Systematic discovery and functional analysis of the PARKIN modified proteome

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Country

USA

Title of project or programme

Systematic discovery and functional analysis of the PARKIN modified proteome

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NIH (NINDS)

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25/09/2013

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1

The project/programme is most relevant to:

Parkinson's disease & PD-related disorders

Keywords

Proteome, Outer Mitochondrial Membrane, PINK1 gene, Mitochondria, Autophagosome

Research Abstract

DESCRIPTION (provided by applicant): Ubiquitin (Ub) ligases are components of dynamic signaling systems whose activation leads to re-sculpting of the proteome through degradative

and non-degradative mechanisms. The Ub ligase PARKIN and its upstream regulatory kinase PINK1 are key components of a signal transduction pathway that controls mitochondrial homeostasis in response to mitochondrial damage via, for example, depolarization. Both of these genes are mutated in early onset Parkinson’s disease (PD). Mitochondrial quality control via this pathway occurs, in part, by altering mitochondrial dynamics and by promoting the degradation of damaged mitochondria by mitophagy. PINK1, a mitochondrially localized kinase, is required for recruitment of PARKIN to the mitochondrial outer membrane (MOM) through a phosphorylation dependent mechanism that is poorly understood at the molecular level. Once associated with the MOM, PARKIN is known to ubiquitylate several MOM proteins including mitofusin and Miro GTPases to alter mitochondrial fission-fusion cycles and trafficking on microtubules, respectively. In the previous funding cycle, we have developed quantitative diGLY capture proteomics as a means by which to identify targets of the Ub system and precisely elucidate the sites of ubiquitylation. Using this method, we have performed a series of studies that have revealed the PARKIN-modified proteome, including hundreds of ubiquitylation sites on dozens of proteins, including known and novel targets. The many candidate PARKIN targets located on the MOM are ubiquitinated on their cytoplasmic face, while other PARKIN targets appear to be primarily cytoplasmic. Parallel interaction proteomic and in vivo functional studies revealed signal dependent association of PARKIN with a cohort of MOM proteins in a manner that depends upon the integrity of the active site of PARKIN. Thus, this work provides the first topological and molecular framework for understanding the mechanisms by which PARKIN controls mitochondrial fate and by which damage activates PARKIN activity. In this renewal, we propose two thematic, yet integrated aims that exploit both the PARKIN target landscape we have elucidated and several proteomics tools that allow quantitative decoding of signaling mechanisms. AIM 1 seeks to understand how site-specific ubiquitylation of proteins on the MOM control mitochondrial clustering and recruitment to autophagosomes. AIM 2 seeks to employ in vivo and in vitro systems to elucidate the mechanistic basis for PARKIN activation through what appears to be a multi-step mechanism, using engineered and patient-derived mutations, and to discover the functional basis for chain-linkage specific poly-Ub synthesis by PARKIN using proteomic and genetic approaches. Together, these studies will provide a much deeper understanding of the molecular mechanisms underlying PARKIN function and how disease mutants affect mitochondrial homeostasis.

Lay Summary
PUBLIC HEALTH RELEVANCE: Parkinson’s disease (PD) represents a major health problem world-wide. Accumulating data indicate that PD may largely reflect defects in mitochondrial homeostasis, particularly in neuronal subpopulations where there is a heavy metabolic load for energy production. This proposal addresses basic mechanisms in mitochondrial quality control that are regulated by two early onset PD genes, the ubiquitin ligase PARKIN/PARK2 and the protein kinase PINK1/PARK6.

Further information available at:

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Investments > €500k

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United States of America

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Parkinson's disease & PD-related disorders

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2016

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