Regulation of cellular polyamines and elucidation of their role in regulating cellular 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, malignant transformation and apoptosis. However, the explicit mechanism by which they affect these cellular processes is mostly unknown. Multiple pathways such as biosynthesis, catabolism, uptake, and excretion regulate their intracellular concentration. However, under most circumstances the major sources for cellular polyamines are synthesis from amino acid precursors and transport across the plasma membrane. The first and key enzyme in the polyamine biosynthesis pathway is ornithine decarboxylase (ODC) that decarboxylates ornithine to form putrescine. ODC is characterized by an extremely short intracellular half-life that enables its efficient regulation by upstream regulatory mechanisms. Interestingly, ODC is degraded by the 26S proteasome without requiring ubiquitination. Instead, ODC is degraded by the 26S proteasome without requiring ubiquitination. Interestingly, ODC is degraded by the 26S proteasome without requiring ubiquitination.

ODC is degraded by the 26S proteasome without requiring ubiquitination. Instead, ODC is marked for rapid degradation by interaction with a unique polyamine induced protein termed, antizyme (Az) (Fig 1). Synthesis of Az requires a +1 translational frameshifting, which results in bypassing a stop codon located shortly downstream to the initiation codon. This frameshifting event that is stimulated by polyamines serve as a cellular polyamine sensing mechanism. Az binds to ODC subunit to form inactive heterodimers. The affinity of Az to ODC subunits is higher than the affinity ODC subunits display to each other. Interaction between Az and ODC subunits results in inactivation of ODC and in targeting ODC subunits to degradation by the 26S proteasome (Fig 1). Az was also demonstrated to negatively regulate the process of polyamine transport across the plasma membrane via a yet unresolved mechanism (Fig 1). Mammalian cells contain yet another protein termed antizyme-inhibitor (AzI) that appear to be a central regulator of cellular polyamines. AzI displays high homology to ODC but it lacks decarboxylating activity. It binds to Az with higher affinity than ODC thus it can release active ODC from the inactive Az-ODC heterodimer and save it from degradation (Fig 1). We have demonstrated that despite being degraded as rapidly as ODC, Az is not degraded while presenting ODC to the proteasome. Az is actually released and either recycled to mediate the degradation of additional ODC molecules or is degraded by the proteasome in a reaction that requiring an active ubiquitin system (Fig 1). Interestingly, AzI is also a rapidly degraded protein. However despite its homology to ODC their modes of degradation are entirely distinct. Our studies revealed that the signals that target ODC for degradation are not required for AzI degradation and that AzI is targeted to degradation by ubiquitination. Furthermore, not only that Az is not required for AzI degradation, actually interaction with Az actually inhibits AzI degradation by interfering with its ubiquitination. 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.

Since AzI has the potential to negate Az functions it may provide cells with growth advantage. In this respect we have demonstrated that overexpression of AzI in NIH3T3 cells increased growth rate, enabled growth in low serum, and permitted anchorage-independent growth in soft agar. In contrast, reduction of AzI levels by AzI siRNA reduced cellular proliferation. Moreover, AzI overproducing cells gave rise to tumors when injected into nude mice. AzI overexpression resulted in elevation of ODC activity and of polyamine uptake. These effects of AzI are a result of its ability to neutralize Az, as overexpression of an AzI mutant with reduced Az binding failed to alter cellular polyamine metabolism and growth properties. We also demonstrate upregulation of AzI in Ras transformed cells, suggesting its relevance to some naturally occurring transformations. Finally, increased uptake activity rendered AzI overproducing and Ras-transformed cells more sensitive to toxic polyamine analogues. Our results therefore imply that AzI has a central and meaningful role in modulation of polyamine homeostasis, and in regulating cellular proliferation and transformation properties. The involvement of AzI in these processes and in tumor development is under investigation.

As mentioned above studies on the degradation of ODC demonstrated that poly-
ubiquitin is not the only signal recognized by the 26S proteasome. Interestingly, together with Y. Shaul’s lab we have recently demonstrated that ODC undergoes another type of ubiquitin-independent degradation by the 20S proteasome that is regulated by NAD(P)H quinone oxidoreductase 1 (NQO1) (Fig. 2). Considering the prevalence of the ubiquitin system in the process of cellular protein degradation it is rather remarkable that a key cellular enzyme such as ODC is subjected to two different proteolytic pathways that are different from the ubiquitin dependent one. While the Az mediated degradation of ODC by the 26S proteasome is tightly related to the cellular polyamine balance, the NQO1 regulated degradation of ODC by the 20S proteasome seems independent of the polyamine metabolism. The detailed mechanism of this novel degradation process and its physiological relevance are under investigation.

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’s considerable potential as therapeutic agents. 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. Based on the sensitivity of yeast to spermine we have performed a genetic screen in yeast in order to identify and characterize transporters of polyamines and regulators of the transport process. We have identified SKY1 as a key regulator of polyamine transport in yeast. Sky1p is a 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 also involved in regulating ion homeostasis. Using, biochemical proteomic and bioinformatics tools we have identified cellular proteins that are phosphorylated by Sky1p. However none of these proteins are involved in regulating spermine tolerance. Conversely, few additional genes that were recently demonstrated by us to regulate spermine tolerance are not Sky1p substrates. Understanding the mechanism that regulates polyamine transport across the plasma membrane is therefore still a main goal of our lab.

Selected publications

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