MATEMAATIKA-LOODUSTEADUSKOND
GEENITEHNOLOOGIA INSTITUUT
TEADUS- JA ARENDUSTEGEVUSE AASTAARUANNE 2014

1. Instituudi struktuur

Geenitehnoloogia instituut, Department of Gene Technology
Instituudi direktor Andres Veske

- Geenitehnoloogia õppetool, Chair of Gene Technology, Cecilia Sarmiento
- Molekulaarbioloogia õppetool, Chair of Molecular Biology, Tõnis Timmusk
- Molekulaardiagnostika õppetool, Chair of Molecular Diagnostics, Lilian Järvekülg
- Genoomika ja proteoomika õppetool, Chair of Genomics and Proteomics, Peep Palumaa

2. Instituudi teadus- ja arendustegevuse (edaspidi T&A) iseloomustus

(NB! punktid 2.1- 2.6 täidab struktuuriüksus)

2.1 struktuuriüksuse koosseisu kuuluvate uurimisgruppide

2.1.1 teadustöö kirjeldus *(inglise keeles*);

Kuna õppetoolides tehtav teadustegevus kujutab endast ette erineva temaatikaga tegelevate teadusgruppide töid on nad toodud eraldi jäädes samas ühe õppetooli teadustegevuse kirjelduse alla.

Chair of Gene Technology

Plant genetics group (K. Järve) was dealing with fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat (*Triticum militinae*) introgression line. The group is the partner for sequencing wheat chromosome 4A in the frames of the Wheat Genome Initiative. In order to find functional marker(s) for the light blight resistance in the Estonian potato cultivar 'Ando', an analysis of expressed sequences comprising the conserved NB-ARC domain of R-genes has started.

Plant virology group (M. Sõmera) sequenced sobemoviruses SNMoV and CnMoV. Analysis of grass and cereal field samples was started using next-generation sequencing technology.

RNA silencing group (C. Sarmiento) continued with the research on human and *Arabidopsis* RNase L inhibitor (ABCE1 and AtRLI2). The involvement of ABCE1 in cellular growth, cell cycle and translation was further investigated. C*o-immunoprecipitation* and mass spectrometry combined with SILAC method was used for the identification of interactors of RNA silencing suppressors.

The group of *Arabidopsis* motor proteins (H. Paves) has completed the studies on the role of myosins in gravitropic behaviour of *Arabidopsis* stem. The work on systematic characterization of the expression of individual *Arabidopsis* myosins in different organs and developmental stages has also reached to the stage of publication.

The maize genetics group (L. Timofejeva) continued the research on meiotic mutants, mapping and cloning meiN2415 in maize. In addition, the complementation for the maize ems63089 in *Arabidopsis* was carried out. A point mutation in *Arabidopsis* EMS1 gene was generated in the region encoding kinase domain, simulating the mutation identified in ZmEMS1 gene in the maize ems63089 mutant.

Chair of Molecular Biology

Tõnis Timmusk group**.** We are studying the molecular mechanisms of the regulation of gene expression and signaling in mammalian nervous system. Specifically we study: (I) Molecular mechanisms controlling the tissue-specific and neural activity-regulated expression of the neurotrophic factor BDNF gene; (II) Signaling of neurotrophin receptors TrkA and TrkB; (III) Transcriptional dysregulation in Huntington's disease; (IV) basic helix-loop-helix transcription factor TCF4, its functions in mammals (rodents, human) and invertebrates (Drosophila), and its dysregulation in Pitt-Hopkins syndrome and schizophrenia; (V) Synaptic functions of dendritically localized Neuralized1 as an ubiquitination ligase and transcriptional regulator.

Kaia Palm group. We are studying molecular mechanisms of stem cell differentiation and cancer, including transcriptional mechanisms, alternative splicing and molecular markers.

Mart Speek group. Transcriptional interference (TI), either activation or repression, is defined as influence of one transcriptional process or an RNA polymerase II (RNAPII) complex on a second transcriptional process. Most of the TI occurs between two genes and depends on their transcriptional orientation and/or genomic arrangement. We have demonstrated that TI induced by intronic retroelements L1s and nested genes could be characterized by intron retention, forced exonization and cryptic polyadenylation. These molecular effects were originally revealed from the analysis of endogenous prematurely terminated transcripts. Recent data also suggest that retroelement insertions into exons and introns of genes induce different types of genetic disease, including cancer. Retroelements interfere with the expression of genes by inducing alternative splicing via exon skipping and exonization using cryptic splice sites, and by providing polyadenylation signals. We categorized these mutagenic effects according to eleven different mechanisms and showed that most of them may be explained either by traditional exon definition or TI. Studies of the molecular mechanisms of TI provide important insight into the complexity of gene regulation at chromatin level and help to understand the basic mechanism of genes' expression in normal as well as in diseased conditions.

Urmas Arumäe group is studying the mechanisms of cell death and survival, in particular the neuronal apoptotic machinery and its control by survival-promoting neurotrophic factors. Two survival-promoting proteins, Mesencephalic Astrocyte-derived Neurotrophic Factor (MANF) and Cerebral Dopamine Neurotrophic Factor (CDNF), are of special interest as they can promote neuronal survival via two modes of action: intracellularly as the resident endoplasmic reticulum proteins, and extracellularly, at least when applied to the brain as extracellular proteins. In the latter approach, both MANF and CDNF efficiently protect and restore the neurons damaged in the animal models of Parkinson's disease and cerebral ischemia, making MANF and CDNF as the most potent therapeutic candidates for these diseases. However, both intra- and extracellular mechanisms of neuroprotection of MANF and CDNF are poorly characterized. In collaboration with the University of Helsinki we are studying the anti-

apoptotic mechanisms of MANF and CDNF. We have identified two structural motifs on MANF that are required for its survival-protecting potency. We have found that one of these motifs – the CXXC motif can protect the cells also when applied as a tetrapeptide, and are studying the anti-apoptotic properties of this peptide on different cells. We have started a new research line, studying the mechanisms of neuronal maturation. We have performed a microarray study of the young and adult sympathetic neurons and found several interesting genes and signaling pathways that are significantly changed during maturation of the sympathetic ganglia. The expression and function of these genes during maturation of the peripheral and motoneurons will be studied in the future. We also started, in collaboration with the Faculty of Pharmacy, University of Helsinki, the studies of the anti-apoptotic mechanism of action of the inhibitors of Protein Kinase C, discovered by the collaborators.

Teet Velling group. Our studies focus on the role of filamin A (FLNa) in the function of integrin-type collagen receptors, EGF receptor (EGFR), and in the regulation of PKB/Akt and ERK1/2 kinases by these receptors. FLNa is a ubiquitously expressed cytoskeletal protein that links transmembrane receptors, e.g. integrins, to filamentous actin, functions as an intermediate in signal transduction and has a role in receptor recycling.

Andres Veske group. We study semaphorins and plexins, which are implicated in a host of cellular responses including regulation of cell migration, immune response, tumor progression and tissue organisation during development. Despite to the fact that semaphorins and their receptors are essential players in the nervous system development and maintenance during adulthood almost nothing is known how expression of above mentioned molecules is regulated in different levels. Semaphorins and their receptors plexins are implicated in various processes in the nervous system, but how B-plexins regulate the growth of dendrites remains poorly characterized. The microtubule cytoskeleton is a key determinant in generating and maintaining neuronal morphology and function. The remodelling and reorganization of microtubules in the growth cone is required for persistent growth cone advance as well as for the recognition of guidance cues. EB proteins are central adaptor proteins at growing MT tips that have been shown to form complexes with a variety of other proteins, but among these only a few transmembrane receptors have been described so far. We want systematically reveal the role of B-type plexins in the regulation of MT tip dynamics and dendrite growth.

Chair of Molecular diagnostics

Merike Kelve group. 2’,5’-oligoadenylate synthetases from marine sponges and other phylogenetically distant animals were characterized for their activities and genomic structures of their genes in order to elucidate the origin and evolution of OASs. In parallel the research of several other sponge enzymes was carried on. The purification of the novel 2’,5’-specific endoribonuclease from sponges, discovered by us in 2012, was continued to establish its amino acid sequence and in order to identify its genomic structure and to express it as a recombinant protein. Previously we had established the activity of another novel enzyme, ATP N-glycosidase, in the marine sponge *Axinella polypoides* as well as in the freshwater sponge *Ephydatia muelleri* but the protein structure is still unknown. The protein purification has proven to be unsuccessful due to the loss of the enzymatic activity during the purification process. Therefore a bioinformatic search for this enzyme in the *E. muelleri* SRA database was initiated. By now several candidate genes have been selected for the further studies and the

characterisation of the enzymatic activities of the respective recombinant proteins has been started. Finally, our general interest for the whole purine metabolism in sponges has led to the conclusion about the absence of the purine *de novo* biosynthesis pathways in demosponges. The studies about the role of symbiotic bacteria in sponge metabolism are going on.

Sirje Rüütel-Boudinot group. We have been working on RGS16 during the past 5 years, and showed that this gene was involved in inflammation and antiviral immunity by different approaches. We have developed relevant tools and protocols, and we now have a precise view of the expression of RGS16 by different types of immune cells, including myeloid cells. Group aimed at understanding the interactions between host cells and PCV2, focusing on RGS16.

Lilian Järvekülg group. L. Group focused mainly on plant potyviruses. Investigation of the relations between the structure and function of the viruses was aimed at:

a) Biological and molecular characterization as well as genetic diversity analyses of PVY strains in Estonia.

b) A detailed study of structural characteristics of potyvirus (PVA) virions and virus coat protein (CP).

c) Developing an epitope presentation system based on PVA CP VLPs as carriers for melanoma associated antigen peptide(s).

d) Apart from the foregoing, a research conducted under the project "Resist" must be mentioned as important. The research deals with the detection, characterization and spread analysis of the most relevant potato viruses (PVX, PVY, PVA, PVM, PVS, and PLRV) in the seed-potatoes grown in different regions of Estonia in 2005–2015. The research is a joint work with the Agricultural Research Centre (ARC) and the Estonian University of Life Science. At present, data collected are being systematized, analysed and an article due in summer 2015 is being written.

Chair of Genomics and Proteomics

Peep Palumaa group. During year 2014 we continued our ongoing research projects by focusing to following topics: 1. Role of oxidative and nitrosoactive stress in functioning of zinc finger proteins. 2. Structure and functioning of iron-sulphur cluster assembly proteins and their role in mitochondrial functioning 3. Role of zinc and copper ions in aggregation of amyloidogenic peptides. 4. Investigation of cellular toxicity of different oligomeric and metalloforms of Alzheimer's amyloid peptide.

2.1.2. aruandeaastal saadud tähtsamad teadustulemused (*inglise keeles*);

Chair of Gene Technoogy

Based on the recently published potato genome sequence (DM1-3 516R44), a set of primers was designed to amplify the 3' ends of the expressed R-gene sequences in 'Ando' and in susceptible cvs 'Agra' and 'Frila'. The sequences generated by next-generation sequencing technology are being aligned in order to identify specific candidate sequence(s) which correspond to Ando's resistance.

The complete genomes of sobemoviruses SNMoV and CnMoV were described for the first time. Utilization of next-generation sequencing technology for wheat virus field monitoring revealed BYDV-PAV, BYDV-MAV and BYDV-RPV in Estonia.

ABCE1 role as suppressor of RNA silencing is conserved in *Nicotiana benthamiana*, in HEK293 cells and in *Caenorhabditis elegans*. Potential ABCE1-interacting proteins that might support its function as an endogenous suppressor were identified. ABCE1 depletion in human cells has a strong effect on cell proliferation and cell cycle progression even at conditions when total protein synthesis is not significantly affected.

Abnormal gravitropic behaviour was described in *Arabidopsis* myosin triple, quadruple, and quintuple mutant lines.

A premature stop codon was revealed in TRIP13/PCH2 gene in meiN2415 meiotic mutant plants. The hypothesis that the point mutation in maize EMS1 gene region encoding kinase domain causes plant sterility in ems63089 mutant has been proven by complementation tests.

1.Olspert A., Kamsol K., Sarmiento C., Gerassimenko J., Truve E. Cocksfoot mottle virus coat protein is dispensable for the systemic infection. *Virol J*. 2014, Feb 4;11:19.

2.Mikelsaar R., Paves H., Org K., Mikelsaar AV. Chromosome variant 1qh- and its influence on the 3D organization of chromosome 1 heterochromatin in interphase nucleus of patients with endometriosis.*J Genet*. 2014 Apr;93(1):219-23.

3. Wang Y., Cheng Z., Lu P., Timofejeva L., Ma H. Molecular cell biology of male meiotic chromosomes and isolation of male meiocytes in Arabidopsis thaliana. *Methods Mol Biol*. 2014;1110:217-30.

4. Kärblane K., Gerassimenko J., Nigul L., Piirsoo A., Smialowska A., Vinkel K., Kylsten P., Ekwall K., Swoboda P., Truve E., Sarmiento C. ABCE1 is a Highly Conserved RNA Silencing Suppressor. *PLOS ONE* (Accepted)

5. Sõmera M., Truve E. Rottboellia yellow mottle virus is a distinct species within the genus Sobemovirus. *Archives of Virology* DOI 10.1007/s00705-015-2336-z

Chair of Molecular Biology

Tõnis Timmusk group. (1) We showed that the forkhead transcription factor FOXO3a levels are increased in HD cells as a result of overactive positive feedback loop (Kannike et al., J. Biol. Chem., 2014). (2) Using modified bacterial artificial chromosome, we generated several transgenic cell lines expressing humanised Renilla luciferase-EGFP fusion reporter gene under the control of rat BDNF gene regulatory sequences in HeLa background and showed that the generated cell lines respond to known modulators of BDNF expression and could be used for screening of compounds/small molecules or transcription factors altering BDNF expression (Jaanson et al., BMC Neuroscience, 2014). (3) In collaboration with Anne E. West from Duke University Medical Center we wrote a review on the transcriptional and translational control of neurotrophin expression (West et al., 2014).

Kaia Palm group. (1) We showed that LXXLL-motif of the human SRC-1 nuclear receptor box 1 peptide converts transportan 10 to a potent inducer of apoptosis in breast cancer cells (Tints et al., Int J Mol Sci. 2014). (2) We wrote a review paper on the role of TATA-box associated factor 4 (TAF4) and its isoforms generated by alternative splicing in controlling

lineage-specific differentiation of different stem cells (Kazantseva and Palm, Int J Mol Sci. 2014).

Mart Speek group. We further analysed transcriptional interference (TI) effects induced by retroelements L1, Alu and SVA, and nested non-coding RNA and protein-coding genes. These molecular effects included intron retention, forced exonization and cryptic polyadenylation. Using different experimental approaches we determined nucleosome occupation and positions of transcription bubbles of transcriptionally engaged/terminated RNAPII in L1- *NCAM1* TI experimental model system. The results of these experiments showed that (1) insertion of L1 into intron of NCAM1 could change the nucleosome occupation and depending on position could influence the level of TI and (2) RNAPII transcription terminates at discrete chromatin positions, in accordance with experimentally determined locations of trancription bubbles. These data suggest the importance of nucleosome occupation and RNAPII termination of transcription in relation to intronic retroelement position. We explained these novel features with the RNA polymerase kinetic model and suggested that intronic retroelements are not just "speed bumps" in regulation of RNA polymerase traffic.

Urmas Arumäe group. In collaboration with the Institute of Biotechnology, University of Helsinki, we have shown that Mesencephalic Astrocyte-derived Neurotrophic Factor (MANF) has two structural motifs that are required for its survival-promoting activity. We found that a deletion of C-terminal tetrapeptide RTDL of MANF (a KDEL-type endoplasmic reticulum retention signal) leads to accumulation of MANF to Golgi in the sympathetic neurons that is accompanied with loss of intracellular cytoprotective activity. The same mutant protein still protected the cortical neurons against cerebral ischemia when applied extracellularly to the rat brain. During these studies, it was noticed that the protein factors can be efficiently targeted to the area of stroke when injected to the striatum, not to the area of infarction itself, most probably via the flow caused by edema. This observation was published as a methodical paper. Mutation of another motif, a CXXC peptide, also inactivates MANF, both intra- and extracellularly. This motif, when applied to the cultured cells as a tetrapeptide, counteracts different cell death programs via neutralization of reactive oxygen species, when studied on Jurkat cells (manuscript in preparation).

Neuronal maturation is an essential but poorly studied process that involves the last steps of terminal differentiation, and the ending of programmed cell death period. We studied the differences in gene expression between young and mature sympathetic neurons, using the Affymetrix Exon arrays and identified several interesting genes whose expression was significantly changed during neuronal maturation and the ending of the programmed cell death. This is the first global analysis of gene expression changes during neuronal maturation. Several interesting genes whose expression was significantly changed were selected for the further studies of the maturation of peripheral neurons and spinal motoneurons.

Teet Velling group. Using the M2 human melanoma cell line, and the same cells expressing EGFP-FLNa (M2F cells), we found that only the M2F cells can effectively adhere to and spread on type I collagen whereas adhesion and spreading on fibronectin were not affected. Out of the integrin-type collagen receptors $\alpha1\beta1$ and $\alpha2\beta1$ expressed on these cells only $\alpha1\beta1$ was found to affect cell adhesion and migration on type I collagen possibly owing to an impact of FLNa on either activation of integrin $\alpha1\beta1$, or transport of $\alpha1\beta1$ back to the cell membrane. The EGF induced disassembly of focal contacts in cells on type I collagen, which was

counteracted by EGFR and PI3K inhibitors, and localisation of PKB/Akt and ERK1/2 to cell nucleus and lamellipodia, respectively in FLNa-dependent manner. Moreover, we found that EGFR stimulation triggered the co-localisation and -precipitation of FLNa with ERK1/2 in a manner dependent on ERK1/2 phosphorylation. Taken together our data demonstrate a role of FLNa in the regulation of the function of integrin $\alpha 1\beta 1$, PKB/Akt and ERK1/2 kinases, and identify ERK1/2 as a putative novel interaction partner of FLNa.

Andres Veske group. We had previously observed that Plexin-B1 and B3 interact with microtubule end-binding proteins (EBs) that are central adaptors at growing microtubule tips, and this interaction is involved in neurite growth. Therefore, we hypothesized that plexins regulate microtubule dynamics and through that also dendritogenesis. The role of all three B-plexins was systematically examined in these processes. B-plexins and their ligand Semaphorin-4D influence the dynamics of microtubule tips both EB-dependently and independendently. EB3 as well as Plexin-B1, B2 and B3 turned out to have a significant role in the development of dendritic arbour of rat hippocampal neurons. Our results clearly indicate that semaphorin-plexin-EB pathway is one molecular mechanism how extracellular guidance cues are translated into intracellular mechanics. Taken together, Semaphorin-4D and B-plexins modulate the dynamic behaviour of microtubule tips, and are therefore important in neurite growth. As a result we provide a novel insight into molecular mechanisms how semaphorin signals are transmitted to the cytoskeleton.

1. West A. E., Pruunsild P., Timmusk T. Neurotrophins: transcription and translation. *Handb. Exp. Pharmacol.*, 2014, 220, 67-100.
2. Jaanson K, Sepp M, Aid-Pavlidis T, Timmusk T. BAC-based cellular model for screening regulators of BDNF gene transcription. *BMC Neuroscience*, 2014, 15:75.
3. Kannike K., Sepp M., Zuccato C., Cattaneo E., Timmusk T. Forkhead transcription factor FOXO3a levels are increased in Huntington disease because of overactivated positive autofeedback loop. *J. Biol. Chem*., 2014, 289, 32845-32857
4. Mätlik K, Abo-Ramadan U, Harvey BK, Arumäe U, Airavaara M (2014) AAV-mediated targeting of gene expression to the peri-infarct region in rat cortical stroke model. *J Neurosci Meth* 236C: 107-113
5. Laht P, Otsus M, Remm J., Veske A. B-plexins control microtubule dynamics and dendrite morphology of hippocampal neurons. *Exp Cell Res*., 2014;326(1):174-84.
6. Tints K, Prink M, Neuman T, Palm K. LXXLL peptide converts transportan 10 to a potent inducer of apoptosis in breast cancer cells. *Int J Mol Sci*. 2014, 15, 5680-5698.
7. Kazantseva J, Palm K. Diversity in TAF proteomics: consequences for cellular differentiation and migration. *Int J Mol Sci*. 2014, 15,16680-16697.

Chair of Molecular Diagnostics

Merike Kelve group. Bioinformatically predicted OASs from several distantly related multicellular animals as well as a unicellular organism were produced as recombinant proteins. The activity assays proved that the OASs from the marine sponges *Geodia cydonium* and *Tedania ignis*, the mollusk *Mytilus californianus* and the little skate *Leucoraja erinacea* were capable of catalysing the synthesis of 2',5'-oligoadenylates from ATP. These results show that enzymatically active OASs are widely distributed among multicellular animals,

even in the Protostomia branch. The recombinant OAS protein from the unicellular organism, the choanoflagellate *Monosiga brevicollis* did not exhibit enzymatic activity.

The characterization of enzymatic properties of the recombinant OASs demonstrated that the sponge OASs differ from the mammalian enzymes. Their activation requirements, as well as product pattern were different from those of mammalian OASs. Surprisingly, the sponge OASs catalysed the formation of 3',5'-phosphodiester linkages in addition to 2',5'-linkages. The linkage tolerance of the sponge OASs may be due to the differences in their active site structures if compared to those of mammalian OASs. Some evidence was found that the cofactor, dsRNA may also have a role in determining the 2'- and 3'-specifities of sponge OASs. The variation in the enzymatic characteristics, observed between the OASs from two sponge species, *G. cydonium* and *T. ignis*, was not unexpected considering the diversity of their primary structures. The other OASs studied, those from the mollusk and the little skate, resembled animalian OASs in their activation properties and linkage specificities.

Sirje Rüütel-Boudinot group. We have invested a lot of efforts to establish a stable colony of RGS16 KO mice in TTU, which allowed us to characterize the importance of RGS16 in antiviral immunity. This situation constitutes a very good context to study the involvement of RGS16 in autoimmune diseases of the CNS. Importantly, we have now clearly showed that RGS16 is part of the type I IFN system. As IFN remains the main treatment of MS in human patients, this link is potentially important for the present project. Using human monocytic cell line (THP-1) as a model, we could show that RGS16 restricts the pro-inflammatory response of monocytes.

We showed that RGS16 binds the viral protein ORF3 in infected porcine cells and that RGS16 is strongly modulated by the IFN response, suggesting a role in antiviral immunity. To get insights into the function of RGS16, we put a lot of efforts in establishing a colony of RGS16KO mice in TTU and started to investigate the impact of RGS16 on immunity. We have now established that PCV2 titer is much higher in RGS16 KO compared to WT especially in the thymus, which has consequences on T cell development. In parallel I investigated the genomic environment of RGS16 and other RGS, and discovered that they cluster close to an MHC paralogous region, with genes potentially involved in antiviral immunity. Morever, using RGS16 region as a genetic marker we could identify a proto-MHC in the genome of the Placozoan Trichoplax and showed that this region is specialized in stress and ubiquitination/proteasome pathways.

Hence, RGS proteins have an important impact on inflammation, we now started to investigating the implication of RGS in autoimmunity. Very promising preliminary results were produced in collaboration with key teams in the field of multiple sclerosis. We have established the protocol of EAE induction in collaboration with Dr S Fillatreau (DRFZ, Berlin); we have clearly established that RGS16 has a significant impact on the course of EAE, in a gender dependent manner; these experiments took advantage of the RGS16 KO mice. In human patients we have undertaken the study of RGS16 expression in human PBMC, and RGS16 haplotype sequences from MS patients, in collaboration with Dr Toomsoo (East Tallinn Hospital) and Dr Gross–Paju (West Tallinn Central Hospital). We have already analysed modest numbers of patients (by RGS16 expression 48 and sequence 14 patients). While no SNP was identified yet, expression studies show a trend towards lower expression in untreated patients, which calls for confirmation and further investigations. Thus, based on these preliminary data and established collaborations, we propose to study whether RGS16 is a

relevant biomarker candidate for MS and/or an important factor to understand the disease and develop treatments.

Lilian Järvekülg group. The following results were obtained from the research into the structure and functions of potyviruses.

a) The study of PVY strains prevalence and distribution in potatoes in different locations of Estonia was, for the first time, conducted concurrently at biological, serological, and molecular levels. Three virus strains (PVY^N, PVY^0 and PVY^C), recombinant strains PVY^{NTN} and PVY^{N-W}, and some new recombinant forms were detected and characterized. As the experimental part has been remarkably labour intensive and time-consuming, experiments and data analysis are being continued this year. The first article on this theme is being written.

b) In our previous communication we have reported that virions of plant potyvirus potato virus A (PVA) have a peculiar structure characterized by high content of disordered regions in intravirus coat protein (CP). In the new article we describe other unusual properties of the PVA CP. With the help of a number of physico-chemical methods we observe that PVA CP just released from the virions by heating at 60–70°C undergoes association into oligomers and transition to beta (and even cross-beta) conformation. Transition to beta-structure on heating has been recently reported for a number of viral and non-viral proteins. The PVA CP isolated by LiCl method was also transformed into cross-β-structure on heating to 60°C. With the help of algorithms for protein aggregation prediction we found that the aggregation-prone segment should be located in the central region of PVA CP molecule.

It must be mentioned that a detailed investigation and comparison of two potyviruses, PVA and PVY, is an extremely interesting model system, important from both theoretical and practical aspects. Two very similar potato viruses of the same group, so alike in their basic characteristics and structure, still behave so differently: PVY giving a great number of different recombinant forms, incl. highly aggressive ones, spreading quickly all over the world, and leading to great economic losses; at the same time PVA no develops recombinants and regarding its characteristics is rather a mild potato virus. We expect that the results of the research will explain some essential structure/function/disease relationships within potyvirus group.

c) Previously it was shown by us that PVA CP VLP-mel immunoparticles have a potential to remarkably delay melanoma development. In this domain, we have done some supplementary experiments. Still, as this promising project will not be financed this year, the activity is suspended.

d) The research conducted under the project “Resist” deals with the analysis, characterization and spread of the most relevant potato viruses (PVX, PVY, PVA, PVM, PVS, and PLRV) in the seed-potatoes grown in different regions of Estonia in 2005–2015.

1. Suurväli J, Robert J, Boudinot P, Rüütel Boudinot S. R4 regulators of G protein signaling (RGS) identify an ancient MHC-linked synteny group. *Immunogenetics* 2014, 65, 145-156.

2. Suurväli J, Jouneau L., Thepot D, Grusea S, Pontarotti P, Du Pasquier L, Rüütel Boudinot S, Boudinot P.. The Proto-MHC of Placozoans, a Region Specialized in Cellular Stress and Ubiquitination/Proteasome Pathways. *Journal of Immunology*, 2014, 193, 2891 – 2901.

3. Suurväli, J.; Pahtma, M.; Saar, R.; Paalme, V.; Nutt, A.; Tiivel, T.; Saaremäe, M.; Fitting, C.; Cavaillon, J-M.; Rüütel Boudinot, S. RGS16 restricts the pro-inflammatory response of monocytes. *Scandinavian Journal of Immunology* 2014. doi: 10.1111/sji.12250

4. Päri M, Kuusksalu A, Lopp A, Kjaer KH, Justesen J, Kelve M**.** Enzymatically active 2',5'-oligoadenylate synthetases are widely distributed among Metazoa, including protostome lineage. *Biochemie* 2014, 97, 200-209.

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Chair of Genomics and Proteomics

Peep Palumaa group. Redox potentials for CCHH and CCCH type of zinc finger proteins have been determined by ESI MS based methodology for apo- and Zn(II)-bound proteins, which demonstrates that apo zinc finger proteins are most probably oxidized in cellular conditions and Zn(II) ions protect zinc finger proteins from oxidation.

Structure and functioning of iron-sulphur cluster assembly proteins GRX5, ISCA1 and ISCA2 has been determined by multitechnique approach using NMR, ERP and MS. We have structurally characterized the Fe/S cluster binding properties of human ISCA2 and investigated in vitro whether and how a [4Fe-4S] cluster is assembled when human ISCA1 and ISCA2 interact with the physiological $[2Fe\text{-}2S]^{2+}$ cluster-donor human GRX5. We found that (i) ISCA2 binds either [2Fe-2S] or [4Fe-4S] cluster in a dimeric state, and (ii) two molecules of $[2Fe\text{-}2S]^{2+}$ GRX5 donate their cluster to a heterodimeric ISCA1/ISCA2 complex. This complex acts as an "assembler" of [4Fe-4S] clusters; i.e., the two GRX5-donated $[2Fe\text{-}2S]^{2+}$ clusters generate a $[4Fe\text{-}4S]^{2+}$ cluster. The formation of the same $[4Fe\text{-}4S]^{2+}$ cluster-bound heterodimeric species is also observed by having first one $[2Fe\text{-}2S]^{2+}$ cluster transferred from GRX5 to each individual ISCA1 and ISCA2 proteins to form $[2Fe\text{-}2S]^{2+}$ ISCA2 and $[2Fe\text{-}2S]^{2+}$ ISCA1, and then mixing them together. These findings imply that such heterodimeric complex is the functional unit in mitochondria receiving [2Fe-2S] clusters from hGRX5 and assembling [4Fe-4S] clusters before their transfer to the final target apo proteins.

Thermodynamic binding constants for zinc ions to monomeric insulin have been determined. The dissociation constant value of the monomeric 1 : 1 Zn-insulin complex is equal to 0.40 μM. The apparent binding affinity decreases drastically at higher insulin concentrations where the peptide forms dimers. Cu(2+) ions also bind to monomeric insulin, whereas the apparent Cu(2+)-binding affinity depends on HEPES concentration. The conditional dissociation constant of the Cu(2+)-insulin complex is equal to 0.025 μM. The analysis demonstrates that insulin cannot form complexes with zinc ions in circulation due to the low concentration of free Zn(2+) in this environment.

The accumulation of the Aβ peptides into amyloid plaques is considered as the key step in the pathology of Alzheimer's disease (AD). Copper as well as zinc and iron ions are enriched within these extracellular fibrillar Aβ aggregates. The electrochemically active copper ions can catalyze a large variety of unspecific redox reactions that substantially contribute to the oxidative stress in the brains of AD patients. Copper ions can also directly contribute to the plaque formation since they can enhance the peptide aggregation and fibril formation in vitro. The estimates to K_D values for copper binding range from 10 pm to 100 nM. We determined that the Y10 fluorescence titration curves correspond to the affinity at the lower limit, however, as the peptide co-aggregates with two copper ions, higher affinities cannot be ruled out.

The toxicity of several Aβ molecular forms on the SHSY cell cultures was determined. Since Aβ peptides showed surprisingly low toxicity with dose-response curves suggesting heterogeneity we proceed the experiments with differentiated cells.

1. Brancaccio D, Gallo A, Mikolajczyk M, Zovo K, Palumaa P, Novellino E, Piccioli M, Ciofi-Baffoni, S, Banci L. Formation of [4Fe-4S] clusters in the mitochondrial iron-sulfur cluster assembly machinery. *J. Am. Chem. Soc.* 2014,136, 16240 - 16250.

2. Gavrilova J, Tõugu V, Palumaa P. Affinity of zinc and copper ions for insulin monomers. *Metallomics.* 2014, 6(7), 1296-300.

3. Tõugu V, Friedemann M, Tiiman A, Palumaa P. Copper(II) ions and the Alzheimer's amyloid-β peptide: Affinity and stoichiometry of binding. *In: AIP Conference Proceedings: International Conference of Computational Methods in Sciences and Engineering (ICCMSE) 2014, Athens, Greece, 4-7 April 2014. (Toim.) T. E. Simos, Z. Kalogiratou, T. Monovasilis* American Institute of Physics, 2014, (1618), 109 - 111.

2.2 Loetelu struktuuriüksuse töötajate rahvusvahelistest tunnustustest.

Tõnis Timmusk ja Mari Sepp. Research grant from Pitt Hopkins Research Foundation, USA.

Mart Speek. Baltic American Freedom Fund (BAFF) Research Scholar Award.

2.3 Loetelu struktuuriüksuse töötajatest, kes on välisakadeemiate või muude oluliste T&A- ga seotud välisorganisatsioonide liikmed.

Peep Palumaa, EMBO liige.

Sirje Rüütel Boudinot, Society for Developmental and Comparative Immunology liige.
Erkki Truve, International Committee on Taxonomy of Viruses, Plant Virus Sub-Committee and Chair of the Sobemovirus Study Group liige.

2.4 Soovi korral lisada aruandeaastal saadud T&A-ga seotud tunnustusi (va punktis 2.3 toodud tunnustused), ülevaate teaduskorralduslikust tegevusest, teadlasmobiilsusest ning anda hinnang oma teadustulemustele.

Laura Tamberg, Eesti Teaduste Akadeemia üliõpilaste teadustööde võistluse preemia uurimistöö ”Pitt Hopkinsi sündroomi modelleerimine Drosophila melanogaster’is” eest.
Julia Gavrilova, Eesti riiklikul üliõpilaste teadustööde riiklikul konkursil kolmas preemia terviseuuringute valdkonnas magistrite kategoorias.