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Regulation of intracellular polyamines, polycations that are essential for cellular viability and proliferation

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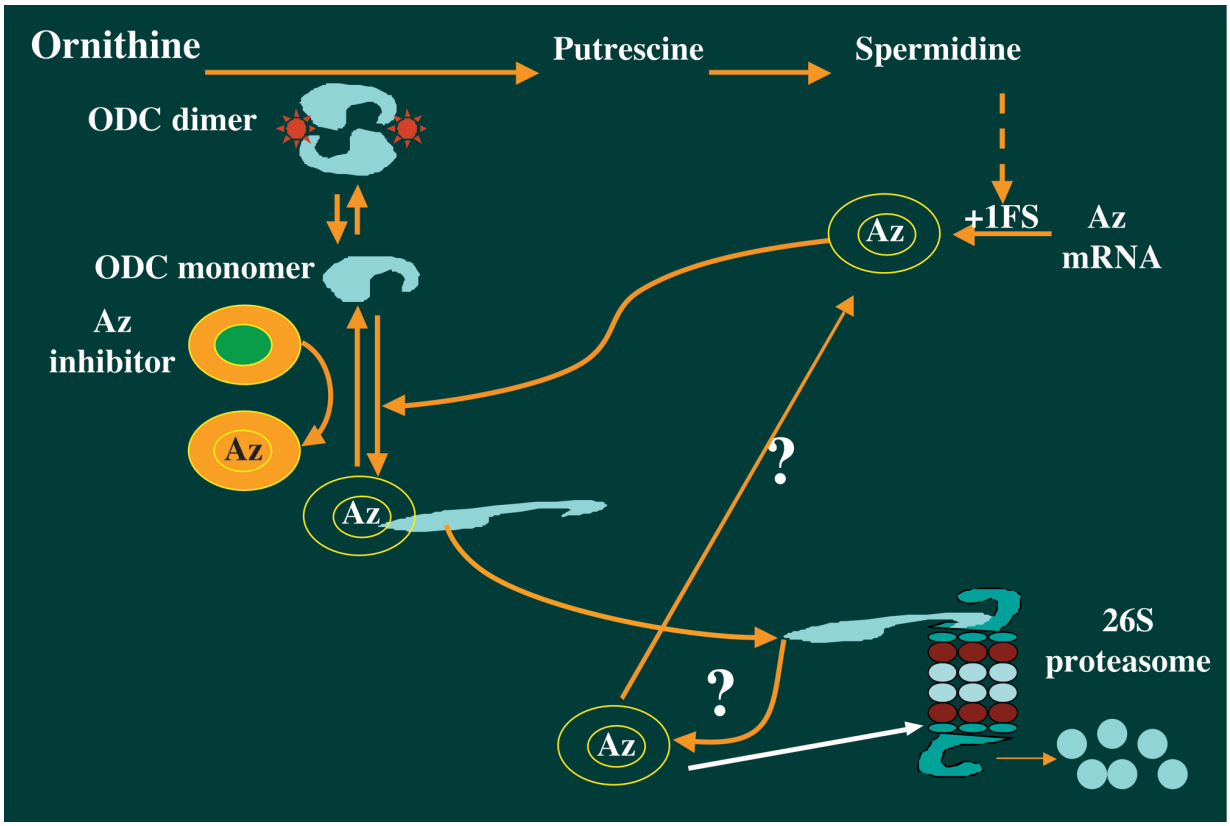
The polyamines spermidine and spermine and their precursor putrescine are ubiquitous aliphatic polycations with multiple cellular functions. Polyamines are essential for fundamental cellular processes such as growth, differentiation, transformation and apoptosis, though their explicit role in these cellular processes is mostly unknown. Due to the critical role of polyamines in important cellular functions, multiple pathways such as biosynthesis, catabolism, uptake, and excretion tightly regulate their intracellular concentration. It is widely accepted that under most circumstances the major sources for cellular polyamines comes from their synthesis from amino acid precursors. In this pathway ornithine is decarboxylated to form putrescine by the action of ornithine decarboxylase. Next an aminopropyl group generated by the action of S-adenosylmethionine decarboxylase on S-adenosylmethionine is attached to putrescine and spermidine to form spermidine and spermine respectively. Both enzymes are highly regulated and are subjected to feedback control by cellular polyamines. While these two highly regulated enzymes constitute the control points of the biosynthesis, the catabolic pathway is controlled predominantly by the action of spermidine/spermine N1-acetyltransferase (SSAT). Control of cellular polyamines by rapid regulated degradation of ODC constitutes an important feedback regulatory mechanism. ODC is one of the most rapidly degraded proteins in eukaryotic cells. Interestingly, it is degraded without requiring ubiquitination. Instead, ODC is marked for rapid degradation by interaction with a unique polyamine-induced protein termed antizyme. Although not requiring ubiquitination, the degradation of ODC occurs by the action of the 26S proteasome, as is the degradation of ubiquitinated proteins. Synthesis of antizyme requires translational frameshifting, which results in bypassing a stop codon located shortly downstream to the initiation codon. High concentration of polyamines subverts the ribosome from its original reading frame to the +1 frame to encode a second ORF and synthesize complete functional antizyme protein. Antizyme binds to ODC subunit to form inactive heterodimers. The affinity of antizyme to ODC subunits is higher than the affinity that ODC subunits have to each other. Interaction between antizyme and ODC subunits results in two outcomes; ODC is inactivated, and the ODC subunits are targeted to degradation. Antizyme was also demonstrated to negatively regulate the process of polyamine transport by a yet unresolved mechanism. Mammalian cells contain another relevant regulatory protein, antizyme inhibitor, and a protein that displays homology to ODC, which lacks decarboxylating activity. It binds to antizyme with higher affinity than ODC thus it may release active ODC from the inactive-antizyme-ODC heterodimer. While it is clear that interaction with antizyme is absolutely required for marking ODC for rapid degradation, it is not clear what happens to antizyme during this proteolytic process. Our present studies focus on the mechanism that regulates the synthesis of antizyme and on the metabolic fate of this regulatory protein.

The range of cellular polyamines is determined at the lower limit by their absolute requirement for cellular proliferation, and at their upper limit by their toxicity. Drugs interfering with polyamine biosynthesis possess considerable potential as therapeutic agents. The most notable example of such inhibitors is α-difluoromethylornithine [α-DFMO, a suicide inhibitor of ODC]. α-DFMO dramatically inhibit cellular proliferation by preventing intracellular production of polyamines. However, since cells, tumor cells in particular, respond to α-DFMO treatment also by increasing their polyamine uptake activity, it is clear that protocols minimizing uptake of polyamines will reveal the entire therapeutic potential of such inhibitors. Conversely, protocols increasing selective uptake of polyamines will enhance the usefulness of toxic polyamine derivatives that are transported by the polyamine transport system.

Polyamine transport is an energy requiring process that is capable of transporting polyamines against significant concentration gradient. However, the mechanisms by which polyamines are transported across the plasma membrane are still poorly understood. We have recently performed a genetic screen in yeast and identified SKY1 as a key regulator of polyamine transport. Sky1p is a recently identified SR protein kinase of the budding yeast that similar to its metazoan counterparts, may function in mRNA maturation by regulating splicing or transport of mRNA from the nucleus to the cytoplasm. Interestingly, similar to the recently identified kinase that regulates polyamine transport, Ptk2p, also Sky1p is involved in



regulating ion homeostasis. Using biochemical proteomic tools we try to identify the cellular proteins that are phosphorylated by these two kinases.

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