【Grant-in-Aid for Scientific Research (S)】

Integrated understanding of proteasomal degradation and the ubiquitin code

Principal Investigator	The University of Tokyo, Institute of Medical Science, Professor SAEKI Yasushi　Researcher Number:80462779
Project Information	Project Number : 23H05479　Project Period (FY) : 2023-2027 Keywords : Proteolysis, Ubiquitin, Proteasome, Mass spectrometry, LLPS

Purpose and Background of the Research

●Outline of the Research

The ubiquitin-proteasome system (UPS) plays an essential role in the regulation of various cellular functions such as gene expression and signaling as well as protein homeostasis (proteostasis) by selectively targeting abnormal proteins and functional proteins that have completed their roles. As malfunctions in UPS components are causative to cancer, neurodegenerating diseases, and aging, drug discovery targeting UPS such as proteasome inhibitors and protein degraders is currently progressing worldwide (Fig. 1). We have previously found that multiple molecules are responsible for selecting and delivering proteasome substrates and that cells utilize liquid-liquid phase separation (LLPS) for proteasomal degradation. However, the UPS is a large and complex system consisting of approximately 1,300 regulatory molecules, and the molecular principles for efficient proteolysis by the proteasome have not been fully understood. This research project aims to elucidate the molecular mechanisms and spatio-temporal regulation underlying proteasomal degradation to understand the pathogenesis of UPS-related diseases for future drug development.

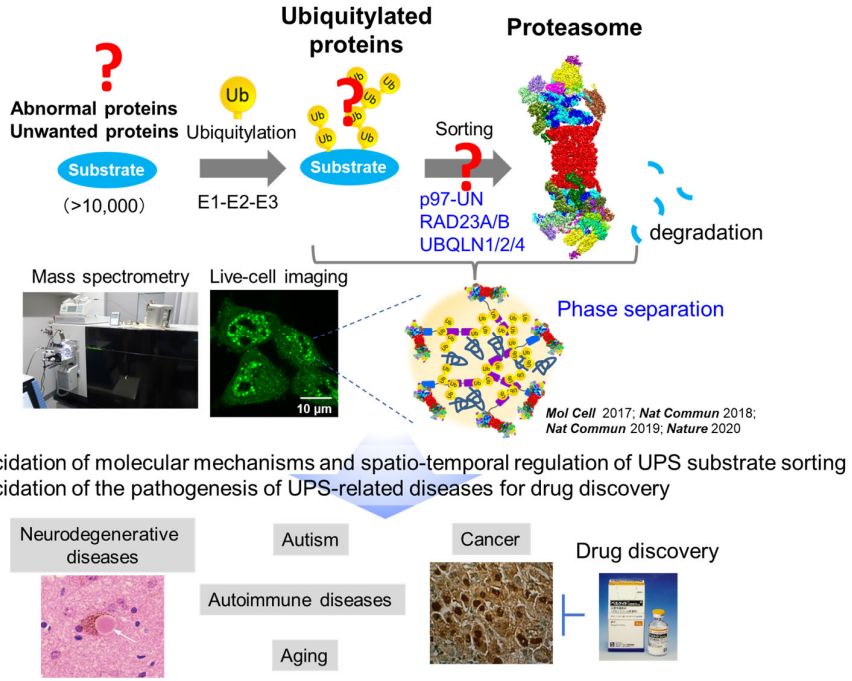

Figure 1. The ubiquitin-proteasome system and the outline of the research

●Ubiquitin code

Ubiquitin modifications often function by forming ubiquitin chains: There are eight different ubiquitin chains (M1, K6, K11, K27, K29, K33, K48, and K63 chains), each with a different function, so the functional information embedded in the ubiquitin chains is called the ubiquitin code. In particular, the K48 chain is involved in proteasomal degradation. However, it has become clear that mono-ubiquitination and branched ubiquitin chains also induce proteasomal degradation, and therefore an integrated analysis as described below is necessary to understand the molecular rules of proteasomal degradation.

●Ubiquitin decoders for proteasomal degradation

Individual ubiquitin codes exert their function through recognition by specific ubiquitin-binding proteins (decoders). AAA-ATPase p97 and its ubiquitin-binding cofactors, and multiple shuttling molecules are often involved in substrate selection for proteasomal degradation. The shuttling molecules also induce the formation of proteasomal droplets by liquid-liquid phase separation with ubiquitylated proteins. These ubiquitin decoders have been implicated in neurodegenerative diseases such as Alzheimer's disease and ALS, but their precise functions especially selectivity to specific ubiquitin chains and ubiquitylated substrates are not fully understood.

Expected Research Achievements

●Elucidation of the molecular mechanisms of proteasome substrate sorting

For a total of 11 shuttling molecules, we will identify ubiquitylated substrates and their regulatory proteins by mass spectrometry (MS)-based deep proteomics. We will also analyze their subcellular localization and involvement of liquid-liquid phase separation to elucidate how individual ubiquitylated substrates are sorted by these shuttling molecules to execute efficient proteasomal degradation in cells (Fig. 2).

●Understanding the ubiquitin code for proteasomal degradation

By injecting defined ubiquitylated proteins into cells, we will elucidate the ubiquitin code that induces proteasomal degradation. This will reveal the functional units of ubiquitin chains. We will also analyze the differences in degradation rates in the nucleus and cytoplasm, and the requirements of p97 and shuttling molecules. These integrated approaches will provide a deeper understanding of the ubiquitin code and decoders that drive proteasomal degradation.

●Pathogenesis of UPS-related diseases using mice models

We have generated mice with systemic proteasome dysfunction by introducing patient-derived mutations (Fig. 3). These mice exhibit multifaceted phenotypes such as developmental delay, abnormal pain perception, mild hepatic dysfunction, and loss of Purkinje cells. To gain our understanding of UPS-related diseases, we will analyze proteome changes and functions of shuttling factors in the mutant mice by tissue-proteomics approaches.

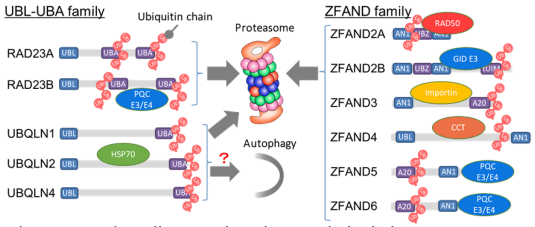

Figure 2. Shuttling molecules and their interactors

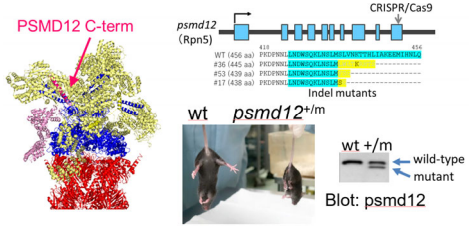

Figure 3. Proteasome mutant mice

Homepage Address, etc.	https://www.cbms.k.u-tokyo.ac.jp/lab/saeki.html https://researchmap.jp/myportal_saeki-ys