氏　　名	崔　松岿 (CUI Songkui)
学位(専攻分野)	博士(理学)
学位記番号	総研大甲第1610号
学位授与の日付	平成25年3月22日
学位授与の要件	生命科学研究科　基礎生物学専攻 学位規則第6条第1項該当
学位論文題目	Proteomic identification and characterization of novel factors involved in peroxisomal biogenesis
論文審査委員	主　査　教授　長谷部　光泰 教授　西村　幹夫 教授　皆川　純 教授　三ツ井　敏明　新潟大学

論文内容の要旨

Peroxisomes are single membrane bound organelles that are found in virtually all eukaryotic cells. In flowering plants, they are highly dynamic and their functions change upon developmental stages and environment. During postgerminative growth, peroxisomes are termed as glyoxysomes in the cotyledons of storage organs, and contain enzymes for fatty acid β-oxidation and the glyoxylate cycle playing essential roles in the conversion of seed reserve lipids into sucrose. After greening, peroxisomes undergo transition from glyoxysomes to leaf peroxisomes accompanied by gaining the enzymes for photorespiration. The reverse process of transformation from leaf peroxisomes to glyoxysomes occurs in senescing cotyledons during late stages of plant growth.

Because peroxisomes lack their own genome, all of their constituent proteins are encoded in the nuclear genome and translated on cytosolic ribosomes before being imported into peroxisomes. The transport of peroxisomal matrix and membrane proteins is mediated by various PEX proteins. PEX7 is a cytosolic receptor for proteins containing peroxisomal signal type2 (PTS2). PEX16 is a peroxisomal membrane protein that controls the peroxisomal morphology as well as the balance between oil and starch in seeds. In yeast and mammals, PEX16 is also involved in the biogenesis of peroxisomes from endoplasmic reticulum (ER). Both PEX7 and PEX16 are essential for seed maturation in plants because each loss-of-function mutation causes embryonic lethal in seeds. Despite their pivotal roles for peroxisomal biogenesis, their regulatory mechanisms on peroxisomal biogenesis are not clear at the molecular level. To understand the molecular mechanism of PEX7 translocation into and out of peroxisomes and of PEX16-mediated peroxisomal membrane regulation, the author identified the binding partners of PEX7 and PEX16 and characterized their functions.

Chapter 1 describes the identification of binding partners of PEX7 and PEX16 using proteomic analysis. *Green fluorescent protein* (*GFP*) gene was fused to each gene and introduced into *Arabidopsis thaliana* to generate the GFP-PEX7 and GFP-PEX16 transgenic plants. Fusion proteins were purified with their potential binding partners from transgenic plants with antibody against GFP and applied to mass spectrometry analysis. Of many proteins, 14 and 2 proteins were specifically identified with PEX7 and PEX16, respectively.

Chapter 2 describes the binding proteins of PEX7 including functional characterization of RabE1c as one of PEX7-binding proteins. Of 14 proteins, PEX5, which is a known binding protein of PEX7 in the cytosol was identified, suggesting

the successful isolation of PEX7 complex. All subunits of T-complex, which is a cytosolic chaperonin involved in ATP-dependent protein folding, was also identified with PEX7. In mammals and yeast, T-complex is known to interact with WD40 repeats. The middle region of PEX7 consists of WD40 repeat motif, therefore the result suggests that T-complex may recognize WD40 repeat motif of PEX7 in Arabidopsis. A small GTPase family protein, RabE1c, was also identified as a protein of PEX7 complex. *In vivo* experiments showed that GTP-bound but not GDP-bound form of RabE1c interacted with PEX7 on peroxisomes. Some population of RabE1c localizes peroxisome. The data indicate that active form of RabE1c interacts with PEX7 on the peroxisomes. Unlike endogenous PEX7 localizes mainly in the cytosol, GFP-PEX7 is abnormally accumulated on peroxisomal membrane and induces degradation of endogenous PEX7, along with the reduction of PTS2 protein import and peroxisomal β-oxidation in the GFP-PEX7 seedlings. This suggests that GFPPEX7 on peroxisomal membrane has a dominant negative effect by leading endogenous PEX7 degradation. The mutation in RabE1c in the GFP-PEX7 seedlings recovered endogenous PEX7 protein level and PTS2-protein import as well as peroxisomal β-oxidation. In addition, the treatment of proteasome inhibitors recovered the endogenous PEX7 protein level in the GFP-PEX7 seedlings. The author concluded that RabE1c binds PEX7 to facilitate PEX7 degradation rather than recycle back to the cytosol in the presence of immobile, membrane accumulated GFP-PEX7.
Chapter 3 describes two novel proteins that bind to PEX16. PEX16 was previously shown to regulate peroxiosmal morphology and positioning in Arabidopsis, based on the facts that suppression of PEX16 by RNAi induces enlarged but fewer peroxisomes and overexpression of GFP-PEX16 results in aggregation of peroxisomes, respectively, comparing to wild-type. In this chapter, PEX16-interacting proteins, termed PEX16-binding protein 1 (PBP1) and PBP2, were analyzed by their localization and mutant phenotype. PBP1, which is localized in the cytosol and tubular structure, was recruited to the aggregated peroxisomes when PEX16 was coexpressed, suggesting that PEX16 recruits PBP1 to peroxisomes. However, the mutant analysis in Arabidopsis showed that the peroxisomal aggregation induced by GFP-PEX16 overexpression was not altered in *pbp1* mutant. The *pbp1* mutant had no effect on the peroxisomal morphology based on the observation of peroxisomes by GFP-PTS1 overexpression. The absence of the effect on peroxisomal morphology in *pbp1* mutant is probably due to the protein redundancy as there is a homolog that has a conserved domain in the C-terminus. The *pbp2* mutant was lethal during the stage of seed production as well as the *pex16* knockout mutant, raising the possibility that

PBP2 has similar function as PEX16 toward peroxisomal biogenesis during seed development.

Taken together, this study makes an advance in our understanding of the molecular mechanisms of PEX7 and PEX16 on the regulation of peroxisomal biogenesis, by providing the information on their associating proteins. With the advantage of short manipulation time, this proteomic study can be applied to other peroxisomal proteins and thus helpful to clarify their molecular mechanisms toward peroxisomal biogenesis in plants.

植物において，ペルオキシソームは，脂肪酸のβ酸化や光合成の光呼吸に必要なオルガネラであり，植物の生育にともなってその機能を大きく変える。ペルオキシソームは一重膜で囲まれたオルガネラであり，植物においてその形成や機能維持の過程には不明な点が多い。申請者は，ペルオキシソームのタンパク質輸送，または膜形成に関わる因子等に着目し，それらの因子と相互作用する新規因子を見いだすことにより，ペルオキシソームの形成と機能維持の分子機構の解明を目標として研究を行った.

申請者はまず，ペルオキソームタンパク質の輸送レセプター，PEX7 に着目し，GFP-PEX7 融合タンパク質を発現させたシロイヌナズナを材料にして，PEX7 と結合する因子をプロテオーム解析により調べ，14 個のタンパク質を同定した。これらのタンパク質にはサイトゾルのシャペロンである T-complex のすべてのサブユニットや，植物においてPEX7 と結合することが既に知られているペルオキシソームタンパク質輸送レセプター，PEX5 が含まれていた。興味深いことに，シロイヌナズナの低分子 GTPase の一つである RabE1c を同定した. RabE1c は細胞礎質やゴルジと思われるオルガネラ以外に，ペルオキシソームにも局在することを示した。一方，PEX7 と RabE1c の複合体はペルオキシソーム膜に局在することを見いだし，RabE1c がペルオキシソーム膜上で PEX7 の機能維持に関わると考えられた。GFP-PEX7 融合タンパク質を発現させたシロイヌナズナでは，β酸化の活性やペルオキシソームタンパク質の輸送が低下する．この植物では GFP-PEX7 融合タンパク質がペルオキシソーム膜に過剰に蓄積していること，内在性の PEX7 の量が減少していることを発見した。この結果は，GFP-PEX7 融合タンパク質の過剰蓄積によるストレスが引き金となり，内在性の PEX7 が分解されると考えられた．GFP-PEX7 融合タンパク質を発現させたシロイヌナズナから，RabE1c を欠損させた植物を作成したところ，この欠損株では，β酸化の活性や，ペルオキシソームタンパク質の輸送は野生株と同じレベルにまで復帰し，内在性の PEX7 の分解は抑えられていた．このことから，RabE1c は PEX7 の機能維持に重要であり，ストレスによる PEX7 の分解に関わることを明らかにした。

次に，ペルオキシソーム膜形成に関わると考えられる PEX16 に着目し，GFP-PEX16 融合タンパク質を発現させたシロイヌナズナを材料にして，PEX16 と結合する因子をプロテオーム解析により調べ，2 個のタンパク質，PEX16-BINDING PROTEIN 1 (PBP1)，PBP2 を同定し，PBP1 や PBP2 が PEX16 と協調してペルオキシソームの膜形成に関わる可能性を示した。

以上のように，申請者の研究から，ペルオキシソームの形成，機能維持に関わるタンパク質因子の理解が深まり，その全貌が明らかになりつつあることが示された．特に，今まで明らかにされてこなかった輸送レセプターのメンテナンスに関わる因子が初めて同定されたこと，さらに，その因子が従来は小胞輸送に関わると考えられてい

た因子であったことなどは驚くべき発見であり，研究成果は高く評価できる。これらの内容をふまえ，審議の結果，本研究は博士号授与にふさわしいと判断した。