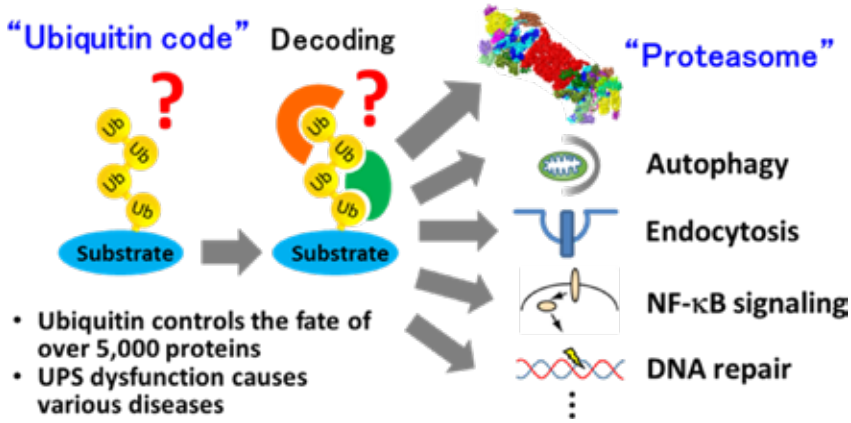
Laboratory Head **Yasushi Saeki** Protein Metabolism Laboratory

The Ubiquitin-Proteasome System "Elucidation of Fundamental and Pathophysiological Mechanisms"



The ubiquitin-proteasome system (UPS) plays a pivotal role in proteostasis and controls almost all of cellular functions by selective protein degradation. As the maintenance of protein homeostasis is essential to human health, dysfunction of the UPS due to stresses, age-associated changes, or gene mutations causes various diseases such as cancers, inflammation, and neurodegeneration. However, we do not yet know the overall principle of the ubiquitin signaling, decoding mechanism, and the proteasome. We aim to elucidate the fundamental mechanisms of the ubiquitin code as well as proteasome function and to integrate it into pathophysiology, and then to develop therapeutic strategies for UPS-related diseases.

Research Projects

1. Proteasome Dynamics and Pathophysiology

The proteasome is a highly organized proteolytic machinery that degrades ubiquitylated proteins in an ATP-dependent manner. We have characterized the structure, assembly pathway, and substrate targeting mechanism of the proteasome. We also found that the proteasome dynamically changes its intracellular localization and its accessory proteins under various stresses to restore proteostasis. Currently, we are generating knock-in mice to visualize proteasome localization and activity to analyze physiological changes of the proteasome accompanying stress and aging. Furthermore, we have generated model mice of proteasomal gene mutation derived from patients with neurodevelopmental disorders. Using the mutant mice, we will elucidate the pathophysiology of the proteasome mutation at the whole-body level.

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Ohtake F, Tsuchiya H, Saeki Y, and Tanaka K. (2018) "K63 ubiquitylation triggers proteasomal degradation by seeding branched chains." ***Proc. Natl. Acad. Sci. USA.*** 115, E1401-E1408.

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Pack Chan-Gi, Yukii H, Toh-e A, Kudo T, Tsuchiya H, Kaiho A, Sakata E, Murata S, Yokosawa H, Sako Y, Baumeister W, Tanaka K, and Saeki Y. (2014) "Quantitative live-cell imaging reveals molecular dynamics and cytoplasmic assembly of the 26S proteasome." ***Nature Commun.*** 5, 4396

Saeki Y, Toh-e A, Kudo T, Kawamura H, and Tanaka K. (2009) "Multiple proteasome-interacting proteins assist the assembly of the yeast 19S regulatory particle." ***Cell*** 137, 900-913.

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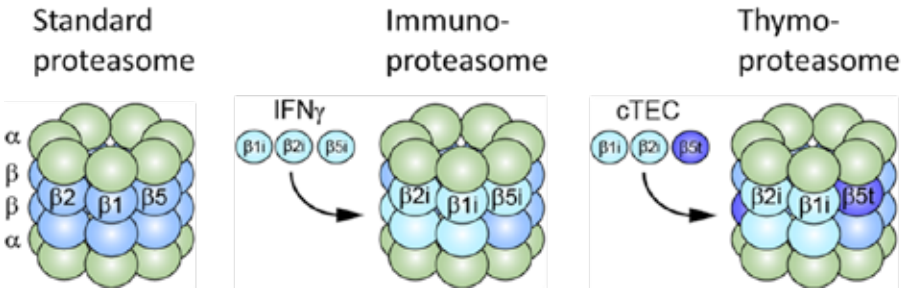
2. Roles of Specialized Proteasomes in Cell-Mediated Immunity

The proteasome has acquired diversity of the catalytic β subunits, which have evolved during the acquisition of adaptive immunity. To date, we have discovered the vertebrate-specific alternative proteasomes, which we named the "immunoproteasome" and the “thymoproteasome”. Whereas the immunoproteasome plays a specialized role as a professional antigen-processing enzyme in cell-mediated immunity, the thymoproteasome is involved in the development of CD8⁺T cells in thymus; i.e., it has a key role in the generation of MHC class I-restricted CD8⁺T cell repertoire during thymic selection called “positive selection”. Currently, we are conducting a deep proteomic screen to validate the positive selection model.



Keiji Tanaka
(The chairperson of TMIMS)

Murata S, Takahama Y, Kasahara M, and Tanaka K. (2018) “The immunoproteasome and thymoproteasome: functions, evolution and human disease.” ***Nature Immunol.*** 19, 923-931.

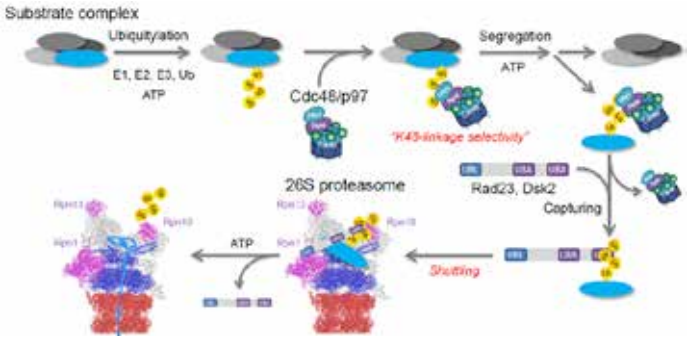
Murata S, Sasaki K, Kishimoto T, Niwa S, Hayashi H, Takahama Y, and Tanaka K. (2007) “Regulation of CD8⁺ T cell development by thymus-specific proteasomes.” ***Science*** 316, 1349-1353.

3. Deciphering the Ubiquitin Code

Different polyubiquitin chain linkages direct substrates to distinct pathways, as referred to as ‘ubiquitin code’. We have developed a highly sensitive MS/MS-based quantification method for ubiquitin chains. The method allows us to analyze linkage-type selectivity of ubiquitin decoder proteins at endogenous experimental setting. We recently identified the main pathway targeting the K48-linked ubiquitylated substrates for proteasomal degradation. We also identified more complexed ubiquitin chains branched at K48 and K63, which act as a unique coding signal to enhance NF-κB signaling. We are further analyzing the decoder proteins throughout the ubiquitin-mediated pathways to reveal the ubiquitin network.

Fumiaki Ohtake

Hikaru Tsuchiya



Members

Keiji Tanaka	Ai Kaiho
Yasushi Saeki	Naoko Arai
Fumiaki Ohtake	Yuko Okamoto
Hikaru Tsuchiya	Arisa Kawano
Sayaka Yasuda	Marcel Diallo

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