GEORGE E. PALADE LECTURE

HUMAN BODY AS A MULTICRINE SYSTEM, WITH SPECIAL REFERENCE TO CELL PROTEIN SECRETION: FROM VASCULAR SMOOTH MUSCLES TO ADIPOSE TISSUE

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Part 2: <https://www.youtube.com/watch?v=pfcDor3G70M>

***It is lyuboznanie
(from Bulgarian, love to knowledge)
that drive us to find our Teacher***

Magister dixit (Teacher said), used by Pythagoras' students, expresses a respect to the Teacher. Socrates was the Teacher of Plato, Plato of Aristotle, Rita Levi-Montalcini of Luigi Aloe, Harry Jellinek of Anna Kadar, Albert Claude of George Palade, George Palade of Günter Blobel and of many cell biologists worldwide.

In 1981 I met with Palade during a coffee break of World Physiology Congress held in Budapest, Hungary, and said to him that I know each of *words and micro-graphs* published in his articles on pancreatic exocrine secretion. They have driven my research on the secretory pathway in vascular smooth muscle cells studied by electron microscopy (1972-1992).

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Paradigm shifts in cell biology

According to the Stanford Encyclopedia of Philosophy, epistemology (Greek *epistēmē*, meaning „knowledge“) is the study of knowledge and justified belief. Also, the creation and dissemination of knowledge. An epistemological paradigm shift was called a „scientific revolution“ by Thomas Kuhn in his book *The Structure of Scientific Revolutions*, its first edition being released in 1932 in the USA. Kuhn challenged the then prevailing view of progress in *normal science*, which was viewed as „development-by-accumulation“ of accepted facts and theories. Kuhn argued for a model in which periods of such conceptual continuity in normal science were interrupted by periods of *revolutionary science*. A paradigms shift then emerged.

In the present lecture I will focus on three paradigm shifts, namely (i) the transition from light to transmission electron microscopy (TEM) in studying cell structures and functions made by Albert Claude, George Palade, Christian de Duve and Günter Blobel from the famous Rockefeller group in New York, (ii) the transition from contractile to secretory phenotype of vascular smooth muscle cells (VSMC), and (iii) the transition from lipid storage to secretory function of adipose tissue cells.

Seldom has a field owed so much to a single man

In his paper published in 1971 George E. Palade wrote these words for Albert Claude, the founder of biological electron microscopic methodology (1). Now, I would like to articulate the same words for George Palade, thus expressing my in-depth homage to him, the scientific father of many generations in cell biology research and teaching.

George E. Palade was born on 19 November 1912 in Jassy, Northeastern Romania to a family that greatly valued education. His mother was a teacher and his father a professor of philosophy, which explains why he acquired “a great respect for books, scholars, and education early in life” (2 and references therein). He graduated *summa cum laude* from Bucharest University Medical School in 1936. In 1945 his *sapere aude* (from Latin, *dare to know*) encouraged him to jump over the Iron Curtain and land in the United States of America. There, at Rockefeller Institute for Medical Research (later renamed Rockefeller University) in New York, Palade joined Laboratory of Albert Claude (1945-1973), which he later dubbed “The American Cradle of Cell Biology”. Indeed, by this time and in this place was the blossom of TEM. Onward, Palade moved to Laboratory of Cell Biology, Yale University Medical School in New Haven, Connecticut (1973-1990) and Department of Cellular and Molecular Medicine, University of California at San Diego, La Jolla, California (1990-2002). For his discoveries of a new world

of cell structures and their functions, George Palade was awarded 1974 Nobel Prize for Physiology or Medicine shared with Albert Claude and Christian de Duve.

In her concluding remarks Marilyn Farquhar wrote (2): “George Palade was truly “A Man for All Seasons.” He had broad interests and knowledge of science, the arts, and history. He died on October 7, 2008, at the age of 95 after a very full and productive life. Palade believed that the most important thing in life is to “leave something behind for future generations,” and indeed he did. He is considered by many to be the father of modern cell biology and leaves behind an unparalleled legacy of discoveries. All these accomplishments and qualities made him, in the words of David Sabatini, “one of the most admired and beloved figures of our time”.

GEORGE PALADE’S EUREKA!

- RIBOSOMES
- MITOCHONDRIA (Fig. 1)
- ROUGH ENDOPLASMIC RETICULUM (RER)
- GOLGI COMPLEX AND GOLGI-DERIVED SECRETION VACUOLES (Fig. 2)
- SPECIFIC GRANULES IN ATRIAL MUSCLE CELLS
- PLASMALEMMA VESICLES (CAVEOLAE)
- WEIBEL-PALADE BODIES IN ENDOTHELIAL CELLS
- NEURONAL SYNAPSES
- JUNCTIONAL COMPLEXES IN EPITHELIAL CELLS
- PROTEIN SECRETORY PATHWAY

Overview of the secretory pathway

The secretion is a fundamental process in all cells, from the simple yeast to cells in human brain. On April 1898 Camillo Golgi communicated to the Medical-Surgical Society of Pavia, Italy, the discovery of the *internal reticular apparatus*, a novel intracellular organelle which he observed in nerve cells with the silver impregnation (*la reazione nera*) he had introduced for the staining of the nerve cells (3). However, the real existence of this organelle (apparatus or complex) was seriously questioned until it was finally identified by electron microscopy in the mid-1950s, mainly due to the excellent work of George Palade (1, 2, 4, Fig. 2). According to Palade’s classical concept (5) and Günter Blobel’s signal hypothesis (6), the protein secretory pathway constitutes of several intracellular processes: synthesis, target-

Figure 1. Electron micrograph of a section through a mitochondrion in rat hepatocyte showing the cristae, or "cristae mitochondriales," which represent infoldings of the inner mitochondrial membrane. x100 000). Image from the George E. Palade EM Collection.

Figure 2. Electron micrograph of the Golgi complex in a pancreatic exocrine cell. x 25 000 Image from the George E. Palade EM Collection.

ing, sorting, storage (in case of regulated *versus* constitutive secretion), translocation and, finally, exocytosis including porocytosis (7) mediated by porosomes (8). As mentioned, in 1974, the Nobel Prize was awarded to the Three Great „for their discoveries concerning the structural and functional organization of the cell“, and in 1999 to the Fourth Great, Günter Blobel - „for the discovery that proteins have intrinsic signals that govern their transport and localization in the cell“.

The secretory proteins are three major subtypes: lysosomal, plasmalemmal (including endosomal recycling proteins) and exported, the latter being the focus of present lecture. The vast majority of exported proteins are processed by the RER-Golgi complex-TGN (trans-Golgi network)-plasmalemma secretory route including sorting processes using signal recognition particle (SRP)-polysomes-signal peptide (SP) receptor-mediated interactions. These latter being Blobel's topology of cell proteins (6). However, such a SRP-SP dependent protein secretion cannot explain the increasing number of SP-lacking proteins which are also exported such as growth factors and cytokines. The process by which such SP-lacking, leaderless secretory proteins gain access to the cell exterior is termed unconventional protein secretion, which does not use Golgi-TGN processing (9). Intriguing examples of this secretion are extracellular vesicles (a sort of signalosomes, also dubbed nanosomes): (i) exosomes, 30-200 nm vesicles derived from multivesicular bodies (10), and (ii) ectosomes (also named microparticles), 100-900 nm vesicles shed from plasma membrane (11), carrying important bioactive molecules, e.g. DNA, mRNA, microRNA, cytokines and immunoglobulins, to communicate among cells *via* endocrine and paracrine way. Today, these talented vesicles are intensively studied by scientists in basic, translational and clinical research.

Although the clearance of unfolded and misfolded proteins *via* the ubiquitin-proteasome pathway (UPP) is very important for cell protein biology and this has been recognized with the Nobel Prize for Chemistry awarded to Avram Herskko, Aaron Ciechanover and Irwin Rose in 2004, it is outside the scope of present lecture.

Taken together the above data are illustrated in Table 1, 2, Fig. 3, 4.

Table 1. The intracellular protein secretory pathway.

PALADE DIXT	
The protein secretory pathway	
• Synthesis	Ribosomes, RER
• Post-translational modification	Golgi complex
• Sorting	Günter Blobel's signaling amino acid sequence
• Storage	Golgi complex, Secretory vacuoles
• Transport	Microtubules, Kinesin, COP I, COP II Actin filaments, Myosin II
• Exocytosis	Plasmalemma/Porosomes

Abbreviations: RER, rough endoplasmic reticulum; COP, coat protein complex, respectively COP I- and COP II-coated vesicles. COP I-coated vesicles transport proteins from cis Golgi complex back to RER, and between Golgi compartments, a transport termed retrograde transport, in contrast to the anterograde transport associated with COP II-coated vesicles, which transport proteins from RER to Golgi complex.

Table 2. The two major protein secretory pathways.

CELL PROTEIN SECRETION
PALADE's RER-Golgi pathway
Rough endoplasmic reticulum-Golgi complex-Microtubules-Exocytosis/Porosomes
GÜNTER BLOBEL
Signal hypothesis of sorting and targeting of proteins
Non RER-Golgi pathway
- Exosomes (MVB-derived microvesicles)
- Ectosomes (plasmalemma-derived microparticles)

Abbreviations: RER, rough endoplasmic reticulum; MVB, multivesicular body.

Intracellular Transport

Figure 3. Diagram of intracellular pathway followed in the pancreatic exocrine cell of the guinea pig. Image from the George E. Palade EM Collection.

Figure 4. Schematic presentation of intracellular targeting of proteins.

Abbreviations: RER, rough endoplasmic reticulum; GPI, glycosylphosphatidylinositol – a protein (3) is co-translationally anchored via a GPI to the inner surface of RER membrane. Other proteins are indicated 1, 2, 4. From George N. Chaldakov's Textbook of Cell Biology, 2nd edition. 2014 (in Bulgarian).

All cells secrete some proteins

The human body may be considered composed of multiple types of secretory cells delivering a large number of (neuro)peptides, proteins, proteoglycans, steroids and other signaling molecules, which control many biological processes in health and disease (12). Selected examples of such *bona-fide* multicrine cellular system are: (i) skeletal muscles - in response to contraction function as an endocrine and paracrine gland secreting various myokines including interleukin-6 (IL-6), IL-15, irisin, adiponectin, leukemia inhibitory factor, brain-derived neurotrophic factor (BDNF), myostatin (growth differentiation factor 8, GDF-8), (ii) bone – osteocytes secrete the hormone osteocalcin, which modulates glucose metabolism and testosterone production, and fibroblast growth factor-23 (FGF-23) that is involved in phosphate and vitamin D metabolism, (iii) stomach mucosal cells secrete gastrin (GKN1), GKN2, GKN3, also TFF1, TFF2, TFF3 (trefoil factor family domain containing proteins), ghrelin (growth hormone-releasing peptide) and leptin, (iv) diffuse neuroendocrine system (DNES), e.g. intestinal mucosal cells (enteroendocrine cells) secrete the hormones incretins (glucagon-like peptide - GLP-1, GLP-2), glucose-dependent insulinotropic polypeptide (GIP), also peptide tyrosine tyrosine (PYY), cholecystokinin, vasoactive intestinal peptide (VIP), somatostatin, motilin, nurotensin, serotonin, and the testicular Leydig-Davidoff cells, a new member of DNES, secrete testosterone, (v) hepatic stellate cells (Ito cells, perisinusoidal cells, fat- and vitamin A-storing cells) in liver injury modulate into fibrogenic (matrix protein-secreting) cells, critically involved in the pathogenesis of liver cirrhosis, (vi) gut microbial endocrine organ (12a, b) releases trimethylamine-N-oxide, short chain fatty acids, bile acids, 4-ethyl phenyl sulfate, and mycotoxins (fungal toxins) (12c, d) which affected many organs, (vii) fibroblasts, chondrocytes, osteocytes, vascular smooth muscles and other fibrogenic cells secrete matrix proteins (procollagens, proelastin, fibrillin, fibronectin, etc), proteoglycans and glycosaminoglycans, and (viii) adipose tissue cells secrete more than 600 signaling proteins collectively termed adipokines.

Figure 5 illustrates schematically multicrine nature of a secretory cell.

Figure 5. A drawing illustrating multicrine and receptor feature of a secretory cell. Depending on its nature, the cell synthesizes, stores and releases, that is, secretes, some of the signalling proteins listed in the square. These communicate via multiple pathways, such as endocrine (arrows 1, 4 and 5, from top to bottom), paracrine (arrow 2) and autocrine (arrow 3, curved); also shown are exosomes and ectosomes. Cell receptors for different ligands are depicted at right side of the figure. From (12).

Vascular biology: secretory phenotype smooth muscle cells

Paradigm shift in the research on vascular smooth muscle cells

In 1960 Maria Daria Haust and colleagues (13) published their seminal article suggesting the fibrogenic potential of VSMC as related to extracellular matrix production. Since then VSMC secretion and proliferation have been increasingly studied as key cellular phenomena in the initiation and development of atherosclerosis. This concept was further sophisticated by Russell Ross (reviewed in 14). Of note, in the last 10-15 years a new paradigm shift is emerging in the field, for which we are preparing a paper tentatively entitled “Quo vadis, atherogenesis?”

In 1973 in Heidelberg, Germany, a Symposium on *The Smooth Muscle of the Artery* was held. There, as an invited speaker I have presented our own TEM findings about (i) Golgi-derived secretion granules and coated vesicles, and (ii) cytoplasmic microtubules in the secretory pathway of VSMC (15). Based on these and our further results (16-18), the term “secretion” was for the first time linked to VSMC function, and the concept of secretory process as viewed by TEM (Fig. 6-11) introduced in VSMC biology. Later on it was appreciated by the vascular biology community, thus replacing the terms “modified SMC” and “synthetic SMC”,

Figure 6. Electron micrographs of secretory-state (secretory phenotype) aortic smooth muscle cells of the rabbit. a. Well developed Golgi complex (G), b-e. Vacuoles with a fuzzy (probably not clathrin) coat (arrowheads and arrows). e. Vacuole-associated microtubules. a, x11 000; b, x30 000; c, x20 000; d, 45 000; e, x80 000. From (17).

Figure 7. Electron micrographs of secretory-state (secretory phenotype) aortic smooth muscle cells of the rabbit. **a-e.** Spherical-shaped (arrows) and elongated-shaped (arrowheads) secretion granules. **a-e**, x20 000. From (17).

Figure 8. Electron micrographs of secretory-state (secretory phenotype) aortic smooth muscle cells of the rabbit. **a.** Golgi-associated clathrin-coated vesicles (arrows). **b.** Elongated vacuole linked via filamentous arms (two arrows) to microtubule. **c.** Plasmallema-derived coated pits (three arrows). **d.** Group of Golgi vesicles are sandwiched between microtubules. **e.** Rough endoplasmic reticulum-associated microtubules. **a, c, d**, x50 000; **b**, 80 000; **e**, 20 000. From (17).

which were not conceptually correct in sense of Palade's data and understanding of cell protein secretion. Here is the great significance to find the Teacher, and to creatively follow Him.

Microtubules are essential for the secretion in vascular smooth muscle cells

Our colchicine study for the first time aimed at the possible role of microtubules (MT) for VSMC secretory process in aortic arch, thoracic aorta and pulmonary trunk of 20-day-old rabbits. The rabbits were treated with a sub-antimitotic dose of colchicine, a tubulin-binding (antitubulin) substance leading to the disassembly of cytoplasmic MT. The VSMC of control, untreated rabbits showed well-developed RER and Golgi complex, and MT associated with Golgi-derived secretion granules (vacuoles) and with cisternae of RER (Fig. 6-8). Colchicine treatment affected (i) MT in a monotypic way (all VSMC contained no MT after colchicine), and (ii) Golgi complexes and RER in a dual way, resulting in two major structural subpopulations of VSMC. One subpopulation displayed a significant accumulation of Golgi-derived secretion granules, whereas another subpopulation showed a vacuolar dilation of RER cisternae (16-18, Fig. 9-11). Moreover, these results may gain insight into a new pharmacotherapeutic approach directed to MT-disassembling agents (16-19).

Adipobiology: most cell types of (white) adipose tissue are secretory in function

Leptin, Jeffrey Friedman and Adipose's Big Bang

On 1 December 1994 the *ob* gene in mice and its homolog in humans were "cloned officially" in *Nature* 372: 425-432, and 1995 *ob* gene product purified and named leptin (from Greek *leptos* – thin), the first adipocyte-secreted hormone. In fact, adipisin was the first adipokine discovered (Cook KS, et al. *Science* 1987; 237: 402-405). The seminal results related to *ob* gene and leptin were achieved by Jeffrey Friedman and coworkers in **Laboratory of Molecular Genetics** at Rockefeller University in New York. Leptin is released "classically" from white adipocytes (now also from gastric mucosal cells and cardiomyocytes) into the bloodstream and acts on the brain. There leptin stimulates hypothalamic anorexigenic neurons (mediated by cocaine- and amphetamine-regulated transcript - CART peptide and α -melanocyte-stimulating hormone - α -MSH), whereas inhibits orexigenic neurons (mediated by *agouti* gene-related protein - AgRP, melanin-concentrating hormone - MCH, and neuropeptide tyrosine - NPY). This adipose-brain circuit maintains homeostatic control of food intake-energy expenditure, and adipose mass and body weight respectively.

Figure 9. Electron micrographs of secretory-state (secretory phenotype) aortic smooth muscle cells of the rabbit treated with a sub-antimitotic dose of colchicine. The cells responded to the treatment by (a) an accumulation of secretion granules (circles), or (b) vacuolar type dilation of rough endoplasmic reticulum (RER) cisternae, some of them approaching the cell periphery (1, 2, 3), suggesting of a direct RER, not dependent on MT, way of exocytosis. **a, b**, x10 000. From (17).

Figure 10. Electron micrographs of secretory-state (secretory phenotype) aortic smooth muscle cells of the rabbit treated with colchicine. **a.** An aggregate of secretion vacuoles; arrows show electron dense material within the cavity of some vacuoles. **b.** Encircled are cross-sectioned intermediate filaments; vacuoles (V). **a, b,** x20 000. From (17).

Figure 11. Electron micrograph of secretory-state (secretory phenotype) aortic smooth muscle cell of the rabbit treated with colchicine. This micrograph is a higher magnification of Figure 9b showing vacuolar dilation of rough endoplasmic reticulum cisternae, some of them (1, 2, 3) approaching progressively the cell periphery. From (17).

In essence, the discovery of Jeffrey Friedman's research group opens an exciting scientific avenue of endocrine and paracrine function of WAT. This is what I call Adipose's Big Bang which is expanding through the human body, thus implicated in health and in the pathogenesis and therapy of cardiometabolic, neurodegenerative, cancer, liver, ovary and other diseases.

Periadventitial adipose tissue is, in fact, tunica adiposa of the vascular wall

In 1983 at the Department of Anatomy, University of Chicago Medical School, Chicago, IL, USA, I presented a lecture about the ultrastructure of secretory process in VSMC. During the discussion, a question whether adventitial fibroblasts may migrate into the intima was raised. The answer of the lecturer was "I do not know. It seems impossible." However, what seemed "impossible" in 1983 was proven possible in 1996 by Shi *et al* demonstrating that adventitial myofibroblasts may contribute to neointima formation in coronary arteries (*Circulation* 1996; 94: 1655-1664).

Since 1998 we, together with our Italian BHF (Brain-and-Heart Friends) Luigi Aloe and Marco Fiore, have been moving further "outside-in", and proposed: "If signals and cells can be translocated from the adventitia into the intima, and hence lead to intimal lesions, then why not look for similar reactions from the artery-associated adipose tissue?" (20), the latter being des-

ignated *tunica adiposa* (21; Fig. 12).

Recently, cardiometabolic diseases (atherosclerosis, hypertension, obesity, type 2 diabetes and metabolic syndrome) are among the major physical, social and economic burdens, globally. Arguably, we have learned more about the molecular control of food intake and energy homeostasis, particularly the role played by adipose tissue in the pathogenesis of cardiometabolic and other diseases. This intellectual growth process was conceptualized as adipobiology and adipopharmacology of disease (23).

Today, it is known that about 30 % of genes in adipose cells (adipocytes, stroma-vascular cells, and associated immune cells) encoded for more than 600 exported secretory proteins collectively designated adipokines (20, 23). Altogether, recent studies have shifted the paradigm of WAT from simple lipid and energy storage to the body's major endocrine and paracrine organ. Accordingly, two major sub-fields of adipobiology have emerged, adipoendocrinology and adipoparacrinology (24-26).

The present challenge is therefore to cultivate secreto-centric thinking about how we can make VSMC secretion and adipose secretion work for the benefit of human's health. This may indeed be a step forward but not the whole route in the systems biology of cell protein secretion.

Whatever we move we have to most sincerely thank George Emil Palade. "Palade was an extraordinarily gifted and visionary

Figure 12. Schematic presentation of vascular wall composed of four tissue coats (tunicae): intima, media, adventitia, and adiposa. Arrows show that tunica media is a target for at least two vasorelaxing factors, endothelium-derived relaxing factor (EDRF) and adipocyte-derived relaxing factor (ADRF) respectively. Discontinuous black line positioned at the adventitia-media border illustrates perivascular nerves. Small-sized discontinuous black lines located in tunica adiposa indicate adipose nerves. Black granules (except those linked to arrows) illustrate immune cells – their association with nerves and adipocytes is also depicted. From (22).

scientist, and a generous mentor to several generations of scientists who went on to stellar careers of their own. He was also an esteemed collaborator who earned the widespread respect and affection of colleagues the world over. He considered the training of new generations of scientists to be an important calling, based upon his belief that scientific discovery is “an enterprise that continues generation after generation.” (From George E. Palade lecture presented by Thomas Südhof, 2013 Nobel laureate in Medicine, on 20 May 2014 at UCSD, USA.)

Noroc, Teacher! A photograph taken after the official part of *Doctor Honoris Causa* ceremony for George Palade in Cluj-Napoca, Romania. From left to right: Gheorghe Benga and George Palade. Courtesy of Professor Gheorghe Benga.

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