Autophagy, Heterophagy, Microautophagy and Crinophagy as the Means for Intracellular Degradation

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Summary. It is generally accepted that the lysosomal compartment plays an important role in the degradation of cellular components.

In this communication we discuss various experimental models which have been used to study mechanisms of intralysosomal degradation and also discuss the evidence obtained in support of the following proposals:

1. The autophagosomes can be isolated into high purity and are the subcellular locus of induced protein degradation.
2. Different membrane components such as proteins and lipids are degraded at different rates inside the lysosomes. Intralysosomal hydrolysis is not the rate limiting step in degradation.
3. Lysosomes take up soluble material in vitro by invagination and pinching off of their membranes (microautophagy).
4. Secretory vesicles can degrade their secretory contents by fusing with the lysosomes.

Key words: Lysosomes – Autophagosomes – Isolation – Proteolysis – Microautophagy – Crinophagy.

Mechanisms of Turnover of Cell Constituents
Membranous and cytosolic constituents of all cells are synthesized and degraded continuously. The regulation and interaction of degradation and synthesis are important mechanisms for achieving tissue growth and occur for example, in normal development and in organ regeneration and involution. Blood amino-acids, as well as glucagon and insulin levels, maintain a steady state by regulating the interaction between synthesis and degradation (Mortimore and Mondon 1970; Mortimore and Schworer 1977; Pfeifer et al. 1978). At the molecular level aminoacyl-tRNA may serve as the final determinant in this process (Scornik et al. 1980). The classical studies of de Duve and others demonstrated that
Lysosomes are the principal subcellular site where acid hydrolysis takes place. Work with perfused liver (Neely et al. 1977) and with isolated cells (Seglen et al. 1979; Dean 1979) has shown that the lysosomal apparatus plays an important role in the degradation of intracellular proteins. By the autophagic pathway subcellular components are sequestered into the lysosomal compartment to be degraded in the autophagosomes. In view of the high proteolytic capacity of lysosomes (Barrett and Heath 1977), it has been suggested that intralysosomal hydrolysis is not the rate-limiting step in this process and therefore does not determine the turnover of proteins. It seems, therefore, that the rate and perhaps the selectivity of the uptake of proteins into lysosomes could function as a regulatory mechanism for protein turnover, rather than the proteolytic rate (Neely et al. 1977).

Hydrolysis of protein and smaller polypeptides also occurs extralysosomally (Ballard 1977). The details of this pathway as well as its subcellular locus have not been fully worked out. It is also unclear at present how intralysosomal and extralysosomal pathways of protein degradation interact, and what is their relative contribution to the net turnover. Estimates of the lysosomal contribution to intracellular degradation during physiologic conditions have been in the range 30–70% depending on the experimental conditions. However, the enhanced proteolysis which occurs as a result of nutrient deprivation and of alterations in homeostasis is predominantly, if not exclusively, a lysosomal process due to increased autophagy.

We have used the induction of autophagy, caused by the microtubule inhibitor vinblastine (VBL) (Hirsimäki et al. 1976), as a means of studying induced proteolysis (Marzella and Glaumann 1980a, b) and have shown that the autophagic vacuole (AV) is the subcellular locus for induced degradation (Marzella et al. 1981). In addition, we have obtained experimental data in support of the hypothesis that lysosomes may be able to internalize their membranes in vitro by a microautophagic mechanism (Marzella et al. 1980a). These recently published results will be summarized briefly. In addition, recent studies on crinophagy, the uptake by lysosomes of marker particles and uptake and degradation of proteins during in vitro incubation will also be discussed. Finally, a model for introducing membranes into lysosomes by means of heterophagy will be discussed.

**Induction of Autophagocytosis as a Model to Study Intralysosomal Proteolysis**

In the case of VBL-induced autophagy, the appearance of the organelles sequestered into nascent autophagosomes is normal as far as can be judged by electron microscopy (Fig. 1). Moreover, by labeling cellular proteins with $^{14}$C-leucine at different intervals before the induction of autophagy, it can be shown that both old and newly synthesized proteins are taken up and degraded by the lysosomes (Table 1). VBL induces proteolysis of proteins labelled for both 48 and 96 h. Even after very short $^{14}$C-leucine incorporation interval (2 h) VBL stimulates proteolysis to roughly the same extent (not shown). These observations indicate that VBL-induced autophagic sequestration of cytoplasm is a random process. It follows that cell injury, as defined by Trump and coworkers...