Biochimica et Biophysica Acta 1824 (2012) 3–13

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbapap

Review

Intracellular protein degradation: From a vague idea thru the lysosome and the ubiquitin–proteasome system and onto human diseases and drug targeting☆

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ARTICLE INFO

Article history:
Received 5 February 2011
Received in revised form 11 March 2011
Accepted 14 March 2011
Available online 22 March 2011

Keywords:
Proteolysis
Lysosome
Ubiquitin
Proteasome
Diseases

ABSTRACT

Between the 1950s and 1980s, scientists were focusing mostly on how the genetic code was transcribed to RNA and translated to proteins, but how proteins were degraded had remained a neglected research area. With the discovery of the lysosome by Christian de Duve it was assumed that cellular proteins are degraded within this organelle. Yet, several independent lines of experimental evidence strongly suggested that intracellular proteolysis was largely non-lysosomal, but the mechanisms involved have remained obscure. The discovery of the ubiquitin–proteasome system resolved the enigma. We now recognize that 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. Not surprisingly, aberrations in the system have been implicated in the pathogenesis of human disease, such as malignancies and neurodegenerative disorders, which led subsequently to an increasing effort to develop mechanism-based drugs. This article is part of a Special Issue entitled: Proteolysis 50 years after the discovery of lysosome.
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1. Introduction

The concept of protein turnover is hardly 70 years old. Beforehand, body proteins were viewed as essentially stable constituents that were subject to only minor "wear and tear": dietary proteins were believed to function primarily as energy-providing fuel, which were independent from the structural and functional proteins of the body. The problem was hard to approach experimentally, as research tools were not available. Important research tools that were lacking at that time were stable isotopes. While radioactive isotopes were developed earlier by George de Hevesy (de Hevesy G., Chemistry 1943. In: Nobel Lectures in Chemistry 1942–1962. World Scientific 1999. pp. 5–41), they were mostly unstable and could not be used to follow metabolic pathways. The concept that body structural proteins are static and the dietary proteins are used only as a fuel was challenged by Rudolf Schoenheimer in Columbia University in New York City. Schoenheimer escaped from Germany and joined the Department of Biochemistry in Columbia University founded by Hans T. Clarke [1–3]. There he met Harold Urey who was working in the Department of Chemistry and who discovered deuterium, the heavy isotope of hydrogen, a discovery that enabled him to prepare heavy water, D_2O. David Rittenberg who had recently received his Ph.D. in Urey's laboratory, joined Schoenheimer, and together they entertained the idea of "employing a stable isotope as a label in organic compounds, destined for experiments in intermediary metabolism, which should be biochemically indistinguishable from their natural analog" [1]. Urey later succeeded in enriching nitrogen with ^{15}N, which provided Schoenheimer and Rittenberg with a "tag" for amino acids and as a result for the study of protein dynamics. They discovered that following administration of ^{15}N-labled tyrosine to rat, only ~50% can be recovered in the urine, "while most of the remainder is deposited in tissue proteins. An equivalent of protein nitrogen is excreted" [4]. They further discovered that from the half that was incorporated into body proteins "only a fraction was attached to the original carbon chain, namely to tyrosine, while the bulk was distributed over other nitrogenous groups of the proteins" [4], mostly as an αNH_2 group in other amino acids. These experiments demonstrated unequivocally that the body structural proteins are in a dynamic state of synthesis and degradation, and that even individual amino acids are in a state of dynamic interconversion. Similar results were obtained using ^{15}N-labled leucine [5]. This series of findings shattered the paradigm in the field at that time that: (1) ingested proteins are completely metabolized and the products are excreted, and (2) that body structural proteins are stable and static. Schoenheimer was invited to deliver the prestigious Edward K. Dunham lecture at Harvard University where he presented

Abbreviations: ODC, ornithine decarboxylase; G6PD, glucose-6-phosphate dehydrogenase; PEPCK, phosphoenol-pyruvate carboxykinase; TAT, tyrosine aminotransferase; APF-1, ATP-dependent proteolysis factor 1 (ubiquitin); UBIP, ubiquitous immunopoietic polypeptide (ubiquitin); MCP, multicatalytic proteinase complex (26S proteasome); CP, 20S core particle (of the proteasome); RP, 19S regulatory particle (of the proteasome)

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doi:10.1016/j.bbapap.2011.03.007

his revolutionary findings. After his untimely tragic death in 1941, his lecture notes were edited by Hans Clarke, David Rittenberg and Sarah Ratner, and were published in a small book by Harvard University Press. The editors called the book "The Dynamic State of Body Constituents" [6], adopting the title of Schoenheimer's presentation. In the book, the new hypothesis was 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." However, the idea that proteins are turning over had not been accepted easily, and was challenged as late as the mid-1950s. For example, Hogness and colleagues studied the kinetics of β-galactosidase in *Escherichia coli* and summarized their findings [7]: "To sum up: there seems to be no conclusive evidence that the protein molecules within the cells of mammalian tissues are in a dynamic state. Moreover, our experiments have shown that the proteins of growing *E. coli* are static. Therefore it seems necessary to conclude that the synthesis and maintenance of proteins within growing cells is not necessarily or inherently associated with a 'dynamic state'." While the experimental study involved the bacterial β-galactosidase, the conclusions were broader, including also the authors' hypothesis on mammalian proteins. The use of the term "dynamic state" was not incidental, as they challenged directly Schoenheimer's studies.

Now, after more than seven decades of research in the field of intracellular proteolysis, and with the discovery of the lysosome and later the ubiquitin–proteasome system, it is clear that the field has been revolutionized. We now recognize that intracellular proteins are turning over extensively, that the process is specific, and that the stability of many proteins is regulated individually and can vary under different conditions. From a scavenger, unregulated and non-specific end process, it has become clear that proteolysis of cellular proteins is a highly complex, temporally controlled and tightly regulated process that plays major roles in a broad array of basic pathways. Among these processes are cell cycle, development, differentiation, regulation of transcription, antigen presentation, signal transduction, receptor-mediated endocytosis, quality control, and modulation of diverse metabolic pathways. Subsequently, it has changed the paradigm that regulation of cellular processes occurs mostly at the transcriptional and translational levels, and has set regulated protein degradation in an equally important position. With the multitude of substrates targeted and processes involved, it has not been surprising to find that aberrations in the pathway have been implicated in the pathogenesis of many diseases, among them certain malignancies, neurodegeneration, and disorders of the immune and inflammatory system. As a result, the system has become a platform for drug targeting, and mechanism-based drugs are currently developed, one of them is already on the market.

2. The lysosome and intracellular protein degradation

In the mid-1950s, Christian de Duve discovered the lysosome (see, for example, refs. [8] and [9] and Fig. 1). The lysosome was first recognized biochemically in rat liver as a vacuolar structure that contains various hydrolytic enzymes which function optimally at an acidic pH. It is surrounded by a membrane that endows the contained enzymes latency that is required to protect the cellular contents from their action (see below). The definition of the lysosome was broadened over the years because it had been recognized that the digestive process is dynamic and involves numerous stages of lysosomal maturation together with the digestion of both exogenous proteins (which are targeted to the lysosome through receptor-mediated endocytosis and pinocytosis) and exogenous particles (which are targeted via phagocytosis; the two processes are known as heterophagy), as well as digestion of endogenous proteins and cellular organelles (which are targeted by micro- and macro-autophagy; see Fig. 2). The lysosomal/vacuolar system as we currently recognize it is a discontinuous and heterogeneous digestive system that also includes structures that are devoid of hydrolases—for example, early endosomes which contain endocytosed receptor–ligand complexes and pinocytosed/phagocytosed extracellular contents. On the other extreme it includes the residual bodies—the end products of the completed digestive processes of heterophagy and autophagy. In between these extremes one can observe: primary/nascent lysosomes that have not been engaged yet in any proteolytic process; early autophagic vacuoles that might contain intracellular organelles; intermediate/late endosomes and phagocytic vacuoles (heterophagic vacuoles) that contain extracellular contents/particles; and multivesicular bodies (MVBs) which are the transition vacuoles between endosomes/phagocytic vacuoles and the digestive lysosomes.

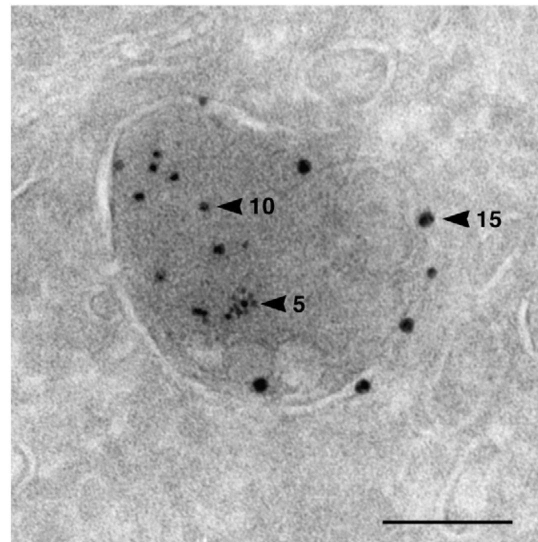

Fig. 1. The lysosome: Ultrathin cryosection of a rat PC12 cell that had been loaded for 1 h with bovine serum albumin (BSA)-gold (5 nm particles) and immunolabeled for the lysosomal enzyme cathepsin B (10-nm particles) and the lysosomal membrane protein LAMP1 (15 nm particles). Lysosomes are recognized also by their typical dense content and multiple internal membranes. Bar, 100 nm. Courtesy of Viola Oorschot and Judith Klumperman, Department of Cell Biology, University Medical Centre Utrecht, The Netherlands.

The discovery of the lysosome along with independent experiments that were carried out at the same time and that have further strengthened the notion that cellular proteins are indeed in a constant state of synthesis and degradation (see, for example, ref. [10]), led scientists to feel, for the first time, that they have at hand an organelle that can potentially mediate degradation of intracellular proteins. 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, exposed to the activity of the lysosomal proteases and degraded. An important discovery in this respect was the unraveling of the basic mechanism of action of the lysosome—autophagy (reviewed in ref. [11]). Under basal metabolic conditions, portions of the cytoplasm, which contain the entire cohort of cellular proteins, are segregated within a membrane-bound compartment, and are then fused to a primary nascent lysosome and their contents digested. This process was called microautophagy. Under more extreme conditions, starvation for example, mitochondria, endoplasmic reticulum membranes, glycogen bodies and other cytoplasmic entities, can also be engulfed by a process called macro-autophagy (see, for example, ref. [12]; the different modes of action of the lysosome in digesting extra- and intracellular proteins are shown in Fig. 2).

However, over a period of more than two decades, between the mid-1950s and the late 1970s, it has become gradually more and more

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