生物医学研究技术丛书

泛素介导的蛋白质降解

主编/邱小波　王琛　王琳芳

中国协和医科大学出版社

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Ubiquitin – Mediated Proteolysis

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序

一百多年来，蛋白质合成的研究受到了广泛重视并取得丰硕的成果，已获得至少五次诺贝尔奖。然而，在很长时间内蛋白质降解的研究并未引起足够重视；直到 2004 年 Ciechanover、Hershko 和 Rose 因发现泛素介导的蛋白质降解而获得诺贝尔奖之后，这一情形才有明显改观。泛素介导的蛋白质降解通路是基因和蛋白质功能的主要调节者和终结者，控制着真核细胞内绝大多数蛋白质的降解。该通路调控着几乎所有动植物的生命活动，包括细胞增殖、分化、凋亡、DNA 修复、转录和蛋白质质量控制等，并参与病原体的入侵、致病和人体的免疫应答等过程。它的异常会导致癌症和神经退行性疾病等人类多种重大疾病。一种该通路的特异性抑制剂 Velcade/PS－341 已于 2003 年成功地用于治疗多发性骨髓瘤。近十年来，与泛素化修饰方式类似的一系列蛋白质类泛素化修饰（包括 SUMO、ISG15、NEDD 等）被发现，它们的生物学意义涉及细胞生命活动的各个方面。总之，该领域已成为现代生命科学研究的热点之一。我国在此领域虽然起步较晚，但正呈现蓬勃发展之势。

作为泛素介导的蛋白质降解领域的第一本中文专著，该书较为系统和深入地介绍了泛素介导的蛋白质降解的基本理论及相关实验技术。在全面介绍本领域研究进展的同时，也在一定程度上反映了我国科学家所取得的相关研究成果。该书的编者几乎都是国内活跃在第一线并在该领域奋斗多年的科学工作者。相信它的出版将会促进我国在这一关键领域的研究，并将惠及整个生命科学的发展。

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Introduction

The idea that proteins in both prokaryotes and eukaryotes are synthesized and destroyed – many rather extensively – is hardly 70 years old. Even along most of this period, scientists focused mostly on translation of the genetic code into proteins: how proteins are removed had remained a neglected area, regarded by many as non – specific, end process of small biological importance. Beforehand, proteins were thought to be essentially stable constituents that were subject only to minor 'wear and tear'. Accordingly, dietary proteins were believed to function primarily as a source of energy, and their metabolism was independent from that of the structural and functional proteins of the body. The concept that the body proteins are static and the dietary proteins are used mostly as a fuel was challenged by Rudolf Schoenheimer who worked at Columbia University in New York City. Schoenheimer, a Jewish scientist who escaped racial Germany, administered ^{15}N – labeled tyrosine to rat and found that a large part of it "*is deposited in tissue proteins*". Later "*an equivalent of protein nitrogen is excreted*'". This and additional experiments carried out by Schoenheimer demonstrated unequivocally that the body structural proteins are in a dynamic state of synthesis and degradation. After his tragic death, his findings and lectures were published (1942) in a small book called "**The Dynamic State of Body Constituents**". In the book, the new hypothesis is clearly presented: "*The simile of the combustion engine pictured the steady state flow of fuel into a fixed system, and the conversion of this fuel into waste products. The new results imply that not only the fuel, but the*

structural materials are in a steady state of flux. The classical picture must thus be replaced by one which takes account of the dynamic state of body structure".

The idea that proteins are turning over was not accepted easily by the scientific community and was challenged as late as the mid1950s. At that time, however, scientists started to change their view which was mostly due to two main findings. First and foremost was the discovery of the lysosome by Christina de Duve in the early 1950s which was a turning point in studies on protein degradation. At that time several independent experiments had already substantiated the notion that cellular proteins are in a constant state of synthesis and degradation, and thus the concomitant discovery of an organelle that contains a broad array of membrane – secluded proteases with different specificities provided, for the first time, an organelle and mechanism that could potentially mediate intracellular proteolysis. The fact that the proteases were separated from their substrates by a membrane provided an explanation for controlled degradation, and the only problem left to be explained was how the substrates are translocated into the lysosomal lumen where they are degraded by the lysosomal proteases. An important discovery in this respect was the unraveling of the mechanism of action of the lysosome under basal conditions – microautophagy: during this process small portions of the cytoplasm (which contain the entire cohort of cellular proteins) are captured in vesicles and tubules that are formed by intraluminal invagination of the endosomal or lysosomal. The contents of these vesicles are digested as the vesicles are consumed by the lysosome. The second discovery was that intracellular proteolysis in both bacterial (Mandelstam, 1958) and mammalian (Simpson, 1953) cells requires metabolic energy. Since proteolysis is thermodynamically exergonic, the energy requirement suggested that the underlying mechanisms must be more complex than simple hydrolysis of peptide bonds, and the energy is required in order to allow control and endow the systems involved with specifici-

ty towards their substrates.

However, over a period of more than two decades, between the mid 1950s and the late 1970s, it has gradually become more and more difficult to explain several aspects of intracellular protein degradation based on the known mechanisms of lysosomal activity: accumulating lines of independent experimental evidence indicated that the degradation of at least certain classes of cellular proteins must be non – lysosomal. Yet, in the absence of any 'alternative' mechanism, researchers came with different hypotheses and experiments, some more substantiated and others much less so, to defend the 'lysosomal' hypothesis.

First was the gradual discovery that different proteins vary in their stability, and their half life times can span three orders of magnitude, from a few minutes to many days. Also, rates of degradation of many proteins were shown to alter with changing physiological conditions, such as availability of nutrients or hormones. It was conceptually difficult to reconcile the findings of distinct and changing half lives of different proteins with the mechanism of action of the lysosome, where the autophagic vesicle contains the entire cohort of cellular proteins that are therefore expected to be degraded at the same rate. Another source of concern about the lysosome as the organelle that carries out proteolysis of intracellular proteins under basal conditions were the findings that specific and general inhibitors of lysosomal proteases had different effects on different populations of proteins, making it clear that distinct classes of cellular proteins are targeted by different proteolytic machineries. Thus, the degradation of endocytosed/pinocytosed extracellular proteins was significantly inhibited, a partial effect was observed on the degradation of long – lived cellular proteins, and short – lived and abnormal/mutated cellular proteins were not affected almost at all by the inhibitors. Interestingly, lysosomal degradation was influenced by changing physiological conditions,

where under stress more cellular proteins were shown to be targeted to the lysosome. Finally, the thermodynamically paradoxical observation that the degradation of cellular proteins requires metabolic energy, and more importantly, the emerging evidence that the proteolytic machinery uses the energy directly, were in contrast with the known mode of action of lysosomal proteases, that under the appropriate acidic conditions and similar to all known proteases, degrade proteins in an exergonic manner. Brian Poole from the Rockefeller University in New York summarized these (1977) some of these concerns in a most poetic manner, arguing that the lysosome is involved mostly in degradation of extracellular proteins, while intracellular proteins are degraded by an as yet to be discovered system: "*The exogenous proteins will be broken down in the lysosomes, while the endogenous proteins will be broken down wherever it is that endogenous proteins are broken down during protein turnover*".

Progress in identifying the elusive, non – lysosomal proteolytic system (s) was hampered by the lack of a cell – free preparation that could faithfully replicate the cellular proteolytic events – degrading proteins in a specific and energy – requiring mode. An important breakthrough was made by Rabinovitz and Fisher who found (1964) that rabbit reticulocytes degrade abnormal, amino acid analogue – containing hemoglobin. Their experiments modeled known disease states – hemoglobinopathies – where mutated hemoglobin chains or excess of unassembled normal hemoglobin chains are rapidly degraded. Reticulocytes are terminally differentiating young red blood cells that do not contain lysosomes, and it was postulated that the degradation is mediated by a non – lysosomal machinery. Etlinger and Goldberg (1977) were the first to establish and characterize a cell – free and energy dependent proteolytic preparation from reticulocytes. The crude extract selectively degraded abnormal hemoglobin, required ATP hydrolysis, and acted optimally at a neutral pH, which further corroborated the assumption that the proteolytic activity was of a

non – lysosomal origin. Yet, the underlying mechanism had not been elucidated. A similar system was isolated and characterized later by Hershko, Ciechanover, and their colleagues (1978). Additional studies by this group and by Irwin Rose (1978 ~ 1983) led subsequently to resolution, characterization, and purification of the major enzymatic components of the system and to the discovery of the ubiquitin signaling system. Degradation of a protein by the ubiquitin system as we currently know it proceeds via two successive steps: (i) covalent attachment of multiple ubiquitin moieties to the substrate, and (ii) degradation of the tagged substrate by the 26S proteasome, followed by release of free and reusable ubiquitin.

We now recognize that ubiquitin – mediated degradation of intracellular proteins is involved in regulation of a broad array of cellular processes, such as cell cycle and division, regulation of transcription factors, and assurance of the cellular quality control. It was later discovered that certain modifications by ubiquitin as well as by the newly discovered family of ubiquitin – like proteins, serve numerous non – proteolytic functions which has broadened the scope of this novel type of post – translational modification well beyond targeting of proteins for destruction. Not surprisingly, aberrations in the system have been implicated in the pathogenesis of human disease, such as malignancies and inflammatory and neurodegenerative disorders, which led subsequently to the development of the first mechanism – based drug, with an expectation for development of many more.

The discovery of the ubiquitin system has added another layer to already known regulatory mechanisms, thus paving the road to the unraveling of numerous novel cellular pathways and explaining the mechanisms that underlie many others. Conceptually, it has divided regulatory mechanisms to those that act in a reversible manner (phosphorylation, for example) and those that act in an irreversible manner (proteolysis), and set the stage for a discussion

on the evolutionary mechanisms and the necessity of such diverse mechanisms. Thus, the discovery of ubiquitin signaling and evolvement of proteolysis as a centrally important regulatory platform is a remarkable example for the evolution of a novel biological concept and the accompanying battles to change paradigms.

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前 言

蛋白质是生物体内细胞和组织的基本构成物质，它还构成机体中参与调节各种细胞和生理活动的生命活性物质，包括催化体内各种生物化学反应的酶，调节机体生长、发育并行使正常生理功能的激素，抵御外来细菌病毒的抗体及免疫类物质，运载许多重要代谢物质、营养素的载体。当机体需要时，蛋白质还可以被代谢分解，释放出能量。几乎所有的蛋白质都处于不断的合成与分解的动态之中，细胞内蛋白质半衰期的差别很大，从几分钟到几年。

泛素介导的蛋白质降解通路控制着真核细胞内绝大多数蛋白质的选择性降解，是基因和蛋白质功能的主要调节者和终结者，并掌控着几乎所有动植物的生命活动。它的异常会导致癌症或神经退行性疾病等多种人类重大疾病的发生。因此，对该通路的调控已成为疾病治疗中的一种创新性方法。2003 年，一种蛋白质降解的特异性抑制剂 Velcade/PS - 341 已成功地应用于治疗多发性骨髓瘤。

虽然国外已有多本外文专著介绍泛素介导的蛋白质降解，但国内尚无同类中文书籍出版，很难满足我国读者的需要。本书系统而深入地介绍该领域的基本理论、实验技术及应用前景，共分八章：①蛋白质降解概论；②泛素、类泛素、泛素化及类泛素化；③泛素激活酶；④泛素载体蛋白；⑤泛素连接酶；⑥去泛素化及去泛素化酶；⑦蛋白酶体；⑧泛素 - 蛋白酶体通路与药物开发。除第一章外，每章包括基本理论和实验技术两个部分。本书可作为从事生命科学研究的研究生、科研人员，以及从事药物或相关产品研发的企业人员的参考书，也可作为有志从事生命科学研究的本、专科学生（包括理、工、农、林、医、环境等相关学科）的课外读物。

本书主要由10位从事泛素介导的蛋白质降解研究的专家合作编写。卫生部部长陈竺院士和因发现泛素化而获2004年诺贝尔化学奖的Aaron Ciechanover教授欣然为本书撰写序和引言。日本东京都临床医学综合研究所的田中启二教授为本书提供了许多珍贵的资料。另外，在本书编写过程中，我们还得到了沈岩院士、蒋澄宇教授及其他老师和学生的支持和帮助。在此一并表示衷心的感谢。

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