**Autophagy and lysosomal proteolysis in the liver**

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**Summary.** Autophagy is defined as any process whereby cellular macromolecules destined for degradation gain access to the lysosomes. A review is presented on the physiological significance, mechanisms and regulation of autophagy in hepatocytes, concentrating on the issue of regulation. The article ends by discussing techniques available for future research.

**Key words.** Autophagy; hepatocytes; protein degradation; lysosomes.

**Introduction**

Autophagy may be broadly defined as any process whereby cellular macromolecules destined for degradation gain access to the interior of lysosomes. In the classical theory of autophagy, specialized membrane structures envelope intracellular components. The vacuoles thus formed, called autophagosomes, contain few if any hydrolytic enzymes. The autophagosomes subsequently fuse with primary lysosomes giving rise to autolysosomes, a type of secondary lysosome. Enzymes contained in the lysosome facilitate the breakdown of internalized macromolecules into their subunits (e.g. proteins to amino acids). The subunits escape back to the cytосol where they may be reutilized.

As will become clear, macromolecules en route to degradation may reach the lysosomes by other mechanisms as well. Any vacuole containing macromolecules en route to degradation will be referred to as autophagic vacuoles. The term comprises autophagosomes and any secondary lysosome carrying endogenous (i.e. cellular) macromolecules (excluding secondary lysosomes containing exogenous macromolecules).

All nucleated cells are believed to have lysosomes, that is specialized vacuoles serving the purpose of macromolecule degradation by carrying a variety of hydrolytic enzymes. It seems reasonable to assume that autophagy occurs in most eucaryotic cells, but in some cells (under certain conditions) the process is much more dramatic than in others.

Among mammalian tissues the liver is, for two reasons, particularly well-suited for the investigation of autophagy. First, the technique of collagenase perfusion facilitates the preparation of a large number of rather homogeneous liver parenchymal cells. Second, the liver has an unusual ability to effect rapid and drastic variations in the rate of autophagy.

Much work has recently been carried out to elucidate the mechanism and regulation of autophagic processes in the liver (most experiments being done with liver from rats, either as a perfused organ or as isolated cells). This review concentrates on rat liver, since autophagy is probably best understood in this tissue. Where nothing else is indicated, the data referred to have been obtained from liver preparations, or the type of system employed is considered unimportant in the present context. Most biochemical work on autophagy is related to proteolysis. The references cited are not meant to be exhaustive, and are chosen to provide convenient leads to current literature rather than to indicate priority. The following review articles carry additional relevant information: on proteolysis, on lysosomes, and on autophagy.

**Physiological significance**

**Supplying substrates**

The lysosomes are the only cellular compartment with sufficient enzymes for the complete degradation of all classes of macromolecules. The function of secluding these enzymes inside a vacuole must be to regulate their activity, and create favorable conditions for hydrolytic reactions.

For many cell types, including liver cells, lysosomal activity depends very much on nutritional status. Under 'basal' conditions, i.e. when the cells receive sufficient nutrition, the degradation of proteins, and probably other macromolecules as well, is maintained at a low rate and occurs partly outside the lysosomes. If the cells are transferred to 'step-down' conditions, either by removing amino acids/serum from cells in culture, or by starving the animal, an increase in the degradation is noted. This activated degradation is lysosomal and is believed to be
due to a rather indiscriminate sequestration of cytoplasm.

Supposedly, the purpose of this increase in lysosomal activity is to secure that the cell or organism possesses a minimal amount of organic substrates (e.g. amino acids).

The liver is involved in regulating the concentration of nutrients in the blood. This may explain why hepatocytes react particularly drastically to nutritional step-down; the rate of proteolysis is reported to vary from nearly 0 to up to 5%/h. At the maximal rate at least 70% of the degradation occurs inside the lysosomes.

Regulating the concentration of macromolecules.

It is believed that some degradation occurs in the lysosomes even under conditions where the cells are supposedly receiving sufficient external nutrition. Apparently lysosomes are involved in the down-regulation of greater than optimal concentrations of macromolecules and organelles.

Although much of the selective degradation of proteins may take place outside the lysosomes, there are probably mechanisms whereby macromolecules are selectively brought into the lysosomes, e.g. protein may interact with the sequestering membrane. There is also evidence indicating that whole organelles can be sequestered discriminately.

Other functions

Lysosomes are possibly involved in regulating the secretion of macromolecules. In a process termed crinophagy, secretory vesicles fuse with lysosomes, resulting in the degradation of their contents. The process may be considered as a form of autophagy.

For cells to divide and tissue to grow, a positive balance between macromolecule synthesis and degradation is required, implying that the rate of degradation influences tissue development. Preneoplastic hepatocytes have a reduced lysosomal proteolysis, indicating that their higher than normal growth rate is connected with a change in the regulation of autophagy.

Mechanisms

Origin of the sequestering membrane

Membranes involved in the engulfing of cytoplasm must necessarily have special properties, most probably mediated by specific membrane proteins. From where these membranes originate is a question which has been debated. Areas of the endoplasmatic reticulum or the Golgi region could be directly involved, or the sequestering membranes originate is a question which has been debated. Areas of the endoplasmatic reticulum or the Golgi region could be directly involved, or the sequestering membranes may be vacuoles budded off from either of these organelles.

Lysosomal enzymes are synthesized on the rough endoplasmatic reticulum, and subsequently transported to the smooth endoplasmatic reticulum, and most probably further to the Golgi region before they undergo the final processing and are packed into lysosomes. Autophagosomes derived from these structures may carry some hydrolytic enzymes. It is assumed, however, that for optimal conditions of degradation to occur, autophagosomes need to fuse with lysosomes.

Factors required

The most obvious requirement for degradation is the presence of hydrolytic enzymes. The role of these enzymes can be studied with inhibitors. More or less specific inhibitors of the main lysosomal proteinases have been developed, some of them acting selectively on lysosomal proteinases, some apparently on both lysosomal and nonlysosomal proteinases. Inhibitors of thiol proteinases are particularly effective in blocking lysosomal proteinases.

Most lysosomal enzymes are optimally active under acidic conditions. The elevated pH-concentration inside the lysosomes is sustained partly by a Donnan equilibrium, i.e. a high concentration of negatively-charged macromolecules inside the lysosomes causes a retention of positive ions. An ATP-driven proton pump in the lysosomal membrane most probably decreases the pH beyond the Donnan equilibrium, down to about pH 5. Experiments with lysosomotropic weak bases and ionophores that cause the breakdown of pH-gradients,