Seeing peroxisomes

At about the same time that Christian de Duve and his colleagues were describing the biochemistry of lysosomes (see “Catching sight of lysosomes” JCB 168: 174), they biochemically identified (Baudhuin et al., 1965) and purified (Leighton et al., 1968) another enzyme-containing organelle. Initially the organelle was known as the microbody, and de Duve declined to give it a more specific name in 1965 because “too little is known of their enzyme complement and of their role in the physiology of the liver cells to substantiate a proposal at the present time” (Baudhuin et al., 1965). But in an abstract presented at the 1965 American Society for Cell Biology annual meeting and a year later in print (de Duve and Baudhuin, 1966), de Duve proposed that the new organelle be called a peroxisome, because it appeared to both generate and break down hydrogen peroxide.

A Swedish graduate student, J. Rhodin, had first described microbodies in his dissertation in 1954, after spotting their distinctive morphology. A year later, they were described in a paper that mistakenly suggested, based on appearance and location, that they were precursors to mitochondria (Rouiller and Bernhard, 1956). Subsequently other researchers observed similar structures by microscopy “but no one knew the function of these particles,” says de Duve. “There were all kinds of wild speculations about what they might do.”

de Duve’s group modified a cell fractionation method devised by Robert Wattiaux and colleagues for separating peroxisomes, lysosomes, and mitochondria, which required injecting animals with Triton WR-1339 (Wattiaux et al., 1963). “The compound accumulates in lysosomes and causes them to float in a sucrose gradient,” says de Duve. This technique led to a full identification of peroxisomes using microscopy and biochemistry (Baudhuin et al., 1965), when they were clearly shown not to be related to mitochondria.

In a landmark paper published in JCB in 1968 (Leighton et al., 1968), de Duve described the first large-scale preparation of peroxisomes—a feat that made possible more conclusive and precise characterization of their biochemical and morphological properties. “The same technique was to be used for many years to come in the study of the biogenesis of peroxisomes,” says de Duve.

The key to scaling up the separation technique was an automated rotor. “That machine was remarkable,” says de Duve. “Belgian scientist Henri Beaufay designed the rotor, and its construction was completed at the Rockefeller [University] instrument lab. It was a transatlantic collaboration.” The automated rotor had several advantages over the conventional swinging bucket rotor. It could accommodate larger sample volumes and allowed loading and unloading of samples while the rotor was running, thereby suppressing artifacts associated with starting and stopping the centrifuge.

With these advantages, de Duve and colleagues were able to use 100 g of liver from mice in a single experiment to obtain significantly more concentrated and cleaner preparations of peroxisomes, lysosomes, and mitochondria. “We were able for the first time to get a sufficiently thick preparation that you could see different colors of the peak fractions,” says de Duve. One of the figures in the paper shows the peroxisomes fraction as having a greenish tinge, presumably reflecting their richness in catalase.

These highly enriched and purified fractions lent themselves to further characterization, thus putting peroxisomes on a much firmer footing as distinct structures in the cell and allowing their identification despite differences in morphology in different cell types. The authors confirmed, for example, that peroxisomes contain essentially all the l-α-hydroxyacid oxidase, ß-amino acid oxidase, and catalase in a liver cell. “The 1968 paper was an upgrading and scaling up of the work we had done before,” says de Duve. “But most importantly it laid the groundwork for subsequent studies on peroxisomes.”

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out, the cables disassembled into their constituent filaments. Colchicine, however, had no obvious effects on the maturing actin filaments of the myofibrils.

By measuring the diameter of individual filaments by electron microscopy, Holtzer and colleagues were able to determine that the free cytoplasmic filaments, and those in the cochicine-induced cables, had a diameter different from that of actin filaments. These filaments were named “intermediate filaments” because their size was between that of myosin and actin filaments in muscle cells.

The 1968 study was followed by an explosion of research that quickly led to the identification of many intermediate filament isoforms, such as the nuclear lamins, vimentin-like filaments, keratins, and neurofilaments. Many of these give mechanical stability to cells (Janmey et al., 1991), but some, says Holtzer, “are almost certain to be involved in cell differentiation and cell maturation.” Changes in the state of their aggregation following stress, infection, or mutation are diagnostic of specific human diseases, and their varied expression profiles in different epithelia make them particularly useful in classifying the tissue of origin of many tumors. JCB

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