Continuing Medical Education

A SAMPLE COPY OF THE TEXTBOOK *PRINCIPLES OF CELL AND TISSUE BIOLOGY*

PRINCIPLES OF CELL AND TISSUE BIOLOGY

A CONCEPTUAL TEXTBOOK FOR STUDENTS OF MEDICINE, DENTAL MEDICINE, CELL BIOLOGY AND ALL UNDERGRADUATES AND GRADUATES WHO ARE THINKING OF DOING RESEARCH.

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This textbook is dedicated with respect and love to my parents, teachers and friends, including my family and all curious students.

George N. Chaldakov

ACKNOWLEDGMENTS

Plato learned from Socrates, Aristotle – from Plato, Friedrich Nietzsche – from Fyodor M. Dostoyevsky, Salvador Luria, Renato Dulbecco and Rita Levi-Montalcini – from Giuseppe Levi, Luigi Aloe – from Rita Levi-Montalcini, George Palade - from Albert Claude, Günter Blobel – from George Palade The author of Cell and Tissue Biology – from his parents, teachers and friends.

Front cover: Micrograph of the mitotic spindle in a human cell showing microtubules in green, chromosomes in blue, and kinetochores in red during metaphase of mitosis. From: Wikimedia Commons.

*That is the high tide of hierarchy in action,
From the groan to the sigh - to the echo...*

Hristo Fotev

The textbook has grown from many years of reading and discussions. Among the numerous colleagues with whom I have exchanged views during the past 59 years, my scientific father Delcho Zhelyazkov, and my colleague-brain-and-heart friends (BHF) (listed chronologically) Michael S. Davidoff, Balyu Balev, Stoyan V. Stoev, Slavi P. Slavov, Kamen G. Uzunov, Maria D. Zhelyazkova-Savova, Stanislav Yanev, Jack P. Strong, Anna Kadar, Harry Jellinek, Peter I. Ghenev, Krikor Dikranian, Alexander K. Stoychev, Yukio Yamori, Yasuo Uehara, Takashi Fujiwara, Geoffrey Burnstock, Ronald L.A.W. Bleys, Kosta V. Kostov, Kamen P. Valchanov, Anton B. Tonchev, Rouzha Z. Pancheva, Wale A.R. Sulaiman, Gorana Rančić, Neşe Tunçel, Dragan Djuric, Vladimir Jakovljevic, Rita Levi-Montalcini, Luigi Aloe, Marco Fiore, Denys N. Wheatley, John E. Heuser, Danko D. Georgiev, Hiroshi Yamamoto, Gheoghe Benga, Marcia Hiriart, Arie Gertler, Bhanu Jena, Jerzy Beltowski, Harpal Buttar, Johan Renes, Oreste Gualillo, and Kelath M. Manoj have been particularly influential.

I. PRINCIPLES OF CELL-MATRIX BIOLOGY

INTRODUCTION

Student – Latin, *studeo* – devoted to do something; *studium* – eager, passion, study.

To be a teacher is profound responsibility.

Emily Dickinson, a paraphrase of *To be a flower is profound responsibility.*

It is *lyuboznanie* (from Bulgarian, love-of-knowledge) that drives us to find our Teacher. The love-of-knowledge to which the growth of science and education is due. The study of being a teacher, *pedagogy* (from Greek “to lead the child”; *paidos*, “child” and *ago*, “lead”), is among the most responsible human duties. Etymologically, in the word “education”, there is also “to lead” (from Latin *ducere*) – to “bring forth what is within”, “bring out potential” of your students.

The textbook contains updated, science-based information-and-knowledge of

Molecules, Structures and Functions (MSF) of cell and extracellular matrix (ECM, briefly, matrix) which build the tissues and organs of the human body. This can enable students to understand the triarchic – MSF – nature of cell-and-matrix life. I want to transmit the knowledge I have been accumulating to students and all curious minds devoted to biomedicine. To enable meaningful understanding the cellular and molecular mechanisms (pathogenesis) of **Diseases** and their **Prevention and Therapy (DPT)**.

The one who sees things in growth all of them, it will have the proper understanding of them.

Aristotle

The principle (Latin *principium* means “first, foremost”) on which is based the present textbook is: **From MSF to DPT**. Accordingly, it is a Bench-to-Bedside (B2B), Translational Cell and Tissue Biology textbook. Even knowing all MSF, without knowledge of the principles that govern them, we will not be able to build our own way of thinking (frame of mind) of the multiplex matter of cell biology and biomedicine in general. Of course, we should know the scientific facts, but I believe

that they are required primarily to incorporate them into knowledge, hypotheses, and concepts. Noteworthy, it is important “to know how”, not only “to know that”, a research-focused teaching illustrated herein by an Chinese saying: “If you give a man a fish, you feed him for a day. If you teach him to fish, you feed him for a lifetime”.

I want to energize *holy curiosity and heuristic potentials* of the students and all devoted to biomedicine, who, after continuous self-improvement, to be able to create new knowledge of MSF and DPT. Ultimately, I tried to write *Cell and Tissue Biology* presenting a conceptual (perhaps uniquely) view of MSF as related to DPT, being away from any descriptive details. My didactics is mediated by Stream of Associations (SOA) (Fig. 1) and KISS (Keep It Short and Simple).

In the same SOA, *KISS* gene (gene's names in *italic*) encodes kisspeptin (initially termed metastin), a protein involved in metastasis suppression and reproductive biology. A chocolate story of the name: 1996, in a research laboratory in Hershey, Pennsylvania, USA was isolated a DNA from cancer (melanoma) cells that was not able to undergo metastasis. This gene was named *KISS* because of the location of where it was discovered - Hershey, PA, home of the famous Hershey's kisses chocolate candy.

Figure 1. A drawing by my grandson Nikifor (when was 11 year-old) presenting Stream of Associations, an emblem of the associative teaching.

This textbook embodies a personal philosophy “standing on the shoulders of giants” in biomedical sciences. It is for students of medicine, dental medicine, cell biology as well as all undergraduates and graduates who are thinking of doing research but have not made up their minds. As any human activities this textbook can and should be made better. Therefore I appeal to readers to propose corrections and novel ideas.

With Respect, Love and Hope,

BHF*-ly yours,

George N. Chaldakov, MD, PhD, DHC, FIACS

*Brain-and-Heart Friend

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In Cell Biology language, my nexuses (communicating junctions) are:

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- Home phone: +359 52 754 394
- YouTube. Lectures George Chaldakov
- Research Gate. George Chaldakov

SCIENCE AND EDUCATION ARE ONGOING PROCESSES

In Cell Biology, also other biomedical sciences, knowledge grows and disseminates as well as changes continuously. Yet, almost everything is “probable” or “most probable”, and “approximately” and “*et cetera (etc)*” at quantitative level – there are no “all”, “always”, “everything” and so forth. However, from didactic viewpoint I use some of these imperatives, whereas “probable”, “most probable”, “approximately”, “*et cetera (etc)*” are rarely mentioned. For example, “All animals, including humans, have eukaryotic cells (cells with karyon/nucleus).” Instead of “Almost all animals, including humans,”, which, in fact, is true. Note, key terms are marked in bold when appeared for the first time in the text.

The teaching maneuver of the textbook reminds that of teaching new foreign language

Definitions of language (according to Encyclopædia Britannica)

“Henry Sweet, an English language scholar, stated: Language is the expression of ideas by means of speech-sounds combined into words. Words are combined into sentences, this combination answering to that of ideas into thoughts.”

Namely “words are combined into sentences.... aimed at the facilitation of communication, in the sense of transmission of information” and knowledge from teacher to students was applied to the present textbook – teaching students to firstly learn alphabet, words and sentences, and thus be ready writing essays on MSF and DPT.

Let us starting then.

Both textbooks and lectures are *condition sine qua non*, but not enough

Search for and read scientific articles, using the following information resource:

- PubMed/Medline, Scopus, Thomson Reuters and other abstract and citation databases – enter the respective website, write one, better two key words or write the family name and initial/s of the author your curiosity searching for, click, and the abstracts of articles published in the prestigious scientific journals will appear with authors’ names, title, journal, year, volume, pages, and DOI (Digital object identifier). Enjoy the regular and creative reading!

Among all the microscopes used in studying the cell, the brain microscope only has heuristic potentials.

- Light microscope (microscopy) - fluorescence, laser-scanning confocal microscopy and other imaging technologies for observations of both fixed and living cells.
- TEM, transmission electron microscope – routine and new technologies such as
- Cryogenic electron microscopy (cryo-EM) - TEM applied on frozen cell specimens to obtain high-resolution, 3-D images.
- SEM, scanning electron microscope
- AFM, atomic force microscopy - obtaining a very-high-resolution (nanoscale) images of the cell.
- STM, scanning tunneling microscope - investigation of biological systems under near-physiological fluid conditions.

A brief history of modern cell biology

In the second half of 1940's there was an explosion in the biological sciences with the rapid emergence of Cell Biology (which had deleted Cytology as a term). These events were affected by the development and use of new technologies, specifically the TEM. Albert Claude was the first who applied methodology for obtaining cell images by using TEM. The first micrographs he obtained were from cultured (*in vitro*) fibroblasts on 6 July 1944 at the Rockefeller Research Institute, New York, NY, USA. Electron microscopes made visible the fine structure (ultrastructure) of cell-and-matrix.

In awarding the 1974 Nobel Prize in Physiology or Medicine to Albert Claude, George Palade and Christian de Duve “for their discoveries concerning the structural and functional organization of the cell”, the Chairman of the Nobel committee said “the trio was largely responsible for the creation of modern cell biology”.

When received Nobel prize, George Palade remarked: “Cell biology finally makes possible a century-old dream*: that of analysis of diseases at the cellular level, the first step toward their control.” Palade, described as “the most influential cell biologist ever”, defined the structure and function of ribosomes, mitochondria, rough endoplasmic reticulum (RER), and Golgi complex, and characterised the cell protein secretory pathway. (I have learned a lot from the Palade's concept of cell protein secretion (in exocrine pancreatic cells) I applied to my study on the ultrastructure of secretion in vascular smooth muscle cells.)

George E. Palade (November 19, 1912 – October 7, 2008) was a Romanian-American cell biologist graduated in 1940 from the Carol Davila School of Medicine, Bucharest.

In the early 1970s Palade's student Günter Blobel discovered that newly synthesized proteins have an intrinsic signal that is essential for guiding them to and across the membrane of RER; this was designated “signal hypothesis”. For his discovery that “proteins have intrinsic signals that govern their transport and localization in the cell”, Blobel received the 1999 Nobel Prize in Physiology or Medicine. Proteins carrying out essential functions are constantly made in our cells. These proteins have to be transported to their subcellular target compartments such as RER, nucleus, mitochondria or other membrane-bound organelles. Blobel characterized the molecular mechanisms underlying these processes, showing that similar “address tags” comprised specific amino acid sequence, navigate proteins to target destinations within and outside the cell. This discovery also gave birth to a new field in cell biology, **molecular cell biology**.

Further, Palade's grand student Bhanu Jena at Wayne State University, Detroit, MI, USA, using of new nanotechnologies, such as AFM, also made pioneering contributions. Bhanu Jena and his research team have discovered a new cellular structure, the "porosome", located at the plasma membrane (plasmalemma), where secretory vacuoles fuse to release their stored proteins. This work has revealed, at nanometer resolution, the molecular structure and dynamics of the porosome in live cells. This *Eureka* has given birth to yet another important field in cell biology, **nano cell biology**

(• Singer MV. **Legacy of a Distinguished Scientist: George E. Palade**. *Pancreatology* 2003;3:518–519. DOI:10.1159/000076328

• Jena B. Porosomes: The universal secretory portal in cells. *Biomed Rev* 2010;21:1-15. DOI:10.14748/bmr.v21.42).

*Two examples of "a century-old dream" are presented in Figure 1 and Table 1.

Figure 1. Schematic illustration of adipose(fat) tissue (AT) as an example of secretory organs. Adipose tissue is consisted of adipocytes, fibroblasts, mast cells, macrophages and other immune cells (not depicted). All these cells are bona fide secretory, that is, they synthesize, fold, target, store, and release more than 500 different proteins collectively designated **adipokines**. The arrows left, from up-to-down, indicate **endocrine, paracrine and autocrine pathway**; other two arrows show the extracellular vesicles **exosomes and ectosomes**. At right, depicted are **receptors** for various **signaling molecules** (collectively named **ligands**).

From: Chaldakov GN, Stankulov IS, Hristova M, Ghenev PI. *Adipobiology of disease: adipokines and adipokine-targeted pharmacology*. *Curr Pharm Des* 2003;9:1023-1031. DOI:10.2174/1381612033455152

Table 1. A list of cardiometabolic diseases

Atherosclerosis, Hypertension, Acute coronary syndromes (coronary heart diseases)
Congestive heart failure, Atrial fibrillation
Stroke (ischemic and hemorrhagic), the major example of cerebrovascular diseases
Obesity
Type 2 diabetes mellitus
- Diabetic neuropathy
- Diabetic retinopathy
- Diabetic erectile dysfunction
- Diabetic nephropathy
Metabolic syndrome, Metabolic-cognitive syndrome
Type 3 diabetes mellitus (Alzheimer's disease)*

* The term "**cardiometabolic diseases**" (CMD) covers a larger list of diseases than that of "**cardiovascular diseases**" (CVD). In the USA, diabetes cost an estimated \$174 billion in 2007. In CVD, according to the American Heart Association and the American Stroke Association the total medical cost moves from \$318 billion in 2005 to \$749 billion in 2035. The major complications of CMD – myocardial infarctions and stroke – are accountable for nearly half of all deaths of non-communicable diseases (NCD) making them the world's number one killer of humans, claiming 17 million lives each year. We must educate people as well as politicians that by controlling risk factors, at least 80% of premature deaths from CMD could be avoided. If not, in 2035, the number of Americans with CMD is projected to rise to 131.2 million – 45 percent of the total USA population.

*Nothing **a cell** does is without significance – Dennis N. Wheatley*
*Nothing **a molecule** does is without significance.*

There is a concept that “form follows function.” Likewise, structure follows molecule’s function - biomolecules transmit their properties into the structures that build and these properties become structures’ functions.

Cells are Molecules they synthesize, Structures they build up, and Functions they perform. The combination of MSF distinguishing one type of cells from another is **cell differentiation** (Table 2).

Table 2. A selected list of names of cells*

Nondifferentiated (“baby”) cells	Differentiated (mature) cells
Neuroblast	Neurocyte (neuron, neural cell, neuronal cell)
Myoblast	Myocyte
Erythroblast	Erythrocyte
Lymphoblast	Lymphocyte
Osteoblast	Osteocyte
Chondroblast	Chondrocyte
Tenoblast	Tenocyte
Adipoblast	Adipocyte
Enteroblast	Enterocyte

* Man gave names to all the cells

In the beginning, long time ago.

A paraphrase from **Bob Dylan’s** “Man gave names to all the animals”

You were studentoblasts, now you are studentocytes. Examples for molecules: pro-insulin, insulin; nerve growth factor (NGF), pro-NGF; brain-derived neurotrophic factor (BDNF), pro-BDNF.

Students learning Cell Biology have to ask Questions and give Answers

Which Molecules, How, Where (cell, matrix, tissue, organ), When (interphase, mitosis, meiosis), What Structures they form, and What Function perform. Hence, the more the Student knows cell-and-matrix MSF, the better could understand DPT – the mission of the physician.

As it was mentioned, we will use the term “matrix” for ECM. Some intracellular organelles however also have their own matrix – then, we will say: “mitochondrial matrix, centrosomal matrix, nuclear matrix”.

One picture is worth a thousand of words.

Chinese saying meaning that information, ideas and knowledge can be conveyed by a single image, which conveys its essence more effectively than a mere verbal description.

BASIC IMAGE TERMINOLOGY

The following set of figures starting with some topological (Fig. 2-4) and structural (Fig. 5-8) terms.

Figure 2. Some cellular terms are shown.

Figure 3. An illustration of Cell Biology language.

Figure 4. Examples of words in Cell Biology language.

Figure 5. Words for cell and matrix structures in Cell Biology language. See at right: 3F includes filament, fibril, and fiber.

Structure	Longitudinal cut	Transversal cut	Diameter/nm
vesicle			60-80
endosome			300-500
secretory granule			300-500
lysosome			300-500
peroxisome			300-500
multivesicular body			500-700
tubule			60-80
smooth bag			-
granular bag...			-
Golgi-bag			-

Figure 6. Membrane-bound structures. Smooth cistern (cisterna), rough cistern, Golgi cistern are more appropriate terms than smooth bag, granular bag, Golgi-bag as indicated in the figure.

Figure 7. Membrane-enclosed (membrane-bound) and membrane-nonenclosed (nonmembrane-bound) structures. Note, clathrin coat is, in fact, composed of two molecular components: clathrin and clathrin adaptor proteins (AP1-4; adaptins). Thus, it should be described as “clathrin-adaptin coat”; however, for brevity, “clathrin coat” is commonly used. COP, COated Protein.

Figure 8. Schematic illustration of the morphogenesis (“origin of form”) of membrane-bound vesicles. COP, COated Protein, GER (a wrong abbreviation for RER – rough endoplasmic reticulum). Note, (i) **invaginations** - membrane protrusions toward the interior of the cavity covered with membrane, and (ii) **evaginations** - membrane protrusions toward the exterior of the cavity covered with membrane.

Imagine a world in which each of us knows a part of alphabet only.

Jamey D. Marth. A unified vision of the building blocks of life.
Nature Cell Biololgy 2008;10(9):1015–1016. DOI:10.1038/ncb0908-1015

The language of Cell Biology has its own alphabet, terms (words), and syntax - rules for the arrangement of the words in the sentences discussing MSF and DPT.

INTRODUCTORY TERMINOLOGY

Key terms

Plasmalemma (PL) - synonyms: plasma membrane, cell surface

Ligands - Latin *ligare* means “to connect, to bind”. **Ligands are signaling molecules that bind to receptors.**

Receptors - molecular antennas for a vast number of ligands. Receptors are plasmalemmal proteins, glycoproteins, or proteoglycans. There are three main places the receptors are localized in the cell: (i) plasma membrane, (ii) endomembranes (covering intracellular organelles), and (iii) cytosol (you also may say “cytoplasm”; the difference between “cytosol” and “cytoplasm” is subtle).

Molecules, structures, function, organelle, ligands, receptors, signal transduction, signaling pathway, receptor-mediated, actin, microtubules, assembly, disassembly, ECM (matrix) are among the most important words used in Cell Biology language. In cell biology, everything is important, but not everything is most important.

Biomorphogenic principle (BMP) – Greek *morphê* meaning shape, *genesis* - creation, literally „the generation of form“. BMP addresses the cell process that leads a structure to develop its shape (• Chaldakov GN. Biomorphogenic principles of cell-matrix biology: A plectics insight. *Biomed Rev* 2019;30:143-147). BMP does not deal with **morphogenesis** which is a biological process that causes a cell, tissue, and organism to develop their shape; that is the topic of **developmental biology**.

Biomorphogenic principles

First biomorphogenic principle: Membrane compartmentalization

Second biomorphogenic principle: Membrane fractalization

Third biomorphogenic principle: Membrane fission-fusion

Fourth biomorphogenic principle: Assembly and disassembly

Fifth biomorphogenic principle: Assembly and package of matrix molecules

Receptor-mediated signal transduction – a down stream (propagation) of signals induced by ligand-receptor interaction.

Receptor-mediated endocytosis – internalization of proteins using plasma membrane-derived clathrin pits/vesicles and caveolin pits/vesicles

Clathrin-coated pits (Latin *clathrum* meaning “lattice”) – 150-200 nm in 2R invaginations of the plasma membrane coated with clathrin and adaptin.

Clathrin-coated vesicles – pinching off formation of clathrin/adaptin-coated pits.

Caveolin-coated pits (caveolae, „little caves“) - 50–80 nm in 2R invaginations of plasmalemma covered with caveolin and cavin proteins. **Caveolae and exosomes** (a type of extracellular vesicles) are the smallest in size multifunctional membranous structures.

Caveolin-coated vesicles (caveolosomes) - pinching off formation of caveolin-coated pits.

Cytoskeleton – a group of **nonmembrane-bound organelles**

- AF, actin filaments (5 nm in 2R)
- AAP, actin-associated proteins
- AF-based motor proteins (myosin II, V-VIII, X)
- IF, intermediate filaments (10 nm in 2R)
- MF, myosin filaments (15 nm in 2R)

- MT, microtubules (25 nm in 2R)
- MAP, MT-associated proteins
- MT-based motor proteins (kinesin, dynein, dynamin)

Cytosol - the liquid medium contained within a cell.

Cytoplasm includes the cytosol, all the organelles, and the liquid contents inside the organelles.

Extracellular matrix (ECM; briefly, matrix)

Focal adhesion contact (FAC) – a matricellular structure

Focal adhesion kinase (FAK)

Cell adhesion molecules (CAM)

Key learning features

Classification of cell organelles (structures)

Note, the terms “organelles” and “structures” are relatively convertible in both meaning and usage.

A human cell (e.g. hepatocyte) contains: one nucleus, approx. 1700 mitochondria, 400 peroxisomes, 300 lysosomes, 200 endosomes, and 20 Golgi complexes.

Nuclear structures

- **Nucleus (karyon)** - a double membrane-bound structure.
- **Nucleolus**, a nonmembrane-bound organelle – birth place of the ribosomes.
- **Nucleosome**, an octamer of histone (H1) encircled by DNA “rope”, the repeated units of DNA architecture.

- **Nucleolemma** (Latin *lemma* – coat) – a double membrane-bound envelope of the nucleus.
- **Nuclear lamina** (Latin *lamina* - sheet) – composed of **lamin** protein attached on the inner side of the inner nuclear membrane, and working in tandem with DNA in the process of **gene transcription**.
- **Nuclear pore complex (NPC)** – a supramolecular structure embedded in the nucleolemma. The protein building blocks of NPC are **nucleoporins (NUP)**, a family of 30 proteins (NUP35, NUP50, NUP107, NUP205, *etc*). For instance, NUP 62 is nucleoporin with molecular weight of 62 kD (kilo Dalton). NUPs translocate at a very high rate proteins from the cytoplasm across NPC into the nucleus. A single NPC can do that for approx. 70 000 protein molecules per minute.
- Spliceosomes – a large ribonucleoprotein complex composed of spliceosomal RNAs (snRNAs) and multiple proteins. They remove noncoding segments (introns) from pre-messenger RNAs (pre-mRNAs), and splice its coding segments (exons), a process critical for mRNAs maturation and for subsequent translation by polyribosomes (polysomes).

Membrane-bound organelles

• Rough endoplasmic reticulum (RER)

- RER is composed of a large group of cisterns with polyribosomes attached on the cisternal surface; vesicles are also components of RER:
- COated Protein (COP) – COP I and COP II vesicles originate from RER cisternal membranes; both types of COP vesicles function as **cargosomes** - they realize a bidirectional transport of secretory proteins (cargo) between RER-Golgi

subcompartments. COP II vesicles export selectively newly synthesized proteins from RER. COP I vesicles mediate a retrograde transport that selectively recycles proteins from *cis*-Golgi into the RER (Fig. 9).

Figure 9. Schematic representations of the secretory – RER-Golgi - membrane flow. **1.** COP II vesicle originates from RER and is translocated to Golgi complex (GC) through membrane fission-fusion way. **2.** GC-derived clathrin-coated vesicles. Note, GC delivers both secretory vacuoles (granules) and lysosomes; their content (exportable proteins) discharged via exocytosis (remember Jena’s porosomes, the portals of exocytosis – see “A brief history of modern Cell Biology” at the beginning of the textbook).

• Golgi complex (briefly, Golgi)

Note, from Latin, *cis* means “on the same side”; *trans* - “on opposing sides”.

- CGN, *cis* Golgi network – a subcompartment containing cisterns, tubules and COP I vesicles located at *cis* pole of Golgi complex, nearby the RER.
- TGN, *trans* Golgi network – a subcompartment of Golgi complex containing cisterns, tubules, vacuoles and clathrin-coated vesicles located at *trans* pole of Golgi complex (Fig. 10, 11), closed to the centrosome (central body).
- RER, Golgi complex and AF-based and MT-based motor proteins are the main players in the protein secretory pathway.

Figure 10. TEM micrograph of the Golgi complex in a pancreatic exocrine cell. x 25 000. **From:** George E. Palade’s TEM Collection.

Figure 11. TEM 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:** Chaldakov GN. Colchicine, a microtubule-disassembling drug, in the therapy of cardiovascular diseases. *Cell Biol Int* 2018;42(8):1079-1084. DOI:10.1002/cbin.10988

• Smooth endoplasmic reticulum (SER)

- SER is composed of cisterns (lacking attached polyribosomes), tubules and vesicles (tubulovesicles).
- storage and release of calcium ions (Ca^{+2})
- biosynthesis of steroid hormones (estrogens, androgens, vitamin D)
- drug- and endogenous substance-metabolizing function performed by the superfamily of
- cytochrome P450 (CYP450) enzymes. “P450”, as a term, derived from its maximum absorbance at 450 nm. CYP450 enzymes catalyze the oxidation and metabolism of a large number of **xenobiotics** (exogenous compounds) and endogenous compounds. CYP450 enzymes are located in SER and mitochondria, mostly in hepatocytes (liver cells).

In the human body, there are three immune (defence) systems: two against microbes (bacteria, viruses, alike) and one against exogenous and endogenous toxic compounds:

- (i) **cellular immunity (cell-mediated immunity)** that involves immune cells - T helper (Th) lymphocytes and natural killer cells,
- (ii) **humoral immunity** – a defence with antibodies (immunoglobulins - Ig) circulating in the blood and other extracellular fluids,
- (iii) **enzyme immunity** mediated by CYP450 enzymes that metabolize endogenous compounds as well as detoxify and/or metabolize **xenobiotics** (drugs and pollutants). Note, **exposome** is a collection of environmental factors, such as pollution, stress, lifestyle, to which an individual is **exposed** and which can have pathogenic effects on health

(• Wild CP. Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiology, Biomarkers and Prevention* 2005;14:1847–1850. DOI:10.1158/1055-9965.EPI-05-04).

• Intracellular vesicles and vacuoles

- Clathrin-coated vesicles, Caveolin-coated vesicles, Caveolosomes, Early endosomes, Late endosomes, Multivesicular bodies (MVB), COP I- and COP II vesicles, Secretory vacuoles (secretory granules), Lysosomes, Phagosomes, Autophagosomes*, Peroxisomes, and Synaptic vesicles (see Fig. 6-8), also Liposomes (lipid droplets) and Weibel-Palade bodies, a signature structure of the vascular endothelial cells**.

*Nucleus, mitochondria, and autophagosomes are covered with double membrane. The remainder membrane-bound organelles are covered with single membrane.

The cells that form the inner lining of the blood vessels and heart. Weibel-Palade bodies store and release two principal molecules, **von Willebrand factor and **P-selectin**, thus play a role in **hemostasis** (“state of the blood”) and **inflammation** (a protective response-to-injury aimed at the elimination of cell damages and initiate tissue repair, a healing process termed **resolution of inflammation**). **Note**, we should stimulate the resolution rather than the inhibition of inflammation (• Perretti M, Leroy X, Bland EJ, Montero-Melendez T. Resolution Pharmacology: Opportunities for Therapeutic Innovation in Inflammation. *Trends Pharmacol Sci* 2015;36(11): 737-755. DOI:10.1016/j.tips.2015.07.007).

• Extracellular vesicles

- Exosomes – MVB-derived vesicles, 50–100 nm in 2R; when the MVB fuses with the plasmalemma, intraluminal vesicles of MVB are released from the cell and become exosomes.
- Ectosomes (microparticles, microvesicles) – 100-500 nm in 2R vesicles derived *via* plasma membrane budding-pinching off mechanism.
- Apoptotic bodies – remnants of **apoptosis** (from Greek, “falling off”), a type of **program cell death**.

Exosomes and ectosomes carry a lot of signaling molecules within their cavities (DNA, mRNA, microRNA, cytosolic proteins – Ig and proteinases). Hence, exosomes and ectosomes may functionally be classified as **signalosomes** (signals-carrying bodies).

Examples of signalosomes

(i) primary cilia (9+0 MT, single, non-motile cilia), (ii) plasmalemmal lipid rafts and caveolae, and (iii) exosomes and ectosomes* (Fig. 12).

***3E** (exosomes, ectosomes, exposome); **3A** (apoptosis, anoikis, autophagy) – the first two, types of program cell death; the third, a “program protein death”; **3F** (filaments, fibrils, fibers; also see Fig. 5); **3T** (troponin-C, tropomyosin, tropomodulin) – building proteins of AF; **3MC** (three levels of Membrane Compartmentalization: plasmalemma, nucleolemma, endomembranes).

Figure 12. A. Number of publications on extracellular vesicles. **B.** Schematic illustration of the formation of microvesicles (ectosomes), exosomes, and apoptotic bodies.

- **Mitochondria** – double membrane-bound organelles
 - TIM, transporter of the inner (mitochondrial) membrane
 - TOM, transporter of the outer (mitochondrial) membrane
 - MOMP, mitochondrial outer membrane permeabilization
 - energy production through the action of ATP-ase
 - protein synthesis by own mtDNA and mitoribosomes
 - biosynthesis of steroid hormones – together with SER
 - critical role in **program cell death (apoptosis)** - release of **caspases** (cysteine-aspartic proteases) by mitochondrial **cytochrome c** is a key step in the apoptosis pathway, MOMP being the key trigger of that pathway.
 - storage and release Ca^{+2}

Nonmembrane-bound organelles

- Polysomes (polyribosomes) – (i) free in the cytoplasm, and (ii) attached on the outer surface of nucleolemma and on RER membrane.
- Cytoskeleton – AF, IF, MF, MT
- Nucleoskeleton – **lamin*** filaments and LAP (lamin-associated proteins) compose the nuclear lamina

***Lamin** is the main building protein of the **nuclear lamina**. **Laminin** is one of the proteins composed **basal lamina** of epithelial and muscle cells, adipocytes, and some glial cell types.

Plasmalemmal-cytoskeletal organelles (fractalosomes)

A human cell (e.g. hepatocyte) has 110 000 μm^2 total membrane surface, about 30 % belonging to the **fractalization** (Latin *fractus* means “broken”) of the inner mitochondrial membrane – an excellent example of the **fractality principle of cell biology** (• Chaldakov GN. *Biomed Rev* 2019;30:143-147).

Theory of „surface roughness and self-similarity“ (fractal geometry) of the Nature was founded by the mathematician Benoît Mandelbrot who coined the term „fractal“ in 1975 (• Gomory R. Benoît Mandelbrot (1924–2010). *Nature* 2010;468:378. DOI:10.1038/468378a).

We have introduced the term “**fractalosomes**” in 2015 (• Chaldakov GN. *Cell Biology*. 2nd revised and enlarged edition. In Bulgarian, Varna Medical University Press. 2015).

Plasmalemmal-actin fractalosomes

- Microvilli (PL + AF + AAP) – finger-like evagination of the plasma membrane of, for example, enterocytes (lining cells of small intestine) aimed at more effective resorption of nutrients.
- Steriocilia (sensocilia; cilia, from Latin “eyelash”).
Steriocilia of hair cells in inner ear $\equiv \text{PL} + \text{AF} + \text{AAP} = \text{Hearing}$.

As structures, both microvilli and cilia are finger-like evaginations (protrusions) of the plasmalemma on the apical surface of the cell. Stereocilia convert the vibrations induced by sound into nerve impulses which are taken up to the brain to be interpreted – a process termed **mechanochemical transduction**; that is a research topic of **mechanobiology**.

Plasmalemmal-microtubule fractalosomes

- Kinocilia (motile cilia); PL + 9+2 MT + MAP + multiple scaffold proteins. In the core of plasmalemmal evaginations (forming the **axoneme**) are located nine doublets of MT around a single doublet in central position, which is transcribed 9+2 or 9+2 MT (Fig. 12a).

Figure 12a. Schematic representations of plasmalemma-derived vesicles (A) and plasmalemma-cytoskeletal organelles formed by plasmalemmal evaginations, not invagination as indicated (B).

Plasmalemmal-cytoskeletal organelles (continued)

- Membrane nanotubules (cytonemes) (PL + AF or MT)
- Dendritic spines (PL + AF + MT + multiple scaffold proteins)

Plasmalemmal-microtubule non-fractalosomes

Primary cilia (sensory, single, non-motile cilia; PL + 9+0 MT + MAP + multiple scaffold proteins) (• **Denys N. Wheatley**. Nanobiology of the primary cilium - paradigm of a multifunctional nanomachine complex. *Methods Cell Biol* 2008;90:139-156. DOI:10.1016/S0091-679X(08)00807-8).

- Flagellum of sperm cells (PL + 9+2 MT + MAP)

Non-plasmalemmal, non-fractal, microtubular structures

- Kinetosome (basal body) – a cylindrical structure, its wall comprised nine peripheral triplets of MT arranged in a ring. Kinetosome is situated at the base of each kinocilium in a ciliated cell; kinetosomes are roots of kinocilia.

Plasmalemmal-cytoskeletal structures (adhesiosomes)*

- Zonulae adherentes (Cell 1 PL + AF + Cell 2 PL + AF)
- FAC [PL + Receptors (R, integrins) + AF + matrix proteins] (Fig. 13)
- Lamellipodium (plural lamellipodia) (PL + R + AF + matrix proteins) - from Latin *lamina*, “thin sheet”; *pod*, “foot” (Fig. 14) - Filopodia (PL + R + AF + matrix proteins) (Fig. 14)
- Podosomes (PL + R + AF + matrix proteins)
- ~~Invadopodia (AF-rich plasmalemmal protrusions involved in cancer invasiveness and metastasis) – feet of the cancer invasiveness~~
- Desmosomes (Cell 1 PL + IF + Cell 2 PL + IF)
- Hemidesmosomes (PL + IF + matrix proteins)
- Basal lamina – a link of matrix to basal plasmalemma in epithelial, muscle and other cells.

* Synonym: Matricellular organelles

Figure 13. Schematic illustration of the unity of cell-and-matrix represented by the focal adhesion contacts (FAC). ECM, extracellular matrix; AAP, actin-associated proteins.

Figure 14. Schematic illustration of cell walking on an ECM road via the “molecular feet” of ligands-and-receptors. We may call these lamellipodia and filopodia “**Johnnie Walker structures**”. Further studies on Mechanobiology of Cell Walk are waiting for you, Dear colleagues.

Other cellular fractals

- Inner mitochondrial membrane
- Sarcolemmal folds at the nerve-skeletal muscle junction (synapse) (Fig. 15, 16).

Figure 15. Schematic representation of an TEM micrograph showing nerve-striated muscle synapse (motor end plate). The sarcolemma (SL) being fractalized reveals many finger-like invaginations with attached small granules (neurotransmitter receptors). Thus, the fractal geometry of the sarcolemma (here postsynaptic membrane) ensures more effective nerve transmission resulting in more effective muscle contraction. In effect, the more developed SL fractalization, the larger the number of neurotransmitter receptors, the more effective muscle contraction-relaxation cycle, the better life for both the nerve and the muscle. What is it? - an excellent ergonomic design created by membrane fractalization. Hence, a synonym for fractasomes may be “**ergosomes**” (Greek ergon, work).

Figure 16. SEM micrograph showing skeletal muscle fibers (M) processed by the method of Uehara. J, neuro-muscle junction (synapse); N, neuronal axon; b, branches of the axon; C, capillary; P, pericyte nucleus. **From:** Fujiwara T, Uehara Y. The cytoarchitecture of the wall and the innervation pattern of the microvessel in the rat mammary gland: A scanning electron microscopic observation. *Am J Anat* 1984;170:39-54. DOI:10.1002/aja.1001700104

FOOD FOR THOUGHT

THE NEED FOR CELL INTELLIGENCE

Guenter Albrecht-Buehler

Nobody in his right mind would believe that the contractile protein molecules in a person's throat speak English. Clearly, the molecules follow orders issued ultimately by the person's brain. This is not a matter of the size of the organism. The contractile proteins in the muscle cells of a small nematode are not gliding or swimming, either. They, too, receive orders from the nervous system of the worm. In short, the interactions between the molecules of any organism generally do not create the functions of the organism, but it is the other way around: The functions of the organism initiate and control the interactions between its molecules. The necessity for such control is obvious. Using the example of contractile proteins, the molecules can only polymerize, depolymerize or slide along each other, but they would not know when and with what force and when to stop. A signal-integrating mechanism is required.

Why should the situation be different for single cells? After all protozoa are in effect small, but quite universal organism and the above conclusion should apply to them as much as to a fly, a frog or the author of this website. Yet, the vast majority of today's biologists devote their efforts to prove the opposite, namely that specific molecular interactions create the cellular functions such as cell division, directed locomotion, differentiation, design of the extracellular matrix, and so forth.

My Q: The functions of the cell initiate and control the interactions between its molecules ($F \rightarrow M$) or specific molecular interactions create the cellular functions ($M \rightarrow F$)?

Albrecht-Buehler's Q: May the 9 triplets of MT of a pair of centrioles suggest their function as cellular eyes (Fig. 17, 18).

Figure 17. Schematic representation of a cell showing a centrosome (“central body”) composed of two centrioles arranged at right angles to each other, and positioned in trans-Golgi zone. Centrioles are surrounded by **pericentriolar material** (black granules) comprised γ -tubulin ring complex (γ -TuRC – not depicted) and several proteins (e.g., **pericentrin, ninein**). There are around 50 γ -TuRC per centriole. **1.** subplasmalemmal skeleton; **2.** microtubules born from MTOC; **3.** nuclear lamina; **4.** bundle of intermediate filaments links nucleolemma and plasmalemma, ensuring a stable position of the nucleus. Now you can say: “Centrosome is localized in trans-Golgi network, the physical center of the cell. The centrosome, particularly its related γ -TuRC, functions as MTOC (microtubule organizing center).”

Figure 18. TEM micrograph of centrosome composed of pair of centrioles positioned perpendicularly to each other. Each centriole is composed of nine triplets of microtubules assembled in a cylindrical structure of 400-500 nm in 2R. **From:** Guenter Albrecht-Buehler’s website.

KEY LEARNING FEATURES (CONTINUED)

Cell biology in number

- The estimated number of cells in the human body ranges from 5 billion to 20 trillion. In the nucleus of each cell there are 23 pairs of chromosomes, a total of 46 chromosomes. Each chromosome is comprised of tightly-coiled DNA (deoxyribonucleic acid), and has 100-1000 genes. DNA is the hereditary multiplex of all living organisms. The Human Genome Project has estimated that humans have 20 000 - 25 000 genes. A genome is the complete set of DNA of an organism.
- Nuclear DNA (ncDNA)** is a polymeric molecule composed of two chains each has purine and pyrimidine bases (nucleotides) - adenin (**A**), thimin (**T**), guanine (G) and cytosin (C); A of of chain always links to **T** of another chain; the same for G and C;

A-T and G-C are base pairs abbreviated as **bp**; 1 kbp = 1000 bp, 1 Mbp = 1000 000 bp. An average human body contains nearly 40 trillion cells, each cell has 3 billion bp (3000 Mbp) in a DNA molecule with length of 2 meters located within the nucleus with 2R of 5-6 μm – that is accomplished by the assembly and packaging DNA and histons (H1-4) into nucleosomes.

- **Chromosomes**

Each chromosome is composed of one DNA molecule. Each chromosome has hundreds of genes. A part of DNA that is transcribed in iRNA is termed “gene” – transcription is made by DNA-dependent RNA polymerases. For example, chromosome 7 has 1000–1400 genes of approx. 160 Mbp – 5-6% of total amount of DNA in the human body’s cells. Each chromosome has short and long arm which are abbreviated “p” and “q” respectively – to these is added a number indicating the locus of the gene in a chromosome. For instance, the gene of p53, tumor suppressor protein, is localized in short arm, position 13 of chromosome 17 (17p13), and (ii) gene of dystrophin (p427) is located in X chromosome (Xp21.2) – it is one of longest gene isolated, composed of 2500 kbp (2.5 Mbp). An average intelligent gene is composed of around 100 kbp, although only 1-2 kbp are required for encoding a protein containing 300-400 amino acids. Adult human has around 100 trillion cells, each cell has 3 billion bp (3000 Mbp) in its DNA.

Chromosomes have **telomeres** (Greek *telos* - end, *meros* - part). Telomere is a region of repetitive repeated nucleotide motifs (the sequences **TTA**, GGG) at the ends of chromosomes. Telomere shortening is involved in all aspects of the aging process on a cellular level. There is a strong connection between short telomeres and cellular aging. The ribonucleoprotein enzyme **telomerase** impacts telomere length. Telomerase restores the length of telomeres, which are shortened when a cell divides *via* mitosis. The **Hayflick limit** is the number of times a normal human cell population will divide before cell division stops, that is, **senescence** - loss of a cell’s power of division and growth.

- **Mitochondrial DNA (mtDNA)** is circular in shape like bacterial DNA – that may be taken in support of endosymbiotic hypothesis. mtDNA is the smallest chromosome containing 37 genes encoding 13 proteins – the remainder 16 551 mitochondrial proteins are encoded in ncDNA (according to date of OMIM). mtDNA is inherited from the mother because an ovocyte has approx. a million mtDNA molecules, whereas a sperm cell has about thousand only.

- **Protein families**

According to structural homology or amino acid sequence homology proteins are living in superfamilies, families and subfamilies. There are around 10-15 million total protein molecules per eukaryotic cell. Most of the proteins exist within a narrow range of between 1000 and 10,000 molecules. Currently, over 20 000 genes and 60 000 protein families comprised more than 500 000 proteins have been identified.

- **Molecular weight of biomolecules**

D or Da; Dalton - unified atomic mass unit; unit of molecular weight named after John Dalton (1766-1844). The molecular weight of large polymers are expressed with kilodaltons (kD), megadaltons (MD), or gigadalton (GD).

In the human genome, the DNA of chromosome 1 has about 249 million base pairs (bp), each with an average weight of about 650 D, or 156 GD total.

Other examples: titin (after Titan in Greek mythology) - protein with molecular weight of

3 - 3.7 MD, obscurin - 800 kD, nebulin - 600-800 kD, dystrophin - 428 kD, all these “obese” proteins belong to striated muscle cells (myocytes), yet dystrophin is found in brain cells too.

- **Macromolecular crowding**

The interior of cells is a crowded environment. For example, an *Escherichia coli* cell is only about 2 micrometres (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 μm^3 . However, *E. coli* contain up to 4 288 different types of proteins. In their natural milieu, proteins are existing together several other proteins and different kinds of small molecules as well as in a highly structured environment within the cell, and this crowded environment can traumatize some of them and, respectively, cause certain type of molecular pathologies. We may call this phenomenon “**molecular traumatology**”.

10-15 million protein molecules and additionally same amount of other molecules in a cell with 2R of 10-150 μm is signifi-

cantly more than 100-400 billion stars and 150-160 billion planets in the Milky route, a galaxy with 2R of 100 thousands light years (ly) – the latter is unit of length in astronomy. One ly is equal of approx. 10 trillion km, exactly 9 454 254 955 488 km. This cell-astronomic calculation was made by my grandson Nikifor when was 11 years of age.

- **To recap**, an average cell contains 10-15 million protein molecules and 10 000 different proteins. Approximately 10% of human genome encodes **receptors** for vast numbers of **ligands**.

How the cell can manage such a huge stream of signals received? And *vice versa*, in that molecular universe, how signal molecules recognize their receptors? One of reasonable answers is: ligands and receptors have a unique ability for self-recognition, binding, and interaction leading to **receptor-mediated signal transduction**. Such a molecular love delivers specific cellular effects.

- Again, a human hepatocyte has 110 000 μm^2 total membrane surface, about 30 % of theme belonging to fractalization of the inner mitochondrial membrane (IMM).

Q: Why does IMM fractalize? **A:** Fractalization of IMM aimed at providing a larger working surface within a relatively small space. The larger the surface of the IMM, the bigger the density of APA-ase, the more energy production, the better functioning of all energy-dependent cellular functions and processes – in effect, an well working ergonomic design.

- Evolutionally, there are three levels of membrane compartmentalization (3MC) - **plasmalemma**, **nucleolemma**, and **endomembranes** (Fig. 19).

Figure 19. Schematic illustration of three levels of membrane compartmentalizations: plasmalemma (arrow at the top), nucleolemma (double membrane), and endomembranes. **5.** Clathrin-coated vesicles. **6.** Caveolin-coated vesicle (caveolosome). The membranous structures shown at left are components of the membrane-bound organelles. Examples of nonmembrane-bound organelles: **2.** Filament; **3.** Microtubule; **4.** Polysomes (polyribosomes) attached on the outer nuclear membrane (**4**) and on a cistern of rough endoplasmic reticulum (**4**). Note, tubule is a membrane-bound, whereas microtubule is a nonmembrane-bound structure.

- Ligands are signaling molecules that bind to receptors. Generally, the receptors are trans-plasmalemmal proteins, glycoproteins or proteoglycans (Fig. 20, 21).

Figure 20. Examples of main types of trans-plasmalemmal proteins. The first three at left are single-pass proteins; the protein at right is seven-pass (heptahelical, 7TM) protein.

Figure 21. Schematic illustration of ligand-receptor interactions.

Receptors

GPCR, G-protein-coupled receptor (Fig. 22, 23)

G-proteins, proteins linked to GTP (guanosine triphosphate) or GDP (guanosine diphosphate).

GPCR is the biggest family of receptors. Of the 367 heptahelical receptors for endogenous ligands, about 330 are potential targets for drug discovery with agonist and antagonist actions.

GPCR are found only in eukaryotes, including humans. The ligands that bind and activate these receptors include light, odors, hormones, neurotransmitters, and a variety of peptides and proteins. GPCR are implicated in the pathogenesis of many diseases and their therapies. Robert Lefkowitz shared the 2012 Nobel Prize for Chemistry with Brian K. Kobilka for the discovery of GPCR.

Figure 22. A heptahelical receptor is coupled to heterotrimeric GTP-binding protein (G-protein) which transduce most signals through their α and $\beta\gamma$ subunits via second messengers to induce diverse cellular responses. **From:** Duman RS, Nestler EJ. *The second messenger hypothesis*. In: George J Siegel, editor. *Basic Neurochemistry. Molecular, Cellular and Medical Aspects*. 6th edition. Philadelphia: Lippincott-Raven, 1999.

Second messengers of various hormones and neurotransmitters

- cAMP, 3'-5'-cyclic adenosine monophosphate
- cGMP, 3'-5'-cyclic guanosine monophosphate

Earl W. Sutherland, Jr. (19 November 1915 - 9 March 1974) was awarded Nobel Prize in Physiology or Medicine in 1971 “for his discoveries concerning the mechanisms of the action of hormones.” Affiliation at the time of the award was Vanderbilt University, Nashville, Tennessee, USA. Imagine a journalists asks you “Who is Earl Sutherland?” - please, do not reply “One of the winners of Horse racing trophy”. Nashville, TN is renowned for Horse racing and Kenny Roger’s country music songs.

Figure 23. (a) Structure of receptor for epidermal growth factor (EGF) - a single-pass transmembrane protein with an **exodomain** (outside of cell) that is EGF binding site, and an **endodomain** (inside of cell). The endodomain functions as the **enzyme tyrosine kinase (TK)**, hence, EGF receptor is, in fact, **receptozyme**. It belongs to a large group of **tyrosine receptor kinase (Trk – pronounced “truck”)**. **(b) Binding of two EGF molecules leads to EGF receptor dimerization and tyrosine (Tyr) phosphorylation**, followed by signal transduction and the corresponding cell effect. Dephosphorylation by protein phosphatase leads to receptor deactivation. Drugs that inhibit (**downregulate**) the activity of Trk have anticancer effects. (•Brown C, Gralla RJ, et al. Impact of EGFR inhibitor in non-small cell lung cancer on progression free and overall survival: a meta-analysis. *J Natl Cancer Inst* 2013;105:595-605. DOI:10.1093/jnci/djt072)

- **Life at intracellular level is mediated by membrane compartmentalization and membrane flows supported by cytoskeletal structures (AF/AAP*, MT/MAP, and myosin V-VIII)**

Two intracellular membrane flows operate inside the cell:

- endocytotic membrane flow (endocytic pathway)** starting from plasmalemma and moves in the following order: coated pits, coated vesicles, caveolae, caveolosomes, early endosomes, late endosomes, dynein [MT-based retrograde (-) motor protein], MVB, and lysosomes,
- protein secretory membrane flow (secretory pathway)** starting on free polyribosomes, RER, COP II vesicles, Golgi complex, COP I vesicles, Golgi-derived coated vesicles and secretory vacuoles, kinesin [a MT-based anterograde (+) motor protein] and, finally, plasmalemma where the release of vacuole-stored proteins through **porosomes** occurred.

***Q-1:** In which cells are there actin and myosin?

A-1: In all cells of a multicellular organism.

Q-2: In which cells is there the highest amount of actin and myosin?

A-2: In the myocytes – smooth muscles and striated muscle (cardiomyocytes and skeletal muscle cells).

- **There are two lysosomal and one nonlysosomal pathway for degradation of exhausted proteins and organelles**

All endocytotic pathways lead to lysosomes. In 1955, Christian de Duve discovered the lysosomes (Greek, *lysis* - destroy, *soma* - body).

- **Phagosomes** together with lysosomes (**phagolysosomes**) eat the phagocytosed bacteria (Greek *phago* - eat, *kytos* - cell). In 1882 Ilya Mechnikov worked in a laboratory in Messina, Italy. He made simple experiment: stuck in the larvae of a starfish

thorns of mandarin, prepared as a Christmas tree for his children. The next morning, he noticed that cells have accumulated around the thorns, wanting to destroy the “enemies.” He then made many experiments that reproduced his first observations. And he calls this protective reaction **phagocytosis**, and the cells that carry it out - **phagosomes**. This was the beginning of the concept of cellular immunity. In 1908 Ilya Mechnikov and Paul Ehrlich were awarded the Nobel Prize in Physiology or Medicine for their discovery of cellular immunity (*via* lymphocytes - T helper cells and natural killer cells) and humoral immunity (with antibodies / immunoglobulins).

- **Autophagy** (“self-eating”) is an evolutionarily conserved process that occurs ubiquitously in all eukaryotic cells and has multiple physiological roles. This is process of (i) direct engulfment of cytoplasm containing exhausted proteins and organelles at the lysosome surface by invagination and protrusion of the lysosome membrane (that is **microautophagy**), or (ii) capture portions of cytoplasm containing nonfunctional proteins and ageing organelles into a *de novo*-formed double membrane-bound vacuoles (**autophagosomes**) and then send them to the lysosome for degradation (that is **macroautophagy**).

Examples of autophagy

- (i) Billion exhausted erythrocytes are eaten per day in the spleen, bone marrow, and liver,
- (ii) Billion apoptotic bodies are phagocytosized daily in different organs during the embryonic life till the arrival of Dr Alois Alzheimer,
- (iii) Thousands of exhausted mitochondria and peroxisome fused with lysosomes and are destroyed therein, processes termed **mitophagy** and **pexophagy**, respectively.

Defective autophagy leads to a wide range of diseases including cancer and neurodegenerative disorders (Alzheimer’s, Parkinson and Huntington diseases)

More than 30 autophagy-related genes (*ATG*) encoding **Atg proteins (Atg-4-6,-12, -16)** are implicated in the regulation of autophagy. **Yoshinori Ohsumi** wins the 2016 Nobel Prize for the discovery of molecular mechanisms in autophagy - how cells degrade and recycle their molecular and structural trash. Autophagy as apoptosis is a type of program cell death (PCD)*, quite different from necrosis (Greek, *nékrōsis* - „death“) (Fig. 24).

Figure 24. The Greek word apoptosis means “falling off”, like the falling of leaves from a tree. Apoptosis functions under physiological and genetic control, which involves a single cell or a small group of cells in a tissue. The released apoptotic bodies are digested by the neighbor macrophages (not depicted), thus apoptosis unlike necrosis does not induce any inflammatory stress.

***Q:** How many types of PCD? **A:** Apoptosis, autophagy, anoikis (Greek, homeless, because detached from the matrix), **ferroptosis** (an iron-dependent form of cell death triggered by the accumulation of lipid peroxides), **necroptosis** (a programmed form of necrosis), **pyroptosis** (a highly inflammatory form of PCD that occurs upon infection and is a part of an antimicrobial response leading to the rapid clearance of various microbes).

Q: Which cellular structures possess both DNA and RNA? **A:** Nucleus and mitochondria.

Nonlysosomal protein degradation

UPS, ubiquitin proteasome system

UPR, unfolding protein response - a process activated in response to an accumulation of unfolded or misfolded proteins in RER's cavity (lumen).

Misfolded and unfolded proteins - folded into an incorrect, nonfunctional 3D shape.

Proteins must achieve and retain a specific 3D conformation in order to function properly. These are correctly folded proteins that play essential and diverse roles in all

living organisms. Protein folding starts as soon as the polypeptide enters into the RER cavity. The proteins destined to exocytosis (plasmalemmal, lysosomal and exportable proteins) or translocation to other cell organelles carrying a specific **signal sequence** of 15-30 amino acids long that interacts with a **signal recognition particle** (SRP). Namely SRP lead the complex of polysomes-iRNA-nascent polypeptide to the RER membrane and then into RER lumen – this is, in fact, the way RER is formed.

Proteasomes and program protein death

Heat shock proteins (e.g., Hsp9, Hsp10, Hsp60, Hsp70). Among them, Hsp9 dubbed **ubiquitin** (from English “ubiquitous”) is found in all eukaryotic cells. **Ubiquitin (Hsp9)** recognizes and links to unfolded and misfolded proteins and then proteases destroy them within the **proteasomes** (Fig. 25). These latter “bodies containing proteases” are supramolecular cleaning machines for incorrectly folded proteins. In analogy with the term **program cell death**, this process was named **program protein death**. **Hsp10 u Hsp60 are foldases**, enzymes that fold proteins properly. **SUMO (small ubiquitin-like modifier)** – like ubiquitin links to unfolded and misfolded proteins.

Figure 25. Schematic illustration of the fate of misfolded and correctly folded protein within the cavity of rough endoplasmic reticulum (ER) – unfolded proteins are released into cytoplasm where they are marked with ubiquitin and enter the proteasome to be destroyed to amino acids – the process designated program protein death. Correctly folded protein exists the ER cavity via vesicle towards the Golgi complex.

Protein misfolding and human disease

Protein misfolding is a common event in living cells. In young and healthy cells, the misfolded protein load is regulated by **protein quality control (PQC)**. In aging cells and in cells from certain individuals with genetic diseases, the load may overwhelm the PQC capacity, resulting in accumulation and aggregation of misfolded proteins. Dependent on the efficiency of the PQC, the misfolded proteins may be degraded or assembled into toxic aggregates. Examples of protein misfolding diseases include Alzheimer's disease (AD) and Parkinson's disease (PD) where amyloid beta (A β) peptide and the MAP *tau* (AD) and α -synuclein (PD) form aggregates (inclusion bodies).

In KISS, unfolded and misfolded proteins are very pathogenic and lead to neurodegenerative and other diseases collectively termed **proteopathies (proteinopathies, protein misfolding diseases)**.

- **Cell-matrix is indivisible unit of life of all multicellular organisms**

It is taken as axiomatic that life is a function of cells, and so strong is the educational belief that cells are "unit of life" and "building blocks of life". However, the cells (except blood and lymph circulation cells) are surrounded and linked - both structurally and functionally - to the matrix. When a cell is separated from the matrix, it becomes "homeless" and dies in a process termed **anoikis**, a type of program cell death. In teaching, to separate a cell from its matrix is conceptually wrong, because not only is the matrix a secretory product of the cell, but it is also connected to the plasmalemma *via* receptor-ligand interactions.

Take in mind message

The soul is a multiplex human phenomenon. It is located in the brain. And in the heart, heart, heart, heart. It is the same for love-of-knowledge.

Educating the mind without educating the heart is no education at all.

Aristotle

DECALOGUE FOR STUDENTS

1. He who learns, he will succeed. (Bulgarian proverb)
2. *Forward! Science is a sun, which in our souls shines!** – not only on May 24, the Bulgarian National Day of Cyrillic Alphabet and Culture, but continuously throughout life (LLP – Lifelong Learning Program; also, *Laboratory, Library, Pub*; CME - Continuing Medical Education).
3. Follow didactic cascade: from information to knowledge to way of thinking. Feel the knowledge like you feel your mother tongue. Translate the information of cell-matrix molecules, structures and functions (MSF) into knowledge of Diseases, Prevention and Therapy (DPT), that is a creative journey from bench-to-bedside (B2B). *Remember:* There are no departments and clinics within the cell.
4. Learning without thinking is useless. Thinking without learning is dangerous (Confucius). *Be thinker and doer, both.*
5. Human cognitive potential is symbiosis of thoughts and emotions. *Think outside the box.*
6. Imagination is more important than knowledge because knowledge is limited (Albert Einstein) – stream of associations (SOA) is an essential tool for creative learning.
7. Develop moral reflexes - mutual respect, love and empathy, create and enjoy brain-and-heart friend/ship (BHF).
8. Follow your parents' advice: WHAT DID I ARRIVE HERE FOR? - to study first, then rejoice - the reverse chronology is meaningless.
9. Healthy lifestyle and good education can ensure your quality of life (QoL).
10. Stay tuned, wise and independent (*sapere aude*), have the intellectual courage to cross (at least once) at a red light the road of science to make your own green path on it. To achieve your *Eureka effects*.

Then you can say: ***Vox Studentium vox Dei!***

* From Stoyan Mihaylovski's 1882 poem, a traditional song of all Bulgarian schools.

INSTRUCTIONS TO AUTHORS

General Information

***Biomedical Reviews (BMR)* is an official Journal of the Bulgarian Society for Cell Biology (BGSCB) published by the Medical University Press, Varna, Bulgaria.** *Biomedical Reviews* publishes articles focused on updated knowledge in disease-oriented molecular cell biology. The following types of contributions are published:

(i) *Review* articles summarize state-of-the-science (SOS) on a given biomedical topic. Contributors to Reviews are, in general, invited by the Editors and the Editorial Board, but idea proposals are welcome. Potential authors are invited to submit a letter of interest to the Editor. Proposals should contain an outline of the contents, including an abstract, a list of 20 relevant articles including from the proposer's own research, and a brief statement on why now is a good time to review the topic in question. Reviews will not be accepted for editorial processing unless pre-approved for submission..

(ii) *Dance Round* articles are short, position papers that are intended to focus observations that seem to point the field in a new direction, to give the author's personal views on a controversial topic, or to direct soundly based criticism at some widely held dogma in biomedicine.

(iii) *Research articles* and (iv) *Topic issues* aimed at clustering contributions to a biomedical cutting edge within one issue. Guest Editors of such issues are, in general, invited by the Editors and the Editorial Board, but idea proposals are welcome. Multiple-part papers are discouraged. Manuscripts submitted under multiple authorship are reviewed with the understanding that all listed authors concur in the submission and that the final manuscript has been approved by all authors. If accepted, the article shall not be published elsewhere in the same form, in either the same or another language, without the written consent of the Editors and Publisher.

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Text of manuscripts must include an abstract, an introduction, followed by the body of manuscript, a conclusion, acknowledgments, a list of references, and, if available, figure legends, and tables. Pages should be double-spaced, Times New Roman should be used throughout, sized at 12 pt. The text file should be submitted in either Word or PDF format to the Editor-in-Chief at chaldakov@yahoo.com and/or to some of the Editors at his e-mail indicated on the inside front cover.

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The abstract (typically about, although not strictly restricted to, 250 words) should provide a concise summary of the data to be reviewed and major conclusions of the study. It should be written in complete sentences, without explicit subheadings. Citing references should be avoided.

Introduction

The introduction should briefly indicate the background of the topic, and explain the objectives of the paper.

Captions should be used within the body of the manuscript to outline important points.

Conclusion

This section should be as concise as possible and should summarize the data discussed in the paper, and possibly, should contain a statement of their significance and future biomedical implications.

References

Only published and "in press" references should appear in the reference list. The latest information on "in press" references should be provided. Any "in press" references that are relevant for reviewers to see in order to make a well-informed evaluation should be included as a separate document text file along with the submitted manuscript. "Submitted" references as well as personal communications should be cited only in text. Authors are responsible for all personal communications and must obtain written approval from persons cited before submitting the paper to the *Journal*. Proof of such approval may be requested by the *Journal*.

References should be each numbered, ordered sequentially as they appear in the text, and cited in parentheses: “text (1)”. In case of with multiple references, these should be cited starting from the smallest number: “text (1-3)”. In the list of references, papers should be listed numerically. The name (surname first) of the author(s) should be followed by the full title of the paper as it appeared in the original, the source of the reference, together with the year, volume number, and the first and last pages. If the author list for a paper exceeds 6, et al (in oblique font) should be added after the sixth author. References to web-only journals should also provide URL in full or DOI if known. Book titles are in oblique font with all main words’ first letter being capitalized. The following illustrates the format to be used:

- **Journal article**

Iwamoto Y, Koide H, Ogita K, Nishizuka Y. The protein kinase C family for the regulation of cellular functions. *Biomed Rev* 1992; 1: 1-6.

- **Book**

Author A. *Book Title*. Publisher name, 2000.

- **Chapter in a book**

Author A. Chapter title. In: Author A, editor(s). *Book Title*. Publisher name, 2000; 1-10.

Abbreviations of journal titles should follow those listed in the *Index Medicus*. Responsibility for the correctness of the references lies with the author(s). After manuscript revisions, authors should double check that all in-text citations are in the reference list and that all references on the reference list have at least one corresponding in-text citation.

Illustrations

All figures must be cited in the text and numbered consecutively (Fig. 1, Fig. 2, etc.). Each figure should be submitted as a separate file. For vector graphics, EPS (Encapsulated PostScript) files are the preferred format. TIFF (Tagged Image File Format) is the recommended file format for bitmap, greyscale and colour images. When supplying TIFF files please ensure that files are supplied at the correct resolution:

- line artwork = minimum of 1000 ppi
- halftone artwork = minimum of 300 ppi
- combination artwork (line/tone) = minimum of 500 ppi

Authors should be aware that using color figures will incur an additional charge for color in their reprints.

Preparation of Tables

Each table should be double-spaced with an explanatory title and will appear at the end of the text of the manuscript. All tables must be cited in the text (e.g. “Table 1”).

Figure Legends

Each figure should be accompanied by a title and an explanatory legend. The title should be part of the legend and not lettered onto the figure itself. Legends should be concise.

Abbreviations

Use abbreviations if a term appears three or more times. Spell out all abbreviations at first occurrence, and then introduce them by placing the abbreviation in parentheses. The metric system should be used for all volumes, lengths, weights, etc. Temperatures should be expressed in degrees Celsius (centigrade). Units should conform to the International System of Units (SI).

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