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学 位 論 文 題 目　Physiological Significance of Autophagy in Protein Turnover

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論文内容の要旨

Cellular activities require the maintenance of a balance between the synthesis and degradation of proteins. Macroautophagy (hereafter referred to as autophagy) is an intracellular bulk degradation system, which is well conserved in eukaryotes; autophagy transports cytoplasmic components to the lysosome/vacuole for degradation. This degradation is a cellular response to starvation and also plays a role in the recycling of cytoplasmic components, which may be important for cellular remodeling, development and differentiation. A total of 16 genes, which are essential for autophagy, and which are named *APG* and *AUT* (current nomenclature is *ATG*), have been identified by genetic screens in the yeast, *Saccharomyces cerevisiae*. Much progress has been made in the functional analysis of these genes.

Autophagy is initiated by the sequestration of cytoplasmic components in a double-membrane structure termed the autophagosome. Immuno-electron microscopy has shown that ribosomes and typical cytosolic marker enzymes are present in the autophagosome and autophagic bodies at the same densities as in the cytosol. It is indicated that autophagy is a nonselective degradation. If degradation of long-lived proteins is exclusively mediated by autophagy, all proteins might be expected to have similar lifetimes. However, long-lived proteins have a variety of lifetimes; therefore, the autophagic pathway might have some selectivity.

To investigate the possibility of selective autophagic degradation, he attempted to compare the amounts of each intracellular protein under growth and starvation conditions in the yeast, *S. cerevisiae*. He performed a systematic analysis using two-dimensional PAGE and MALDI-TOF mass spectrometry to detect the autophagy dependent degradation of intracellular proteins. During this analysis, he detected that the Mg^{2+}- and NADPH-dependent cytosolic acetaldehyde dehydrogenase (Ald6p) decreased under nitrogen starvation. This enzyme catalyzes the conversion of acetaldehyde to acetate in the cytosol (acetaldehyde + $NADP^+$ → acetate + NADPH). As assessed by immuno-blot, Ald6p was reduced by greater than 82% after 24 h of nitrogen starvation. This reduction was dependent on Atg/Apg proteins and vacuolar proteases, but was not dependent on the proteasome degradation, the Cvt pathway or the Vid protein.

He hypothesized that the decrease in Ald6p levels was the result of degradation during nitrogen starvation. To examine this possibility, the kinetics of Ald6p degradation was measured by pulse-chase experiments, which suggest that Ald6p is degraded much more rapidly than typical cytosolic proteins. Ald6p was visualized by Ald6p-GFP fusion protein and immuno-electron microscopy analyses. In Δ*pep4* vacuolar proteinase deficient cells, Ald6p or Ald6p-GFP was localized in autophagic bodies in the vacuole under nitrogen starvation. These results indicate that Ald6p is degraded in the vacuole under nitrogen starvation. Furthermore, using subcellular fractionation and pulse-chase experiments, he also demonstrated that Ald6p was preferentially transported to the vacuole via autophagosome.

To address the physiological significance of this preferential degradation, he analyzed cells of Ald6p over-producer and its disruptant. Δ*atg7* Δ*ald6* double mutant cells were able to maintain higher rates of viability than Δ*atg7* cells under nitrogen starvation, and *ALD6*

overexpressing cells were not able to maintain high rates of viability. Furthermore, the Ald6p^{C306S} mutant, which lacks enzymatic activity, had viability rates similar to Δ*ald6* cells. Ald6p enzymatic activity may be disadvantageous for survival under nitrogen starvation; therefore, yeast cells may preferentially eliminate Ald6p via autophagy.

These results show that Ald6p is one example of a preferential substrate for autophagic degradation. Ald6p was the only major protein on the two-dimensional PAGE gel to decrease during starvation; however, it is still possible that other minor proteins behave like Ald6p. If further studies were able to find such proteins, it would help clarify the molecular mechanisms of selective autophagy and the physiological significance of the preferential degradation.

He also found several specific proteins are induced under nitrogen starvation on the above-mentioned screening using two-dimensional PAGE. These proteins included typical proteins of environment stress responses (Eno1p/Hsp48p and Hsp26p), enzyme of amino acid biosynthesis (Arg1p), quenching enzyme of reactive oxygen species (Sod2p) and so on. These proteins did not increase in Δ*atg7* mutant cells; however, their mRNA levels were high as wild-type cells under nitrogen starvation. Thus, it is possible that these proteins synthesis are inhibited in the translational step.

It is generally thought that autophagic protein degradation supplies significant amounts of free amino acids under nitrogen starvation. From this perspective, he quantified the free amino acids in yeast cells. Wild-type cells could maintain the constant level of amino acids pool during nitrogen starvation, while Δ*atg7* cells depleted free amino acids after a few hours starvation. This result may indicate that Δ*atg7* cells cannot keep free amino acids enough to synthesize starvation-induced proteins. To ensure this consequence, he assessed *in vivo* protein synthesis using [^{14}C]valine; protein synthesis of Δ*atg7* cells was even lower level than that of wild-type cells after 6 h starvation. However, when nitrogen starved cells fed free amino acids beforehand, protein synthesis of Δ*atg7* cells was high level as well as that of wild-type cells. These results suggest that the pool size of free amino acids should limit the protein synthesis.

It is often presumed that the ubiquitin-proteasome degradation is the most important for protein turnover in all phase. In this study, he showed the direct evidence that autophagy is essential for protein turnover and formation of amino acids pool under nitrogen starvation condition.

論文審査結果の要旨

オートファジーは、真核細胞に普遍的なバルクなタンパク質分解を担う生理現象であり、その機構の解明は細胞を理解する上で必須である。オートファジーの最も重要な段階であるオートファゴソーム形成に係る分子群が明らかになり、新奇の膜新生機構としても注目されている。オートファジーをめぐる重要な研究課題の一つは、オートファジーにより分解される分子、つまりオートファジーの基質についての問題である。従来、生化学的手法、および免疫電顕解析による形態学的手法により、細胞質酵素を指標としてオートファジーによる分解は非選択的であることが示されてきた。しかしながら近年、オートファジーに類似した細胞質から液胞への選択的なタンパク質の輸送経路が明らかになり、オートファジーの基質特異性についての詳細な検討が必要であると考えられる。

申請者である小野寺純は、出芽酵母の可溶性タンパク質について、２次元電気泳動法による大規模な分離系を確立した。この系を利用して、細胞質型アセトアルデヒド脱水素酵素である Ald6p が、窒素飢餓条件下にオートファジーによって顕著にその存在量が減少することを見出した。Ald6p はオートファゴソームを介して液胞に輸送された後に分解されていること、また優先的にオートファゴソームに隔離されることを示した。さらに、窒素飢餓条件における生存率の解析から、Ald6p の酵素反応に伴う NADPH の生成が飢餓条件下の生存に不利となるモデルを提唱し、窒素飢餓条件において Ald6p をオートファジーにより優先的に分解する現象に対する理由を考察した。これらの研究成果は本年２月４日付の *J. Biol. Chem.* 電子版に発表された。

後半の研究でオートファジーによるタンパク質分解の生理学的な重要性についてのさらなる解析を進めた。上記の２次元電気泳動法による解析から、オートファジー機能欠損株では、一群の飢餓誘導タンパク質が増加しないことを見出した。その理由を解析する過程でオートファジー欠損株は、そのバルクなタンパク質分解の欠失から、窒素飢餓条件下では遊離アミノ酸プールを維持できないことを示し、タンパク質合成が進行しないことを実証した。これらの結果から、オートファジーは窒素飢餓条件下での細胞内のアミノ酸プールの維持に必須であり、窒素飢餓条件での細胞の生存を支えていることを明らかにした点で高く評価される。

以上の論文は博士論文として十分な内容を有していると審査員全員一致で判断した。

提出された博士学位論文の研究成果について口頭発表をさせた後、審査委員による論文内容、およびその背景となる基礎知識について口頭試問を行った。発表された研究は、独創的であり、かつ論理的であり、得られた結果はその結論を導くのに十分なものであり、審査委員の質問に対する応答も、的確なものであった。提出された約 100 ページにわたる学位論文は英語で書かれていること、また申請者は欧文学術誌に論文を発表しており、英語に関する能力についても十分であると判断された。これらの結果をもとに、審査委員会は申請者の持つ研究能力および学力は、博士の学位に値すると判断した。