LYSOSOMES: Their Role in Protein Breakdown

Edited by
HANS GLAUMANN
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Foreword

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To the present generation of biochemists it may come as a surprise that the concept of protein turnover is less than 50 years old. Until the late 1930s, proteins were viewed as basically stable body constituents, subject only to some inevitable 'wear and tear', whose magnitude could be estimated from the fasting nitrogen excretion. Alimentary proteins were believed to be used in priority to compensate for such 'endogenus' metabolism. Amounts in excess of this requirement were taken to serve as fuel supporting the energy-yielding 'exogenous' protein metabolism.

This dual concept was shattered by the historic experiments of Rudolf Schoenheimer using ^{15}N-labeled amino acids. To quote from Schoenheimer's book, *The Dynamic State of Body Constituents*, published after his tragic death in 1941: 'The simile of the combustion engine pictured the steady 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 dynamic picture has now become 'classical', but the mechanisms underlying turnover and its regulation still provide a rich matter for research – as well as debate – especially as concerns the proteins. A first important

distinction must be made, depending on whether the degradative processes that are estimated affect entire protein molecules or only parts of them. It has become clear in recent years that many proteins are synthesized as larger-size precursors which undergo a fair amount of cotranslational and/or post-translational trimming. It would be interesting to know to what extent the pieces that are trimmed off contribute to the 'short-lived proteins' that the late Brian Poole found to be degraded outside the lysosomes.

Another important distinction depends on whether proteins are actually degraded in the cell where they were synthesized or elsewhere. Secretory proteins, including extracellular structural constituents, belong to the second category, as do the proteins of red blood cells and of many other cells subject to destruction in the body. As is known, a large part of the proteins in this category are degraded inside lysosomes, functioning in their heterophagic capacity. Some are degraded extracellularly, with the help of lysosomal (as in bone) or nonlysosomal proteases.

After correcting for biosynthetic processing and external breakdown, we are left with turnover in the more narrow meaning often given to the word and implying the continual on-site renewal of cell constituents, by complete breakdown and *de novo* synthesis in the same cell. Lysosomes undoubtedly play an important role in the degradative arm of this kind of turnover, subsequent to segregation of its substrates by cellular autophagy (sometimes by crinophagy). The autophagic mechanism is known to vary quantitatively in response to nutrient supply, hormones, and other environmental factors. But its ability to discriminate qualitatively at the molecular level remains problematical. Some of the nonlysosomal proteolytic systems that have been described could be more selective, but their physiological functions are still far from clear.

Thus the field of protein turnover still offers many challenges, which, judging from the number of specialized publications and symposia devoted to the subject, are attracting an increasing number of investigators from every biological discipline. These workers, together with all who are interested in basic cellular mechanisms, will welcome the publication of this book. It covers many aspects of cellular protein degradation, surveyed by experts in the field. Although focused mainly on lysosomes, it does not disregard some of the other mechanisms involved in protein breakdown. No doubt it will remain a major source of information for years to come, while at the same time stimulating the kind of research most likely to render it obsolete.

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MORPHOLOGY, ISOLATION METHODS AND PROPERTIES OF LYSOSOMES

1

Functional Morphology of the Lysosomal Apparatus*

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I. INTRODUCTION

Lysosomes represent one of the best examples in cell biology where a concept has been developed as a collaborative effort on the basis of biochemical and morphological observations. The history of the morphological background has been considered by de Duve (1969) and Aterman (1979). At the time of birth of the idea of lysosomes it was merely the knowledge of intracellular membrane systems and compartments – just discovered

* Dedicated to Professor Dr H.-W. Altmann on the occasion of his seventieth birthday.

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by the early electron microscopists – that justified the ingenious explanation, namely structure-linked enzyme latency (de Duve *et al.*, 1955). Meanwhile, a rapid glance at the literature reveals that the "lyosomal market" is dominated by biochemists, at least insofar as the number of publications is taken as a measure. One of the aims of this chapter then is to point out *morphological* aspects of the problem to those who are working mainly with the biochemical ones. At the same time, those who tend to trust only pictures should be prevented from underestimating the highly dynamic state of the lysosomal system.

Functional morphology has to be based primarily on known functions of the topic of concern. In the case of lysosomes the well-known basic function is intracellular degradation of high-molecular-weight compounds to low-molecular-weight split products. Thus basic structural requirements are the membrane (Section II), which forms a closed compartment, i.e. separates inside from outside, and as contents (Section III) degradative enzymes as well as substrates. While the enzymes are transported to the lysosomes by a kind of "internal secretion", the substrates are delivered by prelysosomes (Section IV), which by fusion (Section V) feed the lysosomal compartment with substrates. Alterations in shape of lysosomes (Section VI) will be considered as an example showing that the consideration of morphological data may still lead to controversial functional interpretations. Finally, the appearance of the compartment in pathological or artificial expansion (Section VII) will be shown to contribute considerably to understanding the sequential steps in normal lysosomal function.

Although the basic morphological definition of lysosomes is well established, it can be difficult to identify a given organelle as part of the lysosomal system. Such difficulties are mainly due to functional and morphological heterogeneity and plasticity of the lysosomes. As an attempt to take account of different functional states, the terms *primary lysosome*, *secondary lysosome* and *telolysosome* have been defined (de Duve and Wattiaux, 1966). There is no doubt that primary lysosomes, i.e. those that contain only newly synthesized enzymes and have not yet been engaged in digestive events, must exist at least temporally in each cell having lysosomes. However, since it may be impossible to distinguish them from "recirculating" lysosomes (see p. 36), the term *primary lysosome* may be dispensable for our purposes, as it does not make very much difference in terms of the functional result whether a prelysosome (see p. 14) fuses with a primary or with a secondary (preexisting) lysosome. Similarly, dealing with the main aspect of intracellular degradative processes, the question of whether or not telolysosomes (i.e. end stages that will never be used for further digestive events) exist as an essential part of the system will be of minor functional importance.

Before going into details, one should remember that the degradation of proteins is only part of lysosomal function. The enzymatic equipment as well as the morphology clearly show that in most lysosomal digestive events substrates of very different chemical classes are degraded simultaneously. Care therefore has to be taken not to make the partial aspect of protein degradation the whole thing, in order to avoid artificial notions. Nevertheless, it is the intention of the following to adduce mainly those examples that are not too far from the systems investigated for protein turnover with biochemical methods.

II. THE LYSOSOMAL MEMBRANE

II.A. Structural Aspects

Most of our knowledge of the functional properties of the lysosomal membrane (i.e. primarily its permeability) is not immediately evident from its morphological appearance. The main statement to be made from electron-microscopical observations is that the membrane with its thickness of 9 nm belongs to the thick type of biomembranes. In this respect the lysosomal membrane resembles the plasma membrane and the membranes of the maturing, or trans face of the Golgi apparatus (Yamamoto, 1963; de Duve and Wattiaux, 1966; Grove *et al.*, 1968). The trilamellar profile (Fig. 1a) sometimes appears to be asymmetric, having a thicker osmiophilic layer on the outside (Fig. 1b).

The membrane is separated from the remaining lysosomal components by a characteristic halo (Daems and van Rijssel, 1961; Daems *et al.*, 1969), i.e. a 7–10 nm layer of low electron density at the inner side (Figs. 1a, b). It has not been definitively proved whether this halo belongs to the essential equipment of the membrane; its visibility obviously depends on the density of the lysosomal contents and can be seen only when the latter exceed a certain threshold of electron density, e.g. lipopigment (Fig. 23), iron particles (Figs. 1a, 25) or appropriately stained glycogen (Fig. 15). This halo zone has been investigated by a number of cytochemical methods and has been shown to contain high concentrations of hydroxyl, vicinal glycol, and α-amino alcohol groups (Neiss, 1984) (Fig. 2). At the inner side of isolated lysosomal membranes sialic-acid-containing residues have been detected by colloidal iron staining (Henning *et al.*, 1973). As discussed in detail by Neiss (1984), this inner coat of the lysosomal membrane seems to be of similar nature to the surface coat of the plasma membrane – the glycocalyx.

In contrast, the majority of lysosomal membrane proteins have been found to be exposed to the cytosolic space (Schneider *et al.*, 1978). This