LABORATORY 12

Laboratory of Bioinformatics of Cell Processes and Motocontrol

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The leading researchers of the laboratory include:

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Dr.Sc. (Biology)	M. Berkinblit	Dr.Sc. (Biology)	S. Minina
Dr.Sc. (Biology)	V. Bozhkova	Dr.Sc. (Biology)	N. Samosudova
Dr.Sc. (Math.)	V. Dunin-Barkowsky, President of the RNNS	Dr.Sc. (Biology)	Yu. Panchin
Dr.Sc. (Biology)	Z. Khashaev	Dr.	J. Burmistrov
Dr.Sc. (Biology)	E. Liberman	Dr.	L. Kudina
Dr.Sc. (Math.)	I. Lukashevich	Dr.	D. Voronov

DIRECTIONS OF ACTIVITY

The general theme of the scientific work in the laboratory is: analysis of the information processes in cellular systems and in motor control. The main directions of theoretical researches in this realm: developmental biology (analysis of principles for the realization of genetic information in the developmental systems), neurobiology (neural communication and biochemical modulation in neural centers), motor control (study of geometry of manipulative space and of control goal-directed moving). Also some new bioinformatical principles are worked out including the building of computerized system for the analysis of expert knowledge.

MAIN RESULTS

Developmental biology. In the framework of the theme "Analysis of principles of the genetic information realization in developmental systems" d-r V. P. Bozhkova continued the investigation of the signal mechanisms in early development which depend on the physical as well as molecular regulators. In particular the significance of G-proteins for fish embryogenesis was studied. Earlier using the activators and inhibitors of G-proteins it was shown that they have important role in control of the morphogenetic movements during epiboly. The main point of the recent work was the analysis of the G-protein influence on the cytoskeleton organization. The work was held together with the Institute of Theoretical and Experimental Biophysics, RAS on the Danio rerio embryos, the model which is recognized all over the world as the best one for the molecular-genetic studies. Injection of G-protein activator GTP-gamma-S in the yolk cell, which directs the epiboly in fish, has two phases in its action. At the first (fast) stage pushing the cell surface and appearance of the surface protrusions near the injection site took place. The second phase (10-25 min after the first one) was pointed by the local contraction of cell surface. The data suggest that the protrusive response was related with the activation of the actin filaments polymerization under GTP-gamma-S action and that there are several different sites in the cytoskeleton of embryonic cells regulated by G-proteins. The second tested question was which signal systems were involved in G-proteins control of cytoskeleton. It was

found that the components of the phosphatidylinositol cycle didn't influence on the first phase of the GTP-gamma-S action. But the contraction phase strengthened markedly with inositol-1,4,5-trisphosphate and calcium. According to a new hypothesis for the epiboly mechanism G-proteins act as regulation factors for the actin cytoskeleton polymerization and contraction.

Neurons can communicate with each other via exchange of specific molecules at synapses or by direct electrical connections (EC) between the cytoplasm of either cell. Although electrical connections are abundant in nervous systems, little is known about the mechanisms that govern specificity of their formation. Dr Y. V. Panchin and his collaborators have recently defined a new group of transmembrane molecules (pannexins) which incorporates invertebrate gap junction proteins (innexins, OPUS) and their vertebrate homologous proteins. They also developed a reliable model system to study how the neurons choose the correct counterparts for electrical coupling in vitro. It was hypothesized, that specificity of electrical coupling in the nervous system may be determined by combinations of different gap junction proteins expressed in distinct cell types. This hypothesis is supported by recent data on differential expression of pannexins in the mollusc neurons and in the experiments where the specificity of gap junctions was drastically altered by intracellular injection of mRNA encoding the mollusc innexin Panx1. As a main model system for these studies pteropod mollusc Clione limacin well studied by Y. V. Panchin and his associates was using. Although the homology of the vertebrate pannexins with gap junction molecules of invertebrates is apparent, the question of the actual function of mammalian pannexins in human and animal cells remains unclear and its study is in progress.

D-r D. A. Voronov studied the mechanics of cardiac looping in the invertebrate heart in collaboration with Prof. Larry A. Taber (Washington University in St. Louis, USA). The work was carried out with the chicken embryo, a standard object in biomechanics of heart development. D. A. Voronov found that in previous experiments in this field of embryology the influence of surface tension was not taken into account. He developed a new method of chicken embryo cultivation in the liquid medium enriched by oxygen that allows excluding completely surface tension. Using this new technique, surface tension was found to distort the results of some classic experiments in mechanics of heart development. The development of heart asymmetry was shown as a very redundant process. It depends mostly not on the mechanical properties of primitive heart tube, as it follows from standard models and experiments in this field of science, but on asymmetry of the left and right rudiments that are forming the heart tube by fusion on its rear end. Spatial restrictions imposed to the heart by surrounding tissues are also very important in cardiac looping. On the basis of experiments that were made by D. A. Voronov, in the laboratory of Prof. L. A. Taber a computer 3-D model of cardiac looping was developed.

D-r N. V. Rozanova is working on the MARCKS protein project in the Laboratory of Signal Transduction (National Institute of Environmental Health Sciences, USA) under the supervision of Dr. Perry J. Blackshear. MARCKS is a protein kinase C substrate that is distributed widely in early embryogenesis. However, its precise developmental role is yet to be established convincingly. Rozanova studies MARCKS protein using the Xenopus embryo as a model, using an antisense approach that allows her to deplete specific proteins from the early embryonic stages well before zygotic gene transcription is activated. Rozanova's data show that depletion of MARCKS protein during cleavage stages leads to abnormal cell division and adhesion, resulting in delayed development of the blastula and delayed morphological movement at gastrula. As a result, the experimental embryos are arrested at the end of gastrula-

tion. Depletion of MARCKS protein significantly reduces expression of several mesoderm genes such as MyoD, Xbra, Goosecoid, and FGF8. In contrast, expression of endoderm genes Mix1 and Mix2 was increased. These significant changes in expression pattern may be the underlying cause of arrested development at gastrulation. The data imply an important role for MARCKS protein in determination of mesoderm and endoderm fates in the Xenopus embryo.

Dr. I. M. Plonsky explores the structure and function of persistent and temporary intercellular contacts.

Gap junctions between liver cells (hepatocytes) were studied using the double whole-cell recording (DWCR) technique that yields the conductance of the junctional area between cells (G_j) while perfusing cells contents with experimental solutions. G_j is ensured by intercellular channels (gap junctions). Previous studies show that external solution enriched with CO_2 decreases intracellular pH, rises the concentration of free Ca^{2+} in the cytoplasm, and decreases G_j (uncouples cells). Using a combination of extra as well as intracellular treatments Dr. Plonsky has shown that uncoupling effect of CO_2 is mediated by the acidification of the cytoplasm. It has been concluded that intercellular communications through gap junctions are highly sensitive to intracellular pH and can be regulated upon changes of liver cells metabolic status.

The structure of tight junctions was explored by a combination of DWCR and admittance measurements (AM). This approach allowed measurements of cell capacitance while the trans-contact electric field is applied. It has been found that exogenic charged hydrophobic compound can move from one cell membrane to a neighboring cell upon application of the trans-contact potential. Such translocation was detected as a characteristic change in the membrane capacitance. The effect has been attributed to the existence of the persistent hydrophobic pathway in the region of tight junctions. Trans-contact potential-dependent traffic was observed even in the absence of the exogenic compound, signifying that cells can exchange endogenous hydrophobic charged molecules.

The fusion of biological membranes is known to be driven by specific proteins. The temporary contact, a narrow fusion pore, is formed at early stages of this process. The pore yields information on characteristics of the initial fusion site. It is not known if merging membranes or fusion proteins determine features of the pore, such as initial conductance and kinetics of formation. The fusion pore induced by the viral protein was studied using DWCR as well as AM. New algorithms that allow more reliable restoration of the fusion pore conductance from AM data have been introduced. Data on fusion pore conductance and kinetics of pore formation were used for modeling the initial fusion site. Dr. Plonsky has found that at the early stage of the initial pore formation its features are rather determined by fusion proteins. The fusion site has been visualized as a structure comprised by about 6 protein trimers.

Currently Dr. Plonsky works in the field of taste reception and chemotransduction

Neurobiology The neural system, a base of informational interaction of multi-cell animals, was studying in the laboratory at different levels.

The cerebellum studies present the traditional topic in the laboratory. D-r W. L. Dunin-Barkowski working in the Health Centre of Texas Tech University, USA, continued analysis of the experimental data on the neuron activity in the medullary respiratory center of cats in the cycle waking-sleep. A new computer model of the respiratory rhythm generator was worked out. It unites the so called pacemaker and network models taking in account the intraneuron processes. In particular one uses the mechanism of calcium releasing through plasmatic membrane in the excitation process. It is shown that the neurons can use this mechanism to generate the batch fir-

ing. Neuron network from two model neuron groups which mutually inhibit one another presents a behavior spectrum seen in experiment. Raising the connection force between neuron groups from zero to large values one remarks firstly asynchrony of pacemaker neuron activity, then synchronize bursts (synphase for neurons of inspiration and expiration) of pacemaker activity, and at last counter-phase bursts of inspiration and expiration neurons.

D-r N. V. Samosudova, d-r N. P. Larionova and corr. member of RAS L. M. Chailakhian together with d-r V. P. Reutov (Inst. High Neural Activity RAS) showed using electron microscopic methods a possibility of NO participation in microtubules (MT) activity during signal conducting from one neural cell (granular cell) to another (Purkinje cell). Electron-dense precipitation seen at longitudinal sections of MT was localized with period approximately 24-25 run that is characteristic for dynein which possesses the ATP-ase activity and may interaction with the tubulin in presence of Ca^{2+}. The electron-dense precipitation (forming in NO presence during stimulation) was found to be a polymerized protein containing calcium ions, what was revealed by Petrunyaka's method (EGTA, 20 mM, 20 min, 37°C). It is known that both ATP-ase activity and NO-synthase activity need calcium. Thus the Ca-containing precipitation in MT formed by exogenous NO may mark "work" sites of endogenous nitric oxide during transmission of neural signal that possibly mediates a release of the intracellular calcium.

In the work of d-r Ju. Burmistrov it was established that electrical potentials that may be recorded near the specialized ventilatory appendage (scaphognathite) represent the cumulative potentials of the scaphognathite-moving muscles. Sequence of these potentials was investigated in free-moving crayfish Procambarus cubensis. In long-term experiments correlation between the ventilatory activity and functional state of the animal was shown, and a ventilatory component of behavioral reactions to sudden changes of the environment was described.

D-r. Z. Kh.-M. Khashaev studies the influence of the varied pharmacological media, especially the supertoxicants of the dioxin series, on the biomembrane penetrability . The study goes in collaboration with the Institute of experimental and theoretical biophysics RAS (Poushchino), and Institute of biochemical physics RAS.

Motor control. D-r M. B. Berkinblit and d-r S. V. Adamovich (together with Ratgers Univ., USA) continued studying of the sensorimotor integration mechanisms in human with their changing under normal ageing and caused by Parkinson disease. They used two main methods. It was, firstly, the cinematic analysis of the pointing movements in a virtual space to the visual aim without seeing arm and with distortions visual feedback. The examinee resumes the visual information about his mistake when the movement plays after end of movement. It was shown that parkinsonics learn to compensate such a distortion so quick as healthy aged men do, but they cannot do it after the visual distortion changes its sign into inverse one. (When e.g. after learning to show below target they must learn to show higher of it). Secondly, they studied the coordination of trunk and arm movements in pointing with the same groups of examinee, and confirmed the hypothesis that humans may plan arm movements in both coordinate systems, relative to the out space or relative to the body, depending on where the description of the aim is simpler (in particular, where the aim reposes). As it was shown that parkinsonics relearn illy under distortion of the visual field as well as under changing of the force field, one can say that a principally new result is obtained about role of the basal ganglia in motor learning. It is shown that their injuring precludes relearning. It would be important to know what time lasts the difficulty for relearning.

In order to clarify the role of motoneuron recurrent inhibition in motor control, which remains obscure, especially in humans, its distribution and efficacy in motoneurones supplying hand muscles in healthy humans were analyzed. In contrast to previous suggestions from the literature, in some hand muscles the inhibition was revealed whose characteristics (stimulation conditions, the latency and duration) suggest strong evidence for Renshow inhibition. In contrast to recurrent inhibition in soleus motoneurones which has been investigated earlier, recurrent inhibition in motoneurones of hand muscles frequently followed by short excitatory effect as well as long-latency and long-lasting inhibitory one (d-rs L. P.Kudina, R. E. Andreeva and N. M. Zhoukovskya in collaboration with d-rs M. Piotrkiewicz, Inst. Biocybernetics and Biomedical Engineering, Polish Acad. Sci., and I. Hausmanova-Petrusewicz, Medical Research Center, Polish Acad. Sci.).

Bioinformatical principles. D-r I. P. Lukashevich developed a method of the structural organization for the mildly formalized information and professional knowledge, which served as foundation to building knowledge bases in neurology, neuropsychology and electroencephalography. The main principle consists in singling out different levels of knowledge, their structuring and picking out essential bonds inside levels and between them. This principle was used in building several learning systems and in particular a computer system for encephalography studying. In the last case the computer system exists as the computerized automatic system for diagnostics "EEG-EXPERT". One uses this system in practice at neurological hospitals and polyclinics for learning and raise of qualification, and also for investigations.

In the framework of his studies in foundations of science d-r E. A. Liberman has carried out the first series of experiments eliciting mechanisms of cytoskeleton functioning, which decide inside neuron the brain tasks. Problems of the new science are formulated, the solution of which is within the rich to direct experiments.

D-r A. V. Chernavsky (together with the sector 1.1) prepared for printing in the electronic journal "Informational Processes" an article about principles of the informational interactions in framework of bioinformatics.

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