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Regulation Of Polyamines, Polycations Regulating Cell Growth Differentiation And Transformation

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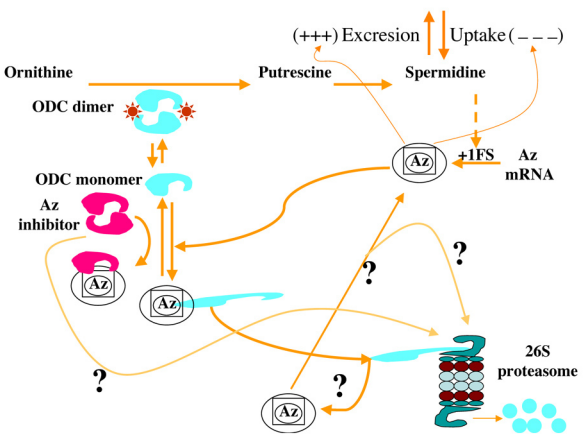
The polyamines spermidine and spermine and their precursor putrescine are ubiquitous aliphatic polycations with multiple cellular functions. Polyamines are essential for fundamental cellular processes such as growth, differentiation, malignant transformation and apoptosis. However, their explicit role in these cellular processes is mostly unknown. In accordance with the critical role polyamines play in important cellular functions, multiple pathways such as biosynthesis, catabolism, uptake, and excretion tightly regulate their intracellular concentration. Under most circumstances the major sources for cellular polyamines are synthesis from amino acid precursors and transport across the plasma membrane. In the biosynthesis 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, ODC is the most notable example of a protein that is degraded by the 26S proteasome without requiring ubuquitination. Instead, ODC is marked for rapid degradation by interaction with a unique polyamine induced protein termed, antizyme (Az). Synthesis of Az 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 Az protein. Az binds to ODC subunit to form inactive heterodimers. The affinity of Az to ODC subunits is higher than the affinity ODC subunits have to each other. Interaction between Az and ODC subunits results in two outcomes; ODC is



inactivated, and the ODC subunits are targeted to degradation by the 26S proteasome. Az was also demonstrated to negatively regulate the process of polyamine transport by a yet unresolved mechanism. Recently it has been demonstrated that mammalian cells contain yet another relevant regulatory protein termed antizyme-inhibitor (AzI). AzI is a protein that display homology to ODC but lacks decarboxylating activity. It binds to Az with higher affinity than ODC thus it can release active ODC from the inactive Az-ODC heterodimer. While it is clear that interaction with Az greatly stimulate ODC degradation, it is not clear what happens to antizyme during this proteolytic process. Our present studies focus on studying the detailed mechanism of Az synthesis

and of the degradation of ODC, Az and AzI and the effect they exert on each other.

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 posses 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 tumor cells display increasing polyamine uptake activity, it is clear that protocols minimizing uptake of polyamines are required for revealing 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 are performing genetic screens in yeast and in mammalian cells in order to identify and characterize structural and regulatory components of the transport process. We have recently identified SKY1 as a key regulator of polyamine transport in yeast. 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.