MATEMAATIKA-LOODUSTEADUSKOND
GEENITEHNOLOOGIA INSTITUUT
TEADUS- JA ARENDUSTEGEVUSE AASTAARUANNE 2013

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 teadusgruppide töid on nad toodud eraldi jäädes samas ühe õppetooli teadustegevuse kirjelduse alla.

Chair of Gene Technology

Plant genetics working group was dealing with fine mapping, phenotypic characterization and validation of non-race-specific resistance to powdery mildew in a wheat–*Triticum militinae* introgression line. A search for functional marker(s) for the late blight resistance in the Estonian potato cultivar Ando which has demonstrated high isolate-specific resistance to most of the local isolates of the oomycete *Phytophthora infestans*, has been initiated.

Plant virology group (M. Sõmera, C. Sarmiento) sequenced sobemoviruses LTSV, TRoV and RoMoV. Surprisingly it was demonstrated that the coat protein of cocksfoot mottle sobemovirus is not essential for the systemic infection.

Gene silencing group (C. Sarmiento) was dealing with mutational analysis of human and Arabidopsis RNase L inhibitor (RLI). Several interactors of these proteins were identified by coimmunoprecipitation and mass spectrometry. The involvement of human RLI in cellular growth and translation was also studied.

Group of *Arabidopsis* motor proteins (H. Paves) continued to study the role of myosins in plant gravitropic behaviour. The systematic characterization of the expression of individual Arabidopsis myosins in different organs and developmental stages was also initiated.

The maize genetics group (L. Timofejeva) published papers on novel genes needed for the normal development of anthers and during the meiosis.

Chair of Genomics and Proteomics

We continued our ongoing research projects, which have proven to be very effective and started new projects in field of structural and medical metalloproteomics by focusing to following topics: 1. Role of oxidative and nitrosoactive stress in functioning of metalloproteins. 2. Structure and functioning of copper chaperones for cytochrome c oxidase and their role in mitochondrial functioning 3. Role of zinc and copper in aggregation of Alzheimer's amyloid peptide and in Alzheimer's disease 4. Application of modern mass spectroscopic techniques for studies of aggregation of amyloidogenic peptides. 5. Investigation of cellular toxicity of different oligomeric and metalloforms of Alzheimer's amyloid peptide 6. Search for new biomarkers of Alzheimer's disease 7. Search for inhibitors of Alzheimer's amyloid formation and amyloid disrupting compounds.

Chair of Molecular Biology

Tõnis Timmusk group. The complex structure of the adult brain is the product of genetic instructions, cellular interactions, and also interactions between the organism and the external environment. 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 (prof. Tõnis Timmusk group)

Urmas Arumäe group started at GTI in August 2012. Our main goal is to study the mechanism of action of novel neurotrophic factors Mesencephalic Astrocyte-derived Neurotrophic Factor (MANF) and Cerebral Dopamine Neurotrophic Factor (CDNF). These factors have thus far the most potent factors to protect the neurons and restore the lost neurological functions in the animal models of Parkinson's disease and cerebral ischemia. The mode of action of these factors is, however, poorly known. We have recently identified a small peptide from the sequence of MANF that promotes neuronal survival by itself, and may have cell-penetrating properties. Such small peptides could potentially have considerable therapeutic benefit in the treatment of neurological diseases, compared to larger parental proteins, as their application into and spreading in the brain tissue is easier. Our goal is to study the anti-apoptotic properties and mechanism of action of this peptide with a long-run goal to apply it in the animal models of neurological diseases. Our in vivo neuroprotective experiment with the peptide in the 6-hydroxydopamine model of Parkinson's disease is promising. In 2012 we started setting up the group and experimental paradigms in the GTI. In particular, we are establishing different cellular apoptotic models to test and analyse the activity and mode of action of MANF-derived neurotrophic peptide. We have also identified two motifs in the MANF protein sequence that are critically required for its intracellular neuroprotective ability (2013, Cell Death Dis, 4: e777).

We also pursue research on the apoptotic Bcl-2 family proteins in the neurons. In particular, we showed that mRNA for N-Bak, a neuron-specific splice variant of pro-apoptotic Bcl-2 family protein Bak, discovered in our group, is translationally repressed in the neurons and identified several motifs in the untranlated regions of the N-Bak mRNA that are responsible for this repression (manuscript in preparation). Part of this study was carried out in the GTI. Continuing on that line, we also started in GTI the analysis of the activity of Bax, another pro-apoptotic Bcl-2 family protein in the sympathetic neurons. Changes in the Bax activity are critical for the ending of programmed death period in these neurons. These are, however, almost not studied. We plan to address this question using Affymetrix Exon Array assay.

Mart Speek group have demonstrated that transcriptional interference (TI) induced by intronic L1s and nested genes could be characterized by intron retention, forced exonization and cryptic polyadenylation (Kaer K, Branovets J, Hallikma A, Nigumann P, Speek M. PLoS One. 2011;6(10):e26099). These molecular effects were revealed from the analysis of endogenous prematurely terminated transcripts derived from different cell lines and tissues and confirmed by the expression of three minigenes in cell culture. While intron retention and exonization were comparably observed in introns upstream to L1s, forced exonization was preferentially detected in nested genes. TI induced by L1 or nested genes was dependent on the presence or absence of cryptic splice sites, affected the inclusion or exclusion of the upstream exon and the use of cryptic polyadenylation signals. Our results suggest that TI induced by intronic L1s and nested genes could influence the transcription of the large number of genes in normal as well as in tumor tissues.

Teet Velling group. Our studies focus on the role of filamin A (FLNa), a ubiquitously expressed cytoskeletal protein that links transmembrane receptors, e.g. integrins, to filamentous actin and functions as an intermediate in signal transduction, in the function of integrin-type collagen receptors, EGF receptor (EGFR), and on the regulation of PKB/Akt and ERK1/2 kinases by these receptors. 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 localise to focal contacts in cells adhering to type I collagen and affect cell adhesion. The EGF induced disassembly of focal contacts in M2F 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. Moreover, we found that EGFR stimulation triggered the interaction 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 $\alpha1\beta1$, PKB/Akt and ERK1/2 kinases, and identify ERK1/2 as a novel interaction partner of FLNa (manuscript in preparation).

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Andres Veske group is studiyng 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. In addition the functions of proteins involved in

the transmission of axonal guidance cues have been expanded to include regulation of blood vessel growth and endothelial cell homing during vessel 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. We investigated in details promoters and transcription factors, which are directing plexin/semaphorin gene family spatial and temporary expression in different tissues. Using modified yeast-two hybrid technique we have found several molecules (small GTPase Rin and microtubule associated proteins MT1, MT2) that are interacting with PLXNB3 intracellular part. We studied the biological meaning of this interactions, map precisely interacting regions, studied their effects to cytosceletal rearrangements.

Chair of Molecular Diagnostics

L. Järvekülg's research group focused 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 physicochemical characteristics of potyvirus (PVA) virions;
c) developing an epitope presentation system based on PVA CP VLPs as carriers for melanoma associated antigen peptide(s).

Sirje Rüütel-Boudinot group studies show for first time that RGS16 is induced in response to various mitogens in both primary monocytes and pro-monocytic cell lines during cell activation. During 2012 we performed endotoxin shock model of RGS16-/- versus WT mice. In this study RGS16-/- mice were found to be more sensitive to lipopolysaccharide (LPS)-induced lethality. This suggests a yet-to be-determined TNF independent role for RGS16 in the endotoxin shock model of mice.
Characterization of the PCV2 pathogenesis in mice. In RGS16 KO model we could show that PCV2 virus, acquires enormous spreading capacities in co-infection with bacteria (Pahtma et al., 2012, manuscript). We analyze the implication of RGS16 and CD44 in the migration of immune cells. We developed an in vivo model of thioglycolate-induced peritonitis to analyze the migration of inflammatory macrophages and characterized the migration capacity of inflammatory macrophages in simple KO and DKO mice compared to WT. We studied its function in B1 cells of peritoneal cavity. We have analyzed porcine proliferative enteropathy and porcine circovirus 2 infection in Estonia.
We analyzed also Influenza A virus to gain a better understanding of the basic mechanism of the epidemiology and spreading of viruses. We could establish that both humans and pigs had been infected by the H1N1 virus during the 2009-2010 outbreak (Saar et al., 2012). In a second study, we could establish that the human population in 2010-2011 had still been infected by the H1N1 virus.
Using a bioinformatic approach, we could show that the genomic region surrounding RGS16 is more or less conserved in most vertebrate groups and can be traced back to *Amphioxus*. We

discovered that the surroundings of RGS16 actually form in fact an extension to a genomic region paralogous to the region surrounding the genes for MHC itself.

Merike Kelve group developed a method that allows for the quantification of the amounts of different alleles containing heterogeneities caused by substitutions as well as by indel events. We used the ITS sequences of the freshwater sponge *Ephydatia fluviatilis* as an example, and described intra-individual heterogeneity in this species for the first time. Our method enables to distinguish between individuals by compiling a specific profile for each individual analysed. Comparing these profiles can help us to assess which specimens are similar enough on a genetic level to be used together for one analysis e.g. for separating an enzyme of interest or compiling a cDNA library. (Karlep et al, Plos One, 2013, 8(6): e66601)

Priit Kogerman group research. Conversion of a normal cell into a malignant tumor cell is a multi-step process requiring several genetic mutations. The first of those mutations that are limiting for further progression cause the loss of normal growth control mechanisms of the cell, either by eliminating the function of tumor suppressor genes or by activating oncogenes that act positively on cell growth. However, what makes cancer a deadly disease is the next set of mutations that lead to malignant tumor progression and the formation of metastases; these latter processes have proven far more difficult to analyze. Successful establishment of metastases requires sequential and coordinated regulation of a whole set of genes that in contrast to growth control genes do not convey a selective advantage for stationary tumor growth and may even be counterproductive. Therefore it has been postulated that important metastasis genes are only transiently activated/inactivated during metastasis. Recently there have been suggestions in the literature that “metastasis genes” as such do not exist. Instead it has been proposed that tumor metastasis is determined by the specific set of mutations in oncogenes/tumor suppressor genes early in tumor development. There is some evidence to support both models. The current project is set up to test these possibilities and to contribute to our understanding of tumor progression and metastasis in a significant manner. Specifically we want to further characterize CD44 as a transient metastasis molecule and study the role of PTCH1 in angiogenesis and metastasis models. The underlying hypothesis is that both metastasis genes and metastasis-suppressing gatekeeper genes exist with CD44 representing an example of the former and PTCH1 of the latter class.

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

Chair of Gene Technology

Recombinations in the translocation on chromosome 4AL responsible for approximately half of the resistance to *Blumeria graminis f. sp. tritici* introduced into hexaploid wheat *Trititcum aestivum* from a wild tetraploid wheat, *Triticum militinae,* were looked for in a population generated from a cross of a irradiated double haploid genotype carrying the translocation and nullisomic for 4A plants. About a thousand of plants were analysed, however no recombination in the translocated region was detected. The introgressive line was now crossed

to a *ph/ph* mutant genotype (nonhomeologous pairing allowed) continuing search for the recombinations in the region of interest.

In order to find functional marker(s) for the late blight resistance in the Estonian potato cultivar Ando, an analysis of expressed sequences comprising the conserved NB-ARC domain of R-genes has been started. The NB-ARC domain is highly conserved between different R-genes and acts as a molecular switch of R protein activity upon pathogen perception. Based on the recently published potato genome sequence (DM1-3 516R44), we have designed a set of primers to amplify the 3' ends of the expressed R- gene sequences in Ando and in susceptible cvs Agra and Frila. By aligning the sequences generated by Illumina next generation sequencing technology, we aim to identify candidate sequence(s) specific to Ando which correspond to Ando's resistance. Cosegregation of candidate resistance genes with late blight resistance will be tested in two segregating populations obtained from crosses of resistant and susceptible parents Ando x Agra and Ando x Frila.

The re-sequencing of TRoV and LTSV showed that there do not exist ORF1b which was only the result of previous sequencing errors leading to the wrong annotation of the genome. Instead, the beginning of ORF2a was found to be situated ca 70 codons upstream. The transmembrane helix prediction tests of ORF2a encoded polyprotein revealed the N-terminal transmembrane segments possibly used for polyprotein processing. The alignment of the N-termini of sobemoviral polyproteins raised a new theory about the site-specificity of a sobemovirus-encoded serine protease. The coat protein of CfMV is a suppressor of RNA silencing and it is dispensable for the systemic infection.

In Nicotiana benthamiana the deletion of FeS domain of human and Arabidopsis RLI enhances the suppression of RNA silencing. Two important human RLI interaction partners have been found, namely translin and Ago2. In addition, human RLI locates in the nucleus and affects the cell cycle arresting it at S phase.

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

Chair of Genomics and Proteomics

Progressive deposition of amyloid beta peptides into amyloid plaques is the pathological hallmark of Alzheimer's disease (AD). The amyloid cascade hypothesis pins this deposition as the primary cause of the disease, but the mechanisms that causes this deposition remain elusive. An increasing amount of evidence shows that biometals Zn(II) and Cu(II) can interact with A beta, thus influencing the fibrillization and toxicity. We have analyzed and reviewed the role of Zn(II) and Cu(II) in AD, and revisited the amyloid cascade hypothesis demonstrating the possible roles of Zn(II) and Cu(II) in the disease pathogenesis. The metal ions can be the main factors causing the formation of initial fibrillar aggregates and Cu(II) ions are generating ROS that may be the main source of the toxicity of amyloid in brain.

Many peptides and proteins can form fibrillar aggregates in vitro, but only a limited number of them are forming pathological amyloid structures in vivo and are causing amyloid diseases. We studied the fibrillization of four peptides Alzheimer's amyloid-beta (AB) 1-40 and 1-42, amylin and insulin. In all cases, intensive mechanical agitation of the solution initiated fast fibrillization. However, when the mixing was stopped during the fibril growth phase, the fibrillization of amylin and insulin was practically stopped, and the rate for AB40 substantially decreased, whereas the fibrillization of AB42 peptide continued to proceed with almost the

same rate as in the agitated conditions. The reason for the different sensitivity of the in vitro fibrillization of these peptides towards agitation in the fibril growth phase remains elusive, however requirement for agitation in the initial phase confirms the self-propagationg nature of Abeta fibrils and the high importance of secondary nucleation events in in vitro amyloid formation.

Oligomers are commonly observed intermediates at the initial stages of amyloid fibril formation. They are toxic to neurons and cause decrease in neural transmission and long-term potentiation. We describe an in vitro study of the initial steps in amyloid fibril formation by human stefin B, which proved to be a good model system. Due to relative stability of the initial oligomers of stefin B, electrospray ionization mass spectrometry (ESI MS) could be applied in addition to size exclusion chromatography (SEC). These two techniques enabled us to separate and detect distinguished oligomers from the monomers: dimers, trimers, tetramers, up to decamers. The amyloid fibril formation process was followed at different pH and temperatures, including such conditions where the process was slow enough to detect the initial oligomeric species at the very beginning of the lag phase and those at the end of the lag phase. Taking into account the results of the lower-order oligomers transformations early in the process, we were able to propose an improved model for the stefin B fibril formation.

Copper chaperones compose a specific class of proteins assuring safe handling and specific delivery of potentially harmful copper ions to a variety of essential copper proteins. Copper chaperones are structurally heterogeneous and can exist in multiple metal-loaded as well as oligomeric forms. Moreover, many copper chaperones can exist in various oxidative states and participate in redox catalysis, connected with their functioning. We have generated an overview of the structural and functional properties of copper chaperones and their partners, which allowed us to define specific regulatory principles in copper metabolism connected with copper-induced conformational control of copper proteins

Chair of Molecular Biology

Tõnis Timmusk: Histone deactylase (HDAC) inhibitors show promise as therapeutics for neurodegenerative and psychiatric diseases. Increased expression of brain-derived neurotrophic factor (BDNF) has been associated with memory-enhancing and neuroprotective properties of these drugs, but the mechanism of BDNF induction is not well understood. We compared the effects of a class I/IIb selective HDAC inhibitor SAHA, a class I selective inhibitor MS-275, a class II selective inhibitor MC1568 and a HDAC6 selective inhibitor tubacin on Bdnf mRNA expression in rat primary neurons. We show that inhibition of class II HDACs resulted in rapid upregulation of Bdnf mRNA levels, whereas class I HDAC inhibition produced a markedly delayed Bdnf induction. In contrast to relatively slow upregulation of Bdnf transcripts, histone acetylation at BDNF promoters I and IV was rapidly induced by SAHA. Bdnf induction by SAHA and MS-275 at 24 h was sensitive to protein synthesis inhibition, suggesting that delayed Bdnf induction by HDAC inhibitors is secondary to changed expression of its regulators. HDAC4 and HDAC5 repressed Bdnf promoter IV activity, supporting the role of class II HDACs in regulation of Bdnf expression. In addition, we show a critical role for the cAMP/Ca(2+) response element (CRE) in induction of Bdnf promoter IV by MS-275, MC1568, SAHA and sodium valproate. In contrast, MEF2-binding CaRE1 element was not necessary for promoter IV induction by HDAC inhibition. Similarly to Bdnf, the studied HDAC inhibitors differentially induced expression of neuronal activity-

regulated genes c-fos and Arc. Together, our findings implicate class II HDACs in transcriptional regulation of Bdnf and indicate that class II selective HDAC inhibitors may have potential as therapeutics for nervous system disorders (Koppel et al., 2013).

Urmas Arumäe: Identification of a neurotrophic peptide from MANF sequence and setting up the systems to study its activity. Identification of two motifs in the sequence of MANF that are essential for its intracellular neurotrophic activity. Demonstrating that the mRNA for neuron-specific Bcl-2 family member N-Bak is translationally repressed (both in the Institute of Biotechnology and GTI).

Mart Speek: 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. In the commentary article, Kaer K and Speek M. Mob Genet Elements. 2012 May 1;2(3):154-157, 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. We discussed the complexity of the regulation of gene transcription by intronic retroelements and predicted that in addition to transcriptional activity, transcription factor binding and nucleosomal occupancy play a significant role in the regulation of host genes. We also analysed 85 instances of retroelements causing human genetic diseases and showed that exon definition and TI could be used to explain the disruption of normal gene expression (Kaer and Speek, “Retroelements in human disease” Gene. 2013 Apr 15;518(2):231-41). In addition, we wrote a chapter about determination of transcription factor binding sites in long fragments of genomic DNA published in Methods Mol Biol. 2013;977:169-81, Humana Press.

Teet Velling: Our more important findings were briefly as follows: expression of FLNA in M2 cells (M2F cells) seems to specifically influence the function of collagen receptors; the expression levels of collagen receptors identified on these cells (□1□1, □2□1, and □□1□1) were differentially regulated dependent on the expression of FLNA and on adhesion substrates of the cells; PKB/Akt, a well-charcterised cytoplasmic protein kinase with a major role in the regulation of cell survival and an elevated expression in various tumours, was found to localize to the cell nucleus only in M2F cells that express FLNA upon the activation of EGFR but not integrins; upon EGFR stimulation a sustained activity of PKB/Akt was detected in M2F cells but not in M2 cells suggesting a positive regulatory role of FLNA; a method to identify proteins selectively associating with FLNA upon stimulation of EGFR or integrins has been developed and optimized, experiments have been performed by immunoprecipitating EGFP-FLNA from stimulated and control cells and the samples have been analysed by mass spectrometry. The results show that the complexes are indeed different depending on the treatment of cells, and contain both known FLNA binders and a number of putative novel interactors. Stable Isotope Labelling of Amino Acids In Cell Culture (SILAC) methodolgy is currently being set up to assess the quantitative differences in these complexes.

In co-immunoprecipitation experiments, MAP kinase Erk1/2 and, Ric8, have been found to associate with FLNA upon stimulation of EGFR. Work on characterizing the signalling pathways the control these interactions, and on the role of the association in regulation of cell migration, is currently in progress.

Andres Veske: We show that the N-terminal segment of intracellular domain of Plexin-B3 interacts with microtubule plus end binding proteins EB1, EB2 and EB3. The corresponding region in human plexins A2, B1 and B3 contains the conserved EB-binding motif SxIP and these plexins also associate with EBs indicating the specificity of plexin-EB binding. As to the EB proteins, their N-terminal microtubule-binding domain is dispensable for plexin interaction. Plexin-EB interaction is involved in neurite growth as the synthetic peptide corresponding to the EB-binding region of Plexin-B1 increases significantly the number of neurite tips. We found out howPlexin-B1 alters microtubule +TIP dynamics in response to Sema4D treatment and how B-plexins influenced dendrite growth and dendritic spine formation .

Chair of Molecular Diagnostics

L. Järvekülg**:** The following results were obtained from the reasearch 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 recombinants were detected and characterized. Analysis of the data obtained and some experimental work are still in progress.

b) The first detailed study of physicochemical characteristics of potyvirus virions was published by us (Ksenofontov et al 2013, PLOS ONE vol 8, e67830, pp. 1-7). We suggest that the structure of PVA virions below 55^{0}C is stabilized by interactions between the remaining structured segments of intravirus coat protein (CP). It is not improbable that the biological efficiency of PVA relies on the disordered structure of intravirus CP.

c) It was shown that PVA CP VLP-mel immunoparticles have a potential to remarkably delay melanoma development.

Sirje Rüütel-Boudinot: Characterizing the immunomodulating role of RGS16 in human monocytes (Suurväli et al., submitted). Characterization of the lipopolysaccharide (LPS)-induced lethality on RGS16-/- versus WT mice (Pahtma et al.,manuscript). Characterization of PCV2 pathogenesis in mice (Pahtma et al., 2012, manuscript). Characterization of PCV2 pathogenesis in pig (manuscript). Characterization of the 2009-2011 H1N1 (H1N1pandemic09) Influenza outbreak in Estonia (Saar et al., 2012). Characterizing the genomic context of RGS16 as a conserved region containing genes involved in antiviral defenses, and as part of the proto-MHC, an ancient synteny group containing MHC–related markers. (Suurväli et al., Immunogenetics, 2012).

Merike Kelve. We provided an approach for the characterization of a set of internal transcribed spacer sequences found within every rDNA repeat unit by implementing direct sequencing methodology. The prominent allelic variants and their relative amounts characterizing an individual can be described by a single sequencing electropherogram of the mixed amplicon containing the variants present within the genome. We propose a method for

rational analysis of heterogeneity of multicopy genes by compiling a profile based on quantification of different sequence variants of the internal transcribed spacers of the freshwater sponge *Ephydatia fluviatilis* as an example. In addition to using conventional substitution analysis, we developed a mathematical method, the proportion model method, to quantify the relative amounts of allelic variants of different length using data from direct sequencing of the heterogeneous amplicon. This method is based on determining the expected signal intensity values (corresponding to peak heights from the sequencing electropherogram) by sequencing clones from the same or highly similar amplicon and comparing hypothesized combinations against the values obtained by direct sequencing of the heterogeneous amplicon. (Karlep et al, Plos One, 2013, 8(6): e66601)

Priit Kogerman: In order to better understand the mechanisms of tumor progression and metastasis we have foused our attention on SHH-PTCH signalling. Here we have studied the structure and function of the repressor domain of the GLI3 transcription factor and a new manuscript describing our results has been submitted. We have also been studying the kinases phosphorylating the GLI transcription factors with a particular focus on DYRK1 and ULK3. While studying the the functions in CD44 on endothelial cells and its role in inhibiting tumor angiogenesis and metastasis, we have identified the receptor for CD44 on endothelial cells as cell surface vimentin. We have also optimized the recombinant CD44 for the inhibition of angiogenesis by covalently modifying it with polyethylene glycol (PEG). This modification significantly increases the stability of CD44 in the organism. A manuscript describing the generation and properties of PEGylated CD44 has been submitted for publication

2.2 Uurimisgrupi kuni 5 olulisemat publikatsiooni läinud aastal.

Instituudi töötajate osalusel publitseeritud teadustulemused on loetletud allpool.

2.3 Loetelu struktuuriüksuse töötajate rahvusvahelistest tunnustustustest.

Mart saarma, Alfred Kordelin Foundation Science Achievement Prize 2013

2.4 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

Mart Saarma, ERC Scientific Council liige, EMBO Council liige

2.5 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.

Mari Palgi, Eesti üliopilaste teadustööde riiklikul konkursil II preemia bio-ja keskkonna teaduste valdkonnas doktoriõppe üliopilaste astmes.
Julia Geller, teadustööde riiklikul konkursil III kolmas preemia terviseuuringute valdkonnas doktorõppe üliõpilaste astmes.
Aljona Kotšubei, Eesti üliopilaste riiklikul teadustööde konkursil bioteaduste magistrate kategoorias teise koha.
Teadlasmobiilsus. Ljudmilla Timofejeva töötab pool aastat külalisteadurina Cornelli Ülikoolis. Kahepoolne teadlasmobiilsus toimub K. Järve rühma ja M. Valariku rühma vahel Olmoucist, Tšehhi Vabariik. Tõnis Timmusk töötas 5 kuud külalisõppejõuna Waseda Ülikoolis, Tokyos, Jaapanis