#### *Questionnaire* Summary of the main activities of a research institute of the Slovak Academy of Sciences

Period: January 1, 2012 - December 31, 2015

#### 1. Basic information on the institute:

#### 1.1. Legal name and address

Institute of Animal Biochemistry and Genetics Slovak Academy of Sciences Moyzesova 61 900 28 Ivanka pri Dunaji



#### 1.2. URL of the institute web site

www.ubgz.sav.sk

#### 1.3. Executive body of the institute and its composition

Directoriat	Name	Age	Years in the position
Director	RNDr. Ľubor Košťál, CSc., 2014-2016	60	2
	RNDr. Ivan Hapala, CSc., 2002-2014	62	12
Deputy director	RNDr. Ivan Hapala, CSc., 2014-2016	62	2
	RNDr. L'ubor Košťál, CSc., 2002-2014	60	12
Scientific secretary	RNDr. Jana Antalíková, PhD.	50	7

#### 1.4. Head of the Scientific Board

RNDr. Peter Griač, PhD

#### 1.5. Basic information on the research personnel

1.5.1. Number of employees with university degrees (PhD students included) engaged in research projects, their full time equivalent work capacity (FTE) in 2012, 2013, 2014, 2015, and average number of employees in the assessment period

	20	12	20	13	20	14	20	15	total			
	number	FTE	number	FTE	number	FTE	number	FTE	number	averaged number per year	averaged FTE	
Number of employees with university degrees	26.0	17.700	27.0	17.400	24.0	17.600	25.0	18.900	102.0	25.5	17.900	
Number of PhD students	9.0	9.000	9.0	9.000	9.0	9.000	11.0	11.000	38.0	9.5	9.500	
Total number	35.0	26.700	36.0	26.400	33.0	26.600	36.0	29.900	140.0	35.0	27.400	

Bassarah staff	2012		2013		2014		2015		average	
Research stan	No.	FTE	No.	FTE	No.	FTE	No.	FTE	No.	FTE
Organisation in whole	35.0	26.7	36.0	26.5	33.0	26.6	36.0	29.9	35.0	27.425
Department of Membrane Biochemistry	18.0	14.400	16.0	11.500	15.0	11.600	17.0	12.500	16.5	12.500
Department of Physiology and Ethology	11.0	8.300	14.0	10.700	12.0	9.700	12.0	10.400	12.3	9.775
Department of Immunogenetics	6.0	4.000	6.0	4.300	6.0	5.300	7.0	7.000	6.3	5.150

## 1.5.2. Institute units/departments and their FTE employees with university degrees engaged in research and development

#### 1.6. Basic information on the funding of the institute Institutional salary budget and others salary budget

Salary budget	2012	2013	2014	2015	average
Institutional Salary budget [thousands of EUR]	382.384	383.489	377.296	405.116	387.071
Other Salary budget [thousands of EUR]	44.429	61.252	25.09	21.835	38.152

#### 1.7. Mission Statement of the Institute as presented in the Foundation Charter

- The mission of the Institute of Animal Biochemistry and Genetics of the Slovak Academy of Sciences activity is to perform fundamental research in biological and agricultural sciences oriented at molecular-biological, physiological, biochemical and genetic processes undergoing in animals and microorganisms. The main focus is on neurobiological and physiological mechanisms of animal behaviour in normal and extreme conditions, cell biology of membranes and the role of membrane in energy transformation, the role of membrane antigens inimmunological and reproduction processes, as well as at the application of the knowledge on membrane processes in nanotechnologies, biotechnologies and biomedical disciplines.
- The Institute provides consulting and expertise services related to its main activities.
- The Institute participates in the PhD. studies according to the legal regulations in force.
- The Institute administers the publication of the results of the research and development activities and their popularization via periodic press and other information media.

# 1.8. Summary of R&D activity pursued by the institute during the assessment period in both national and international contexts, (recommended 5 pages, max. 10 pages)

Membranes and their functions under normal and pathological conditions remained the unifying concept of the research program of the Institute during the assessed period 2012-2015. We were studying membrane phenomena at various levels of complexity, such as molecular interactions at membrane-like interfaces, formation and maintenance of membranes in eukaryotic cells, participaion of selected membrane antigens in cell-cell recognition, and the role of membrane receptors in neural signalling and control of behavior (Fig. 1). Activities of the Institute during the assessed period are summarized below according to the organizational structure.

Although many traditional research programs continued, research themes of the Institute of Animal Biochemistry and Genetics underwent several significant changes during the period 2012-2015. The research in two laboratories, the Laboratory of Bioenergetics involved in the studies of energy metabolism in Methanoarchaea, and Laboratory of Gravitational Physiology which was involved in space research has been terminated due to the retirement of group leaders. On the other hand,

both affected departments, Department of Membrane Biochemistry and Department of Physiology and Ethology, extended their research activities into new areas of cell division and the quail chorioallantoic membrane as an *in vivo* angiogenesis model, respectively.



Fig. 1 Membrane processes at different levels of biological complexity – the unifying concept of the institute's research activities.

#### **Department of Membrane Biochemistry**

Major research activities in the Department were concentrated into the following research areas:

#### Neutral lipid homeostasis in yeast

Neutral lipids (e.g. triacylglycerols, TAG; diacyl glycerols, DAG; and sterol esters, SE) constitute an important class of energy-rich lipids stored mostly in lipid droplets. Using the model eukaryotic organism, the yeast *Saccharomyces cerevisiae*, we focused on the following roles of lipid droplets in cellular lipid homeostasis:

<u>Lipid droplets in cellular defense from lipotoxicity.</u> It is generally accepted that the formation of TAG is an important cellular mechanism for seclusion of potentially toxic free fatty acids (FFA). Using the lipid droplet-less mutant deleted in four acyltransferases involved in the synthesis of TAG and SE we studied how the yeast cell is coping with the lipotoxic effect of FFA. We have found two mechanisms helping yeast to survive in the absence of lipid droplets – metabolic transformation of FA to the less toxic form, namely transformation of palmitoleic acid to cis-vaccenic acid (1) and excretion of accumulating FFA to the medium (Sec et al., submitted). We also showed that high levels of the sterol intermediate squalene are lipotoxic to yeast cells with impaired storage capacity in lipid droplets (2).

<u>Functional interactions of lipid droplets with other cellular organelles.</u> The importance of lipid droplets in cellular metabolism is supported by their interactions with several intracellular organelles. In collaboration with the Institute of Biophysics, Chinese Academy of Sciences, we have studied two lipid droplet proteins, Erg6p and Pet10p, involved in the interactions of lipid droplets with mitochondria and peroxisomes. Our results show that these functionally and structurally unrelated proteins are expressed in a complementary way as inactivation of one protein stimulates the expression of the other. Their simultaneous deletion disturbs TAG and SE turnover, illustrating the importance of physical interactions of lipid droplets with mitochondria and/or peroxisomes for lipid homeostasis (Garaiova et al., manuscript in preparation).

<u>Biotechnological production of valuable lipids in yeast.</u> Yeasts are promising microorganisms to produce biofuels and various high-value lipids. The accumulation of such lipids at biotechnologically interesting levels depends on the storage capacity of lipid droplets. We have

focused mainly on the role of lipid droplets in squalene production in yeast. Squalene is an intermediate in eukaryotic sterol synthesis with numerous applications in cosmetics, pharmacology, and food industry. We have shown that modification of squalene epoxidase activity is a promising approach for high squalene production in *S. cerevisiae* (3). Accumulating squalene is stored in the lipid droplets and affects their amount and morphology. Our results indicate that the storage capacity of lipid droplets is indeed the limiting factor of squalene production (3). We have also shown that partial inhibition of squalene epoxidase increased squalene accumulation in *Kluyveromyces lactis,* a biotechnologically relevant yeast able to utilize whey as the food industry waste (4).

#### Regulation of phospholipid metabolism

Phosphatidylinositol transfer proteins. Characteristic lipid composition of cell membranes implies the existence of precise mechanisms for its maintenance, including monitoring of membrane lipid composition and intracellular lipid transport. Phosphatidylinositol transfer proteins (PITPs) are characterized by their ability to transfer phosphatidylinositol (PI) between membranes across the hydrophilic environment. Defects in PITPs of higher eukaryotes have serious consequences manifested in metabolic diseases and disorders of the nervous system. In the assessed period we focused mainly on the role and mechanism of action of one of the yeast PITPs, Pdr16 protein. The absence of Pdr16p results in enhanced susceptibility of yeasts to azole antifungals. We have shown that enhanced azole susceptibility in S. cerevisiae is not caused by the elevated levels of azole antimycotics inside  $pdr16 \Delta$  cells, but rather by the impact of Pdr16p on sterol metabolism (5). Our other important finding was that the ability of Pdr16p to bind PI is an essential feature of this protein to provide protection against azole antifungals (6). Studies in S. cerevisiae were complemented by studies in another yeast model organism, Kluyveromyces lactis, where absence of KIPdr16p influenced the plasma membrane properties that lead to subsequent changes in susceptibility not only to azoles but to a broad range of xenobiotics (7, 8). Importantly, in the major fungal pathogen, the yeast Candida albicans, CaPDR16 is a known factor involved in clinical azole resistance. Thus, we believe that our studies of Pdr16p will also contribute to the fight against increasing resistance of pathogenic yeasts and fungi to antifungal drugs.



Fig. 2 Mitochondria in CL deficient yeast strains form large flat sheets. Fluorescence confocal microscopy and 3D reconstruction (adapted from (10)).

<u>Mitochondrial phospholipids.</u> Mitochondrial membranes contain a unique anionic dimeric phospholipid, cardiolipin (CL). CL plays an important role in bioenergetics of eukaryotic cells by optimizing the activities of oxidative phosphorylation enzymes and by stabilization of respiratory chain supercomplexes. It is involved in mitochondrial biogenesis and in apoptotic processes. Consequently, impaired homeostasis of mitochondrial anionic phospholipids leads to a broad range of human diseases, mostly myopathies and neuropathies. Considering the important role anionic mitochondrial phospholipids play in the cellular physiology relatively little is known how eukaryotic cell controls their homeostasis. Our current research is based on the original observation from our laboratory that the amount of phosphatidylglycerol (PG), the biosynthetic precursor of CL, is controlled by a specific phospholipase C, product of the *PGC1* gene (9). Yeast

strain without a functional phospholipase C, Pgc1p, enabled us to study the effects of the disbalance in anionic phospholipids in an efficient and specific way. We have found that accumulation of PG in yeast with otherwise normal membrane lipid composition lead to increased fragmentation of mitochondria and abnormally increased respiration rate due to the increased activity of cytochrome c oxidase. Our results indicate that not only the absence of CL (or anionic phospholipids), but also excess of PG, or unbalanced ratio of anionic phospholipids in mitochondrial membranes, have harmful consequences on mitochondrial morphology and function (10). The results were obtained in collaboration with the Institute of Experimental Medicine, Czech Academy of Sciences and Johns Hopkins University, Maryland.

#### **Cell division**

Sexual reproduction is primarily dependent on meiosis, a special type of cell division, in which the number of sets of chromosomes is reduced to half. It is absolutely essential that the halving of chromosome sets is achieved accurately. Errors in this process can lead to various genetic diseases such as Down syndrome in humans. "Characterization of novel genes involved in meiotic chromosome segregation" is the project financed by the SASPRO Programme under which Dr. Bagelova-Polakova relocated from Vienna Biocenter to our institute in April 2015. Her research is based on original screening of a collection of the yeast Schizosaccharomyces pombe knock-out mutants for genes required for proper segregation of chromosomes. At least 30 of these mutants represent novel genes, which have not yet been implicated in meiosis. In the most recent paper we showed that dbl21 mutant has no defect in cohesin metabolism, and is not involved in DSB formation or Holliday junction processing, but that Dbl2 is required for the timely removal of Rad51, presumably by recruiting the F-box DNA helicase Fbh1 (11). Our results clearly demonstrate that many of the key regulators of meiotic chromosome segregation have not yet been described opening exciting possibilities for future research. In addition to study meiosis in a yeast model organism, our laboratory was involved in the study of eukaryotic cell cycle control, especially in characterization of the role of evolutionary conserved cyclin-dependent kinase (CDK) subunits, CKS1 and CKS2, during the cell cycle. The major finding of this work lead by V. Yu (Imperial College, London) was that the balance between CKS1 and CKS2 modulates p27 CDK inhibitor degradation, and with it cyclin A/CDK2 activity to safeguard replicative fidelity and to control neuronal differentiation (12). Additionally, Dr. Holic from our laboratory was also involved in description of the first human case of CDKN1B deficiency, which recapitulates features of the murine CDKN1B knockout mouse model, including gigantism and neurodevelopmental defects (13).



Fig. 3 Deletion of *dbl2* causes failure of chromosome segregation during meiosis (adapted from (16)).

#### Development of novel biosensors

Development of novel biosensors for early detection of serious human diseases is in the focus of the Laboratory of Biosensors. Early detection of prion neurodegenerative diseases such as Alzheimer's or Creutzfeldt–Jakob disease is especially challenging due to the low levels of prions in bodily fluids outside the brain (down to pM levels in the blood). Our laboratory in collaboration with the Department of Biophysics of the Comenius University developed a biosensor based on the surface plasmon resonance (SPR) method for the study of the binding kinetics and detection of human cellular prions (PrP<sup>c</sup>). The biosensor was formed by immobilization of various biotinylated DNA aptamers on a surface of conducting polypyrrole modified by streptavidin. PrP<sup>C</sup> interaction with DNA aptamers was detected by SPR method and by guartz crystal microbalance method (14, 15). We employed the most modern analytical methods, including analysis of surface topology by atomic force microscopy, also in study of interactions of thrombin, an important marker of blood vessels injury, with DNA aptamers (16). In addition to human disease markers we were interested in monitoring of some serious food contaminants, including aflatoxins. An important achievement in this area was development of aflatoxin B1 aptamer-based biosensor using PAMAM dendrimers as immobilization platform (17, 18). Taken together, our results show highly promising potential for DNA aptamer-based biosensensors in analytical biomedicine and food control. Work in the Laboratory of Biosensors was supported by participation of the laboratory in the EU 7th Framework Programme "Ultra-sensitive, stable and easy to use AFM bio sensor tips".

#### **Department of Physiology and Ethology**

The Department of Physiology and Ethology is for several decades focused on studying various biological problems using avian models, including Japanese quail, domestic chickens and the most recently also zebra finches.

#### Poultry Behaviour and Welfare

Animal welfare is one of the key issues of animal production and was also the goal of the Coordination and Support Action of the 7th Framework Programme AWARE (March 2011 -February 2014, <u>www.aware-welfare.eu</u>). The aim was to promote integration and to increase the impact of European research on farm animal welfare (FAW) the project was organised in four mutually supportive Work Packages (WPs). Our institute (Dr. Košťál) coordinated the WP1 Research. Within the AWARE our institute organized a lot of activities, such as the Workshop for Research Leaders in Bratislava for the specialist from the East Central Europe and Baltic Countries in May 2012, or similar workshop for colleagues from the Eastern and Western Balkan region in Athens in October 2012 (co-organized by Dr. Košťál). Both activities brought the top European specialists in field of farm animal behaviour and welfare to regions where FAW research is less developed. We also co-organized so called road shows, e.g. in Slovakia and Hungary or the Joint East and West Central Europe ISAE Regional Meeting in October 2013 in Skopie. Macedonia. The results of the survey of European education in field of FAW organized by AWARE (19), and the results of the similar survey of research (20) mapped the current situation across Europe. AWARE successfully used several instruments (road shows, workshops for research leaders, satellite symposia at conferences, seminars aimed at improving skills in networking and proposal writing for international research projects) to stimulate animal welfare research in less active regions of Europe and to support the networking in animal welfare research in parts of Europe with shorter tradition in this field. AWARE with these instruments created a positive impulse for more Europe-wide development of animal welfare science. We are very proud that the network built by AWARE contributed to the 'visibility' of its members from the new member and candidate countries of the EU and that the connections established by the AWARE has been used by several consecutive projects, such as the Coordinated European Animal Welfare Network (EUWelNet), or the COST Action CA15134 Synergy for preventing damaging behaviour in group housed pigs and chickens (GroupHouseNet).

In March 2012, the European Commission launched a call (SANCO 2012/10293) to study the feasibility of a network of resources in order to improve the implementation of the EU legislation on animal welfare through knowledge strategies. The call was granted to the EUWelNet team who carried out the project during 2013 (January – December 2013, <u>www.EUWelNet.eu</u>). We are hounoured that we were invited as the only one representative from Slovakia to join the

consortium. Societal demands for improved farm animal welfare are increasingly important and must be realised within economically viable and environmentally friendly production systems. Effective knowledge transfer and innovative strategies are essential to satisfy the welfare requirements under these constraints. EUWelNet demonstrated that a coordinated network of universities and research institutes can work successfully together and deliver valuable support for the implementation of European legislation on animal welfare. EUWelNet effectively identified difficulties and bottlenecks and created innovative knowledge strategies to overcome them.

Our research in field of poultry behaviour and welfare was focused on several topics. In collaboration with the Department of Animal Physiology and Ethology, Faculty of Science, Comenius University in Bratislava, we were studying the problem how avian mother can adjust phenotypic development of her progeny to actual environmental conditions through differential deposition of hormones into the egg. Our experimental approach is based on the use of two genetic lines of Japanese quail that we have divergently selected for yolk testosterone concentrations. These lines served us as a unique model for exploring mechanisms of maternal hormone transfer into the egg and epigenetic effects of maternal hormones on offspring physiology, immunological response or behaviour (21-25). This approach can provide relevant data for better understanding of trans-generational maternal effects with potential application for poultry breeding and welfare.

The interaction between cognition and emotions represents previously unforeseen source of information for the animal welfare science. To study these phenomena in poultry, we introduced completely new methodology. We have developed Skinner boxes for Japanese quail and domestic chicken with touchscreen monitors for the presentation of stimuli and the collection of pecking data enabling us to study operant discrimination learning (discrimination of visual cues). We have also developed custom automated mealworm dispensers enabling us to use highly palatable food (mealworms) as a reward. In a series of experiments in both species and in case o domestic chicken even in two breeds (Dominant and Dekalb White) we have tested in so called judgment bias tests based on the response of animals to ambiguous cues the effects of various housing conditions (cages vs. deep litter pen) on emotions induced cognitive bias. However, it seems that in both species the differences in the environment were not big enough to induce persistent changes in emotions reflected by changed response to ambiguous cues, or that the method is not sensitive enough to reflect these changes (26, 27). We have also tested how food related enrichment can affect behaviour of broilers in order to increase their welfare via increased activity. We have shown that the food items with high motivation value (mealworms) scattered on the litter affect activity of broiler chickens. Nevertheless, this effect was only transient (28).

#### Neurobiology of Birdsong

About 10 years ago the leader of this laboratory Dr. Niederová-Kubíková was the member of the consortium Avian Brain Nomenclature Forum that published studies which changed the nomenclature as well as the view of organization of avian brain. These studies were widely accepted by scientific community (>800 citations). We advanced this knowledge further in later study (29). Using quantitative analyses of 52 constitutively expressed or behaviorally regulated genes in 23 telencephalic brain regions and based on the known connectivity we proposed that the avian pallium has four major cell populations similar to those in mammalian cortex and some parts of the amygdala: 1/ a primary sensory input population (intercalated pallium); 2/ a secondary intrapallial population (nidopallium/hyperpallium): 3/ a tertiary intrapallial population (mesopallium): and 4/ a quaternary output population (the arcopallium). Each population contributes to columns that control different sensory or motor systems. We suggest that this organization of cell groups is formed by expansion of contiguous developmental cell domains that wrap around the lateral ventricle. This study has been met with a good recognition and it was cited till the end of 2015 more than 40 times. We studied also singing behavior in songbirds and neurobiology of its control which has many behavioural, anatomical, neural, genetic as well as molecular parallels to human speech. We have found that that neurotoxic damage to adult song control nucleus Area X within striatum induced changes in singing tempo and global syllable sequencing in all animals, and considerably increased syllable repetition in birds whose song motifs ended with minor repetitions before lesioning. This stuttering-like behavior started at one month, and improved over six months. Unexpectedly, the lesioned region showed considerable recovery, including immigration of newly generated neurons that became active during singing. The timing of the neuronal recovery and stuttering suggests that immature recovering activity of the circuit might be associated with stuttering. Thus, even after juvenile learning is complete, the adult striatum plays a role in higher level organization of learned vocalizations (30). We searched for a method that would enable to visualize the brain recovery in a longitudinal study in the same bird. This would enable to directly correlate brain changes with behavioral changes to find a causal relationship. We employed the non-invasive magnetic resonance imaging (MRI) and compared the striatal lesion size measured using MRI with the size measured by immunohistochemistry. We found that the MRI method is suitable to identify changes in brain recovery volume even in small animals and small brain areas and therefore can be used for further studies (31). We also studied the mechanism regulating the striatal recovery and the role of dopamine D3 receptors that are highly expressed in the subventricular zone where the newborn neurons arise. We found that the striatal lesion leads to increased expression of D3 receptors in the neurogenic zone and activation of D3 receptors accelerates the striatal regeneration (32).



Fig. 4 Neuronal recovery of zebra finch striatum. Adapted from (30).

#### The Quail Chorioallantoic Membrane as an In Vivo Angiogenesis Model

The Department has a long history of physiology and endocrinology research on avian models, with the shift of focus towards behavioural and neurobiological research in the last 15 years. Recently we have added another research topic, when we revived the idea of using Japanese quail embryo as an alternative to *in vivo* animal testing. Chorioallantoic membrane (CAM) of avian embryo is thin and transparent, with dense capillary network which makes it an excellent model for testing of various drugs and for the study of tumor angiogenesis and antivascular therapy. Although chicken embryo is used most often, in our laboratory we introduced and validated Japanese quail CAM assay. Advantages of this model are short embryonic development, high egg production of quail, smaller space required for husbandry and experiments and lower general cost.

In the first period we studied photosensitizing properties of hypericin, a natural extract from the plant *Hypericum perforatum*. Therapeutical formulation (2 µg/g of embryo weight) was topically applied on CAM surface. Hypericin after excitation by diode laser (405 nm) caused damage of quail CAM vasculature ranging from haemorrhage and vanishing of capillary vessels, to thrombosis, lysis and haemorrhage of larger vessels (33).

In the second period we focused on applying fluorescence-based imaging techniques to detect and visualize the tumour. Strong photodynamic activity of hypericin can be improved by various molecular transport systems (e.g. LDL). Our aim was to examine pharmacokinetics of hypericin in the presence of LDL particles with implanted human squamocellular carcinoma spheroids. Spheroids were implanted on CAM surface on embryonal day 7 and after 24 hours formulations of free Hyp and Hyp:LDL 100:1 and 200:1 were topically applied. All experimental formulations in the fluorescent image very well visualized the tumour spheroid position, with gradual increase of fluorescence intensity in the 6-h observation period. LDL transportation system exhibited clear superiority in fluorescence pharmacokinetics to the free Hyp formulation by increasing tumour - normal tissue difference (34).

So far our experimental results show that Japanese qual CAM test is inexpensive and fast *in vivo* test suitable for research and understanding of the tumor angiogenesis and antivascular therapy, as well as to test the effect of photodynamic compounds and their transport systems.

#### **Department of Immunogenetics**

#### CD molecules in the reproductive process of cattle

Important step of the fertilization process in mammals is cell adhesion of gametes, in which some of the CD molecules play an essential role. The main aim of the Department of Immunogenetics in the last period was to study the expression and localization of CD molecules on bovine gametes and tissues, with focus on the analysis of the CD9 molecule, forming complexes with other molecules within the "tetraspanin web", identification of its partner molecules and their participation in the process of fertilization (see 35 for review). By means of immunohistochemistry and western blot analysis, using monoclonal antibody IVA-50 produced at our department, we have shown the expression of the CD9 molecule in the male and female tissues and fluids of bovine reproductive tract (36). By immunofluorescence analysis we detected for the first time the presence of tetraspanin on freshly ejaculated and cryopreserved bull spermatozoa. The presence of CD9 exclusively on the plasma membrane of the bovine sperm has been proved by Western blot analysis of the protein fractions after the discontinuous sucrose gradient fractionation of the bull sperm membranes (37). Process of capacitation is usually accompanied by rearrangement of sperm surface proteins acquired from seminal plasma as well as from epididymal secretion. We have also shown that the pattern of CD9 molecule did not change on freshly ejaculated as well as frozen-thawed sperm after the incubation in medium for sperm cell capacitation. We supposed that the CD9 molecule is "queueing" for the right moment to participate in some of the subsequent events in the fertilization process. Moreover, a role of the sperm CD9 molecule in fertilization process of cattle has been proposed as the sperm treatment with anti-CD9 antibody significantly reduced the number of fertilized oocytes compared to control group in fertilization assay in vitro (37).

The *in vitro* fertilization (IVF) experiments have shown the influence of the sperm CD9 on the fertilization; however, we suppose the common effect of multimolecular complexes within the tetraspanin web in bull sperm. By immunoprecipitation of the sperm lyzates with IVA-50 (anti-CD9) followed by western blot and re-immunoprecipitation analysis of coprecipitated molecule by monoclonal antibody IVA-520 (anti-CD46) we suggested that sperm CD46 could be one of the partners of CD9 in this complex (38).

To date, previous analysis of CD9 tetraspanin was aimed at the inspections of its role in IVF on the zona pellucida (ZP) free oocytes. Using immunofluorescence staining we detected CD9 molecule in addition to the plasma membrane of bovine oocytes on ZP. We tried to investigate whether the anti-CD9 antibody treatment influences the sperm-oocyte interaction during IVF with ZP-intact oocytes, using experimental system closest to *in vivo* fertilization and we have found that bovine sperm binding is partly dependent on the CD9 protein in the ZP (39). Experimental observations and experiences gained during *in vitro* fertilization experiments using mineral oils (used for covering of fertilization drops) were discussed in a review paper (40).

Role of the complement regulatory proteins CD46, CD55 and CD59 in reproduction was summarized in review by Frolíková et al. (41) in collaboration with partners from the Faculty of Science, Charles University in Prague. In addition, within the framework of a joint research program with the Research Institute for Animal Production, National Agricultural and Food Centre in Nitra, the apoptosis and sperm viability in transgenic rabbits was studied (42).

To summarize, the research program of the Institute of Animal Biochemistry and Genetics in the period 2012-2015 reflects the continuing focus on membranes and membrane-related processes as well as the innovative aspects in newly introduced research directions. Research activities during the assessed period resulted in several principal findings that significantly contributed to better understanding of the roles of membranes in normal and pathological physiology. Some of these results have direct potential for practical application in agriculture, medicine and/or technology.

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- 34. Buríková M, Bilčík B, Máčajová M, Výboh P, Bizík J, et al. in press. General physiology and biophysics
- 35. Jankovicová J, Simon M, Antalíková J, Cupperová P, Michalková K. 2015. Physiological Research 64: 279
- Cupperová P, Simon M, Antalíková J, Michalková K, Horovská Ľ, Hluchý S. 2014. Czech Journal of Animal Science 59: 134-9
- 37. Antalíková J, Jankovičová J, Simon M, Cupperová P, Michalková K, Horovská Ľ. 2015. *Reproduction in Domestic Animals* 50: 423-30
- 38. Antalikova J, Cupperova P, Simon M, Horovska L. 2013. *Molecules associated with CD9 tetraspanin in the bull sperm membrane*. Presented at Reproduction in Domestic Animals
- 39. Cupperova P, Antalikova J, Simon M, Horovska L. 2013. *The influence of anti-bovine CD9 monoclonal antibody IVA-*50 on sperm binding to zona pellucida of bovine oocytes. Presented at Reproduction in Domestic Animals
- 40. Cupperová P, Antalíková J, Simon M, Jankovicová J. 2015. Chem. Listy 109: 841-5
- 41. Frolíková M, Stopková R, Antalíková J, Johnson PM, Stopka P, Dvoráková-Hortová K. 2012. Folia Zoologica 61: 84
- 42. Chrenek P, Makarevich A, Simon M. 2012. Zygote 20: 33-7
- 43. Škrobánek P, Baranovská M, Šárniková B, Zeman M, Okuliarová M. 2012. Physiological Research 61: 389

#### 2. Partial indicators of main activities:

#### 2.1. Research output

2.1.1. Principal types of research output of the institute: basic research/applied research, international/regional (ratios in percentage)

The primary mission of the Institute of Animal Biochemistry and Genetics is to perform high quality basic research. Scientific publications in international scientific journals represent thus a principal form of research output.

Basic research – 100 %; International research - 100 %

- 2.1.2 List of selected publications documenting the most important results of basic research. The total number of publications listed for the assessment period should not exceed the average number of employees with university degrees engaged in research projects. The principal research outputs (max. 5, including Digital Object Identifier DOI) should be underlined
- ANTALÍKOVÁ, Jana JANKOVIČOVÁ, Jana SIMON, Michal CUPPEROVÁ, Petra -MICHALKOVÁ, Katarína - HOROVSKÁ, Ľubica. Localization of CD9 molecule on bull spermatozoa : its involment in the sperm egg interaction. In Reproduction in Domestic Animals, 2015, vol. 50, p. 423-430. (1.515 - IF2014). (2015 - Current Contents). ISSN 0936-6768. doi: 10.1111/rda.12508
- HOLIČ, Roman ŠIMOVÁ, Zuzana ASHLIN, T. PEVALA, Vladimír POLONCOVÁ, Katarína - TAHOTNÁ, Dana - KUTEJOVÁ, Eva - COCKCROFT, Samshad - GRIAČ, Peter. Phosphatidylinositol binding of Saccharomyces cerevisiae Pdr16p represents an essential feature of this lipid transfer protein to provide protection against azole antifungals. In Biochimica et Biophysica Acta : Molecular Cell Biology of Lipids, 2014, vol. 1841, p. 1483–1490. (4.495 - IF2013). ISSN 1388-1981. doi: 10.1016/j.bbalip.2014.07.014.
- JARVIS, Erich D. YU, J. RIVAS, M.V. HORITA, H. FEENDERS, G. WHITNEY, O. -JARVIS, S.C. - JARVIS, E.R. - KUBÍKOVÁ, L'ubica - PUCK, A.E. - SIANG-BAKSI, C. -MARTIN, S. - MC ELROY, M. - HOWARD, Ju. - PFENNING, A. - MOURITSEN, H. -CHEN, C.C. - WADA, K. Global view of the functional molecular organization of the avian cerebrum: mirror images and functional columns. In Journal of Comparative Neurology, 2013, vol. 521, no. 16, p. 3614-3616. (3.661 - IF2012). ISSN 0021-9967. doi: 10.1002/cne.23404
- KUBÍKOVÁ, Ľubica PAVUKOVÁ, Eva CVIKOVÁ, Martina LUKÁČOVÁ, Kristína -SCHARFF, Constance - JARVIS, Erich D. Basal ganglia function, stuttering, sequencing, and repair in adult songbirds. In Scientific Reports, 2014, vol. 4, p. 6590. (5.078 - IF2013). ISSN 2045-2322. doi:10.1038/srep06590
- POTURNAYOVÁ, Alexandra ŠNEJDÁRKOVÁ, Maja BÁBELOVÁ, Lenka KORRI-YOUSSOUF, H. - HIANIK, Tibor - EBNER, Andreas. Comparative analysis of cellular prion detection by mass-sensitive immunosensors. In Electroanalysis, 2014, vol. 26, p. 1312-1319. (2.502 - IF2013). ISSN 1040-0397. doi: 10.1002/elan.201400049
- BOSÍKOVÁ, Eva KOŠŤÁL, Ľubor CVIKOVÁ, Martina BILČÍK, Boris NIEDEROVÁ-KUBÍKOVÁ, Ľubica. Song-related dopamine receptor regulation in Area X of zebra finch male. In General Physiology and Biophysics, 2012, vol. 31, no. 3, p. 291-298. (1.192 -IF2011). ISSN 0231-5882.
- <u>ČAVARGA, Ivan BILČÍK, Boris VÝBOH, Pavel ZÁŠKVAROVÁ, Monika</u> CHORVÁT, Dušan - KASÁK, Peter - MLKVÝ, Peter - MATEAŠIK, Anton - CHORVÁTOVÁ, Alžbeta -MIŠKOVSKÝ, Pavol. Photodynamic effect of hypericin after topical application in the ex ovo quail chorioallantoic membrane model. In Planta Medica : an international journal of natural products and medicinal plant research, 2014, vol. 80, p. 56-62. (2.339 - IF2013). ISSN 0032-0943.

- <u>CUPPEROVÁ</u>, Petra SIMON, Michal ANTALÍKOVÁ, Jana MICHALKOVÁ, Katarína -<u>HOROVSKÁ</u>, <u>L'ubica</u> - HLUCHÝ, Svätoslav. Distribution of tetraspanin family protein CD9 in bull reproductive system. In Czech Journal of Animal Science, 2014, vol. 59, p. 134-139. (0.871 - IF2013). ISSN 1212-1819.
- DING, Y. ZHANG, S. LI, Y. HUIMIN, N. ZHANG, P. ZHANG, H. WANG, Y. -CHEN, Y. - YU, J. - HUO, Ch. - XU, S. - <u>GARAIOVÁ, Martina</u> - LIU, P. Isolating lipid droplets from multiple species. In Nature Protocols, 2013, vol. 8, no. 1, p. 43-51. (7.960 -IF2012). ISSN 1754-2189.
- DROZDÍKOVÁ, Eva <u>GARAIOVÁ, Martina</u> <u>CSÁKY, Zsófia</u> OBERNAUEROVÁ, Margita <u>HAPALA, Ivan</u>. Production of squalene by lactose-fermenting yeast *Kluyveromyce lactis* with reduced squalene epoxidase activity. In Letters in Applied Microbiology, 2015, vol. 61, no. 1, p. 77–84. (1.659 IF2014). ISSN 0266-8254
- FROLÍKOVÁ, Michaela STOPKOVÁ, Romana <u>ANTALÍKOVÁ, Jana</u> JOHNSON, Peter M. - STOPKA, Pavel - DVOŘÁKOVÁ-HORTOVÁ, Kateřina. Role of complement regulatory proteins CD46, CD55 and CD59 in reproduction. In Folia Zoologica: International Journal of Vertebrate Zoology, 2012, vol. 61, no. 1, s. 84-94. (0.554 -IF2011). ISSN 0139-7893.
- FRONTINI, Mattia KUKALEV, Alexander LEO, Elisabetta NG, Yiu-Ming -CERVANTES, Marcella - CHENG, Chi-Wai - <u>HOLIČ, Roman</u> - DORMANN, Dirk - TSE, Eric - POMMIER, Yves - YU, Veronica. The CDK Subunit CKS2 Counteracts CKS1 to Control Cyclin A/CDK2 Activity in Maintaining Replicative Fidelity and Neurodevelopment. In Developmental Cell, 2012, vol. 23, no. 2, p. 356-370. (14.030 - IF2011). ISSN 1534-5807.
- <u>GARAIOVÁ, Martina ZAMBOJOVÁ, Veronika ŠIMOVÁ, Zuzana GRIAČ, Peter HAPALA, Ivan</u>. Squalene epoxidase as a target for manipulation of squalene levels in the yeast Saccharomyces cerevisiae. In FEMS Yeast Research, 2014, vol. 14, no. 2, p. 310-323. (2.436 IF2013). ISSN 1567-1356.
- <u>GOFFA, Eduard</u> BALYZFYOVÁ, Zuzana TOTH, Hervay <u>ŠIMOVÁ, Zuzana -</u> <u>BALÁŽOVÁ, Mária - GRIAČ, Peter</u> - GBELSKÁ, Yvetta. Isolation and functional analysis of the *KIPDR16* gene. In FEMS Yeast Research, 2014, vol. 14, p, 337-345. (2.436 -IF2013). ISSN 1567-1356.
- <u>HOLIČ, Roman</u> YAZAWA, Hisashi KUMAGAI, Hiromichi UEMURA, Hiroshi. Engineered high content of ricinoleic acid in fission yeast *Schizosaccharomyces pombe*. In Applied Microbiology and Biotechnology, 2012, vol. 95, no. 1, p. 179-187. (3.425 -IF2011). ISSN 0175-7598.
- ILLMANN, Gudrun KEELING, Linda MELIŠOVÁ, Dana ŠIMEČKOVÁ, M. ILIESKI, Vlatko - WINCKLER, Christoph - <u>KOŠŤÁL, Ľubor</u>. Mapping farm animal welfare education at university level in Europe. In Animal Welfare, 2014, vol. 23, p. 401-410. (1.228 -IF2013). ISSN 0962-7286.
- JANKOVIČOVÁ, Jana SIMON, Michal ANTALÍKOVÁ, Jana CUPPEROVÁ, Petra -<u>MICHALKOVÁ, Katarína</u>. Role of tetraspanin CD9 molecule in fertilization of mammals. In Physiological Research, 2015, vol. 64, p. 279-293. (1.293 - IF2014). ISSN 0862-8408.
- KAŇKOVÁ, Zuzana OKULIAROVÁ, Monika <u>ZEMAN, Michal</u>. Immune responsiveness of Japanese quail selected for egg yolk testosterone content under severe protein restriction. In Comparative biochemistry and physiology.Part A Molecular & integrative physiology, 2014, vol. 177, p. 41-48. (2.371 - IF2013). ISSN 1095-6433.
- KAŇKOVÁ, Zuzana <u>ZEMAN, Michal</u> OKULIAROVÁ, Monika. Growth and innate imunity are not limited by selection for high egg testosterone content in Japanese quail. In Journal of Experimental Biology, 2012, vol. 215, no. 4, p. 617-622. (2.996 - IF2011). ISSN 0022-0949.
- KARMAŽÍNOVÁ, Mária JAŠKOVÁ, Katarína <u>GRIAČ, Peter</u> PEREZ-REYES, Edward LACINOVÁ, Ľubica. Contrasting the roles of the I-II loop gating brake in Ca(V)3.1 and Ca(V)3.3 calcium channels. In Pflugers Archiv-European Journal of Physiology, 2015, vol. 467, iss. 12, p. 2519–2527. (4.101 IF2014). ISSN 0031-6768.

- LANG, B. Franz JAKUBKOVÁ, Michaela HEGEDUSOVÁ, Eva DAOUD, Rachid -FORGET, Lise - BREJOVÁ, Broňa - VINAŘ, Tomáš - KOSA, Peter - FRIČOVÁ, Dominka - NEBOHÁČOVÁ, Martina - <u>GRIAČ, Peter</u> - TOMÁŠKA, Martin - BURGER, Gertraud -NOSEK, Jozef. Massive programmed translational jumping in mitochondria. In Proceedings of the National Academy of Sciences of the United States of America, 2014, vol. 111, no 6, p. 5926-5931. (9.809 - IF2013). ISSN 0027-8424.
- MÁNIKOVÁ, Dominika VLASÁKOVÁ, Danuša LETAVAYOVÁ, Lucia <u>KLOBUČNÍKOVÁ, Vlasta GRIAČ, Peter</u> CHOVANEC, Miroslav. Selenium toxicity toward yeast as assessed by microarray analysis and deletion mutant library screen: a role for DNA repair. In Chemical Research in Toxicology, 2012, vol. 25, no. 8, p. 1598-1608. (3.779 IF2011). ISSN 0893-228X.
- 23. MIODEK, A. <u>POTURNAYOVÁ, Alexandra ŠNEJDÁRKOVÁ, Maja</u> KORRI-YOUSSOUF, H. - HIANIK, Tibor. Binding kinetics of human cellular prions detection by DNA aptamers immobilized on a conducting polypyrrole. In Analytical and Bioanalytical Chemistry, 2013, vol. 405, no. 8, p. 2505-2514. (3.659 - IF2012). ISSN 1618-2642.
- OKULIAROVÁ, Monika KAŇKOVÁ, Zuzana BERTIN, A. LETERRIER, C. MOSTL, E. <u>ZEMAN, Michal</u>. Maternally derived egg hormones, antibodies and antimicrobial proteins : common and different pathways of maternal effects in Japanese quail. In PLoS ONE, 2014, vol. 9., p.e112817. (3.534 IF2013). ISSN 1932-6203.
- OKULIAROVÁ, Monika KAŇKOVÁ, Zuzana <u>ŠKROBÁNEK, Peter ZEMAN, Michal</u>. Bidirectional selection for yolk testosterone content in Japanese quail. In Avian Biology Research, 2014, vol. 7, p. 18-24. (0.895 - IF2013). ISSN 1758-1559.
- <u>SEČ, Peter GARAIOVÁ, Martina</u> GAJDOŠ, Peter ČERTÍK, Milan <u>GRIAČ, Peter HAPALA, Ivan HOLIČ, Roman</u>. Baker's yeast deficient in storage lipid synthesis uses cis-vaccenic acid to reduce unsaturated fatty acid toxicity. In Lipids, 2015, vol. 50, no. 7, p. 621-630. (1.854 IF2014). ISSN 0024-4201.
- <u>ŠIMOVÁ, Zuzana POLONCOVÁ, Katarína TAHOTNÁ, Dana HOLIČ, Roman HAPALA, Ivan</u> SMITH, A.R. WHITE, T.C. <u>GRIAČ, Peter</u>. The yeast Saccharomyces cerevisiae Pdr16p restricts changes in ergosterol biosynthesis caused by the presence of azole antifungals. In Yeast, 2013, vol. 30, no. 6, p. 229-241. (1.955 IF2012). ISSN 0749-503X.
- <u>ŠKROBÁNEK, Peter BARANOVSKÁ, Magda ŠÁRNIKOVÁ, Božena ZEMAN, Michal -</u> OKULIAROVÁ, Monika. Effect of Long-Term Simulated Microgravity on Some Sexual Traits of Male Japanese Quail. In Physiological Research, 2012, vol. 61, no. 4, p. 389-394. (1.555 - IF2011). ISSN 0862-8408.
- YAZAWA, H. <u>HOLIČ, Roman</u> KUMAGAI, H. UEMURA, H. Toxicity of ricinoleic acid production in fission yeast Schizosaccharomyces pombe is suppressed by the overexpression of plg7, a phospholipase A2 of a platelet-activating factor (PAF) family homolog. In Applied Microbiology and Biotechnology, 2013, vol. 97, no. 18, p. 8193-8203. (3.689 - IF2012). ISSN 0175-7598.
- <u>ZEMAN, Michal ŠKROBÁNEK, Peter</u> OKULIAROVÁ, Monika. Genetic differences in yolk testosterone levels influence maternal hormone deposition in the second laying cycle in Japanese quails. In Comparative biochemistry and physiology.Part A Molecular & integrative physiology, 2013, vol. 164, p. 271-275. (2.167 - IF2012). ISSN 1095-6433.

#### 2.1.3 List of monographs/books published abroad

#### 2.1.4. List of monographs/books published in Slovakia

KRŠKOVÁ, Lucia - OLEXOVÁ, Lucia - <u>PICHOVÁ, Katarína</u>. Metódy etologického výskumu. (Methods of Ethological Research. In Slovak). ISBN 978-80-969670-7-0, published by ASAP-translation.com, Nitra, 2015, pp. 125.

## 2.1.5. List of other scientific outputs specifically important for the institute, max. 10 items

Two scientists from our institute, Ivan Hapala and Peter Griač were guest editors of the special issue of Current Genetics "Yeast membranes and cell wall: from basics to applications".

## 2.1.6. List of patents, patent applications, and other intellectual property rights registered abroad, incl. revenues

2.1.7. List of patents, patent applications, and other intellectual property rights registered in Slovakia, incl. revenues

#### 2.1.8. Table of research outputs (as in annual reports).

Papers from international collaborations in large-scale scientific projects (Dwarf team, ALICE Collaboration, ATLAS collaboration, CD Collaboration, H1 Collaboration, HADES Collaboration, and STAR Collaboration) have to be listed separately.

		2012			2013			2014			2015			to	tal	
Scientific publications	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	No. / FTE	No. / salary budget	number	averaged number per year	av. No. / FTE	av. No. / salary budget
Scientific monographs and monographic studies in journals and proceedings published abroad (AAA, ABA)	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.0	0.000	0.000
Scientific monographs and monographic studies in journals and proceedings published in Slovakia (AAB, ABB)	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.0	0.000	0.000
Chapters in scientific monographs published abroad (ABC)	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.0	0.000	0.000
Chapters in scientific monographs published in Slovakia ( <i>ABD</i> )	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.0	0.000	0.000
Scientific papers published in journals registered in Current Contents Connect (ADCA, ADCB, ADDA, ADDB)	8.0	0.300	0.021	9.0	0.341	0.023	12.0	0.451	0.032	11.0	0.368	0.027	40.0	10.0	0.365	0.026
Scientific papers published in journals registered in Web of Science Core Collection and SCOPUS (ADMA, ADMB, ADNA, ADNB)	2.0	0.075	0.005	0.0	0.000	0.000	1.0	0.038	0.003	0.0	0.000	0.000	3.0	0.8	0.027	0.002
Scientific papers published in other foreign journals (not listed above) (ADEA, ADEB)	0.0	0.000	0.000	1.0	0.038	0.003	1.0	0.038	0.003	0.0	0.000	0.000	2.0	0.5	0.018	0.001
Scientific papers published in other domestic journals (not listed above) (ADFA, ADFB)	1.0	0.037	0.003	1.0	0.038	0.003	5.0	0.188	0.013	0.0	0.000	0.000	7.0	1.8	0.064	0.005
Scientific papers published in foreign peer- reviewed proceedings (AEC, AECA)	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.000	0.000	0.0	0.0	0.000	0.000
Scientific papers published in domestic peer- reviewed proceedings (AED, AEDA)	2.0	0.075	0.005	5.0	0.189	0.013	10.0	0.376	0.027	0.0	0.000	0.000	17.0	4.3	0.155	0.011
Published papers (full text) from foreign and international scientific conferences (AFA, AFC, AFBA, AFDA)	3.0	0.112	0.008	0.0	0.000	0.000	1.0	0.038	0.003	1.0	0.033	0.002	5.0	1.3	0.046	0.003
Published papers (full text) from domestic scientific conferences (AFB, AFD, AFBB, AFDB)	0.0	0.000	0.000	1.0	0.038	0.003	0.0	0.000	0.000	9.0	0.301	0.022	10.0	2.5	0.091	0.006

## • Supplementary information and/or comments on the scientific outputs of the institute.

The policy of the institute's management is for a long time focused on support of high quality publications in international journals. This is our philosophy, which we are trying to transfer also to our students. Low salary budget gives us only limited opportunity to stimulate production via differential financial reward of publications according to their quality. Nevertheless, we are using also this instrument in the possible extent. Positive trend of increasing the mean impact factor of our production from the previous assessment periods continued. The average impact factor increased from 2.11 during the previous assessment period to 2.93 in current assessment period. Nevertheles, we did not manage to overcome the problem of relatively low quantity of our scientific outputs.

As we have mentioned in the introduction to this section (see 1.8.), some research directions of major importance in the past have been terminated during the assessed period. Avian gravitational physiology or cosmic biology was since the seventies of the last century the flagship of research at our institute (or its predecessor, branch of the Institute of Farm Animal Physiology in Ivanka pri Dunaji). The milestones of research with the greatest achievements (the first quails hatch in the space on the Mir orbital station in 1990, the flight experiment aboard the Mir with the participation of the Slovak astronaut Lt. Col. Bella) are shown on the Fig. 5. Nevertheless, after the retirement of the founder of this research Koloman Bod'a (\*1927 - †2005) and his main collaborators, Peter Škrobánek remained the only active investigator left in the group. He retired in early 2015. The last publication of this group was dedicated to the effects of long term simulated microgravity on some sexual traits of male Japanese quail (43).



Fig. 5 Overview of the milestones of research on quails in cosmos and the political changes in Slovakia and Eastern block on the bottom half of the timeline adapted from: Birds in Space II: Quails in the Cosmos (<u>https://finchwench.wordpress.com/2011/09/06/cosmoquails/</u>).

Similar fate has met the research on the bioenergetics of *Methanoachaea* at the Department of Membrane Biochemistry. In the period of more than 30 years the laboratory headed by Peter Šmigáň has applied several unique approaches in the studies of bioenergetics of *Methanoarchaea* (e.g. introduction of genetic analysis in the study of methanogenesis and ATP synthesis in *Methanoarchaea*) which brought priority discoveries such as identification of the role of H<sup>+</sup> and Na<sup>+</sup> ions in the methanogenesis of *Methanothermobacter thermoautotrophicus*, the discovery of Na<sup>+</sup>/H<sup>+</sup> translocating ATP-ase or mutational analysis of the Na<sup>+</sup>-H<sup>+</sup> antiport in this organism.

#### 2.2. Responses to the research outputs (citations, etc.)

#### 2.2.1. Table with citations per annum.

Citations of papers from international collaborations in large-scale scientific projects (Dwarf team, ALICE Collaboration, ATLAS collaboration, CD Collaboration, H1 Collaboration, HADES Collaboration, and STAR Collaboration) have to be listed separately.

	20	011	20	)12	20	013	20	)14		total	
Citations, reviews	number	No. / FTE	number	averaged number per year	av. No. / FTE						
Citations in Web of Science Core Collection (1.1, 2.1)	239.0	8.951	226.0	8.561	254.0	9.549	215.0	7.191	934.0	233.5	8.522
Citations in SCOPUS (1.2, 2.2) if not listed above	35.0	1.311	53.0	2.008	55.0	2.068	47.0	1.572	190.0	47.5	1.734
Citations in other citation indexes and databases (not listed above) (3.2,4.2,9,10)	0.0	0.000	1.0	0.038	0.0	0.000	0.0	0.000	1.0	0.3	0.009
Other citations (not listed above) (3, 4, 3.1, 4.1)	0.0	0.000	3.0	0.114	8.0	0.301	20.0	0.669	31.0	7.8	0.283
Reviews (5,6)	0.0	0.000	0.0	0.000	0.0	0.000	0.0	0.000	0.0	0.0	0.000

## 2.2.2. List of 10 most-cited publications, with number of citations, in the assessment period (2011 – 2014).

- BEOPOULOS, Athanasios <u>MROZOVA, Zuzana</u> THEVENIEAU, France LE DALL, Marie-Thérese - <u>HAPALA, Ivan</u> - PAPANIKOLAOU, Seraphim - CHARDOT, Thierry - NICAUD, Jean-Mare. Control of Lipid Accumulation in the Yeast Yarrowia lipolytica. In Applied and Environmental Microbiology, 2008, Vol.. 74, No.č. 24, 7779-7789. (4.004 - IF2007). ISSN 0099-2240 (68 citations - 55 WoS, 4 Scopus, 9 not listed in WoS/Scopus)
- HARA, Erina <u>KUBÍKOVÁ, Ľubica</u> HESSLER, Neal A. JARVIS, Erich D. Role of the midbrain dopaminergic system in modulation of vocal brain activation by social context. In European Journal of Neuroscience, 2007, Vol. 25, No. 11, p. 3406–3416. ISSN 0953-816X (48 citations – 46 WoS, 2 Scopus)
- ENNACEUR, A. <u>MICHALÍKOVÁ, Simona</u> BRADFORD, A. AHMED, S. Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. In Behavioural Brain Research, 2005, Vol. 159, No. 2, p. 247–266. ISSN 0166-4328. (38 citations – 35 WoS, 3 Scopus)
- BILČÍK, Boris KEELING, Linda J. Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. In British Poultry Science, 1999, Vol. 40, No. 4, p. 444–451. ISSN 0007-1668 (29 citations – 18 WoS, 10 Scopus, 1 not listed in WoS/Scopus)
- <u>KUBÍKOVÁ, Ľubica</u> WADA, Kazuhiro JARVIS, Erich D. Dopamine receptors in a songbird brain. In Journal of Comparative Neurology, 2010, Vol. 518, No. 6, p. 741-769. (3.718 -IF2009). ISSN 0021-9967 (29 citations – 27 WoS, 2 Scopus)
- <u>ŠNEJDÁRKOVÁ, Maja</u> <u>SVOBODOVÁ, Lenka</u> EVTUGYN, G. BUDNIKOV, H. -KARYAKIN, A. - NIKOLELIS, D.P. - HIANIK, Tibor. Acetylcholinesterase sensors based on gold electrodes modified with dendrimer and polyaniline : a comparative research. In Analytica Chimica Acta, 2004, Vol. 514, No. 1, p. 79-88. ISSN 0003-2670 (**24 citations** – 17 WoS, 7 Scopus)
- <u>ŠNEJDÁRKOVÁ, Maja</u> <u>POTURNAYOVÁ, Alexandra</u> RYBÁR, Peter LHOTÁK, Pavel -HIML, Michal - FLÍDROVÁ, Karolína - HIANIK, Tibor. High sensitive calixarene-based sensor for detection of dopamine by electrochemical and acoustic method : High sensitive calixarenebased sensor for detection of dopamine by electrochemical and acoustic method . In Bioelectrochemistry, 2010, Vol. 80, Special Issue, p. 55-61. (2.652 - IF2009). ISSN 1567-5394. (22 citations – 15 WoS, 7 Scopus)
- <u>KUBÍKOVÁ, Ľubica</u> <u>KOŠŤÁL, Ľubor</u>. Dopaminergic system in birdsong learning and maintenance. In Journal of chemical neuroanatomy, 2010, Vol. 39, No. 2, p. 112-123. (1.753 -IF2009). ISSN 0891-0618 (**21 citations** – 12 WoS, 9 Scopus)
- NEWBERRY, Ruth C. KEELING, Linda J. ESTEVEZ, Inma <u>BILČÍK, Boris</u>. Behaviour when young as a predictor of severe feather pecking in adult laying hens: The redirected foraging hypothesis revisited. In Applied animal behaviour science. 2007, Vol. 107, No. 3-4, p. 262–274. ISSN 0168-1591 (**21 citations** – 17 WoS, 4 Scopus)
- <u>HAPALA, Ivan</u> MARZA, Esther FERREIRA, Thierry. Is fat so bad? Modulation of endoplasmic reticulum stress by lipid droplet formation. In Biology of the Cell, 2011, Vol. 103, No. 6, p. 271-285. (4.898 - IF2010). ISSN 0248-4900. (20 citations – 19 WoS, 1 not listed in WoS/Scopus)

## 2.2.3. List of most-cited authors from the Institute (at most 10 % of the research employees with university degree engaged in research projects) and their number of citations in the assessment period (2011–2014).

- 1. Hapala Ivan: 193 citations (162 WoS, 15 Scopus, 16 not listed in WoS/Scopus)
- 2. Zeman Michal: 172 citations (125 WoS, 45 Scopus, 2 not listed in WoS/Scopus)
- 3. Kubíková-Niederová Ľubica: 137 citations (119 WoS, 18 Scopus)
- 4. Šnejdárková Maja: 131 citations (97 WoS, 34 Scopus)

## • Supplementary information and/or comments on responses to the scientific output of the institute.

Although not ideal, the number of citations of published papers is still the most objective indicator of the acceptance of the research outputs by scientific community. This indicator may be biased in comparisons of institutions or scientists working in different research areas, and it does not reflect the quality of the very recent research. However, it can be used for assessment of long-term trends in the quality of research outputs. Also in this evaluation period we confirmed the increasing acceptance (and quality) of our published results and maintained the positive trend of increasing number of citations. The total number of citations (1156 in years 2011-2014) increased significantly when compared to two previous evaluation periods (994 citations in years 2007-2010 –increase by 16 %; 672 citations in years 2002-2005 – increase by 72 %). Very positive is also the fact, that highly cited were the results of relatively recent research (737 citations of the papers published during the last 10 years, 261 citations of papers published during the last 5 years).

#### 2.3. Research status of the institute in international and national contexts

#### • International/European position of the institute

- 2.3.1. List of the most important research activities demonstrating the international relevance of the research performed by the institute, incl. major projects (details of projects should be supplied under Indicator 2.4). Max. 10 items.
- 1. Animal Welfare Research in an Enlarged Europe, AWARE, KBBE 265686RE, FP7, <u>www.aware-welfare.eu</u>
- Coordinated European Welfare Network, EUWelNet, SANCO/2012/G3/EUWELNET/ SI2.635078, <u>www.euwelnet.eu</u>
- 3. Ultra-sensitive, stable and easy to use AFM bio sensor tips, IntelliTip, MNT-ERA.Net II, ID 431, FP7, <u>http://www.mnt-era.net/mnt-era-net-success-stories/2009-iNTELLITIP%20\_Public\_.pdf</u>
- 4. Precursors of cardiolipin biosynthesis: reasons for aberrant accumulation, effects on mitochondrial function and morphology, Agreement between the Academies of Sciences of the Slovak and Czech Republics, SAS-AS CR 15-02
- 5. Study of CD molecules on mammanlian sperm, Agreement between the Academies of Sciences of the Slovak and Czech Republics, SAS-AS CR 15-05
- 6. Euro-Bioimaging (EuBI), <u>http://www.eurobioimaging.eu/content-page/euro-bioimaging-node-candidates</u>

#### 2.3.2. List of international conferences (co)organised by the institute.

- 1. 40th Annual Conference on Yeast, 8. -11. 5. 2012, Smolenice, Slovakia <u>http://yeastconference.sk/</u>
- 2. 41th Annual Meeting on Yeast, 20. 23. 5. 2014, Smolenice, Slovakia <u>http://yeastconference.sk/</u>
- 3. 42th Annual Conference on Yeast, 20. 22. 5. 2015, Smolenice, Slovakia <u>http://yeastconference.sk/</u>
- 4. 30th International Specialized Symposium on Yeast, 18. 22. 6. 2013, Stará Lesná, Slovakia
- 5. Farm Animal Welfare Research and Education in an Enlarged Europe and Beyond, Satellite symposium of the 46th Congress of the International Society for Applied Ethology, Vienna, Austria 1. 8. 2012, <u>http://www.aware-welfare.eu/aware/45587/9/0/60</u>
- 6. AWARE Workshop for Research Leaders in Field od Farm Animal Welfare, Bratislava, Slovakia 31. 5. 2012 <u>http://www.aware-welfare.eu/aware/45498/9/0/60</u>

- 7. AWARE Workshop for Research Leaders in the Field of Farm Animal Welfare, Athens Greece, 4. 10. 2012, <u>http://www.aware-welfare.eu/aware/46016/9/0/60</u>
- 8. The Joint East and West Central Europe ISAE Regional Meeting, 8.-10.10. 2013, Skopje, Macedonia, <u>http://www.aware-welfare.eu/aware/53175/9/0/60</u>

#### 2.3.3. List of edited proceedings from international scientific conferences.

- Programme and Abstracts, 40<sup>th</sup> Annual Conference on Yeast, Smolenice, 8-11 May 2012, ISSN: 1336-4839
- 30<sup>th</sup> ISSY: Cell Surface & Organelles in Yeasts, Stara Lesna June 18-22, 2013, ed: Peter Polcic, Chairs of the conference: Peter Griac, Ivan Hapala, ISSN: 1336-4839
- Programme and Abstracts, 41<sup>st</sup> Annual Conference on Yeast, Smolenice, 20-23 May 2014, eds: Ivan Hapala, Emilia Breierova, ISSN: 1336-4839
- Programme and Abstracts, 42<sup>nd</sup> Annual Conference on Yeast, Smolenice, 19-22 May 2015, eds: Ivan Hapala, Emilia Breierova, ISSN: 1336-4839

#### 2.3.4. List of journals edited/published by the institute:

- 2.3.4.1. WOS (IF of journals in each year of the assessment period)
- 2.3.4.2. SCOPUS
- 2.3.4.3. other databases
- 2.3.4.4. not included in databases
- National position of the institute
  - 2.3.5. List of selected projects of national importance

University.

- 2.3.6. Projects of the Slovak Research and Development Agency (APVV)
- APVV-0785-11 Lipotoxicity in yeast: mechanisms of protection against accumulated fatty acids and squalene. PI: Roman Holič Project duration: 1.7.2012 / 31.12.2015 Project was coordinated by the IABG SAS.
- APVV-0410-10 The mechanisms of interaction small molecules with DNA aptamers. PI: Maja Šnejdárková Project duration: 1.5.2011 / 31.10.2014 IABG SAS was a participating organisation. Project was coordinated by the Faculty of Mathematics, Physics and Informatics, Comenius
- APVV-0123-10 Molecular architecture, dynamics and evolution of chromosomes in yeast mitochondria. PI: Peter Griač Project duration: 1.5.2011 / 31.10.2014 IABG SAS was a participating organisation. Project was coordinated by the Faculty of Science, Comenius University.
- LPP-0291-09 Mitochondrial lipid homeostasis: the yeast Saccharomyces cerevisiae as a model organism PI: Peter Griač Project duration: 1.9.2009 / 30.9.2013 Project was coordinated by the IABG SAS.

APVV-0137-10 Effect of body condition and some immunological factors (CD molecules) on fertilization process in cattle PI Michal Simon Project duration: 1.5.2011 / 30.4.2014 IABG SAS was a participating organisation. Project was coordinated by the Animal Production Research Cerntre Nitra

APVV-0047-10 Epigenetic, physiological and neurobehavioural aspects of poultry welfare PI: L'ubor Košťál Project duration: 1.5.2011 / 30.4.2014 Project was coordinated by the IABG SAS.

APVV-0242-11 Towards increased sensitivity of cancer detection and selectivity of cancer treatment: biophotonic nanotechnology applications PI: Boris Bilčík Project duration: 1.7.2012 / 31.12.2015 IABG SAS was a participating organisation. Project was coordinated by the Pavol Jozef Šafárik University in Košice

APVV-14-0267 Development of novel diagnostic method for clinical oncology based on the interaction of DNA aptamers with proteins
 PI: Alexandra Poturnayová
 Project duration: 1.7.2015 / 31.12.2019
 IABG SAS was a participating organisation. Project was coordinated by the Faculty of Mathematics, Physics and Informatics, Comenius University in Bratislava.

- 2.3.7. Projects of the Scientific Grant Agency of the Slovak Academy of Sciences and the Ministry of Education (VEGA)
- VEGA 2/0058/11 Neutral lipid homeostasis in yeast: from cell biology to biotechnology Pl: Ivan Hapala

Project duration: 1.1.2011 / 31.12.2014 Project was coordinated by the IABG SAS.

- VEGA 2/0180/12 Fatty acid secretion in the yeast Saccharomyces cerevisiae. PI: Roman Holič Project duration: 1.1.2012 / 31.12.2015 Project was coordinated by the IABG SAS.
- VEGA 2/0168/14 Study of mitochondrial lipids in the yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe PI: Mária Balážová Project duration: 1.1.2014 / 31.12.2017 Project was coordinated by the IABG SAS.
- VEGA 2/0185/14 Sterol uptake: An adaptation mechanism to hostile conditions in yeast PI: Martin Valachovič Project duration: 1.1.2014 / 31.12.2016 Project was coordinated by the IABG SAS.
- VEGA 2/0055/14 The study of the mechaisms of interaction DNA aptamers with cancer markers at the surface of leukemic T-cells PI: Alexandra Poturnayová

Project duration: 1.1.2014 / 31.12.2016 Project was coordinated by the IABG SAS.

- VEGA 2/0111/15 Yeast phosphatidylinositol transfer proteins: lipid homeostasis and resistance to azole antimycotics PI: Peter Griač Project duration: 1.1.2015 / 31.12.2018 Project was coordinated by the IABG SAS.
- VEGA 2/0006/12 The study of tissue distribution, biochemical properties and functions of CD molecules in the reproductive process of cattle PI Jana Antalíková Project duration: 1.1.2012 / 31.12.2015 Project was coordinated by the IABG SAS.
- VEGA 2/0196/14 Emotions, cognition and poultry welfare PI Ľubor Košťál Project duration: 1.1.2014 / 31.12.2016 Project was coordinated by the IABG SAS.
- VEGA 2/0177/14 Regeneration of specific brain regions of adult songbirds studied by *in vivo* magnetic resonance PI Ľubica Niederová Project duration: 1.1.2014 / 31.12.2017 Project was coordinated by the IABG SAS.
- VEGA 2/0102/15 Japanese quail chorioallantoic membrane as in vivo model for testing hypericin induced fluorescence diagnosis of tumor lesions PI Boris Bilčík Project duration: 1.1.2015 / 31.12.2017 Project was coordinated by the IABG SAS.

#### 2.3.8. Projects of SAS Centres of Excellence

#### 2.3.9. National projects supported by EU Structural Funds

Centre of Excellence for Translational Research in Molecular Medicine (TRANSMED 2), 26240120030, Principal investigator: Institute of Virology SASci, principal coinvestigator for IABG: Ivan Hapala, 2010 - 2012

## 2.3.10. List of journals (published only in the Slovak language) edited/published by the institute:

- 2.3.10.1. WOS (IF of journals in each year of the assessment period)
- 2.3.10.2. SCOPUS
- 2.3.10.3. Other databases
- 2.3.10.4. Not included in databases

#### • Position of individual researchers in an international context

## 2.3.11. List of invited/keynote presentations at international conferences, as documented by programme or invitation letter

- **1. Holič R**. 11th International Symposium on Biocatalysis and Agricultural Biotechnology (ISBAB) in Banff, Alberta, Canada, September 13-16, 2015 at the Banff Centre
- **2. Holič R.** 10th International Symposium on Biocatalysis and Agricultural Biotechnology (10th ISBAB) on Oct. 28-31, 2014 at I-Shou University, Kaohsiung, Taiwan
- **3. Holič R**. 9th International Symposium on Biocatalysis and Agricultural Biotechnology (9th ISBAB) in Spa Resort in Piešťany, Slovak Republic from October 13th to October 16st, 2013.

## 2.3.12. List of researchers who served as members of the organising and/or programme committees

#### Hapala Ivan

- head of the organising committee, member of the programme committee, 40<sup>th</sup> Anual Conference on Yeast, Smolenice, 2012
- head of the organising committee, member of the programme committee, 41<sup>st</sup> Annual Conference on Yeast, Smolenice, 2014
- head of the organising committee, member of the programme committee, 42<sup>nd</sup> Annual Conference on Yeasts, Smolenice, 2015

#### Hapala Ivan, Valachovič Martin

 members of the organising committee, 30th International Specialized Symposium on Yeast, Stará Lesná, 2013

#### Griač Peter

 head of the organising committee, 30th International Specialized Symposium on Yeast, Stará Lesná, 2013

#### Bilčík Boris, Košťál Ľubor

 members of the programme and organising committee, East Central Europe and Baltic Countries Worksop for Research Leaders in field of farm animal welfare, Bratislava 2012

#### Košťál Ľubor

- member of the programme and organising committee, AWARE Workshop for Research Leaders, Athens Greece, 2012

#### • Position of individual researchers in a national context

2.3.13. List of invited/keynote presentations at national conferences, as documented by programme or invitation letter

## 2.3.14. List of researchers who served as members of organising and programme committees of national conferences

#### Hapala Ivan, Griač Peter

 members of the organising committee, 7<sup>th</sup> Ivanka Days of Young Biologists, Ivanka pri Dunaji, 2012

#### Hapala Ivan, Griač Peter, Bilčík Boris.

 members of the organising committee, 8th Ivanka Days of Young Biologists, Ivanka pri Dunaji, 2014

## • Supplementary information and/or comments documenting the international and national status of the Institute

During previous assessment period (2008-2011), IABG has acquired the position of 'organizational centre' of the biological membrane research in Slovakia. The Institute coordinated educational program supported by the European Social Fund 'Biomembranes: cross-sectional educational programme for graduate students and young scientists in life sciences' (13120200072, 2006-2008) and the project Research and Education Centre of Excellence 'Biomembranes membrane structure and dynamics in relation to cell functions' (VVCE-0064-07, 2008-2011). This Centre of Excellence project was exceptional with respect to number of institutions involved (consortium of 15 laboratories consisting of 4 SASci institutes, 3 Faculties of the top Slovak universities, 1 institute of the Ministry of Agriculture) as well as by excellent results and outcomes. Although the Centre of Excellence "Biomembranes" has not received direct funding during the evaluated period, the close collaboration continued supported by 11 'elicited' projects with the participation of at least 2 consortium members that were funded by the Slovak grant agencies (8 APVV, 3 VEGA). To strengthen the material basis of membrane research in Slovakia, IABG submitted as the coordinating institution project proposal 'Biomembranes: establishing the infrastructure for a modern research of biological membranes' for support from the Structural funds of the EU. The project included two partners from the Biomembranes consortium (Faculty of Science Comenius University, and the Faculty of Chemical and Food Technology, Slovak Technical University) and although the project proposal has not been funded, we plan to resubmit similar application in the close future.

IABG SASci plays an important role in the yeast science in Slovakia with respect to experimental research and organisational issues. The Department of Membrane Biochemistry represents the leading center of the research on yeast lipids and membranes in Slovakia and its activities are broadly accepted also internationally. Regarding the organisational activities, dr. Hapala is the chair of the Czechoslovak Committee on Yeast at the Czechoslovak Microbiological Society and on behalf of this function he is the main organizer of the Annual Conferences on Yeast, a series of international conferences taking place for more than 40 years in the Conference Centre of SASci in Smolenice Castle (http://yeastconference.sk/). Dr. Hapala is active also internationally as the elected member of the International Commission on Yeast (ICY), scientific body organizing the worldwide cooperation between yeast scientists and organizing scientific conferences on topics related to yeasts (International Congresses on Yeast, International Specialized Symposia on Yeasts). We were highly honoured when the ICY accredited IABG SASci and the Czechoslovak Committee on Yeast to organize in 2013 the 30<sup>th</sup> ISSY: Cell Surface & Organelles in Yeasts. The symposium took place in the Congress Centre of SASci in Stara Lesna (High Tatras) and was evaluated by all participants as a scientifically and socially very successful event.

IABG SASci is the founding member of the Slovak Biolmaging Network (SKBIN). This network of expert laboratories involved in application of advanced bioimaging techniques in biological and biomedical research has been founded in 2014 by the Center for Interdisciplinary Biosciences, Faculty of Science of the UPJS Kosice together with IABG, International Laser Centre Bratislava and Institute of Molecular Physiology and Genetics SAS, Bratislava. In 2015 SKBIN became a partner and national node in the consortium Euro-Biolmaging (<u>www.eurobioimaging.eu</u>) aimed at providing access to a broad range of cutting-edge biological and medical imaging technologies as well as advanced training in these technologies for life scientists in Europe.

As already mentioned in the Summary of R&D activity (see 1.8), research in field of poultry behaviour and welfare at our institute was during the evaluated period largely affected by participation in two European projects consortia, AWARE (FP7) and EUWelNet (DG Sanco). Both projects strengthened the national and international status of our institute in field of animal welfare science. Our role especially in AWARE (work package leader of the WP1 Research) helped us not only to stimulate animal welfare research in Slovakia (Workshop for Research Leaders, Lecturers Helping Lecturers in Bratislava, Road Show in Košice), but in a broader perspective, our activities contributed also to the support of this type of research in the whole Eastern Europe and Balkans (Workshop for Research Leaders in Athens, or the Joint East and West Central Europe ISAE Regional Meeting initiated by the AWARE and co-organized by the International Society for Applied Ethology in Skopje, Macedonia). The fact that we were approached by several new

projects consortia (European Training Network, several COST actions) gives evidence that we proved to be reliable partner.

Another example of the positive role of the participation in larger European project in gaining national and international status is the participation of the research group focused on biosensors in the ERA-Net project Intellitip together with the top laboratories from Austria and Finland. The collaborations established within the project continue beyond the end of the project.

#### 2.4. Tables of project structure, research grants and other funding resources

#### • International projects and funding

2.4.1. Major projects within the European Research Area – Framework Programmes of the EU, ERA-NET, European Science Foundation, NATO, COST, INTAS, etc. (here and in items below please specify: type of project, title, grant number, duration, total funding and funding for the institute, responsible person in the institute and his/her status in the project, e.g. coordinator, work package leader, investigator),

Start	Project title	Project number	Duration in months	Funding for theOrganisation (EUR)	Role of the Organisation
	Ultra-sensitive, stable and easy to use AFM bio sensor tips (2010- 2012)	MNT-ERA.Net II	24	150000	Maja Šnejdárková, I
2012	Animal WelfAre Research in enlarged Europe(2011-2014)	KBBE - 265686	36	59920	Ľubor Košťál, W
	Coordinated European Welfare Network	SANCO/2012/G 3/EUWELNET/S 12.635078	12	27178	Ľubor Košťál, I
2013					
2014					
2015	Characterization of novel genes involved in meiotic chromosome segregation(2015-2018)	SASPRO 0070/01/02	36	81713	Silvia Bágeľová, C
2015					

#### 2.4.2. Other international projects, incl. total funding and funding for the institute

- Precursors of cardiolipin biosynthesis: reasons for aberrant accumulation, effects on mitochondrial function and morphology, bilateral agreement between the Czech and Slovak Academies of Sciences, SAV-AV ČR 15-02, Mgr. Mária Balážová, PhD., 2015-2017, funding – 1517 EUR
- Study of CD molecules on mammanlian sperm, bilateral agreement between the Czech and Slovak Academies of Sciences, SAV-AV ČR 15-05, Ing. Jana Jankovičová, PhD., 2015-2017, funding 1517 EUR

## 2.4.3. Other important projects and collaborations without direct funding (max. 10 projects)

- collaboration with prof. S. Cockcroft, Department of Neuroscience, Physiology, and Pharmacology, University College, London, UK on phosphatidylinositol transfer proteins. Collaboration was initiated during a postdoctoral stay of Dr. Holič in prof. Cockcroft's laboratory and continues in exchange of ideas, methods and experimental material. Collaboration resulted in recent common scientific paper (Holic et al. (2014) Biochim Biophys Acta 1841, 1483).
- collaboration with prof. H. Uemura, Bioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Japan on ricinoleic acid production and its lipotoxicity in the fission yeast *Schizosaccharomyces pombe*. Collaboration was initiated during a postdoctoral stay of Dr. Holič (JSPS Postdoctoral fellowhsip) in prof. Uemura's laboratory. It continued later on during postdoctoral stay of Dr. Garaiová (JSPS Postdoctoral fellowship) and Dr. Holič (JSPS Invitation fellowship). Collaboration resulted so far in two scientific papers (Holic et al., 2012, Appl Microbiol Biotechnol 95(1):179-87) and Yazawa et al., 2013, Appl Microbiol Biotechnol. 97(18):8193-203).
- collaboration with prof. R. Weselake, Alberta Innovates Phytola Centre, Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Canada on direct evolution of enzymes involved in triacylglycerol biosynthesis. Collaboration was initiated during a stay of Dr. Holič as a visiting professor in prof. Weselake's laboratory and continues in exchange of ideas and methods. Collaboration resulted in recent common abstracts at conference abstract books (107th American Oil Chemists' Society (AOCS) Annual Meeting & Expo, May 1–4, 2016, Salt Lake City, Utah, USA and 22nd International Symposium on Plant Lipids (ISPL) 03-08 July 2016, Goettingen, Germany).
- collaboration with the team of Dr. A. Ebner from Johannes Kepler University in Linz (JKU), Austria on the application of AFM. This collaboration started under the joint project "IntelliTip" in framework of EU funded program mnt-era.net (2010-2012) and continues to the benefit of both participating laboratories. The major outcome is a common publication (Poturnayova et al. (2014) J. Nanomed. Nanotechnol. 5: 202)
- collaboration with prof. E.D. Jarvis, Howard Hughes Medical Institute and Department of Neurobiology, Duke University Medical Center, Durham, USA on avian brain organization as well as neurogenesis in songbirds. Dr. Niederova spent 4 years in the laboratory of Dr. Jarvis as a postdoctoral fellow and the collaboration continued thereafter. It resulted during the assessed period in two papers (Jarvis et al., 2013, J Comp Neurol 521(16): 14-65 and Kubikova et al., 2014, Sci Rep 4: 6590).
- collaboration with prof. A. Van der Linden, Bio-Imaging Lab, University of Antwerp, Belgium on magnetic resonance imaging techniques used in the study of brain regeneration. The collaboration was initiated by 6 months long fellowship in 2015 (covered by travel grant from the Slovak Academic Information Agency) of the doctoral student K. Lukáčová in Belgium and her following joint PhD. study. The collaboration provided the advantage of the performing experiments with the state-of-the-art MRI

techniques such as diffusion tensor imaging for tractography as well as future collaboration based on the obtained results.

 collaboration with prof. K. Riebel, Institute of Biology, Leiden University, Netherlands, on biological significance of birdsong. The collaboration was initiated by the postdoctoral stay of Dr. Pavúková (Bosíková) in 2015 in Netherlands. During the collaboration we obtained the know-how of performing preference experiments, exchange of ideas and learn methods to manipulate song.

#### National projects and their funding

2.4.4. Projects supported by the Slovak Research and Development Agency (APVV)

Start	Project title	Project number	Duration in months	Funding for theOrganisation (EUR)	Role of the Organisation
	Towards increased sensitivity of cancer detection and selectivity of cancer treatment: biophotonic nanotechnology applications (2012- 2015)	APVV-0242-11	42	14459	I
	National monitorities of protections2012-2015)Mitochondrial lipid homeostasis: the yeast Saccharomyces cerevisiae as a model organism (2009-2013)LPP-02Lipotoxicity in yeast: mechanisms of protection against accumulated fatty acids and squalene. (2012- 2015)APVV-01The mechanisms of interaction small molecules with DNA aptamers (2011-2014)APVV-04Epigenetic, physiological and neurobehavioural aspects of poultry welfare (2011-2014)APVV-04Molecular architecture, dynamicsAPVV-04	LPP-0291-09	49	27017	С
2012		APVV-0785-11	42	130094	С
2012	The mechanisms of interaction small molecules with DNA aptamers (2011-2014)	APVV-0410-10	42	57000	I
	Epigenetic, physiological and neurobehavioural aspects of poultry welfare (2011-2014)	APVV-0047-10	36	128340	С
	Molecular architecture, dynamics and evolution of chromosomes in yeast mitochondria (2011-2014)	APVV-0123-10	42	30000	I
	Effect of body condition and some immunological factors (CD molecules) on fertilization process in cattle (2011-2014)	APVV-0137-10	36	80942	I
2013					
2014					
2015	Development of novel diagnostic method for clinical oncology based on the interaction of DNA aptamers with proteins (2015-2019)	APVV-14-0267	54	59625,-	I

2.4.5. Projects supported by the Scientific Grant Agency of the Slovak Academy of Sciences and the Ministry of Education (VEGA) for each year, and their funding

VEGA	2012	2013	2014	2015
Number	8	7	10	10
Funding in the year (EUR)	57 868	48951	63532.44	75359

#### • Summary of funding from external resources

#### 2.4.6. List of projects supported by EU Structural Funds

Centre of Excellence for translational research in molecular medicine (TRANSMED 2), 26240120030, Principal investigator: Institute of Virology SASci, principal coivestigator for IABG Ivan Hapala, 2010 - 2012

Year	Project title	Project number	Duration in months	Funding for the Organisation (EUR)	Role of the Organisation
2012					
2013					
2014					
2015					

#### 2.4.7. Summary of external resources of the EU Structural Funds (ERDF/ESF)

External resources	2012	2013	2014	2015	total	average
External resources (milions of EUR)	0.015	0.036	0.011	0.019	0.081	0.020
External resources transfered to coooperating research organisations (milions of EUR)	0.000	0.000	0.000	0.000	0.000	0.000

• Supplementary information and/or comments on research projects and funding sources

#### 2.5. PhD studies and educational activities

#### 2.5.1. List of accredited programmes of doctoral studies, period of validity

Animal Physiology 4.2.10 131/2002 Act, contract with Comenius University, Faculty of Science, validity "not limited until performed in accordance with law".

# 2.5.2. Summary table on doctoral studies (number of internal/external PhD students; number of foreign PhD students, number of students who successfully completed their thesis, number of PhD students who quit the programme)

PhD study	31	1.12.20	12	31	.12.20	13	31	1.12.20	14	31	.12.20	15
Number of potential PhD supervisors												
PhD students	number	defended thesis	students quitted									
Internal	5.0	1.0	1.0	7.0	0.0	0.0	7.0	0.0	0.0	7.0	1.0	0.0
External	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other supervised by the research employees of the institute	4.0	1.0	0.0	2.0	3.0	0.0	2.0	0.0	0.0	4.0	1.0	0.0

#### 2.5.3. Summary table on educational activities

Teaching	2012	2013	2014	2015
Lectures (hours/year) <sup>3</sup>	62	62	64	60
Practicum courses (hours/year) <sup>3</sup>	4	4	4	2
Supervised bachelor thesis (in total)	2	2	2	8
Supervised diploma thesis (in total)	4	0	3	11
Supervised PhD thesis (in total)	6	7	10	11
Members in PhD committees (in total)	4	5	2	3
Members in DrSc. committees (in total)	0	0	0	0
Members in university/faculty councils (in total)	0	0	0	0
Members in habilitation/inauguration committees (in total)	1	0	1	0

#### 2.5.4. List of published university textbooks

KRŠKOVÁ, Lucia - OLEXOVÁ, Lucia - <u>PICHOVÁ, Katarína</u>. Metódy etologického výskumu. (Methods of Ethological Research. In Slovak). ISBN 978-80-969670-7-0, published by ASAP-translation.com, Nitra, 2015, pp. 125.

#### 2.5.5. Number of published academic course books

#### 2.5.6. List of joint research laboratories/facilities with universities

- Faculty of Science, Comenius University Bratislava, collaboration with the Department of Animal Physiology and Ethology
- Faculty of Science, Comenius University Bratislava, collaboration with the Department of Biochemistry, the Department of Microbiology and Virology and the Department of Genetics
- Faculty of Mathematics, Physics and Informatics, Comenius University Bratislava, collaboration with the Department of Nuclear Physics and Biophysics
- Faculty of Chemical and Food Technology, Slovak Technical University, Bratislava, collaboration with the Institute of Biochemistry, Nutrition and Health Protection
- Faculty of Chemical and Food Technology, Slovak Technical University, Bratislava, collaboration with the Institute of Biotechnology and Food Science
- Faculty of Agrobiology and Food Resources, Slovak Agricultural University, collaboration with the Department of Veterinary Disciplines

## • Supplementary information and/or comments on doctoral studies and educational activities

In accoradance with the agreements on cooperation between the IABG and the Comenius University and Slovak Technical University our employees are involved in lecturing, holding seminars and laboratory courses, or in supervising the diploma theses of students who carry out their experimental work at our Institute. We consider these teaching activities as very important for the Institute with respect to the recruitment of motivated and talented PhD. students from the universities.

PhD. education is one of the long-term priorities of our institute. The institute is involved in PhD. studies in two subjects – Animal Physiology and Biochemistry. The quality of the PhD education in our institute is traditionally high. We give talented graduate students opportunity to get experience in foreign competetive research laboratories during their PhD. studies whenever possible. Here is the list of students that spent part of their PhD studies in research laboratories abroad:

#### 2012

Martina Garaiová: 5 month stay at the National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China. The stay was supported by the stipend from the National Stipend Program of the Ministry of Education, Science, Research and Sports of the Slovak Republic.

Alexandra Poturnayová: 3 week stay at the Biophysics Institute, Johanes Kepler University, Linz, Austria. The stay was supported by the ERA-Net FP7 project ID 431 "Ultra-sensitive, stable and easy to use AFM bio sensor tips (IntelliTip)".

#### 2014

Daniela Blahutová: 2 week stay at Unité de Recherches Avicoles, INRA Centre de Tours, Nouzilly, France. The stay was supported by the FP7 project "Animal Welfare Research in Enlarged Europe (AWARE)".

Mária Horváth: 5 week stay at the UMR CNRS 6552 Ethologie animale et humaine, Université de Rennes 1, Rennes, France. The stay was supported by the FP7 project "Animal Welfare Research in Enlarged Europe (AWARE)".

Katarína Pichová: 4 week stay at the Norwegian School of Veterinary Science, Department of Production, Animal Clinical Sciences, Oslo, Norway. The stay was supported by the FP7 project "Animal WelfAre Research in enlarged Europe (AWARE)".

#### 2015

Kristína Lukáčová: 5 month stay in the Bio-Imaging Laboratory, University of Antwerp, Belgium. The stay was supported by the stipend from the National Stipend Program of the Ministry of Education, Science, Research and Sports of the Slovak Republic. This stay resulted in dual PhD - Doctor of Biomedical Sciences study (IABG SAS - supervisor dr. Ľ. Niederová; University of Antwerp, Belgium - supervisor prof. Annemie Van der Linden).

Daniela Blahutová: 8 month stay at Unité de Recherches Avicoles, INRA Centre de Tours, Nouzilly, France). The stay was supported by the stipend from the National Stipend Program of the Ministry of Education, Science, Research and Sports of the Slovak Republic.

High level of the PhD. studies at the IABG SAS is manifested by the fact that during the evaluation period PhD. students from our institute have won 7 prices on domestic or international conferences ('Best student poster award' or 'Best student lecture award'). Three PhD students have been awarded the competetive grants of the Slovak Academic Information Agency for long-term stays in top-level international laboratories. The high quality of the PhD. studies at our institute is demonstrated also by the fact that two students that graduated in the assessment period have won 2nd and 3rd price in the 2015 SASci competition for the best young scientists.

One of the important activities of the IABG representing an integral part of our contribution to the PhD. studies is the organization of a bi-annual series of minisymposia "Ivanka Days of Young Biologists". These minisymposia are aimed specifically on PhD. students from the SASci institutes and universities. The students have the chance to present their results in the form of short talks and to discuss them with fellow students and senior scientists. The presentations in 3 sections (Biochemistry and molecular biology; Cell biology and microbiology; Physiology and general biology) are evaluated by an independent committee and best ones in each section are awarded. The minisymposium is already a traditional event (the 7<sup>th</sup> and 8<sup>th</sup> minisymposium took place in 2012 and 2014, respectively).

#### 2.6. Social impact

- 2.6.1. List of the most important results of applied research projects. Max. 10 items
- 2.6.2. List of the most important studies commissioned for the decision-making authorities, the government and NGOs, international and foreign organisations
- 2.6.3. List of contracts and research projects with industrial and other commercial partners, incl. revenues
- 2.6.4. List of licences sold abroad and in Slovakia, incl. revenues
- 2.6.5. List of most important social discourses under the leadership or with significant participation of the institute (max. 10 items)
- 2.6.6. Summary of relevant activities, max. 300 words

The European Commission is encountering difficulties to implement animal welfare legislation. Some of these difficulties have been associated to a lack of knowledge. Stakeholders (farmers, producers' organisations, supply chains, NGOs, etc.) may face difficulties in interpreting the legislation or may not be sufficiently familiar with the range of options available for implementing it. Furthermore, competent authorities may not be aware of the bottlenecks in the implementation of EU legislation. Those difficulties are compounded by the fact that Member States differ in how and to what extent animal welfare legislation is implemented. Being aware of these problems, the European Commission is considering the possibility of establishing a coordinated European animal welfare network to ensure that competent authorities receive coherent and uniform information on the way the EU legislation should be implemented. The EUWelNet project, carried out in 2013, provided a set of recommendations and a clear model for the future establishment of such a European network. Different strategies were developed to transfer knowledge to competent authorities and other stakeholders to improve the level of understanding and implementation of the animal welfare legislation. EUWelNet demonstrated that a coordinated network of universities and research institutes can work successfully together and deliver valuable support for the implementation of European legislation on animal welfare.

Our Institute was one of the members of the EUWelNet consortium as a single representative of Slovakia. In collaboration with the competent authority (State Veterinary and Food Administration of the Slovak Republic) we contributed mainly to the analysis of the implementation process and procedures and identification of major bottlenecks and the relevance of remedial knowledge strategies using an example of the implementation of the Council Directive 2007/43/EC laying down minimum rules for the protection of chickens kept for meat production.

#### 2.7. Popularisation of Science (outreach activities)

#### 2.7.1. List of the most important popularisation activities, max. 20 items

- 1. Košťál Ľ.: Vajcia od šťastných sliepok (Eggs from happy hens), magazin article, Profit, november 2012, pp. 20-23
- 2. Košťál Ľ.: TV JOJ The first news (short shot about poultry behaviour and welfare research), 11.12.2012.
- 3. Košťál Ľ.: TV Markíza Reflex (short shot about poultry behaviour and welfare research), 12.12.2012
- 4. Košťál Ľ.: Radio-FM Morning on FM (interview about the poultry welfare research Pláničkova metóda v praxi), 23.10.2012
- 5. Bilčík B: short article about the activity of the Slovak Branch of the World's Poultry Science Association (WPSA). Slovenský chov 2/10/12, p. 40.
- Departement of Immunogenetics and the Department of Physiology and Ethology: Participation at the 39<sup>th</sup> International Agriculture and Food Exhibition Agrokomplex Nitra, poster presentations, 23.8.2012
- 7. Pichová K.: How do animals percieve their environment on farms? Lecture on the Faculty of Science, Comenius University Bratislava, 22. 8. 2013, Nature Week.
- 8. Pichová K.: The birdsong. Lecture on the Faculty of Science, Comenius University Bratislava, 22. 8. 2013, Nature Week.
- 9. Bilčík B.: Are birds stupid? Alumni lecture at the secondary school Gymnázium Jura Hronca, 20.11.2014, <u>https://www.gjh.sk/files/prednasky/56Bilcik2.pdf</u>
- 10. Máčajová M.: Parents in front of blackboard ethology, birds, zebra finches. Lecture for children at the St. Vincent de Paul High & Primary School, 9.5.2014
- 11. Hapala I, Holič R.: What is squalene? (Čo je skvalén?), popular science magazine article, Quark 12, 2015
- 12. Košťál Ľ.: Quail project inspired even the scientists from NASA (Projekt s prepelicami inšpiroval aj vedcov z NASA), newspaper article, Denník N, 14.9. 2015
- 13. Niederová Ľ.: Singing in the brain. Lecture on the Faculty of Science, Comenius University Bratislava, 23. 2. 2015
- Collaboration with the Elementary School in Ivanka pri Dunaji, "Scientists to Children Children to Scientists", excursions to Institute laboratories, simple experiments, children drawings contest "Science trough children eyes" – every year during the Science Week
- 15. Collaboration with the Evangelical Lyceum in Bratislava, visit of students, lectures, hands-on activities every year during the Science Week
- 16. Bilčík B.: photographic exhibitions (Ivanka pri Dunaji, Bratislava), every year during the Science Week

2.7.2.	Table of outreach activities according to institute annual reports
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Outreach activities	2012	2013	2014	2015	total
Articles in press media/internet popularising results of science, in particular those achieved by the Organization	2	0	0	2	4
Appearances in telecommunication media popularising results of science, in particular those achieved by the Organization	3	0	0	0	3
Public popularisation lectures	5	7	4	6	22

## • Supplementary information and/or comments on popularisation activities, max. 300 words

In order to be understood and accepted by the society and to gain the respect and credit, popularization activities represent essential part of scientific work. Besides activities such as newspaper articles, appearences in TV and broadcast, or participation at exhibitions, our popularization activities culminate every year in the Science and Technology Week. During the Science and Technology Week we regularly welcome children from the local elementary school in Ivanka and students from the secondary school in Bratislava. They have the possibility to get an impression of the scientific work through some hands-on activities and popularization lectures prepared especially for them. In return, teachers from the elementary school organize every year the drawing competition for children that thematicaly reflects their visit to our institute and scientific work in general. The winners of the competition are rewarded by prizes donated by our Institute – books related to nature.

## 2.8. Background and management. Human resources and implementation of recommendations from previous assessment

#### 2.8.1. Summary table of personnel

Personnel	2012	2013	2014	2015
All personnel	49.0	50.0	44.0	49.0
Research employees from Tab. Research staff	35.0	36.0	33.0	36.0
FTE from Tab. Research staff	26.700	26.500	26.600	29.900
Average age of research employees with university degree	45.5	44.0	45.6	42.2

FEMALE	AGE								
Number of	< 30	31 - 34	35 - 39	40 - 44	45 - 49	50 - 54	55 - 59	60 - 64	> 65
DrSc. / prof. <sup>5</sup>	0	0	0	0	0	0	0	0	0
II.a / Assoc. prof. <sup>6</sup>	0	0	2	1	0	0	0	0	1
Other researchers PhD./CSc.	2	5	2	0	1	1	0	0	0
doc. / Assoc. prof.	0	0	0	0	0	0	0	0	0

2.8.1.1. Professional qualification structure (as of 31.12. 2015) FEMALE

#### 2.8.1.2. Professional qualification structure (as of 31.12. 2015) MALE

MALE		AGE							
Number of	< 30	31 - 34	35 - 39	40 - 44	45 - 49	50 - 54	55 - 59	60 - 64	> 65
DrSc. / prof. <sup>5</sup>	0	0	0	0	0	0	0	1	1
II.a / Assoc. prof. <sup>6</sup>	0	0	1	1	1	0	2	1	0
Other researchers PhD./CSc.	1	0	0	0	0	1	0	0	0
doc. / Assoc. prof.	0	0	0	0	0	0	0	0	0

#### 2.8.2. Postdoctoral and mobility scheme

## 2.8.2.1. Postdoctoral positions supported by national and international resources

Mgr. Mária Balážová, LPP-0291-09, Mitochondrial lipid homeostasis: the yeast Saccharomyces cerevisiae as a model organism, 2009-2013

#### 2.8.2.2. Postdoctoral positions supported external funding

#### 2.8.2.3. SAS stipends and SASPRO stipends

The SASPRO project: Characterization of novel genes involved in meiotic chromosome segregation PI Silvia Bágeľová-Poláková Project duration: 1.4.2015 / 31.3.2018 Project is coordinated by the IABG SAS

#### 2.8.2.4. Internal funding - the Slovak Academy of Sciences Supporting Fund of Stefan Schwarz

#### **2.8.3.** Important research infrastructure (max. 2 pages)

To perform high quality experimental research, modern sophisticated instruments are required. The current status of Institute's research infrastructure is far from ideal, however, combined with the instruments available at the collaborating institutions of the SAS and universities in Bratislava enable us to perform research at the competitive level. The EU Structural Funds represent the only source for building the research infrastructure in Slovakia. Unfortunately, the possibilities of our institute in this respect are very limited due to low funding for Bratislava region and our location outside of the SASci campus. We nevertheless attempted recently to improve the Institute's infrastructure for membrane research (project application Biomembranes: Building up the Infrastructure for the Modern Membrane Research, in a consortium with the Faculty of Science, Comenius University, and the Slovak Technical University; call OPVaV-2015/3.1/01-SORO), however, this attempt was not successful.

The Institute's research infrastructure, respectively the infrastructure of the established joint laboratories with universities consists of:

- 1. The research infrastructure for the study of interactions between molecules and surfaces: a state-of-the-art joint laboratory between our Institute and the Department of Nuclear Physics and Biophysics, Comenius University to study interactions between surfaces and biologically important molecules was built. The major instruments currently available are Atomic force microscope (Agilent) and Research quartz crystal monitor. These instruments enable to measure motional resistance and changes of frequency on physico-chemical interface (RQCM) and to measure topology of surfaces as well as the forces between individual molecules and surfaces (AFM). Additional state-of-the-art biophysical instruments, including laser elipsometer (Sentech, Germany) to map surfaces, Zetasizer Nano ZS (Malvern, UK) to determine particle size and fluorescence spectrometer Agilent Eclipse (Agilent, USA) are also available.
- 2. The instrumental base for membrane research: the Agilent HPLC apparatus equipped with diode array detector, evaporative light scattering detector and ESA CoronaCAD detector, combined with HPLC fraction collector are available for analysis of membrane lipids. Analytical instrumentation for membrane research was further improved during the current assessment period by purchase of the gas chromatograph Agilent. This instrumentation together with semiautomatic system for Thin Layer Chromatography consisting of CAMAG sample applicator Linomat 5 and a TLC scanner for densitometric evaluation of TLC chromatograms enable us to perform all needed standard analyses of phospholipids, sphingolipids, and sterols. Ultracentrifuge Beckman Optima XP and 2D-electrophoretic apparatus Multiphor II allows us to carry out the whole range of separation and analytical methods used in membrane research. To perform experiments requiring strict anaerobiosis we have available an anaerobic system consisting of anaerobic chamber, fermentor and the gas mixing unit. A semi-automatic pipetting station (Mettler-Toledo) enables to perform large-scale phenotype screening of microbial mutants in 96-well and 384 formats. The infractructure for membrane research is available to collaborating laboratories from other SASci institutes, Comenius University and Slovak Technical University, particularly for experiments in the area of our specific expertise (lipid analysis, anaerobic cultivations, whole genome phenotypic screening of yeast). The positive outcome of sharing the instruments and methodologies in the assessed period is documented by numerous joint publications including those initiated by the collaborating laboratories.
- 3. Infrastructure for neurobiological and behavioural research. The inevitable prerequisite for this research is animal facility. Our accredited small animal facility consists of enriched cages and deep litter pens for laying hens, cages and deep litter system for Japanese quail and aviary and breeding cages for zebra finch. We have the birdsong recording studio, with 20 soundproof boxes, enabling to accommodate 1-2 zebra finches. Each box is equipped with air supply, light, IP camera (D-Link, Taiwan) and highly sensitive microphone (Shure, USA), connected to amplifiers and computers with sophisticated software for song recording and analyses. The operating theatre is equiped with small animal inhalation anesthesia Matrix<sup>™</sup> VIP 3000 Isoflurane Vaporizer (Midmark, USA) using Oxygen Concentrator OXYMAT 3 (Weinmann, Germany) as oxygen supply. This, together with the stereotaxic apparatus Kopf 900 (David Kopf Instruments, USA) allows us to do targeted brain surgery on our avian models. With the help of automated microprocessor controlled microinjection pipette Nanoject II (Drummond Scientific,

USA;) lesions are produced and then studied with fluorescent microscope Leica DM5500 B and documented with digital camera Leica DFC340 FX (Leica, Germany. Our custom built operant conditioning chambers enable us to test the cognitive abilities in birds, using touchscreen monitors (ELO 1529 L, Elo Systems, USA) and the Biopsychology Toolbox, an open source Matlab toolbox for the control of behavioral experiments. Infrared thermography (IRT) is a non-invasive tool for evaluating the stress response, for the detection of fear and/or pain related responses of animals to different handling procedures or for the assessment of the feather cover of laying hens. The FLIR E5 camera (FLIR System AB, Sweden) and the Fluke 3-Point IR Calibrator (Fluke Corporation, USA) represent available IRT technology. In collaboration with the Slovak University of Technology, we use for brain MRI their 4.7 Tesla small animal scanner with the 400mT/m gradient system, DDR INOVA (Agilent) console and sensitive proton and phosphorous RF coils The lab is equipped with animal monitoring system (SA instruments) essential for monitoring of vital functions such as body temperature, EEG, respiration rate during anesthesia.

- 4. Infrastructure for research on quail chorioallantoic membrane. As mentioned above, key prerequisite for studies using quail ebryo is our own Japanese quail breeding colony providing fertilized eggs and hatcheries Bios midi. For incubation of *ex ovo* embryos and cell cultures we use Binder BF 115 and ESCO CCL-050B CO2 incubator. Photoactivation is done by 405 nm diode laser (Ocean Optics) and the results are documented either by macro photo setup (camera Canon 5D II, lens MP-E 65mm) or fluorescence microscope Leica DM 5500 B.
- 5. Infrastructure for research of the role of CD molecules in reproductive prcesses in cattle. Fully equipped and functional sterile box, CO<sub>2</sub> incubator BINDER C150, stereomicroscopes, and container for liquid nitrogen ARPEGE 100 are used for production and cryopreservation of hybridoma and for gamete membrane research in *"in vitro*" fertilization experiments.

## 2.8.4. Description of how the results and suggestions of the previous assessment were taken into account

Previous assessment formulated four recommendations:

#### 1. It is highly advisable to relocate the institute to campus of SAS in Bratislava

The relocation from the current premises in Ivanka to the campus of the SASci in Bratislava has been the priority of the Institute from 2008. At the time of the last evaluation we had already the grounds in the campus, construction project and the building license from local authorities but our plans were obstructed by lack of funds. Moreover, in 2012 the Presidium of the SASci changed its priorities and decided to build a biomedical research park supported from EU structural funds on the grounds assigned originally for our institute. Despite a lot of time and energy invested into the relocation and building plans, our efforts seemed to become null and void. However, in 2013 Presidium of SASci offered us to reconstruct an existing building in the campus formerly used as the seat of the SASci campus administration and maintenance. In 2014 the architectural and construction plans were designed with our assistance. Due to obstacles in the procurement process the reconstruction started in early 2016 and should be finalized in July 2016. We have already started the procurement procedure for the furnishing the building and expect to move to the new premises by the end of the 2016. The relocation will bring several benefits: stimulation of the cooperation with other SASci institutes, simpler access to the cutting-edge infrastructure available in the campus and last but not least, more efficient recruitment of undergraduate and graduate students from two major universities residing in Bratislava. All this should have a major positive impact on the quality and efficiency of the research performed at the institute. Successful accomplishment of our relocation plans together with establishing of the Bioscience Centre (see the paragraph 3) will thus be an important milestone in the Institute's history.

#### 2. It is necessary to increase the number of DrSc.

Two scientists fulfilling the criteria for DrSc. (Dr. Košťál and Dr. Hapala) are at advanced stage of writing the theses. However, they did not manage to submit them yet due to various reasons. Nevertheless, they plan to do so in the in the near future.

## 3. It is advisable to increase to the quantity of publication and at the same time remain on the high quality level

We continued the positive trend of increasing the quality of journals we publish our results. This was reflected in a significant increase in the average impact factor of our publications (from IF 2.11 in the assessment period 2007-2011 to IF 2.931 in this period 2012-2015). The negative side of our efforts was higher demand of the reviewers and longer time between submitting and publishing of the manuscripts. Together with other reasons, e.g. high administrative and organizational burden linked to our relocation plans, this resulted in problems in increasing the number of publications. Although the high quality of our papers will remain our major objective, we are aware of the urgent need to publish more papers per scientific worker. We believe that the new institute premises and formation of the Bioscience Centre will increase our productivity in this respect.

## 4. In the future research topics and visions should be discussed and developed by permanent discussions within the institute

Although the communication between the institute administration, scientific board and the body of scientific workers has never been problematic in the past, we feel that the participation of scientists (particularly the young ones) in shaping the research program of the institute and visions for the future has been improved in the assessed period. The scientific board elected in 2014 has 4 new members (out of 5) which should bring new ideas to the research management. The young scientists were more involved in administration of the research at the Institute as 5 VEGA projects and 1 APVV project had the principal investigators younger than 40 years. In addition, all important issues related to our relocation plans and to the planned formation of the Bioscience Centre were discussed in the academic body of the institute before the decisions were made.

## • Supplementary information and/or comments on management, research infrastructure, and trends in personnel development

#### Management and organization of the Institute

Similary to the previous assessment period, also in the period 2012-2015 the Institute experienced major changes in the management and organization. With respect to the management, the new director and the new Scientific Board have been elected in 2014. We hope that both these changes will bring new impulses to the Institute performance while preserving the continuity in positive aspects of our activities. Significant changes took place also in the organization of the Institute and in its research program. As mentioned already in previous sections, two laboratories terminated their activities (Laboratory of Gravitational Physiology and Laboratory of Bioenegetics). Both laboratories were historically among the most successful research groups in our Institute with internationally unique results, personal changes and retirement of their leaders pressed us to make this uneasy decision.

#### **Research infrastructure**

Compared to previous assessment period there were no major changes in the research infrastructure. In 2012, the project of EU Structural funds operational programme Research and Development "Centre of Excellence for translational research in molecular medicine" (TRANSMED 2) has been terminated without further acquisition of instruments in addition to HPLC evaporative light scatter detector and ultracentifuge purchased in 2011. In 2015 we applied with the project proposal "Biomembranes: establishing the infrastructure for a modern research of biological membranes" within a call of the Structural funds as the coordinating institution and the Faculty of Science Comenius University, and the Faculty of Chemical and Food Technology, Slovak Technical University, as partners. This project application was not successful but we plan to resubmit similar application in future calls, if the call in which our institution would be eligible applicant and the suitable topic will arrive. Some improvements in infrastructure have been realized within our limited financial resources. The analytical unit has been consolidated by the purchase of a new gas chromatography instrument. Thanks to the existing collaboration with prof. S. Cockroft (University College London) we obtained an older but functional fluorescence microscope. Our

needs for more sophisticated instrumentation are covered also within the joint laboratories (particularly with the Faculty of Mathematics, Physics and Informatics and the Faculty of Science, Comenius University Bratislava, and the Institute of Biotechnology and Food Science, Slovak Technical University) and within international colaborations and study stays.

#### Personal policy

The long-term personal policy of the Institute is to employ the best PhD graduates as well as to attract young talented scientists from external sources/institutions. Within the SASPRO scheme, one of the aims of which is to support the return of Slovak scientists from abroad back to Slovakia, we are proud to host the SASPRO scholar Silvia Bágeľová, PhD. We believe that her presence at the institute will be mutually stimulating.

The focus on young scientists was reflected also in the very positive trend in decrease of the average age of research staff with university degree from 48 in 2007 to 45 in 2011 and to 42 in 2015 (PhD students not included). The policy of the management to strengthen the involvement of young researchers in shaping the research programme of the Institute brought positive results in this assessment period. Young investigators under 40 were principal investigators in 5 VEGA projects and 1 APVV project. Moreover, 5 young researchers started to supervise their own graduate students. Our focus on young investigators is also reflected by the success of our reserachers in the Young Scientists (under 35 years) Awards awarded by the Presidium of the SASci (in 2015 Eva Bosíková 2nd prize, Alexandra Poturnayová 3rd prize). We hope that the relocation of the Institute to new premises in the SASci campus will bring new impulses in this respect and make the Institute more attractive for students and young researchers not just from Slovakia, but also from abroad.

#### 3. Research strategy and future development of the institute for the next five years (2016-2020) (Recommended 3 pages, max. 5 pages)

With the aim to improve the scientific level and productivity of the institute and in greement with the general trend to integrate the fragmented research in Slovakia, an initiative to establish a new legal entity joining several biological institutes of the SAS with compatible research programs has been launched recently. As the first step, two institutes (Institute of Animal Biochemistry and Genetics and Institute of Molecular Physiology and Genetics) signed The Memorandum of Understanding on the formation of the BioScience Centre of the SAS. The main motivation for this initiative was to improve the efficiency of the research by supporting the horizontal integration of the research in individual teams and sharing the infrastructure and methodologies established in partner institutes. Strengthening the interdisciplinary character of the research should also improve the quality of the research outputs and increase the competitiveness in the European Research Area and in applications for the international research projects. The time schedule of the integration process assumes the creation of the BioScience Centre by January 1, 2017. In the future, the Centre will be open also for other institutes currently having the status of the observer.

#### 3.1. Present state of the art in both the national and the international contexts

#### **Department of Membrane Biochemistry**

Research in the Department of Membrane Biochemistry will be concentrated around the following themes:

#### Lipid droplets: important players in lipid homeostasis

Lipid droplets (LD) are multifunctional organelles involved in lipid homeostasis, protection from toxicity of various hydrofobic substances and proteins, response to cellular stress, etc. This important role in cell metabolism is reflected also in frequent interactions of LD with other cellular organelles. Defects in LD biogenesis and functions are involved in several pathological conditions (e.g. obesity, diabetes, atherosclerosis, or metabolic syndrome). Since LD in the yeast *Saccharomyces cerevisiae* are very similar in structure and composition to LD of higher organisms including humans, this organism is often used as an experimental model for studies on LD biogenesis and functions.

#### Lipid transport: the way to ensure optimal membrane lipid composition

Lipid transport into a eukaryotic cell and inside the cell between subcellular organelles is an important mechanism to supply lipids for optimal membrane composition and function. Uptake of sterols and seclusion of detrimental sterols into lipid droplets in the form of steryl esters is hypothesized to be an important mechanism of protecting membrane integrity. Phospholipids are synthesized in some subcellular organelles, mostly in the endoplasmic reticulum, the mitochondrion, and the Golgi apparatus but are required in all cellular membranes. Lipid transfer proteins play an essential part in intracellular lipid transport. Defects in lipid transfer proteins lead often to serious human diseases, mostly metabolic and deurodegenerative. Proper lipid turnover is also connected to lipid transport.

#### Cell division: understanding the mechanisms of meiosis

It is absolutely essential that segregation of chromosomes during meiosis, a special cell division, is achieved accurately, because errors in this process may lead to meiotic aneuploidy, which is the most common cause of miscarriages and genetic disorders in humans. The yeast *Schizosaccharomyces pombe* is an excellent model organism for studying meiosis as it is amenable to both genetic and cell biology techniques, and highly synchronous meiosis, which facilitates proteomic approaches and biochemistry, can be induced. Moreover, several groups of genes are conserved in *S. pombe* and humans including splicing genes, genes involved in telomere and heterochromatin functions, and components of the RNAi pathway. Therefore, the understanding of the processes underlying meiosis in the fission yeast will pave the way for studies of meiosis in higher eukaryotes, including humans.

#### Biosensors: sensitive diagnostic tools for cancer biomarkers

Application of DNA aptamers is a highly promising method in development of novel biosensors for detection of cancer biomarkers. Aptamers are oligonucleotides that are selected from among a pool of thousands of different sequences, often using a combinatorial chemistry-based technology termed SELEX, for very high binding affinities for their targets. The use of novel biosensor technologies could be instrumental in early cancer detection and could result in more effective treatments and, particularly, in monitoring of those treatments resulting in improvements in patient quality of life and overall chance of survival.

#### **Department of Physiology and Ethology**

#### Poultry Behaviour and Welfare

Poultry behaviour and poultry welfare science are solving both fundamental and applied problems. The fact that animals are sentient beings is recognized already by the EU law (the Treaty of Lisbon). Although the connection between emotions and cognition is broadly accepted in humans, the potential of this interaction as a source of information about the animal emotions has been discovered only recently (cognitive bias approach). Poultry is less frequent subject of studies in this highly dynamic field, nevertheless, this type of studies can contribute to find the ways of the assessment of welfare 'from animal's point of view'. Practical problems of poultry welfare are raised by poultry industry. Consumer demand for high standard of animal welfare is changing European livestock practice. As a consequence of the European ban on battery cages in 2012, hens are now kept in large flocks in non-cage systems or flocks of 9 to 60 birds in enriched cages. Although the new group-housing systems provide the animals with more possibilities of movement and expression of species-specific behavior, at the same time they represent an increased risk of damaging behaviour (feather pecking) compared with traditional systems. Another serious welfare problem facing the industry exacerbated by recent transitions is the extremely high frequency and severity of keel bone damage (keel or carina is an extension of the sternum), particulary in noncage systems.

#### Neurobiology of Birdsong

Songbirds are model for the study of both learned vocal communication and neurogenesis. Learned vocal communication encompasses speech in humans but also less complex vocal learning in large sea mammals such as dolphins and whales, seals, elephants, bats, and three groups of birds, songbirds, parrots and hummingbirds. Among these, songbirds are the most frequently used animals for the study of neurobiological basis of learned vocal communication. Birdsong and speech share remarkable behavioural, neural, genetic as well as molecular parallels. It is controlled by two pathways. The cortical-basal ganglia-thalamic-cortical pathway forms a loop and enables vocal learning. The posterior motor pathway enables production of the learned vocalizations. If the basal ganglia part of the first pathway is out of balance it is associated with speech disorders such as stuttering or speech defficiences at neurodegenerative Parkinson's or Huntington's diseases. To repair such deficiencies, neurogenesis in adult animals.is intensively studied. The new neurons are born either in hippocampus and migrate locally within hippocampus, or they are born in subventricular zone and migrate into olfactory bulb in rodents. However, there are only very few newborn neurons migrating to olfactory bulb in humans. Instead, the proliferative cells migrate to striatum, a part of basal ganglia. In songbirds, new neurons from subventricular zone migrate widely to many regions of the forebrain including striatum. Therefore songbirds are used also to study striatal neurogenesis following brain injury.

#### The Quail Chorioallantoic Membrane as an In Vivo Angiogenesis Model

There is strong pressure from the public to pursuit alternatives in the use of experimental animals for testing in the pharmaceutical industry, toxicity studies, drug discovery and development. One of the alternatives is chorioallantoic membrane (CAM) of avian embryo and the so called CAM assay. The European Center for the Validation of Alternative Methods has accepted the use of the CAM model as an alternative to animal testing. Chicken CAM test and to lesser extent quail CAM can be used to study antivascular therapy and tumor angiogenesis, development of new biophotonic techniques as well as novel drug testing. Especially drug induced tissue fluorescence on CAM

model can be used to develop ways to detect and visualize the tumour configuration and the border with healthy tissue.

#### **Department of Immunogenetics**

#### CD molecules in the reproductive process of cattle

Fertilization process is a very sophisticated and unique process comprising some essential steps resulting in fusion of gametes and formation of zygote. Despite the identification of numerous molecules involved in gamete interaction, the molecular mechanism of sperm-oocyte fusion is not yet elucidated. Our research interests include the study of cell membrane molecules involved in the reproduction process of farm animals. Particularly, our focus on characterization of CD molecules in bovine reproductive system is unique in Slovakia and Czech Republic.

## 3.2. Research strategy of the institute in the national and the international contexts, objectives and methods

#### **Department of Membrane Biochemistry**

Orientation on various aspects of membrane lipid homeostasis, development of novel biosensors and lately on meiotic cell division was in the focus of our research in the last few years. We do not expect any dramatic changes in research topics in coming years, however, we would like to tighten cooperation between individual research groups and broaden national and international collaboration, especially with the research groups from the newly formed BioScience Centre. The research will be oriented on:

#### Lipid droplets: important players in lipid homeostasis

An important cooperation between individual research groups is emerging around lipid droplets (LD) and their role in various aspects of eukaryotic cell physiology and interactions of LD and other cellular organelles. Specifically, we will study:

Cellular control of LD biogenesis, including the factors involved in storage capacity of LD for triacylglycerols, steryl esters, and squalene. We will investigate if accumulation of other lipids found in LD together with expression of specific LD proteins can trigger formation of LD in the absence of TAG and SE synthesis. In addition to obtaining basic knowledge of LD biogenesis we believe that our results will help to develop yeast strains suitable for biotechnological production of squalene.

Role of esterification of foreign sterols and ergosterol biosynthesis intermediates in protection of membrane integrity. Using mutants and conditions enabling the uptake of specific sterol molecules from the medium we will study which structural features predispose sterol molecules for esterification or integration into the membranes and test the hypothesis that seclusion of esterified sterols in LD is one of the mechanisms protecting eukaryotic cells from the detrimental effects of foreign sterols and sterol biosynthesis intermediates on cell membranes.

Interactions between mitochondria and LD. Our results indicate that LD resident protein Pgc1 influenced mitochondrial lipid composition, mitochondrial morphology and function. An important aspect of the study of mitochondrial lipid homeostasis is its connection to understanding of human mitochondria associated disorders. Particularly, we plan to co-operate with the Tunghai University (Taiwan) on the molecular mechanisms leading to Barth syndrome.

*Role of LD in yeast meiosis.* During the process of meiosis in yeast novel membranes have to be formed in time of nutrient starvation. LD are organelles that store TAG and SE from which basic membrane building blocks can be derived. We plan to study the role of LD in meiosis, particularly we ask questions whether the presence of LD is required for properly executed meiosis and what is the role of key LD resident proteins in this process, especially the role of PITP Pdr16p.

#### Lipid transport: the way to ensure optimal membrane lipid composition

Role of PITP in homeostasis of phospholipids and sterols. We would like to concentrate on two yeast PITPs, Pdr16p and Pdr17p, with proposed functions in regulation of ergosterol and phosphatidylethanolamine biosynthesis, respectively. Novel aspect of our studies lies in planned

structure – function studies that will be performed in collaboration with the Institute of Molecular Biology of SAS and University College, London.

*Mechanisms of anionic lipids transport to and from mitochondria.* Our preliminary results indicate that mitochondrially synthesized phospholipid, phosphatidylglycerol (PG) is transported from mitochondria to the ER and then turned over as a regulatory aspect of cardiolipin biosynthetic pathway. We will study how is this transport accomplished and how is it regulated.

Sterol import from the medium into cells. We will study plasma membrane sterol transporters, regulation of sterol transport, mechanisms of sterol esterification, and the role of yeast cell wall in sterol uptake.

#### Cell division: understanding the mechanisms of meiosis

We will continue to study meiosis using the yeast *S. pombe* as a model organism using the original collection of yeast deletion mutants defective in proper chromosome segregation. Broad national and international collaboration (Dept. of Genetics, Comenius University; National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic; Vienna Biocenter, Austria) will enable effective studies of molecular mechanisms ensuring proper segregation of chromosomes during meiosis. Taking advantage of lipid biosynthesis expertise of the Department we plan to include studies of the role lipids, especially sphingolipids and phospholipids, play in proper execution of meiosis. The expertise of the Laboratory of Biosensors in atomic force microscopy and single molecular force spectroscopy will guide us in using state-of-the-art methods in study of meiosis.

#### Biosensors: sensitive diagnostic tools for cancer biomarkers

The main objective for the laboratory of biosensors in the coming years will be application of DNA aptamers in diagnostics and therapy of cancer diseases. We will focus on development of new sensitive diagnostics methods for detection of cancer markers on the surface of leukemic T-cells and breast cancer cells by means of DNA aptamers, atomic force microscopy (AFM) and acoustic method based on measuring of oscillation frequency of the piezocrystal (TSM). We expect that using of single molecular force spectroscopy will enable to determine cancer markers density depending on the disease stage. The above-mentioned plans will be realized in collaboration with the Faculty of Mathematics, Physics and Informatics, Comenius University within the framework of a joint laboratory.

#### Department of Physiology and Ethology

#### Poultry Behaviour and Welfare

In field of fundamental research we will continue to work on improving methods of the assessment of emotions in poultry. We will further develop the study of the link between cognition and emotions. We would like also to study the possibilities of the use of cognitive enrichment as a way to induce anticipation of positive events and hence to affect the emotional status of poultry. Neurobiological mechanisms underlying decision making under ambiguity with the emphasis on dopaminergic neurotransmission will be the part of these studies

With respect to practical problems of poultry industry our membership in the consortia of the two COST actions (CA15134 GroupHoseNet and CA15224 KeelBoneDamage, both starting 2016 and ending in 2020) guarantee our collaboration with the top European laboratories in this field and possible contribution to solving these problems at the European level. In field of damaging behaviour we will follow up on our previous work on neurobiology and psychopharmacology of the feather pecking in laying hens. Based on our collaborative work with the Department of Physiology and Ethology, Faculty of Science, Comenius University, with the Japanese quail lines divergently selected for the egg yolk testosterone levels, we would also like to contribute to the elucidation of the possible role of epigenetics in the development of damaging behaviour in poultry.

#### Neurobiology of Birdsong

In field of neurobiology of birdsong, our main collaborators are prof. Erich Jarvis from the Duke University, NC, USA, prof. Annemie van der Linden from the University of Amsterdam, Belgium, prof. Katharina Riebel from the Institute of Biology in Leiden, Netherlands, and Dr. Svatava

Kašparová from the Slovak University of Technology in Bratislava, Slovakia. These collaborations resulted in several joint projects, fellowships, and a joint PhD. study of one doctoral student.

The main goal is to apply state-of-the-art methods of MRI for longitudinal studies of recovery of brain regions as well as circuitry. We will employ brain lesions, immunohistochemistry, *in situ* hybridization, pharmacological manipulation of neurogenesis, and MRI techniques such as diffusion tensor imaging for tractography together with behavioral observations. These methods will be used to determine the role of newborn neurons in brain plasticity after injury. We will also further develop behavioural studies to find biological relevance of changed vocalization after damaging some of the brain vocal nuclei.

#### The Quail Chorioallantoic Membrane as an In Vivo Angiogenesis Model

In the following time period we would like to continue developing and validating quail CAM model mainly for photodynamic diagnosis and treatment of tumours of various origin. We will be continuing in developing and testing a drug delivery system that will optimize the therapeutic action of drugs, increase specificity, while reducing their toxic side effects.

#### Department of Immunogenetics

#### Tetraspanins in the reproductive system of cattle

The analysis of the expression and localization of tetraspanins CD9 and CD81 and their potential partners (CD52, CD46, or others) on tissues and cells of reproductive system of cattle could help to understand the complexity of fertilization process.

The main goals for the next years will be as follows:

1. Detection of expression and colocalization of CD molecules during oocyte maturation and also in the process of epididymal maturation, capacitation and acrosome reaction of bull sperm and sperm from other species (mouse, pig) in collaboration with Biotechnology Institute ASCR; BIOCEV, Faculty of Science, Charles University and Department of Veterinary Sciences, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences in Prague, CR.

2. Analysis of the possible cooperation of CD molecules during the development and maturation of gametes and upon sperm-egg interaction in the *in vitro* fertilization experiments in cattle.

The analysis of the predicted tetraspanin web on spermatozoa and its dynamics during the processes of reproduction could be an important contribution to this field of research. We will also try to extend the knowledge about the function of tetraspanin web on oocytes obtained in other species, since the presence of the web and its function is species-specific. The obtained results could help to clarify the particular steps and mechanisms of reproduction process at the molecular level. Finally, the results could help to explain some causes of fertility disorders of livestock.

3. Assessment of influence of cryopreservation of ovarian tissue on the viability of bovine oocytes. The analysis of the possible changes in cytoskeletal structures of oocytes in association with the expression of CD molecules after freezing and thawing. This research will be aimed to optimize the methodology of long-term cryopreservation of female gametes of cattle as an effective tool for the operating of the National Gene Bank within the cooperating Research Institute for Animal Production Nitra, National Agricultural and Food Centre.

Project proposals submited to 7RP or H2020	2012	2013	2014	2015
Institute as coordinator				
Institute as participant				3

#### 4. Other information relevant for the assessment