Technologies to measure and probe the function of polyamines

by

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Abstract

Polyamines are abundant and evolutionarily conserved metabolites that are essential for life. Dietary polyamine supplementation extends lifespan, and dysregulated polyamine homeostasis is linked to diseases, including Parkinson's disease and cancer, driving interest in therapeutically targeting this pathway. Although polyamines are implicated in diverse processes, including ribosomal translation, cellular proliferation, aging, and disease, their molecular functions remain incompletely understood. This gap reflects, in part, the lack of tools to measure polyamine dynamics in living cells, which has confined most studies to static, population-averaged assays, as well as the absence of systematic approaches to map the cellular pathways that regulate and respond to polyamine homeostasis. The central aim of this thesis was therefore to develop and apply experimental strategies that enable real-time, single-cell measurement of intracellular polyamines and unbiased identification of the molecular networks that control and depend on polyamine metabolism.

First, I developed genetically encoded fluorescent reporters that enable dynamic, single-cell quantification of intracellular polyamine concentrations. These reporters overcome the temporal and population-averaging limitations of conventional biochemical assays and allow systematic perturbation-based analysis of polyamine homeostasis across physiological and disease contexts. Using these tools in combination with genetic screens, I show that polyamine import is actively regulated by mitochondrial metabolism and identify connections with relevance to neurodegenerative disease.

Second, I applied genetic screens to uncover a previously unrecognized functional role for polyamines as endogenous buffers of labile iron. I demonstrate that polyamine depletion increases redox-active iron, enhances lipid peroxidation, and sensitizes cells to ferroptotic cell death, establishing a direct mechanistic link between polyamine homeostasis, iron metabolism, and ferroptosis. To directly visualize this relationship, I developed a genetically encoded fluorescent reporter for redox-active iron and observed a striking inverse correlation between intracellular polyamine levels and labile iron at single-cell resolution. This polyamine-iron axis provides a mechanistic explanation for why cells invest heavily in maintaining polyamines at millimolar levels and reshapes our understanding of metabolic dysfunction in aging, cancer, and other diseases.

Collectively, this work introduces new experimental tools to the field of polyamine biology that shift it toward a dynamic and systems-level understanding, with implications for metabolism, redox regulation, cell fate, and therapeutic intervention.

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