Excess secretory products fuse with lysosomes

Cells known to secrete proteins were studied in the 1960s, but what happened when secretion systems were turned off? Lysosomes were known to degrade foreign proteins taken up by cells and even autodigest intracellular membranous structures—but what was the fate of excess endogenous protein?

To ask that question, Smith and Farquhar (1966) needed a secretion system that could be manipulated in the lab (and without the benefit of today’s inducible gene expression systems). Lactating rats provided prolactin-secreting pituitary cells that fit the bill as “it was easy to cut off secretion by removing the suckling babies and then ask, how would the cells adapt?” says Marilyn Farquhar (University of California, San Diego, CA).

The cells, says Farquhar, were “devoted to pushing out prolactin,” and then the babies were removed. At that point the duo brought in the new and powerful technique of enzyme histochemistry to localize lytic activity with two proteins, hemoglobin and ferritin, that were readily recognizable by their characteristic density or shape; they were also known to end up in two lysosomal structures. Sections of rodent kidney cells were also treated with the enzyme reactions in one of the first examples of combined cytochemistry and EM. The results were plainly obvious when the lead reaction with two proteins, hemoglobin and ferritin, that were readily recognizable by their characteristic density or shape; they were also known to end up in two lysosomal structures. The experiments also led to the observation that the cells did not store lytic enzymes, but rather “the enzyme might be produced when needed...and transported by small vesicles.” That assumption, the authors write, “implies the enzyme may well pass through some elements of the Golgi complex,” but the evidence so far for this theory “cannot be considered sufficient proof in this case.”

The traditional assumption was that cells would simply store excess secretory granules until they were needed again. So the researchers were surprised to observe that immature granules fused with multivesicular bodies (MVBs) and mature granules fused with “dense bodies” or lysosomes. Previous work suggested that MVBs were intermediates in lysosomal degradation of endocytosed proteins in renal and nerve cells (Farquhar and Palade, 1962; Rosenbluth and Wissig, 1964). Furthermore, Smith and Farquhar’s acid phosphatase tests showed lytic activity in the MVBs that contained granules. This led the authors to postulate that MVBs “can take up and digest proteins and are transformed in the process into” lysosomes. The idea was very close to the concept of maturation followed by fusion with lysosomes as suggested 30 years later by Futter et al. (1996).

Because the prolactin granules retained a characteristic size and density for some time after fusion, the paper demonstrated for the first time that endogenous proteins could also enter the lysosomal degradation pathway. The paper also noted that excess ER and ribosomes, ramped up for prolactin production, were downregulated by autophagic structures that also converged on the lysosomal pathway.

Farquhar notes that one of the prolific namers of the times, Christian de Duve, dubbed the observed secretory granule down-regulation “crinophagy.” And she points out that we have yet to answer a key question of the crinophagy pathway: “How does that [granule] membrane get changed in such a way that it goes to the lysosome instead of the plasma membrane?”

From the Archive

The first supper

By 1963, lysosomes were well established as an in vitro degradative entity localized to a few fractions (de Duve et al., 1955; de Duve, 1963). But a corresponding in vivo classification was trickier due to the heterogeneity of structures seen in different cell types and within cells. Christian de Duve had grouped lysosome-like entities into a system of four types of compartments: enzyme-storing granules, digestive vacuoles for reabsorbing proteins, autolytic vacuoles, and residual bodies containing the remnants of digestion.

These compartments had enzymes such as acid phosphatase. But did the same compartments have both enzymes and meaningful protein substrates? The advent of lysosomal enzyme tests, which gave a lead precipitate reaction product visible by EM [Novikoff and Holt, 1957; Essner and Novikoff, 1961], gave Miller and Palade (1964) a method to test for colocalization.

Fritz Miller and George Palade injected rats and mice (Miller and Palade, 1964), which could “bridge the gap between [fractionation] biochemistry and EM.”

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Were mitochondrial contractions driving the cellular energy cycle?

No, but Charles Hackenbrock’s thesis work so elegantly supported the hypothesis—that a mechanochemical mechanism coupled electron transport to ATP synthesis—that it was cited almost 600 times as evidence. It earned him speaking invitations all over the US and Europe, and an assistant professorship at Johns Hopkins University (Baltimore, MD) straight out of graduate studies. “Everyone,” he says, “fell in love with these ultrastructural changes.”

Hackenbrock, now an emeritus professor at the University of North Carolina, Chapel Hill, recalls that the project started as a graduate course project to isolate mitochondria from rat livers and test the effects of snake venom on their function. But when he noticed that his control group of mitochondria underwent a dramatic conformational change—from “knotted up” just after isolation to the “beautiful mitochondria” of intact cells—during a sucrose buffer incubation, he immediately switched his thesis to study how this structural change might correlate with oxidative phosphorylation function.

“In those days, mitochondrial function in terms of making ATP was one of the key questions in biochemistry around the world,” he says. “I realized immediately this was going to be of wide interest.” At the time, the only evidence for structural changes during oxidative phosphorylation came from light-scattering studies that measured the optical density of isolated mitochondria (Chance and Packer, 1958). Lehninger (1959) first proposed that the structural changes might represent, in Hackenbrock’s words, “an energy-linked mechanochimical process which may reside in a multienzyme respiratory assembly which carries out electron transport and oxidative phosphorylation.” In one scenario for such a link, ions might be pumped across the inner membrane to generate osmotic deformation of the mitochondrion, and the resultant mechanical force might then be used by an enzyme to generate ATP.

Hackenbrock developed a unique set-up to correlate ultrastructural changes, light scattering, and metabolic function. Before and after addition of ADP and substrates for the electron transport chain, he measured mitochondrial optical density and oxygen consumption and fixed mitochondria for EM (Hackenbrock, 1966).

The experiment revealed that mitochondria changed from a “condensed” to “orthodox” conformation while incubating in a buffer supporting slow respiration with no added ADP. Once ADP was added, however, the organelles contracted to the condensed form once again. The contractions were reversible (arguing against a fixation artifact) and seemed to be controlled by the inner mitochondrial membrane, which shrank away from the outer membrane and enclosed a more dense matrix in the condensed form. In a follow-up study, Hackenbrock clearly linked the contraction phenomenon to the activity of the electron transport chain by using several electron transport inhibitors and then adding downstream substrates to reinitiate transport and conformational changes (Hackenbrock, 1968).