Department of Basic Medical Sciences

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

Professor	Yasushi Saeki, Ph.D.	教　授	博士(薬学)	佐伯　泰
Associate Professor	Taeko Kobayashi, Ph.D.	准教授	博士(理学)	小林妙子
Assistant Professor	Takuya Tomita, Ph.D.	助　教	博士(薬科学)	冨田拓哉

The ubiquitin-proteasome system and lysosomes are the major proteolytic pathways and play a fundamental role in cellular protein homeostasis (proteostasis). Dysregulation of these pathways 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. Substrate structure determines p97- and RAD23A/B-mediated proteasomal degradation in human cells

Yi Ding, Takuya Tomita, Hikaru Tsuchiya[1,2], Yasushi Saeki: [1]Department of Physiology, Graduate School of Medicine, Juntendo University, [2]Autophagy Research Center, Graduate School of Medicine, Juntendo University

Proteasomal degradation of ubiquitinated proteins involves various accessory factors, including p97 and shuttle factors, but their requirements and relationship with substrate structural properties are not fully understood, especially in human cells. Here, we demonstrate that substrate structure dictates the dependency on p97 and RAD23A/B for proteasomal degradation in human cells, using two ubiquitin-fusion model substrates, Ub-GFP (well-folded) and Ub-GFP-tail (with an unstructured tail). Both substrates exhibited similar ubiquitin chain composition, primarily mediated by the UBR4–KCMF1 E3 ligase. Interactome analyses revealed that Ub-GFP preferentially interacts with p97 and RAD23B, while Ub-GFP-tail binds more strongly with the proteasome. The degradation of Ub-GFP depends on p97 and RAD23A/B, whereas that of Ub-GFP-tail bypasses these accessory factors. RAD23A/B knockdown resulted in a reduction in the apparent lengths of ubiquitin chains on both substrates, yet it only affected Ub-GFP degradation, suggesting that shorter ubiquitin chains are sufficient to drive the degradation of substrates possessing an unstructured tail. Collectively, our findings highlight substrate structure as a key determinant of accessory factor requirement, offering valuable insights for optimizing targeted protein degradation strategies.

2. Development of PSLiP-MS: a novel method for direct identification and quantification of proteasome substrates

Takuya Tomita, Kurumi Asano, Yasushi Saeki

The proteasome controls intracellular protein lifespan through selective protein degradation, thereby regulating diverse biological phenomena. Therefore, the accurate identification of proteasome substrates is critical for elucidating these physiological processes. However, existing methods rely on indirect approaches, such as substrate stabilization by proteasome inhibitors or the detection of ubiquitination. Thus, despite advancements in proteomics, directly measuring proteasomal degradation remained

a challenge. To address this, we developed PSLiP-MS (ProteaSome Limited Proteolysis- Mass Spectrometry), a novel approach that leverages limited proteolysis (LiP) to directly identify and quantify *bona fide* substrates entrapped within the proteasome core particle. Using PSLiP-MS, we successfully identified a broad spectrum of substrates ranging from well-known proteasome substrates to several classes of previously elusive substrates, including highly abundant proteins, missorted organelle proteins, and orphan subunits of protein complexes, providing direct evidence that the proteasome serves as a central hub for eliminating a diverse array of proteins to maintain cellular homeostasis. Furthermore, PSLiP-MS allowed us to dissect ubiquitin-dependent versus ubiquitin-independent degradation pathways, revealing that ubiquitin-independent substrates are notably enriched in transcription factors possessing intrinsically disordered regions. Collectively, PSLiP-MS serves as a powerful fundamental technique for proteasome research, promising to deepen our understanding of the mechanisms behind various biological events.

3. Mechanisms for maintaining proteostasis under hyperosmotic stress

Kouta Nakadai, Hikaru Tsuchiya[1,2], Shungo Adachi[3], Takuya Tomita, and Yasushi Saeki: [3]Department of Proteomics, National Cancer Center Research Institute, Tokyo

Acute changes in osmolality impair various cellular functions and can lead to apoptosis. In response to hyperosmotic stress, cells are known to induce signal transduction pathways such as the p38 MAPK pathway, and we previously reported that the proteasome forms nuclear droplets via liquid-liquid phase separation to degrade ubiquitylated proteins. However, it remains unclear whether hyperosmotic stress induces proteome changes that, in turn, regulate the stress response. Here, we identified a novel hyperosmotic stress response pathway mediated by proteasomal degradation. Using TMT-based or DIA deep proteomics, we revealed that approximately 100 proteins are rapidly degraded by the proteasome under hyperosmotic stress. Strikingly, the linker histone H1 was identified as the major ubiquitylated substrate, modified with heterotypic ubiquitin chains (K11, K29, K48 linkages). Mass spectrometry-based interactome analysis demonstrated that the CRL4-DCAF X E3 ubiquitin ligase complex promoted H1 ubiquitylation, leading to its degradation in both the nucleoplasm and proteasome droplets. Notably, RNA sequencing revealed that depletion of DCAF X, which inhibited H1 degradation, suppressed stress-responsive gene expression. Furthermore, DCAF X depletion sensitized cells to hyperosmotic stress. These findings suggest that hyperosmotic stress-dependent H1 degradation plays a crucial role in cellular stress response by inducing stress-responsive genes.

4. A Stable Bioisostere of Ester-Linked Ubiquitin Chains Enables Decoding of Protein Interactors

Yoshinori Taguchi[4], Takuya Tomita, Takuma Nishizawa[5], Dai Nakamura[6], Showmitra Saha[4], Takanori Oyoshi[6], Kohei Sato[4], Nobuyuki Mase[4], Yasushi Saeki, Tetsuo Narumi[4]: [4]Graduate School of Medical Photonics, Shizuoka University, [5]Department of Engineering, Graduate School of Integrated Science and Technology, Shizuoka University, [6]Department of Science, Graduate School of Integrated Science and Technology, Shizuoka University

Protein ubiquitination is a pivotal posttranslational modification that regulates diverse biological processes depending on the type of ubiquitin chain linkage. Recently, ester-linked ubiquitin chains have been identified, yet their inherent hydrolytic instability has posed a significant challenge for biochemical investigations. To address this limitation, we chemically synthesized a stable and isosteric amide analog of an ester-linked ubiquitin dimer, in which serine (Ser) at position 20 of the proximal ubiquitin is replaced with 2,3-diaminopropionic acid (Dap). We achieved this using a convergent approach involving the sequential chemoselective ligation of three peptide fragments generated through Fmoc-based solid-phase peptide synthesis. By employing this chemically robust ubiquitin probe, we uncovered a previously unrecognized interaction between Ser20-linked ubiquitin chains and spliceosome-associated factors, notably ubiquitin-specific protease 39 (USP39). These findings highlight the potential of the ester-to-amide bioisosteric strategy to unlock mechanistic insights into atypical ubiquitin modifications. Our approach not only circumvents the intrinsic instability of ester-linked ubiquitin chains but also provides a broadly applicable framework for dissecting their biological roles, paving the way for future discoveries in ubiquitin signaling.

5. CAPN15 is a non-proteasomal, ubiquitin-directed calpain protease that regulates cell adhesion by cleaving E-cadherin

Aya Noguchi[7], Shoji Hata[7], Hikaru Tsuchiya[1,2], Hiroshi Shitara[8], Yasushi Saeki, Yasuko Ono[7]: [7]Calpain Project, Tokyo Metropolitan Institute of Medical Science, [8]Animal Research Division, Tokyo Metropolitan Institute of Medical Science

CAPN15, a member of the calpain protease family, contains a unique N-terminal region with five Zn^{2+}-finger domains, including two ubiquitin-binding Npl4-type Zn^{2+}-finger domains. However, the role of these domains in CAPN15 function remains elusive.

Here, we identified CAPN15 as a non-proteasomal, ubiquitin-directed protease that regulates cell surface E-cadherin. In cultured epithelial cells, CAPN15 KO resulted in densely packed morphology accompanied by the accumulation of cell surface E-cadherin, suggesting enhanced cell adhesion. This defect was rescued by expressing WT CAPN15, but not protease-inactive or ubiquitin-binding-deficient CAPN15. Mechanistically, CAPN15 recognizes the ubiquitinated E-cadherin-catenin complex through its N-terminal Zn^{2+}-finger region and cleaves E-cadherin near its transmembrane domain. Since the truncated form is directed for lysosomal degradation, CAPN15 acts as a negative regulator of E-cadherin. E-cadherin accumulation was also observed in epithelial tissues of *Capn15* KO mice, further underscoring the physiological significance of CAPN15 in regulating E-cadherin function. Collectively, these findings reveal a previously unappreciated ubiquitin-dependent proteolytic pathway involving CAPN15 and provide important insight into the regulation of cell adhesion.

6. Caveolin-1 maintains lineage fidelity of adult neural stem cells through lysosomal degradation of PDGFRα

He Zhang, Yusuke Kihara, Mitsuho Sasaki[9], Takuya Tomita, Yasushi Saeki, Taeko Kobayashi: [9]National Institute of Biomedical Innovation, Health and Nutrition

Caveolae are cell-surface signaling hubs that play a role in stem cell proliferation and differentiation. However, the molecular mechanisms by which they regulate stem cell quiescence remain poorly understood. By proteomics screening, we revealed that most caveolae components, especially caveolin-1 (Cav-1), are specifically increased in quiescent neural stem cells (NSCs). We further demonstrated that Cav-1 inhibits platelet-derived growth factor receptor (PDGFR) α signaling, a critical regulator of NSC proliferation and oligodendrocyte differentiation in the adult brain, by promoting lysosomal degradation. Genetic ablation of Cav-1 leads to aberrant NSC activation, impaired neurogenesis, and increased ectopic oligodendrocyte differentiation at the ventricular surface of the ventral ventricular-subventricular zone (V-SVZ), an important NSC niche involved in neuroblast development. Mechanistically, we revealed that Cav-1 deficiency impairs the endo-lysosomal degradation of PDGFRα, causing PDGFRα accumulation and heightened signaling that promotes oligodendrocyte differentiation. Collectively, our results identify a key homeostatic mechanism in which caveolae act as a safeguard to maintain adult NSC quiescence and lineage fidelity by regulating lysosomal turnover of PDGFRα within a niche-specific context.

7. Integrative proteomics and AI-driven structural analysis to identify the state-specific degron landscape in neural stem cells.

Tianquan Cui, Takuya Tomita, Yasushi Saeki, Taeko Kobayashi

Precise regulation of protein degradation is essential for neural stem cells (NSCs), but the core principles remain poorly understood. To address this challenge, we established an integrated workflow to map the degron landscape of activated (aNSC) and quiescent (qNSC) mouse NSCs. We leveraged quantitative proteomics to identify short-lived proteins, applied a neural network model to predict potential degron motifs, and incorporated multi-parametric structural validation to generate a map of candidate degrons. A quantitative scoring system then prioritized these degrons based on AI-driven predictions and structural feasibility. Functional analysis revealed distinct degradation programs: short-lived proteins in aNSCs were enriched in cell division and biosynthesis pathways, whereas those in qNSCs were enriched in developmental regulation. Additionally, we identified key candidates that mechanistically exemplify these divergent roles. Overall, this work demonstrates that the NSC state is characterized by a specialized, programmed protein-degradation machinery.

Publications

Iwasa, Y., Miyata, S., Tomita, T., Yokota, N., Miyauchi, M., Mori, R., Matsushita, S., Suzuki, R., Saeki, Y., Kawahara H. TanGIBLE: A selective probe for evaluating hydrophobicity-exposed defective proteins in live cells. J. Cell Biol. 224:e202109010, 2025.

Morita, M., Takao, M., Tokuhisa, H., Chiba, R., Tomomatsu, S., Akizuki, Y., Tomita, T., Endo, A., Saeki, Y., Sato, Y. and Ohtake, F. Combinatorial ubiquitin code degrades deubiquitylation-protected substrates. Nat. Commun. 16:2496, 2025.

Okatsu, K., Kawaguchi, T., Watanabe, K., Taguchi, Y., Takeuchi, R., Okamoto, A., Iwasa, Y., Tomita, T., Saeki, Y., Sato, Y., Narumi, T. and Fukai, S. Adaptor-Specific Peptide Inhibitors of the Ubiquitin-Chain-Dependent Unfolding Activity of the Human p97(VCP)-UFD1-NPL4 Complex. J. Med. Chem. 68:11270-11278, 2025.

Asakawa, K., Tomita, T., Shioya, S., Handa, H., Saeki, Y. and Kawakami, K. Intrinsically accelerated cellular degradation is amplified by TDP-43 loss in ALS-vulnerable motor neurons in a zebrafish model. Nat. Commun. 16:9213, 2025.

Ding, Y., Tomita, T., Tsuchiya, H. and Saeki, Y. Substrate structure determines p97- and RAD23A/B-me-

diated proteasomal degradation in human cells. J. Biochem. 178:341-353, 2025.

Ozawa, A., Toriumi, K., Shimada, T., Endo, A., Tomita, T., Munesue, S., Tomita, Y., Takagi, H., Parida, I.S., Miyashita, M., Inagi, R., Itokawa, M., Yamamoto, Y., Saeki, Y. and Arai, M. Pentosidine modification of neuronal proteins induces dendritic spine enlargement in vitro. Biochem. Biophys. Res. Commun. 793:153032, 2025.

Tomomatsu, S., Matsuo, Y., Ohtake, F., Tomita, T., Saeki, Y. and Inada, T. Polyubiquitin architecture editing on collided ribosomes maintains persistent RQC activity. EMBO J. 44:6051-6077, 2025.

Taguchi, Y., Tomita, T., Nishizawa, T., Nakamura, D., Saha, S., Oyoshi, T., Sato, K., Mase, N., Saeki, Y. and Narumi, T. A Stable Bioisostere of Ester-Linked Ubiquitin Chains Enables Decoding of Protein Interactors. Chembiochem. e202500749, 2025.

Noguchi, A., Hata, S., Tsuchiya, H., Shitara, H., Saeki, Y. and Ono, Y. CAPN15 is a non-proteasomal, ubiquitin-directed calpain protease that regulates cell adhesion by cleaving E-cadherin. J. Biol. Chem. in press.

Furuhata, T., Yoshida, K., Fujita, R., Moriyama, C., Miyamoto, J., Masuda-Ozawa, T., Tsuchiya, H., Saeki, Y. and Okamoto, A. Indirect Ubiquitination Independent of Endogenous Ubiquitination Machinery for Targeted Protein Degradation. Commun. Chem. in press.