Polyamines, regulators of cellular proliferation are subjected to strict regulation at several control levels

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The polyamines, Putrescine, Spermidine, and Spermine are ubiquitous organic polycation that play important role in regulating fundamental cellular proceses especially the process of cell proliferation. Although, their exact molecular function have not yet been identified, various studies have suggested their involvement in a number of cellular processes such as DNA replication, transcription and translation. It is likely that that the polyamines function through special cationic nature exhibiting flexible charge distribution (Figure 1).

Fig. 1 *Structure of the three major cellular polyamines*

The crucial role polyamines play in regulating cellular functions is reflected by the existence of regulatory mechanisms that keep their intracellular concentration within a narrow optimal level. Some of these mechanism that are unique to the polyamine biosynthesis pathway

The biosynthesis of the polyamines is initiated by ornithine decarboxylase that decarboxylates (ODC) ornithine to form putrescine. Putrescine is then converted to spermidine and spermine by the action of spermidine and spermine synthase utilizing an aminopropyl group generated by S-adenosylmethionine decarboxylase.

ODC is a highly regulated enzyme that is characterized by an extremely short intracellular half-life. The half-life of ODC is among the shortest known for mammalian enzymes. Rapid degradation is a central element in regulating cellular ODC levels. ODC is unique in being degraded without ubiquitination. Its degradation is ATP dependent, occurs in fraction-II of reticulocyte lysate that lacks ubiquitin and in cells harboring a thermosensitive ubiquitin-activating enzyme, E1, even when E1 is inactivated. Although there are several proteins that are proclaimed to be degraded in a ubiquitin-independent manner, ODC is unique since its degradation is always ubiquitin-independent and since an alternative mechanism for mediating proteasomal recognition was provided. In the case of ODC the requirement for ubiquitination is replaced by interaction with a polyamine induced protein termed antizyme (Az). Az has higher affinity to ODC compared to the affinity ODC monomer display to each other. ODC subunits that are in a constant state of association/dissociation can be therefore trapped by Az and saved from destruction. Az stimulates ODC degradation by enhancing the interaction of ODC with the 26S proteasome rather than stimulating the degradation rate. This may be an outcome of a conformational change resulting in the exposure of its C-terminal degradation signal that is recognized by the proteasome. As in mammalian cells, also in yeast cells the degradation of yeast ODC is ubiquitin independent and Az dependent.

Two segments of ODC are involved in regulating its degradation. The first is a C-terminal segment encompassing the last 37 amino acids that seems to serve as the proteasomal recognition signal. The second, encompassing amino acids 117-140 mediates the interaction with Az. Deletion of each of these segments significantly stabilize ODC. Interestingly, Trypanosoma brucei ODC that does not bind Az and lacks the C-terminal segment is a stable protein both in trypanosome cells and when expressed in mammalian cells. A chimerical trypanosome ODC containing the C-terminus of mouse ODC is rapidly degraded in mammalian cells. Like trypanosome ODC also the

Fig. 2 *Schematic presentaion of the regulatory players and their role in regulating ODC.*

yeast enzyme lacks the C-terminal destabilizing segment found in the mammalian enzyme. However, while being stable when expressed in mammalian cells, yeast ODC is rapidly degraded in yeast cells. An N-terminal segment that is unique to yeast ODC seems to mediate its degradation in yeast cells as its deletion stabilizes the enzyme. Our recent studies have demonstrated that yeast ODC is not degraded in mammalian cells because it is not recognized by the mammalian proteasome and that in yeast cells the most N-terminal segment serves as the proteasome recognition signal.

Az is a central regulator of the polyamine biosynthesis pathway that is conserved from the budding yeast saccharomyces cerevisiae to man (Figure 2).

Az, which was originally described as an ODC inhibitory activity that is stimulated by polyamines, is a protein that binds to ODC monomers preventing their re-association and targeting them to ubiquitin-independent proteasomal degradation. Az is a central component of an autoregulatory circuit that regulates cellular polyamine levels. Az synthesis is regulated by a polyamine stimulated ribosomal frameshifting. Az is encoded by two different open reading frames (ORFs). Translation initiated at one of two possible initiation codons is terminated shortly thereafter at an in-frame termination codon. The synthesis of a complete Az protein requires that the scanning ribosomes will be subverted to the +1 reading frame. This shift in the translation-reading frame is stimulated by polyamines and serves as a cellular polyamine sensing mechanism. Two segments of Az fulfill two different roles in mediating ODC degradation. The first is a C-terminal segment encompassing amino acids 104-183 that mediates the interaction with ODC resulting in its inactivation. The second, a more N-terminal segment containing amino acids 70-103 is required for targeting ODC to proteasomal degradation.

While inhibition of ODC by Az is stoichiometric, Az stimulated ODC degradation appears to be catalytic, in an *in vitro* degradation assay. Az remains stable while stimulating ODC degradation supporting the notion that Az is recycled to support additional cycles of ODC degradation. Nevertheless, in cells Az is a rapidly degraded protein. Whereas interaction with Az accelerates ODC degradation, this interaction does not affect the degradation rate of Az, suggesting that Az is not degraded together with ODC when presenting it to the proteasome. Az degradation was inhibited in cells harboring a thermosensitive allele of the ubiquitin-activating enzyme E1 when the cells were grown at the non-permissive temperature. It was therefore concluded that Az is degraded in a ubiquitin-dependent manner. Using mutant cell lines dependence on ubiquitination was also demonstrated for the degradation of yeast Az in yeast cells. Interestingly, this degradation of yeast Az is inhibited by polyamines.

Fig. 3 *In its active form ODC is a homodimer. Az that has higher affinity to ODC subunits traps transient ODC monomers and targets them to proteasomal degradation. AzI can rescue ODC from the degradation destiny by sequestering Az.*

Cells contain yet another protein termed antizyme inhibitor (AzI) demonstrated to regulate cellular polyamines and as a consequence cellular growth (Figure 3).

Like Az, AzI was also described as an inhibitory activity capable of inhibiting Az activity. AzI is highly homologous to ODC but it has no ornithine decarboxylating activity. In a recent study it was demonstrated that in contrast to ODC AzI is a monomer, thus providing the basis for the lack of activity. The affinity of AzI for Az is greater than that of ODC partially as a result of its monomeric nature. It can therefore sequester Az and save ODC from degradation. Indeed, forced AzI expression was demonstrated to provide cells with growth advantage via neutralization of Az functions, although Az independent mechanism was also suggested. Of the two segments that regulate ODC degradation, one, the Az binding segment of AzI functions even better than that of ODC. In contrast, the C-terminal segment of AzI differs from that of ODC. Despite this difference AzI is a rapidly degraded protein. Furthermore, an AzI mutant lacking the C-terminal fragment is still rapidly degraded, demonstrating that unlike ODC, AzI does not posses a C-terminal destabilizing signal that is recognized by the proteasome. Although AzI binds efficiently to Az, this interaction is not required for AzI degradation. Furthermore, interaction with Az actually stabilized ODC. This stabilization in complex with Az is a key element in defining AzI as an inhibitor of Az. Based on stabilization of AzI due to lesions in the ubiquitin system and on direct ubiquitination assays it was concluded that AzI is degraded in an ubiquitin-dependent manner. Furthermore, it was demonstrated that interaction with Az stabilized AzI by interfering with its ubiquitination. Interestingly, Az also stabilized an AzI/ODC chimera in which the C-terminal segment of AzI was replaced with that of ODC. The inability of the C-terminal segment of ODC to confer ubiquitin independent/Az dependent degradation on AzI is interesting in light of the high affinity of AzI to Az and of previous observations demonstrating that the C-terminal 37 amino acids of mammalian ODC act as an autonomous degradation signal that confers rapid degradation when appended to stable proteins.

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Acknowledgements

Studies in the Kahana lab are supported by grants from the Israel Academy of Sciences and Humanities.

INTERNAL support

The M.D. Moross Institute for Cancer Research, and the Leo and Julia Forchheimer Center for Molecular Genetics at the Weizmann Institute.