible for the stringent effect in *E. coli* [35]. The accumulation of ppGpp leads to the cessation of stable RNA synthesis and appears to have a bearing on the fidelity of protein synthesis [36]. Treating recombinant ODC with calf intestine alkaline phosphatase leads to inactivation of ODC that can be allosterically reversed only by guanosyl or uridyl phosphate analogues at a concentration of 10^{-4} or 10^{-3} M [37]. Nucleotides are effective in activating ODC, in the order (G,U)TP>(G,U)DP>(G,U)MP. [8-³H]GTP binds specifically to ODC since cold GTP but not ATP can dissociate the radioactive analogue. High concentration of GTP can dissociate the ODC-Az complex and either reactivate or liberate ODC [37].

Regulation of ODC by phosphorylation

Biosynthetic ODC of *E. coli* can be phosphorylated both *in vivo* as well as *in vitro* [37]. An ODC-phosphorylating kinase was purified from middle-log growing *E. coli* K-12 strain MG1655, containing plasmid pODC [38]. The kinase requires 10 mM Ca⁺⁺ for optimal activity.

In vivo phosphorylation of ODC

E. coli K-12 strain MG 1655+pODC, was labeled *in vivo* with [³²P] orthophosphate, ODC was immunoprecipitated by ODC antibody and proteins were then analyzed by SDS-PAGE followed by autoradiography. In the autoradiogram one radioactive band appeared at 82 kDa, corresponding to the M_r of the subunit of the biosynthetic ODC [37].

In vitro phosphorylation of ODC

The *in vitro* phosphorylation of ODC depends on the state of phosphorylation of the ODC molecule [37]. Therefore, the difficulties of the negative data on the ODC phosphorylation in different bacterial systems can be explained by the above observation. A partially purified kinase from *E. coli* was capable to phosphorylate the dephosphorylated preparation of ODC (ODCb). This was the first indication

that a homologous kinase can *in vitro* phosphorylate ODC in bacteria.

Regulation of ODC by Az

Two macromolecular effectors of ODC were identified in the ODC-*E. coli* mutant, MA255 [28]. One was an inhibitor of ODC, with characteristics similar to the Az of eukaryotic cells. It was a noncompetitive inhibitor of ODC; its complex with ODC could be dissociated with salt to yield active ODC and active ODC inhibitor. This *E. coli* inhibitor also inhibited ODC activity of eukaryotic cells. The other effector was a thermostable, nondialyzable molecule that activated the ODC of *E. coli* 6- to 7-fold. Similar macromolecular inhibitors and activators have been identified in the parent ODC⁺ strain, MA197. Those results suggested that ODC activity might be controlled post-translationally by positive and negative macromolecular effectors whose intracellular levels also fluctuate in response to the extracellular putrescine, the end product of the ODC reaction [28].

The mode of Az induction

The induction of macromolecular inhibitors of ODC by putrescine, spermidine, and spermine, as well as by other diamines, has been now amply verified by a number of laboratories, which has been reviewed elsewhere [9,15]. Initial attempts to demonstrate the existence of Az, by mixing extracts, each of which had ODC activity failed [39]. The reasons of failure which have been presented in detail [40], rest upon the fact that the bound inhibitor cannot be assayed by activity measurements. Only excess or free unbound inhibitor can be assayed by such mixing experiments; therefore, in order to assay for free Az, the test sample should have no measurable ODC activity.

Following the published methodology from Canellakis' laboratory for the induction of Az [9,17,18,28], extensive purifications of rat liver and of *E. coli* Az have been accomplished [9]. Increasing concentrations of diamino-octane progressively inhibit ODC activity and eventually produce free assayable ODC antizyme. Since diamino-octane is an analogue of spermidine-compare $\text{NH}_2(\text{CH}_2)_3\text{NH}(\text{CH}_2)_4\text{NH}_2$ and $\text{NH}_2(\text{CH}_2)_8\text{NH}_2$ - a possible explanation is that the very low concentrations of diamino-octane compete for certain spermidine sites and release the inhibition exerted by spermidine; the higher diamino-octane concentrations then inhibit ODC activity through the production of Az, as do all diamines and polyamines that have been tested [9,40].

The evidence in favor of Az participating, at least in part, in the regulation of ODC is as follows:

a) Free Az can be induced by 10^{-7} – 10^{-6} M putrescine in cells that have been depleted of ODC activity. Originally,

it was necessary to use higher concentrations of putrescine in order to elicit free Az in cells that either contained ODC activity or were being induced concurrently for ODC activity. Higher concentrations of the inducer were required, because large amounts of Az were necessary to neutralize the existing intracellular ODC activity [40].

b) Az is a normal component of the nuclei and sub cellular particles of uninduced rat liver cells [41,42].

c) Putrescine also induces an Az in *E. coli*, which cross-reacts with rat liver ODC and functions like the eukaryotic Az1 [28,29].

Overproduction of Az by *E. coli* transformed with a plasmid carrying the Az gene is inhibitory to ODC and ADC (arginine decarboxylase). Indeed the ODC of an inactivated ODC-Az complex can be reactivated by the addition of ADC; the converse also occurs [43]. This technique enables the demonstration of much inactive ODC and ADC in *E. coli* extracts.

Reversal of Az inhibition by nucleic acids

Huang *et al.* [44] found that the inhibition of *E. coli* ODC by Az from the same source is relieved to different degrees by DNA and by a variety of synthetic nucleic acid polymers, including ribo- and deoxyribo-nucleotide polymers. Preferences for certain nucleic acid sequences were observed. The inhibition of rat liver ODC by the *E. coli* Az was also relieved by nucleic acids.

The ability of *E. coli* genomic DNA or other nucleic acids to relieve the inhibition of ODC by the acidic Az [28] was originally attributed to possible binding of Az to certain sequences of DNA [45]. For this reason the relief of the Az inhibition of ODC activity was used as an assay of the relative effectiveness of various nucleic acid sequences. Furthermore, the relative effectiveness of single-stranded deoxynucleotide and ribonucleotide homopolymers in relieving the inhibition of *E. coli* ODC by Az was compared. Since both the acidic Az and the nucleic acids are negatively charged at the assay pH, these interactions could not be ascribed to charge effects alone. Moreover, the nucleic acids were not equivalent in their abilities to relieve the inhibition of ODC by antizyme. The differences in the degree of relief of inhibition exerted by various segments of *E. coli* DNA, suggested that there was a specificity of interaction between nucleic acids and Az. The main property that differentiates Az from many of the double or single-stranded DNA or RNA binding proteins is that Az also binds and inhibits ODC and subsequently, upon its binding to DNA, this inhibition of ODC is reversed.

Recently, the interplay between the bacterial Az (AtoC) and nucleic acids has been determined by attributing an additional physiological function to Az, that of the response regulator of the bacterial two-component signal transduction system AtoS-AtoC regulating expression of *atoDAEB* operon encoded enzymes [16,46-53].

The two-component regulatory system AtoS-AtoC

The *E. coli* Az gene has been identified and found to share significant homology with bacterial transcriptional activators of the two-component regulatory system family [16]. These systems consist of a "sensor" kinase and a response regulator, which often is a transcriptional factor [54-56]. By sensing an appropriate signal, the sensor kinase autophosphorylates on a histidine residue. Following physical contact of the phosphorylated sensor kinase with the response regulator, the phosphoryl group is transferred, usually to an aspartate residue of the response regulator, which leads to its activation.

Recently, it has been reported that Az is the *atoC* gene product [47,48,53] possessing a second function as the regulator of the expression of genes encoding enzymes involved in short chain fatty acid metabolism (*atoDAEB* operon) [49-51]. Today, the protein is referred to as AtoC, functioning both as a transcriptional and post-translational regulator. The gene located just upstream of *atoC* encodes the sensor kinase that modulates AtoC activity [16] and has since been named *atoS*, to reflect the role of its product, AtoS, as a sensor kinase [48,52,53]. Recently, direct biochemical data have been obtained proving that the gene products of *atoS* and *atoC* constitute a two-component signal transduction system involved in the regulation of the *atoDAEB* operon [46]. Moreover, the DNA binding sequences of AtoC on *atoDAEB* operon promoter have been determined [46,57]. Thus, the novel physiological function that has been ascribed to bacterial Az (AtoC) as a transcriptional regulator is in accordance with Az's DNA specific binding on the *E. coli* genome and can clearly explain the fore mentioned data describing the relief of ODC inhibition upon Az binding to DNA.

The role of the AtoS-AtoC signal transduction system in *E. coli* on the positive regulation of the levels of poly-(R)-3-hydroxybutyrate (PHB) biosynthesis has been recently identified [58]. Increased amounts of PHB are synthesized in *E. coli* upon exposure of the cells to acetoacetate, the inducer of the AtoS-AtoC two-component signal transduction system, when both components of the system are overproduced (our unpublished data).

A recent transcriptome analysis of the *E. coli* two-component systems [59] revealed possible roles, positive and/or negative, of the AtoS-AtoC system in a number of processes including flagellar synthesis and chemotactic behav-

our of *E. coli*. The same study also highlights the interplay between two-component systems, as mutations in the *ompR-envZ* system were also found to affect the expression of *atoC*. Moreover, a phenotype microarray analysis of *E. coli* mutants with deletions of all two-component systems showed that *atoSC* mutants presented susceptibility to specific osmolarity conditions, some membrane agents, aminoglycoside and a respiration inhibitor indicating other putative roles for the two-component system under study [60].

Gene structural analysis of antizymes

Eukaryotic Az genes require ribosomal frame shifting for their expression. Twelve nucleotides around the frame shift site are identical between *S. pombe* and the mammalian counterparts. The core element for this frame shifting is likely to have been present in the last common ancestor of yeast, nematodes and mammals [24], but not in *E. coli* [61]. The existence of homology between the mammalian antizymes, Az-1, -2 and -3, and the bacterial antizyme AtoC was more thoroughly investigated. It was revealed that conserved domains are present at the C-terminal parts of all antizymes (data obtained and will appear shortly) as it has been similarly reported for the *S. pombe* counterpart [62]. This region is responsible for binding and inactivating ODC, while it cannot accelerate its degradation by the 26S proteasome [13,20]. AtoC was found to belong to the NtrC-NifA family of sigma⁵⁴-RNA polymerase transcriptional activators [52], while it presents 40% identities and 58% positives with the *E. coli* NtrC transcriptional regulator [63-67]. The *E. coli* AtoC protein which has been biochemically characterized [46] was found to have homologues in the proteobacteria, g-proteobacteria and enterobacteria as indicated by BLAST analysis [68-88], while an AtoC homologue was also present in *T. thermophilus*, whose complete genome analysis was recently published [69] [Table 1]. The latter is in agreement with previous biochemical data reporting the presence of antizyme activity in *T. thermophilus* [31]. BLAST analysis also indicated that AtoC structurally comprises three conserved domains, i.e. the N-terminal receiver domain containing the phosphorylation site, the central sigma⁵⁴ interaction domain and the C-terminal DNA-binding domain.

A working hypothesis for the metabolic role of Az (AtoC)

A working hypothesis for the bacterial Az (AtoC) role and the cascade of reactions that are triggered by its activation/induction is shown in Fig. 1. Acetoacetate or its metabolic counterpart acetoacetyl-CoA, is the signal upon which the kinase AtoS is autophosphorylated [49-52,46]. Subsequently, the phosphoryl group is transferred by protein-protein interaction to the response regulator AtoC. AtoC is thus activated and able to bind to the *atoDAEB* operon promoter at two "dyad symmetry" sequences [46], as it

Table 1: Best hits of BLAST analysis of the *E. coli* K-12 *atoC* gene against the genomes of all microorganisms

Locus or Accession No	Genome annotation	Microorganism [Taxa]	Hits ^a	Id./Sim. ^b (%/%)	Ref.
Q06065 ATOC ECOLI	Acetoacetate metabolism regulatory protein <i>atoC</i> (Ornithine/arginine decarboxylase inhibitor) (Ornithine decarboxylase antizyme)	<i>Escherichia coli</i> K12 [enterobacteria]	2	95/95	16, 47, 48, 70
NP 754649.I	Acetoacetate metabolism Regulatory protein <i>atoC</i>	<i>Escherichia coli</i> CFT073 [enterobacteria]	3	95/95	71
ZP 00015409.I	COG2204: Response regulator containing CheY-like receiver, AAA-type ATPase and DNA-binding domains	<i>Rhodospirillum rubrum</i> [alpha-proteobacteria]	1	52/69	72
NP 953090.I	Sigma-54 dependent DNA binding response regulator	<i>Geobacter sulfurreducens</i> PCA [d-proteobacteria]	14	45/61	73
NP 972572.I	sigma-54 dependent transcriptional regulator/response regulator	<i>Treponema denticola</i> ATCC 35405 [spirochetes]	1	44/64	74
ZP 00079669.I	COG2204: Response regulator containing CheY-like receiver, AAA-type ATPase and DNA-binding domains	<i>Geobacter metallireducens</i> [d-proteobacteria]	13	42/63	72
YP 012430.I	sigma-54 dependent transcriptional regulator/response regulator	<i>Desulfovibrio vulgaris</i> subsp. <i>vulgaris</i> str. <i>Hildenborough</i> [d-proteobacteria]	5	43/61	75
NP 218960.I	response regulatory protein (<i>atoC</i>)	<i>Treponema pallidum</i> subsp. <i>pallidum</i> str. <i>Nichols</i> [spirochetes]	1	43/63	76
AAQ63912.I	mutant NtrC-like activator	<i>Myxococcus xanthus</i> [d-proteobacteria]	8	44/62	77
YP 008363.I	probable two-component response regulator	<i>Parachlamydia</i> sp. UWE25 [chlamydias]	1	44/62	78
ZP 00128642.I	COG2204: Response regulator containing CheY-like receiver, AAA-type ATPase and DNA-binding domains	<i>Desulfovibrio desulfuricans</i> G20 [d-proteobacteria]	4	42/61	72
NP 968400.I	regulator protein <i>piIR</i>	<i>Bdellovibrio bacteriovorus</i> HD100 [d-proteobacteria]	3	40/63	79
AAL27375.I	HydG	<i>Yersinia pestis</i> [enterobacteria]	1	43/62	80
NP 709798.I	response regulator of hydrogenase 3 activity (sensor HydH)	<i>Shigella flexneri</i> 2a str. 301 [enterobacteria]	2	44/61	81
NP 992358.I	Response regulator containing CheY-like receiver, AAA-type ATPase and DNA-binding domains	<i>Yersinia pestis</i> biovar <i>Medievalis</i> str. 91001 [enterobacteria]	1	43/63	82
NP 290636.I	response regulator of hydrogenase 3 activity (sensor HydH)	<i>Escherichia coli</i> O157:H7 EDL933 [enterobacteria]	2	44/60	83
NP 243814.I	two-component response regulator in acetoacetate metabolism	<i>Bacillus halodurans</i> C-125 [eubacteria]	1	41/62	84
Q9APD9 ZRAR KLEOX	Transcriptional regulatory protein <i>zraR</i>	<i>Klebsiella oxytoca</i> [enterobacteria]	1	43/61	85
NP 312817.I	GlnG; response regulator for <i>gln</i>	<i>Escherichia coli</i> O157:H7 [enterobacteria]	1	40/58	86
P25852 ZRAR SALT	Transcriptional regulatory protein <i>zraR</i>	<i>Salmonella typhimurium</i> LT2 [enterobacteria]	2	43/60	87
NP 458044.I	Two-component system, response regulator	<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhi</i> str. CT18 [enterobacteria]	2	40/58	88
YP 005107.I*	two-component response regulator	<i>Thermus thermophilus</i> HB27 [eubacteria]	22	33/58	69

^aHits: Number of homologues found in each microorganism (only the one with the highest homology is presented). ^bId./Sim.: Identities/Similarities.

* The *E. coli* K-12 *atoC* gene was aligned against the *T. thermophilus* genome independently since its homology was not detected among the first 100 best BLAST hits (threshold 0.005).

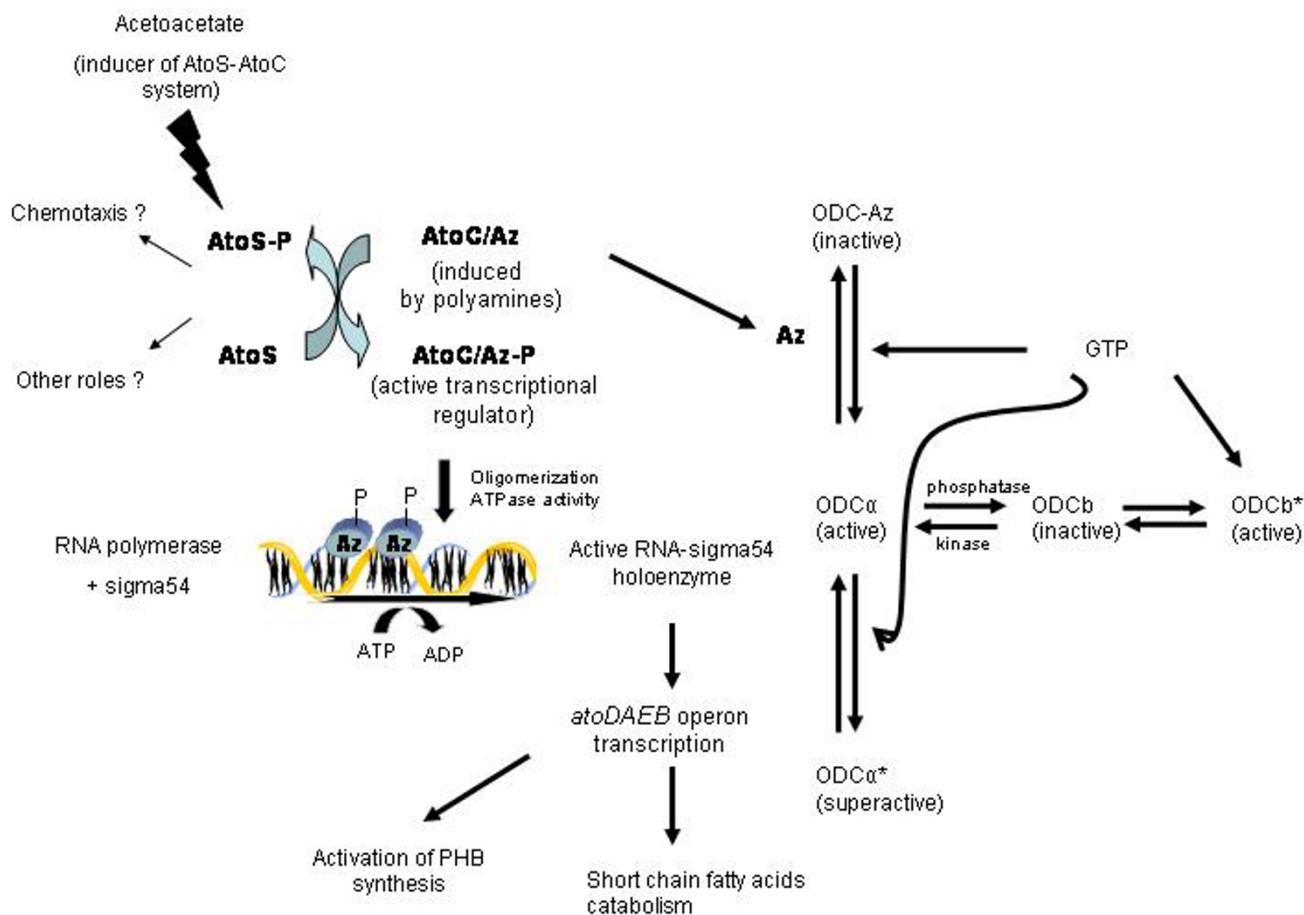


Figure 1
The involvement of antizyme (AtoC) in various regulatory mechanisms in *E. coli*.

has been reported for the NtrC response regulator [63-67]]. Thereafter, AtoC is probably oligomerized, a process that triggers its ATPase activity (our unpublished data). Upon ATP hydrolysis, the "closed" form of the RNA-sigma54 holoenzyme is transformed into an "open" form [67]. The activated open form is able to induce *atoDAEB* operon transcription which leads to short chain fatty acid catabolism and activation of the PHB biosynthetic pathway, via the AtoDA molecule (acetoacetyl-CoA transferase) [58, our unpublished data]. A functional AtoS-AtoC system has also been reported to affect *E. coli* chemotactic behavior [59], while some other putative roles are still under investigation [60]. AtoC whose production is stimulated by polyamines in *E. coli* as well, plays a central role in ODC regulation by binding to ODC and inhibiting it non-competitively [28]. We still don't know whether

the ODC inhibiting activity of AtoC/Az is activated upon AtoC/Az phosphorylation and thus affected by the two-component system under study. Moreover, native ODC can be reversibly inactivated by a dephosphorylation-phosphorylation reaction. Therefore, phosphorylation of the bacterial ODC is one of the possible regulating mechanisms for this enzyme, as was proved for the mammalian and protozoan ODC [15,37]. Finally, nucleotides can convert allosterically the inactive, phosphorylated ODCb to an active form of ODCb, suggesting that nucleotides may be physiological regulators of ODC in *E. coli*. Active ODC can be recovered from the ODC-Az complex by high concentrations of salts, DNA or GTP [28,37,44].

Do antizymes exist for other enzymes?

The antizyme may constitute part of a normal control mechanism that defines the levels of ODC activity. However, it is a noncompetitive protein inhibitor and cannot be detected in free form when there is measurable ODC activity. Consequently, to prove the participation of the Az in the normal control of ODC, methodology has been developed permitting the isolation of the ODC-Az complex, separation of the complex into the component parts and assaying the respective activities [9,28,29].

We do know that the cell will produce free Az in the presence of high diamine or polyamine concentrations. Under these conditions, the tissue culture cells are in a relatively "unhappy" state; in the rat, the amount of putrescine required to elicit high levels of Az makes the animal extremely sick. Consequently, in the search for Azs to other enzymes, we believe the following criteria should be kept in mind:

1. The product should be added in high enough concentrations so that no enzyme activity can be detected.
2. The inhibition should be maintained over relatively long periods of time to maximize the amount of Az.
3. Inhibition, by the protein which will be induced by the end product of the enzymic reaction must be of a non-competitive manner.

We believe that under these extreme conditions the cell is responding to the excess product by what may be its last available defense mechanism producing additional non-competitive inhibitory proteins to neutralize the enzyme in order to lower the level of the product. So far, not any antizyme to other enzyme has appeared in the literature. The above question needs to be addressed at the biochemical as well as genetic level.

Conclusions and future directions

This review has evaluated some of the information available for the ornithine decarboxylase antizyme and has attempted to summarize the regulatory mechanisms in which it may be involved. Our understanding of the functions of Az derives from different biological sources. In eukaryotes three antizymes have been detected: i) Az1 functions as an inhibitory protein that targets its enzyme for degradation as well as a negative regulator of polyamine transport, ii) Az2 possesses very similar properties to Az1, except that it does not stimulate degradation of ODC and iii) Az3 is expressed only during spermatogenesis. The bacterial Az (AtoC) functions as an inhibitory protein to ODC, as well as transcriptional regulator for the two component AtoS-AtoC system. This two component system regulates the expression of *atoDAEB*

operon encoded enzymes, participates in the mechanism of chemotaxis and regulates the biosynthesis of polyhydroxybutyrate, while some other possible roles remain to be elucidated. Today, under the term antizyme one can find 22 entries in the SwissProt database and 21 in the TrEMBL database, respectively. We have proposed possible functions of this unique protein, but a lot of work must still be done, in order to understand its pleiotropic role from mammals to archaea and eubacteria. For example, we still don't know if Az has a function as a transcriptional regulator in higher organisms and whether its nuclear localization can be ascribed to such an activity. Whether phosphorylation of Az plays a role in ODC regulation is also under investigation. Also, a possible role of polyamines or other catabolic products in activating the two-component system AtoS-AtoC, which will bring into interplay two apparently unrelated biological pathways, i.e short chain fatty acid catabolism and polyamine biosynthesis, remains to be elucidated. Uncertainties within some areas concerning the actual role of Az must be further clarified before it can be considered worthwhile to extend the scope of research in this particular area.

Abbreviations

Az, antizyme; ODC, ornithine decarboxylase; ADC, arginine decarboxylase

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