論文 / 著書情報
Article / Book Information

題目(和文)	
Title(English)	Mechanisms of ubiquitination-dependent lysosomal traffic of monomeric and oligomeric plasma membrane proteins
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出典(和文)	学位:博士(理学), 学位授与機関:東京工業大学, 報告番号:甲第10267号, 授与年月日:2016年6月30日, 学位の種別:課程博士, 審査員:駒田 雅之,岩崎 博史,太田 啓之,中戸川 仁,中村 信大
Citation(English)	Degree:Doctor (Science), Conferring organization: Tokyo Institute of Technology, Report number:甲第10267号, Conferred date:2016/6/30, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	Biological Sciences 専攻	申請学位（専攻分野）： Academic Degree Requested	博士 （ Science ） Doctor of
学生氏名： Student's Name	Daocharad Burana	指導教員（主）： Academic Advisor(main)	Prof. Masayuki Komada
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Ubiquitination is a post-translational modification in which a 76-amino-acid protein called ubiquitin (Ub) is covalently conjugated to the Lys residue(s) of various intracellular proteins. Ub conjugation of substrate proteins regulates their degradation, activity, and trafficking. Ubiquitination is a reversible reaction where deubiquitinases (DUBs) remove Ub from substrates and counteract Ub-dependent protein regulation. Conjugation of a single Ub moiety to Lys residue(s) of the substrate is known as monoubiquitination, which modulates protein activity and endocytosis. The Ub molecule carries 7 internal Lys residues that are capable of forming poly-Ub chains on the substrates. While Lys48-linked polyubiquitination is responsible for proteasomal degradation of the substrates, substrates that are conjugated with Lys63-linked Ub chains are targeted for endocytosis and lysosomal degradation.

Cells regulate the composition of plasma membrane proteins and lipids, as well as responses to various extracellular stimuli, through the process called endocytosis. The mechanism involves compartmentalization of cell surface molecules on particular microdomains of the plasma membrane, their incorporation into the endocytic vesicle, and their delivery to the lysosome for degradation via early and late endosomes. Endocytosis of plasma membrane proteins is triggered by ubiquitination. For example, epidermal growth factor receptor (EGFR), a monomeric or dimeric plasma membrane protein, is ubiquitinated upon ligand binding and is recognized by a series of Ub-binding proteins that incorporate EGFR into the endocytic vesicle. On the endosomal membrane, ubiquitinated EGFR is trapped by the Ub-binding protein complexes, ESCRTs, which prevent EGFR from recycling back to the plasma membrane, incorporate it into the intraluminal vesicle of the multivesicular body (MVB)/late endosome, and eventually transport it to the lysosome for degradation. VCP/p97 is an AAA-type ATPase that utilizes the energy from ATP hydrolysis to segregate the complexes of ubiquitinated proteins. The role of VCP has been highlighted in a myriad of cellular activities such as endoplasmic reticulum-associated degradation. Intriguingly, lysosomal traffic of caveolin-1 (Cav-1), an oligomeric transmembrane protein comprising caveora on the plasma membrane, has been shown to require VCP to facilitate the segregation of endocytosed Cav-1 oligomer on the endosomal membrane.

The first half of this thesis describes a novel interaction of VCP with an endosomal Ub-binding protein family, Ankrd13. I demonstrated that the binding of Ankrd13 proteins to VCP depends on their Ub-binding domain; the Ub-interacting motifs (UIMs). Ankrd13 proteins bound indirectly to VCP, and the interaction was mediated by ubiquitinated protein(s). Overexpression of Ankrd13 proteins led to an enlargement of hallow late endosomes, implicating an impaired lysosomal trafficking caused by the inhibition of MVB formation. VCP co-localized with Ankrd13 proteins and ubiquitinated proteins on the peripheral membrane of the enlarged late endosome. In addition, Ankrd13 proteins bound to Cav-1, which is an endosomal substrate of VCP, through their UIMs, with a higher binding affinity to ubiquitinated oligomeric form of Cav-1. Mass spectrometric analysis showed that Cav-1 is primarily decorated with Lys63-linked poly-Ub chains and that the UIMs of Ankrd13 proteins bind preferentially to this Ub chain type. Ankrd13-VCP-Cav-1 formed a ternary complex in the cell, and overexpression of Ankrd13 proteins stabilized ubiquitinated Cav-1 oligomers on the limiting membrane of the enlarged endosomes. Genetic mutations in VCP compromise the lysosomal trafficking of Cav-1, leading to a disease called inclusion body myopathy, Paget disease of the bone and frontotemporal dementia (IBMPFD). Fibroblasts taken from the patients with IBMPFD exhibit enlarged late endosomes which are reminiscent of those in Ankrd13-overexpressing cells. The interaction with Ankrd13 and Cav-1 was abrogated in the IMBPFD-associated VCP mutants, implicating that the failure of the VCP mutants to form the Ankrd13-VCP-Cav-1 complex is causative to IBMPFD. Collectively, these findings suggest that Ankrd13 proteins cooperate with VCP to facilitate the lysosomal trafficking of ubiquitinated Cav-1.

In the second half of the thesis, the mode of Ub conjugation of EGFR was studied. Although the roles of mono- and Lys63-linked poly-ubiquitination are well recognized in lysosomal trafficking of EGFR, mass spectrometric analysis revealed that EGFR is also modified with Lys48-, Lys11-, and Lys6-linked poly-Ub chains. In vitro deubiquitination assay using Ub linkage-specific DUBs suggested that each Ub linkage is mostly formed independently. In addition, I developed a novel method to determine the length of substrate-conjugated poly-Ub chains using EGFR as a prototypic model. The Ub protection from trypsin digestion (Ub-ProT) approach utilizes trypsin-resistant tandem Ub-binding entities (TR-TUBE) to protect poly-Ub chains from trypsin digestion, proteases, and DUBs, facilitating the analysis of substrate-bound poly-Ub chains by SDS-PAGE. I found that EGFR is conjugated with a substantial amount of mono-Ub and Lys63-linked poly-Ub chain with 4-6 Ub moieties and that EGFR continuously undergo ubiquitination at multiple sites along the way from the cell surface to the endosome. These findings indicate that the endocytosis of EGFR is modulated by mono-Ub and long poly-Ub chains with Lys63, Lys48, as well as atypical linkages.

In conclusion, this study provides a better understanding on ubiquitination-dependent sorting and trafficking of monomeric and oligomeric plasma membrane proteins for lysosomal degradation.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).