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OP I_06

PROTEASOME ACTIVATION INCREASES LIFESPAN THROUGH MITOHORMESIS AND UPR^{ER} ACTIVATION

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Bolstering proteostasis has long been considered a promising antiaging strategy. Enhancement of proteasome activity has been repeatedly shown to extend the replicative potential of human primary cells and the lifespan of nematodes and flies. However, until now, the mechanisms underlying these prolongevity effects remain largely elusive. In this study we show that in nematodes, proteasome activation through the overexpression of the *pbs-5* catalytic subunit, causes a mild mitochondrial impairment and promotes the engagement of UPR^{ER}. Proteomics analysis revealed several mitochondrial proteins to be dysregulated in the *pbs-5*-overexpressing nematodes, thus leading to mitochondrial adaptations that underlie the lifespan extension of these animals. Moreover, UPR^{ER} is also engaged and necessary for the observed lifespan extension. Currently, we are validating these findings in human cells overexpressing the β5 proteasome subunit in order to investigate the potential relevance of our findings in higher organisms. Given the attention that proteasome enhancement attracts as an antiaging strategy, a detailed image of the effects of proteasome activation on organismal physiology will fill an important and therapy-related knowledge gap.

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OP II_01

HYPER1 AS A TOOL TO MEASURE THE REDUCTIVE POWER IN CELLULAR COMPARTMENTS

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The formation of disulfide bonds between cysteine residues is a post-translational modification that can provide structural support and confer conformational stability to proteins. In addition, reversible formation of disulfides can impact the activity of metabolic and signaling proteins, thus performing regulatory functions. Disulfides are formed when the thiol groups of cysteine residues are oxidized in reactions mediated by hydrogen peroxide or in thiol-disulfide exchange reactions. In turn, TrxR- or GSH-dependent enzymatic pathways are responsible for the cleavage of disulfides. A bottleneck in redox chemistry research is the limited availability of methods that allow specifically assessing the activity of disulfide reductases in living cells. Here, we advocate for repurposing the genetically encoded biosensor HyPer1, initially designed as a fluorescent probe for H_2O_2, as a tool to measure the reductive power in various cellular compartments. Using the AI tool (AlphaFold2) and verifying its predictions in both cell-free systems and cellular models, we show that in the cytosol and nucleus of human cells, HyPer1 primarily relies on Trx1/TrxR1-mediated reduction with a second-order rate constant of 5.8 x 10^2 $M^{-1}s^{-1}$. On the other hand, within the mitochondria, HyPer1 is predominantly reduced by GSH. The GSH-mediated reduction rate constant is 1.77 $M^{-1}s^{-1}$, and the recovery period for mito-HyPer1 *in cellulo* is twice as long compared to cyto- and nuc-HyPer1. After exploring various human cells (mesenchymal stem cells, induced pluripotent stem cells, fibroblasts, malignant cell lines), we revealed a potent cytosolic Trx1/TrxR1 pathway, particularly pronounced in cancer cells such as K-562 and HeLa. In conclusion, our study demonstrates that HyPer1 can be harnessed as a robust tool for assessing compartmentalized reduction activity in cells.

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OP II_02

PIONEERING PRECISION: UNRAVELING CELLULAR BIOENERGETICS WITH PH-CONTROL

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The correlation between pH levels and diseases such as renal and heart failure, cancer, and stroke has long been acknowledged. However, the precise manipulation of pH in specific cellular locales poses a challenge, impeding the comprehensive understanding of the underlying mechanisms. Addressing this gap, we recently introduced Chemogenetic Operation of iNTRacellular prOton Levels (pH-CONTROL) offering heightened spatio-temporal resolution for pH manipulation. We thoroughly characterized this method through extensive *in vitro* and *in cellulo* investigations utilizing the SypHer3s biosensor. pH-CONTROL exhibits remarkable precision in modulating pH within distinct subcellular compartments, including the mitochondria, nucleus, and cytosol. Preliminary Seahorse data unveiled that the application of pH-CONTROL to cancer cells, particularly those expressing it in the mitochondrial intermembrane space, causes an augmentation in ATP production, as evidenced by an increased oxygen consumption rate. These findings underscore the potential of pH-CONTROL to reshape diverse cellular functions, with a particular emphasis on energy metabolism. The ability to precisely manipulate pH at the subcellular level opens up new avenues in cellular bioenergetics research, offering insights into the intricate interplay between pH dynamics and cellular processes. With its enhanced precision and versatility, pH-CONTROL holds promise for unraveling the connections between redox biology and various health and disease states. This method thus represents a valuable tool in advancing our understanding of cellular bioenergetics in the context of redox biology, paving the way for future investigations and therapeutic interventions.

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OP II_03

DIMETHYL LABELLING ALLOWS IDENTIFICATION OF NOVEL PROTEIN CROSSLINKS BY MASS SPECTROMETRY

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Protein crosslinks (*e.g.,* dityrosine) formed by reactions of different oxidants are associated with multiple pathologies including atherosclerosis, Alzheimer's and Parkinson's diseases. In many cases, proteins on which these are formed, and the exact positions of these within (intramolecular), or between (intermolecular) polypeptide chains is unclear. Whilst some data is available from fluorescence or antibody approaches, this requires prior knowledge of the species formed. To obtain unbiased knowledge, including detection of novel species, and to pinpoint their locations, mass spectrometry is typically performed on peptides arising from proteolytic digestion. Such analyses are challenging since the fragment ion spectra are often more complex than for non-crosslinked species. We hypothesised that highly-efficient and specific labelling of the *two* N-termini in crosslinked peptides (compared to the single N-terminus of linear peptides) would facilitate unbiased identification and validation of crosslinks. We have employed chemical dimethyl labelling of amine groups with "light" and "heavy"