XI. ORGANELLES INVOLVED IN INTRACELLULAR DIGESTION. LYSOSOMES

The identification of the lysosomes as a distinct class of membrane-enclosed cytoplasmatic organelle was originally derived from an extensive series of subcellular fractionation experiments carried out by Christian de Duve and his associates in the early 1950s.

XI.1. DISTRIBUTION

Lysosomes are found in all eukaryotic cells, except for the erythrocytes. Large amounts of lysosomes are found in cells implicated in phagocytosis. In mammals there are two classes of phagocytes, according to the size: microphages-are only the neutrophils, and macrophages - which are monocytes, Kupfer cells, osteoclasts, glial cells, etc. These two types of cells are called "profesional phagocytes", they develop from a common precursor cell, and they defend us against infection by ingesting invading microorganisms. Macrophages also play an important part in scavenging senescent and damaged cells and cellular debris. In quantitative terms the latter function is far more important: macrophages phagocytize more than 10^{11} senescent red blood cells in each of us every day.

XI.2. THE ULTRASTRUCTURE

Lysosomes are membranous bags of hydrolytic enzymes used for the controlled intracellular digestion of macromolecules. They contain about 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. All are **acid hydrolases.** For optimal activity they require an acid environment, and the lysosome provides this by maintaining a pH of about 5 in its interior. In this way the contents of the cytosol are doubly protected against attack by the cell's own digestive system. The membrane of the lysosome normally keeps the digestive enzymes out of the cytosol, but even if they should leak out, they can do little damage at the cytosolic pH of about 7,2.

Like all other intracellular organelles, the lysosome not only contains a unique collection of enzymes, but also has a unique surrounding membrane. Transport proteins in this membrane allow the final products of the digestion of macromolecules, such as amino acids, sugars, and nucleotides, to be transported to the cytosol, from, from were they can be either excreted or reutilized by the cell. An **H^+pump** in the lysosomal membrane utilizes the energy of ATP hydrolysis to pump H^+ into the lysoosme, thereby maintaining the lumen at its acidic pH.

Most of the lysosomal membrane proteins are unusually highly glycosylated, which is thought to help protect them from the lysosomal proteases in the lumen. Also the lysosomal membrane can be broken by physical agents - UV radiation, very low or very high temperature, or by chemical agents - A, K vitamins, detergents.

After the discovery by biochemical fractionations of cell extracts, lysosomes were seen clearly in the electron microscope. They are extraordinarily diverse in shape and size but can be identified as members of a single family of organelles by histochemistry, using the precipitate produced by the action of an acid hydrolase on its substrate to show which organelles contain the enzyme. By this criterion, lysosomes are found in all eukaryotic cells. In electron micrographs, a population of vesicles could be recognized either by their content of partially digested material or by specific histochemical demonstration of one of their contained hydrolytic enzymes. The

demonstration that these two populations were identical earned Dr. Christian de Duve a share of Nobel Prize in Medicine in 1974.

The **heterogeneity** of lysosomal morphology contrasts with the relatively uniform structures of most other cellular organelles. The diversity reflects the wide variety of digestive functions mediated by acid hydrolases, including the breakedown of intra-and extracellular debris, the destruction of phagocytized microorganisms, and the production of nutrients for the cell. For this reason lysosomes are sometimes viewed as a heterogeneous collection of distinct organelles whose common feature is a high content of hydrolytic enzymes. **Acid phosphatase** is a marker enzyme for lysosomes.

XI.3. FORMATION OF LYSOSOMES

Most of the hydrolytic enzymes present in lysosomes are glycoproteins synthesized on ribosomes bound to the endoplasmic reticulum. So, newly synthesized lysosomal proteins are transferred into the lumen of the ER, transported through the Golgi apparatus, and then carried from the trans Golgi network to late endosomes by means of transport vesicles.

The lysosomal hydrolases contain N-linked oligosaccharides that are covalently modified in a unique way in the cis Golgi network so that their mannose residues are phosphorylated. These mannose 6 -phosphate (M6P) groups are recognized by an M6P receptor protein in the trans Golgi network that segregates the hydrolases and helps to package them into budding transport vesicles, which deliver their contents to late endosomes, and thereby to lysosomes. These transport vesicles act as shuttles that move the M6P receptor back and forth between the trans Golgi network and late endosomes. The low pH in the late endosome dissociates the lysosomal hydrolases from this receptor, making the transport of the hydrolases unidirectional.

XI.4. TYPES OF LYSOSOMES

According to the size and content lysosomes can be primary lysosomes, secondary lysosomes, and tertiary lysosomes.

Primary lysosomes -are those lysosomes not yet involved in digestive activity. They contain all the enzymes that are used in digestion in the cell. They are small (5-8µ). Primary lysosomes fuse with the membrane of the structure that contains the material to be digested and release their enzymes, thus forming a ***secondary lysosome.*** Secondary lysosomes may also be called phagosomes, digestive vacuoles, or autophagic vacuoles, depending on the material to be digested. The lysosomal enzymes can now hydrolyze the foreign matter originally present in the endosome, releasing small molecules that diffuse through the vesicle membrane and into the cell sap. Undigested substances are retained and accumulate in the secondary lysosome, eventually converting it to a structure known as a ***residual body*** or ***tertiary lysosome***. Residual bodies may remain in cells for the life of the cell, as in nerve cells, in which they have been called "age pigment" or ***lipofuscin granules***. But in some cells, residual bodies can fuse with the plasma membrane and expel their contents from the cell by exocytosis. When this discharge is slow or absent, the resulting accumulation of residual bodies is believed to contribute to cellular aging.

XI.5. FUNCTIONS

The realization that all liposome enzymes catalyse hydrolysis reactions has naturally fostered the theory that liposome’s serve a digestive role within the cell. So there are at least four

distinct ways in which this digestive function is utilized by cells, these include (1) degradation of foreign matter taken up by endocytosis, (2) destruction of worn-out organelles (autophagy), (3) breakdown of cellular structures associated with cell death (autolysis), and digestion of extracellular materials (Figure 105).

So, phagocytosis is carried out by two types of cells in mammals: macrophages and neutrophils. The function of these cells is to rid the body of senescent or damaged cells and to protect us against pathogenic microorganisms. Macrophages and neutrophils carry out this function by engulfing the cell or particles with their plasma membrane, folowed by internalization of the membrane enclosed ***phagosome***. Phagosomes are the size of the particle they are ingesting, and tend to be more than 1.0 µm in diameter. Once ingested by the cell, the phagosome, with entrapped particle, fuse with the cell's lysosome, forming a ***phagolysosome*** in which the ingested material is digested by acid hydrolases, leaving behind indigestible substances that form residual bodies within the lysosome.

Pathogenic bacteria and damaged cells are seen by the immune system as foreign material and therefore, are coated on their surface by IgG antibodies. The Fc region of the IgG molecule is recognized by Fc receptors on the surface of the macrophage or neutrophil. If the IgG molecules completely surround the particle, the plasma membrane of the phagocyte will completely wrap around and engulf the foreign particle by a membrane-zippering mechanism. If the IgG molecules are localized to one region of the particle, the phagocyte will bind the particle through the IgG-Fc receptor interaction but phagocytosis will not occur.

True endocytosis is quite different from phagocytosis. In endocytosis the plasma membrane invaginates, instead of evaginating, around a foreign particle. The invagination leads to adherence and fusion of the plasma membrane, creating an endocytotic vesicle that is typically 0.1 to 0.2 µm in diameter. There are two forms of invaginating endocytosis - fluid - phase endocytosis (pinocytosis) and receptor - mediated endocytosis. Most eukaryotic cells undergo both fluid-phase endocytosis and receptor -mediated endocytosis, primarily at specialized plasma membrane sites called coated pits. In fluid-phase and receptor-mediated endocytosis, the endocytic vesicles are constantly forming, and all molecules entrapped within the invagination of the plasma membrane are carried into the cell.

Receptor-mediated endocytosis involves the binding of a molecule or small particle to a receptor. The receptor is usually clustered within a coated pit, and the resulting endocytic vesicles that are formed are referred to as coated vesicles. Therefore, receptor-mediated endocytosis differs from fluid-phase endocytosis in the concentrating of ligand within the endocytic vesicles. The polyhedral coat that is found on the cytoplasmic surface of the plasma membrane and endocytic vesicle is composed primarily of the protein clathrin. Because the receptors are clustered within the coated pit they tend to concentrate their specific ligand within the coated vesicle. Once the coated vesicle enters the cortical cytoplasm, clathrin is rapidly removed by an uncoating ATPase, and the clathrin returns to the plasma membrane to form new coated pits. The uncoated endocytic vesicle fuses with a CURL vesicle (compartment that uncouples receptor and ligand) that has an internal pH of approximately 5.5. The resulting peripheral endosome contains an ATP-dependent proton pump that maintains its acidic internal pH. Many ligand -receptor complexes formed at neutral pH become dissociated at pH 5.5. For those complexes that dissociate within the peripheral endosome, the receptors are recycled to the plasma membrane by receptor-enriched vesicles budding off the peripheral endosome and returning to the plasma membrane. The ligands still enclosed within the peripheral endosome are temporally found in endosomes closer to the nucleus, called perinuclear endosomes, and then in immature lysosomes, called endolysosomes. Transport vesicles from the Golgi that are carrying

newly made acid hydrolases fuse with the endolysosome, forming mature lysoosmes. The ligands within the lysosomes are degraded and their building blocks reused or excreted.

XI.6. LYSOSOMAL STORAGE DISEASES

Lysosomal storage diseases are caused by genetic defects that affect one or more of the lysosomal hydrolases and result in accumulation of their undigested substrates in lysosomes, with severe pathological consequences. Most often, there is a mutation in a structural gene that codes for an individual lysosomal hydrolase.

Several genetic diseases afflicting young children are known to be caused by an excessive intracellular accumulation of polysaccharide or lipids. The quantity of polysaccharide or lipid stored is often massive enough to interfere with and even destroy the cells involved. Depending on the particular cell types affected, symptoms such as muscle weakness, skeletal deformities, and mental retardation may result.

The first of these so-called lysosomal storage diseases to have its underlying mechanism unravel was type II glycogenosis, an illness whose victims die at an early age with abnormally large amounts of glycogen in the liver, heart, and muscles. In 1963 H. Hers discovered that type II glycogenosis is caused by a severe deficiency of the lysosomal enzyme alpha- glucosidase, which catalyzes hydrolysis of glycogen to oligosaccharides and glucose. In the absence of the enzyme, undigested glycogen accumulates within the lysosomes. The most dramatic form of lysosomal storage diseases is a very rare disorder called inclusion-cell disease (I-cell disease). In this disease almost all of the hydrolytic enzymes are missing from the lysosomes of fibroblasts, and their undigested substrates accumulate in lysosomes, which consequently form large "inclusions" in the patients' cells. I-cell disease is due to a single gene defect, and like most genetic enzyme deficiencies, it is recessive, it is seen only in individuals in whom both copies of the gene are defective. Other lysosomal storage diseases are Niemann-Pick disease, Gaucher's disease, and Tay-Sachs disease (Table XIII).

Table XIII – Lysosomal storage diseases

Disease	**Symptoms**	**Substance Accumulated**	**Enzyme Defect**
Type II glycogenosis	Muscle weakness	Glycogen	α-Glucosidase
Niemann-Pick disease	Liver-spleen enlargement; Mental retardation	Sphingomyelin	Sphingomyelinase
Gaucher's disease	Liver-spleen enlargement; Bone erosion	Glucocerebroside	β-Glucosidase
Tay-Sachs disease	Mental retardation; Blindnes; Muscle weakness	Ganglioside	β-N-Acetylhexos-aminidase