Department of Basic Medical Sciences

Division of Protein Metabolism
タンパク質代謝制御分野

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The ubiquitin-proteasome system and lysosomes are the major proteolytic pathways and play a fundamental role in cellular protein homeostasis (proteostasis). Their dysregulation causes a plethora of diseases, including neurodegenerative disorders, but the detailed pathogenic mechanisms remain largely unknown. Our goal is to elucidate the basic molecular mechanisms governing proteostasis-related diseases by analyzing the regulation of protein metabolism, thereby providing the basis for therapeutic strategies.

1. Generation and characterization of proteasomopathy model mice

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The proteasome plays a central role in many biological processes, including cell proliferation, transcriptional regulation, inflammation and proteostasis, by selectively degrading ubiquitinated proteins. Nevertheless, proteasome research at the whole-body level has lagged far behind because the proteasome is essential for the viability of all cells and knockout mice of the constitutive subunits are embryonic lethal. Recently, heterozygous mutations in *PSMD12*, a 19S subunit gene of the proteasome, have been identified in patients with developmental disorders and autism. Based on this finding, we generated three lines of heterozygous mutant mice lacking the 3′ end of *Psmd12* at different lengths. The most deficient *Psmd12* mutant mice exhibited growth retardation, impaired liver function, and diaphragmatic hernia. Further histological analysis revealed hepatocyte shedding and accumulation of ubiquitinated substrates in the cerebellum. The other two lines of mutant mice had mild or no phenotypes. Next, we performed a deep proteomic analysis of the liver and unexpectedly found that the mildest *Psmd12* mutant mice showed the largest proteome changes, with accumulation of proteins involved in mitochondrial dysfunction, DNA damage, and oxidative stress responses. Thus, the systemic proteasome mutant mice revealed that proteasome dysfunction exhibits diverse phenotypes, including impaired liver function, and that a slight decrease of the proteasome activity can trigger abnormal proteostasis.

2. A quality control system to monitor the integrity of protein complex

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Approximately half of all proteins form complexes to achieve functional expression in cells. The stoichiometry of the subunits that form a complex must be properly maintained, and failure of assembly or disruption of this balance due to causes such as aneuploidy results in the accumulation of misassembled intermediates and orphan subunits. Recently, multi-

ple protein quality control mechanisms have been discovered that target these incorrectly assembled proteins for degradation. For example, the E3 ligases HUWE1 and STUB1/CHIP are well established as general quality control factors, and some E3 ligases that are dedicated to specific complexes have been identified, such as UBE2O for ribosomal proteins, HERC1 for proteasome intermediates and HERC2 for CCT/TRiC chaperonin. However, it has not been described whether cells have quality control mechanisms that specifically monitors the integrity protein complexes.

We have discovered a quality control system for the disintegrated minichromosome maintenance (MCM) complex generated by depletion of a single MCM subunit using auxin-inducible degron (AID) system. Rapid depletion of MCM2-mAID destabilized the remaining MCM subunits, and we identified the ubiquitin ligase CRL4 (DCAF *X*) for selective removal of MCM5 by the proteasome. Mechanistically, the CRL4 substrate adaptor DCAF *X* recognized the buried region inside the MCM complex and ubiquitinated only after the disintegration. This mechanism appeared to be distinct from the elimination pathway of the orphan subunit during assembly. Thus, CRL4 (DCAF *X*) is an E3 ligase that monitors the integrity of the MCM complex, thus providing insights into the surveillance mechanisms of complex integrity.

3. Liquid-to-solid phase transition of ubiquitylated proteins triggered by ATP depletion

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Ubiquitin-positive inclusions are a hallmark of almost all the neurodegenerative diseases such as Alzheimer's disease and amyotrophic lateral sclerosis. Dysregulation of ubiquitin-dependent phase separation has been implicated in the disease pathogenesis, but little is known about the molecular mechanisms involved. We have shown that proteasomes undergo liquid-liquid phase separation (LLPS) together with ubiquitinated proteins in response to changes of in the cellular environments (Yasuda et al, Nature 2020; Iriki et al, Cell Rep 2023). Recently, we found that ATP depletion also induces LLPS of the proteasome and ubiquitinated proteins. The decrease in ATP levels caused a massive remodeling of the ubiquitin proteome, especially in ATP-binding proteins and components of certain protein complexes, and condensation in a RAD23B- and UBQLN-dependent manner. Restoration of ATP levels resolved this condensation, which required p97 activity and proteasome deubiquitination activity, suggesting that the primary function of the condensates is sequestration of the ubiquitinated proteins rather than their degradation. Interestingly, inhibition of p97 or prolonged ATP depletion resulted in insolubilization of the ubiquitinated protein, that is, a phase transition from the liquid-to-solid phase transition. Thus, ubiquitin-dependent LLPS contributes to the correction of impaired proteostasis upon ATP stress and may be involved in the formation of ubiquitin-positive inclusions.

4. USP8 prevents aberrant NF-κB and Nrf2 activation by counteracting ubiquitin signals from endosomes

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K63-linked ubiquitin chains attached to plasma membrane proteins serve as tags for endocytosis and endosome-to-lysosome sorting. USP8 is an essential deubiquitinase for the maintenance of endosomal functions. Prolonged depletion of USP8 leads to cell death, but the major effects on cellular signaling pathways are poorly understood. Here, we show that USP8 depletion causes aberrant accumulation of K63-linked ubiquitin chains on endosomes and induces immune and stress responses. Upon USP8 depletion, two different decoders for K63-linked ubiquitin chains, TAB2/3 and p62, were recruited to endosomes and activated the TAK1–NF-κB and Keap1–Nrf2 pathways, respectively. Oxidative stress, an environmental stimulus that potentially suppresses USP8 activity, induced accumulation of K63-linked ubiquitin chains on endosomes, recruitment of TAB2, and expression of the inflammatory cytokine. The results demonstrate that USP8 is a gatekeeper of misdirected ubiquitin signals and inhibits immune and stress response pathways by removing K63-linked ubiquitin chains from endosomes.

5. Dynamic changes of lysosomal protein degradation in neural stem cells of the postnatal mouse brain

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Lysosomes are intracellular organelles responsible for degrading diverse macromolecules delivered from several pathways, including the endo-lysosomal and autophagic pathways. Recent reports have sug-

gested that lysosomes are essential for regulating neural stem cells in developing, adult, and aged brains. However, the activity of these lysosomes has yet to be monitored in these brain tissues. Here, we report the development of a new probe to measure lysosomal protein degradation in brain tissue by immunostaining. Our results indicate that lysosomal protein degradation fluctuates in neural stem cells of the hippocampal dentate gyrus, depending on age and brain disorders. Neural stem cells increase their lysosomal activity during hippocampal development in the dentate gyrus, but aging and aging-related disease reduce lysosomal activity. In addition, physical exercise increases lysosomal activity in neural stem cells and astrocytes in the dentate gyrus. We therefore propose that three different stages of lysosomal activity exist: the state of increase during development, the stable state during adulthood, and the state of reduction due to damage caused by either age or disease.

Publications

Akizuki, Y., Morita, M., Mori, Y., Kaiho-Soma, A., Dixit, S., Endo, A., Shimogawa, M., Hayashi, G., Naito, M., Okamoto, A., Tanaka, K., Saeki, Y., Ohtake, F. cIAP1-based degraders induce degradation via branched ubiquitin architectures. Nat. Chem. Biol., 19:311-322, 2023.

Iriki, T., Iio, H., Yasuda, S., Matsuta, S., Kato, M., Kosako, H., Hirayama, S., Endo, A., Ohtake, F., Kamiya, M., Urano, Y., Saeki, Y., Hamazaki, J., and Murata S. Senescent Cells form nuclear foci that contain the 26S proteasome. Cell Rep. 42:112880, 2023.

Zhang, H., Ishii, K., Shibata, T., Ishii, S., Hirao, M., Lu, Z., Takamura, R., Kitano, S., Miyachi, H., Kageyama, R., Itakura, E. and *Kobayashi, T. Fluctuation of lysosomal protein degradation in neural stem cells of postnatal mouse brain. Development, in press.

*Endo, A., Fukushima, T., Takahashi, C., Tsuchiya, H., Ohtake, F., Ono ,S., Ly, T., Yoshida, Y., Tanaka, K., *Saeki, Y., and *Komada, M. USP8 prevents aberrant NF-κB and Nrf2 activation by counteracting ubiquitin signals from endosomes. J. Cell Biol., in press.

Kobayashi, T. Protein homeostasis and degradation in quiescent neural stem cells. J. of Biochem., in press.

佐伯泰、土屋光、遠藤彬則、冨田拓哉．ユビキチン化プロテオーム解析とユビキチン鎖絶対定量．羊土社．実験医学別冊「決定版 質量分析活用スタンダード」．213-224, 2023.

小林妙子．神経幹細胞におけるリソソーム機能．羊土社．実験医学．41(11): 1743-1747, 2023.