In Memoriam: Lelio Orci, 1937–2019

Roberto Montesano[a,1], Randy Schekman[b,c], James Rothman[d], and Alain Perrelet[a]

On October 22, 2019, the international community of cell biologists lost one of its most eminent members, an exceptional scientist whose consummate mastery of cell membrane morphology contributed fundamental insights to our understanding of the microanatomy and function of the endocrine pancreas, as well as the molecular mechanisms of protein secretion.

Born in San Giovanni Incarico (Frosinone, Italy) on March 22, 1937, Lelio Orci obtained his medical doctorate at the Faculty of Medicine of Rome University in 1964. After moving to the Medical School of the University of Geneva in 1966, he was named Assistant Professor at the Institute of Histology and Embryology in 1967. An impressive scientific productivity driven by an outstanding talent earned him a full professorship a few years later, then the Chairmanship of the Department of Morphology (now Department of Cell Physiology and Metabolism) of the University of Geneva, until his retirement in the early 2000 with the title of Professor Emeritus (Fig. 1).

The leading motif of Orci's career was a constant drive to understand in minute detail the relationship between cell structure and function, using light and electron microscopy and a variety of cell biological techniques that were complemented later, in the course of collaborative works, by the application of the tools of biochemistry and molecular genetics.

In the first phase of his career, Orci unraveled, with exquisitely refined immunocytochemical techniques, the complex organization of the pancreatic islets of Langerhans with its four distinctive endocrine cell types present in different relative proportion, depending on the dual embryonic origin of the islets. This epoch was also characterized by the identification of a whole panel of endocrine cells in the gastrointestinal tract, which has been considered since then as a fully equipped endocrine organ.

Orci and his team developed the immunogold technique for the ultrastructural localization of intracellular proteins, and particularly hormones. By combining this methodological approach with radioactive labeling of newly synthesized proteins in pulse-chase experiments, he was able to follow, through time and space, the path of insulin from its site of synthesis (as proinsulin) to its site of release into the extracellular space. During this journey, proinsulin migrated, enclosed in transport vesicles, from the endoplasmic reticulum (ER) to the Golgi apparatus, then to secretory granules, where it underwent enzymatic conversion to mature insulin. At the stage of prohormone maturation, Orci observed clathrin patches on the surface of immature secretory granules that contained both proinsulin and mature insulin. His team documented that proinsulin proteolytic processing is linked to the generation of an acidic clathrin-coated vesicle compartment as a

Fig. 1. Lelio Orci in Paros, Greece (2016). Image courtesy of Roberto Montesano.

[a]Department of Cell Physiology and Metabolism, Centre Médical Universitaire, CH-1211 Geneva 4, Switzerland; [b]Department of Molecular and Cell Biology, University of California, Berkeley, CA 94270; [c]Howard Hughes Medical Institute, University of California, Berkeley, CA 94720; and [d]Department of Cell Biology, Yale University, New Haven, CT 06520-8002

Author contributions: R.M., R.S., J.R., and A.P. wrote the paper.

The authors declare no competing interest.

[1]To whom correspondence may be addressed. Email: roberto.montesano@unige.ch.

First published December 16, 2019.

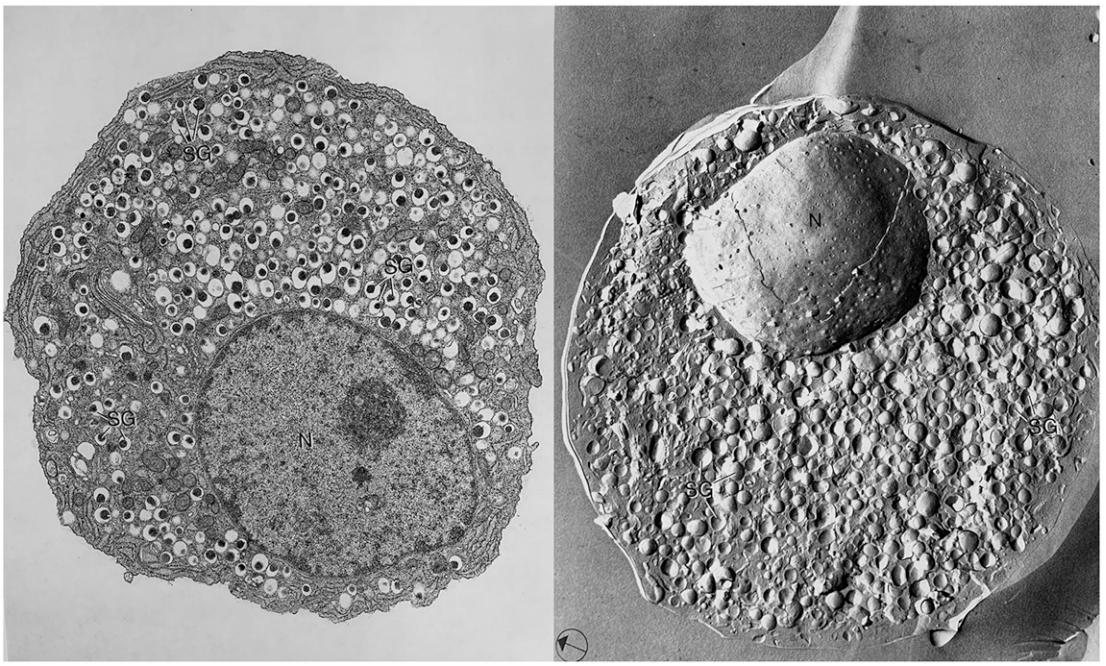

Fig. 2. Beautifully matched images of an insulin-secreting cell as seen on a thin section (*Left*) and a freeze-fracture replica (*Right*). The nucleus (N) and secretory granules (SG) are prominent features. From the book *Freeze-Etch Histology* (1). The initial crispness of the original pictures was a bit lost in the photographic transfer. (Magnification: *Left*, 5,400×; *Right*, 5,000×.) Image courtesy of Alain Perrelet. Reprinted with permission from ref. 1.

prelude to the generation of mature insulin-containing granules. Thus, insulin transport and maturation were revealed with molecular precision.

In another series of innovative experiments, Orci pioneered the use of the freeze-fracture technique to study the internal organization of cell membranes (Fig. 2). Through this novel approach, Orci identified the occurrence of gap junctions (clusters of closely packed intercellular channels) between adjacent islet cells, and demonstrated their crucial role in the regulation of insulin secretion. At this point in his career, Orci was already considered to be among the great masters of membrane morphology, on a par with the pioneering standards of George Palade.

Already well established in the field of hormone transport, Orci then embarked on a multidecade transatlantic collaboration with two of us (J.R. and R.S.), bringing his talents in electron microscopy to the molecular mechanistic approaches we had taken in dissecting the transport of proteins from the ER into and through the Golgi apparatus. The J.R. laboratory had developed a cell-free biochemical approach to probe the traffic of a viral glycoprotein within the cisternal network of the Golgi apparatus. His laboratory had identified two proteins, NSF and α-SNAP, for their role in the transit of the vesicular stomatitis virus glycoprotein between donor and acceptor compartments of the Golgi membrane. Orci observed that inhibition of NSF function led to the accumulation of small, uncoated transport vesicles bound but not yet fused to Golgi cisternae in the cell-free reaction: Thus, the conclusion that NSF and α-SNAP, working together, promoted the fusion of transport vesicles to a target membrane. Independently, the R.S. laboratory had demonstrated a role for two yeast genes, *SEC17* and *SEC18*, in the fusion of secretory vesicles transiting from the yeast ER to the Golgi apparatus. On cloning and DNA sequence analysis, a forge was linked between this fusion step in all eukaryotes with the discovery that *SEC18* encodes NSF and *SEC17* encodes α-SNAP.

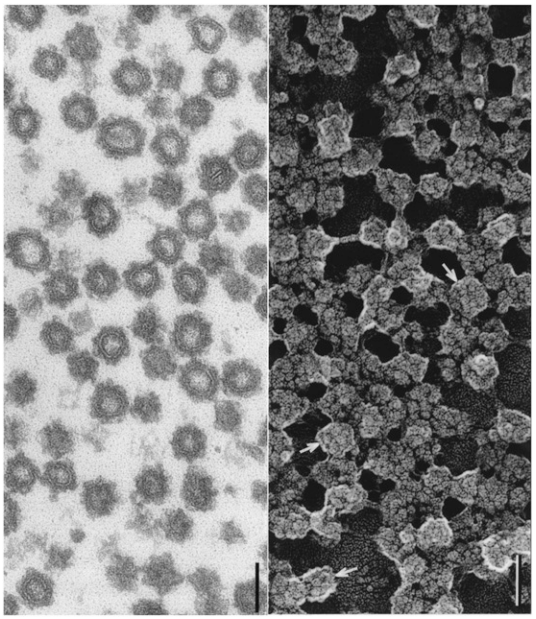

Fig. 3. Purified COPII-coated vesicles as seen on a thin section (*Left*) and on a quick-freeze/deep-etching/rotatory shadowing replica (*Right*). The arrows point to subunits of COPII-coat. (Scale bars, 100 nm.) Image courtesy of Randy Schekman.

But more was in store, again as a result of the interplay of observations from the three laboratories. Peter Novick, who isolated the *sec* mutants as a graduate student in the R.S. laboratory, had then in his own laboratory at Yale discovered that one of the genes required for the fusion of mature secretory vesicles at the cell surface, *SEC4*, encoded a Ras-like small GTP-binding protein. The J.R. team speculated that a similar function may be required for vesicle fusion within the Golgi network, and tested this idea by the application of an inhibitor of GTP hydrolysis to the cell-free reaction. On inspection by electron microscopy, Orci and J.R. observed the accumulation of nonclathrin-coated vesicles, which they showed must be uncoated as a prelude to the membrane fusion reaction. This led to the discovery of the COPI coat complex, now known to be the universal budding coat for vesicles transiting within the Golgi apparatus and in the retrieval of proteins in vesicles that travel from the Golgi back to the ER.

Although one of the original *SEC* genes, *SEC21*, encodes a subunit of the COPI coat, a distinct set of protein requirements for the budding of ER-derived transport vesicles emerged from biochemical studies in the R.S. laboratory. These vesicles, formed with a set of pure Sec proteins, were seen by Orci to be coated with an appearance similar to that of COPI vesicles (Fig. 3). The two coats, COPI and COPII, are molecularly distinct and employ different GTP-binding proteins for coat polymerization and both operate in all eukaryotes. Mutations in the human genes have emerged in several diseases, one of which, a craniofacial disorder, was documented by Orci and R.S. to have a dramatic effect on traffic of collagen from the ER in COPII vesicles. Through this collaborative effort, a personal bond developed among the three groups that advanced the field in ways that could not have been achieved without Orci's indefatigable effort and unique skills. In all of this work, Lelio Orci set the standard with his rigorously quantitative approach and original blend of electron microscopy with cell-free biochemistry.

Many prestigious distinctions honored Orci's outstanding achievements in research, such as the Banting Medal of the American Association for the Study of Diabetes, the King Faisal Award for Medicine, the Otto Naegeli Prize, a foreign membership of the US National Academy of Sciences, and honorary degrees in Medicine from the Universities of Geneva, Leuven (Belgium), McGill and Guelph (Canada), and Padua (Italy).

To conclude, a few words about Lelio Orci as a man. Orci had a strong, flamboyant personality coupled to an insatiable curiosity and a deeply rooted commitment to pursue the truth through rigorous and exhaustive experimentation. Lelio Orci was endowed with an acute sense of observation and an unsurpassed ability to detect fine, yet highly relevant details of cell structure that would have been overlooked by most of his peers. He was extremely sensitive to the beauty of cell organization, which he celebrated with micrographs of legendary aesthetic quality. His "uncompressible enthusiasm" (as he used to call it) was only matched by an almost obsessive autocriticism. In the department he chaired, Orci set very high standards for research, and contagiously disseminated excitement, perseverance, and quest for excellence among young investigators. His academic teaching was guided by the same fervent passion that drove his research activity, and the superbly illustrated lectures he delivered to medical students were often rewarded with a vigorous applause.

Despite his infectious enthusiasm, during much of his career beyond the mid-1980s, Orci seldom left his laboratory to travel to meetings or to deliver seminars. Far from being shy, he was so devoted to the research that he usually worked well into the night and called his collaborators in the United States at times well past midnight in Geneva. He kept voluminous correspondence and many thousands of electron microscopy pictures and took particular pride in a list of seminar invitations that he had declined. Those of us who had the privilege of a collaboration with Orci made the pilgrimage to Geneva, knowing full well that he was unlikely to return the favor with a scientific visit abroad. The pure joy of his personality and gracious family made the trip to his office and home worth any effort.

What legacy is left to us, former pupils, doctorate students, collaborators, or colleagues? We can treasure and perpetuate the values Lelio Orci stood for: Experimental rigor, quest of the highest possible technical quality, unflinching intellectual honesty, and pursuit of excellence in both science and life. We have an affectionate thought for his surviving wife Catherine, his three grown-up children Lelia, Gregory, and Lorenzo, and four baby grandchildren.

1 L. Orci, A. Perrelet, *Freeze-Etch Histology: A Comparison Between Thin Sections and Freeze-Etch Replicas* (Springer Verlag, 1975).