MATEMAATIKA-LOODUSTEADUSKOND
GEENITEHNOLOOGIA INSTITUUT
TEADUS- JA ARENDUSTEGEVUSE AASTAARUANNE 2012

1. Instituudi struktuur

Geenitehnoloogia instituut, Department of Gene Technology
Instituudi direktor Andres Veske

- Geenitehnoloogia õppetool, Chair of Gene Technology, Heiti Paves
- 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.

Plant virology working group propagated LTSV and TRoV in their host plants. Viral genomic RNA was purified from particles for their genome re-sequencing studies. Also, the genomic material of 3 other sobemoviruses was collected and sequenced. Analysis of these data is currently in the pipeline. In addition, localization and systemic spread pathway of CfMV was identified in oat plants.

Gene silencing working group 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.

Working group of *Arabidopsis* motor proteins started a new research direction: the role of myosins in plant gravitropic behaviour.

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

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 2013. 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 (manuscript in preparation).
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. 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: Integrins and receptor tyrosine kinases are cellular receptors that are instrumental for mediating cell adhesion and migration and regulating the underlying protein interactions and cellular signalling. Filamin A (FLNA) is a major structural and signalling protein linking adhesion receptors to the actin network and providing a scaffold for numerous interaction partners including cytoplasmic kinases and phosphatases. Defects in the function of integrins and FLNA, and dysregulation of receptor tyrosine kinases, may lead to different pathologies such as haemophilia, defective wound healing, developmental defects in central nervous system and cancer. Our studies focus on the role of FLNA in the regulation of integrin- and receptor tyrosine kinase-induced cellular signalling, cell migration and protein-protein interactions that govern these processes. More specifically we work on projects such as: a) characterisation of FLNA interactome upon stimulation of integrins or epidermal growth factor receptor (EGFR); b) the regulatory role of FLNA in integrin- and EGFR- induced MAPK and PKB/Akt activation; c) the effect of FLNA on intracellular distribution of signalling molecules in response to stimulation of integrins or EGFR; d) interaction of FLNA with Ric 8, a guanine-nucleotide exchange factor for G-alpha subunits, and MAP kinase Erk ½, and the role of these interactions in the regulation of cell migration.

Andres Veske group is dealing with 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 and 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 rearrangaments.

Chair of Molecular Diagnostics

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 research was focused on the nucleotide metabolic enzymes discovered by them, in salvage and recycling processes of purine nucleotides. To identify the protein having the activity of ATP N-glycosidase they performed the analysis of the first sponge genome available so far, in regard to to the data of the nucleotide phosphorylase/hydrolase family. They cloned a candidate gene from the freshwater sponge *Ephydatia muelleri* that may code for ATP N-glycosidase**.** The enzymatic characterization of the expressed and purified candidate protein will give the answer about its identity.

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

Testing for seedling resistance to 16 different races/mixtures of *Blumeria graminis f. sp. tritici* revealed four highly significant nonrace-specific resistance QTL including the main QTL on chromosome 4AL. The major QTL on chromosome 4AL (QPm.tut-4A) as well as two minor QTLs on chromosomes 5AL and 7AL were also highly effective at the adult stage of plant growth.

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. A localization study about the spread of CfMV (our current sobemovirus model species) in oat plants demonstrated that differently from another monocot-infecting sobemovirus RYMV, CfMV uses phloem pathway to conquer the host.

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 seems to affect the cell cycle arresting it at S fase.

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

Chair of Genomics and Proteomics

Copper chaperone for superoxide dismutase 1 (SOD1), CCS, is the physiological partner for the complex mechanism of SOD1 maturation. We report an in vitro model for human CCS-dependent SOD1 maturation based on the study of the interactions of human SOD1 (hSOD1) with full-length WT human CCS (hCCS), as well as with hCCS mutants and various truncated constructs comprising one or two of the protein's three domains. The synergy between electrospray ionization mass spectrometry (ESI-MS) and NMR is fully exploited. Domain 1 of hCCS is necessary to load hSOD1 with Cu(I), requiring the heterodimeric complex formation with hSOD1 fostered by the interaction with domain 2. Domain 3 is responsible for the catalytic formation of the hSOD1 Cys-57-Cys-146 disulfide bond, which involves both hCCS Cys-244 and Cys-246 via disulfide transfer.(Banci, L et al. 2012, Proc. Natl. Acad. Sci. USA 109 (34), 13555 - 13560)

Insulin-like growth factor 1 (IGF-1) like other growth factors are oxidatively folded in the endoplasmic reticulum and act primarily in the blood, under relatively oxidative conditions. It is known that IGF-1 exists in various intracellular and extracellular compartments in the oxidized form; however, the reduction potential of IGF-1 and the ability of fully reduced protein to bind metal ions is not known. We determined that the redox potential of human IGF-1 is equal to -332 mV and the reduced form of hIGF-1 can bind cooperatively four Cu+ ions. The Cu+ binding affinity of hIGF-1 is approximately 3 times lower than that for the copper chaperones; thus, it cannot compete with known Cu+-binding proteins. (Smirnova, J. et al. 2012, Biochemistry, 51(29), 5851-5859)

We have studied the abnormal fibrillization of amyloidogenic peptides/proteins that has been linked to various neurodegenerative diseases such as Alzheimer's and Parkinson's disease, prion diseases as well as with type-II diabetes mellitus.

The kinetics of protein fibrillization is commonly studied by using a fluorescent dye Thioflavin T (ThT) that binds to protein fibrils and exerts increased fluorescence intensity in bound state. We demonstrated that the interference of small organic molecules that are tested as fibrillization inhibitor with ThT-fluorescence test is a general phenomenon and more attention has to be paid to interpretation of kinetic results of protein fibrillization obtained by using fluorescent dyes. (Noormägi, A., et al. 2012 ***Journal of Peptide Science,*** 18(1), 59-64.)

Proteins involved in the listed diseases are able to form complexes with metal ions and there is a dyshomeostasis and miscompartmentalization of the transition metals in the brain during these diseases. Therefore, it is important to understand the interactions between the key proteins of the neurodegenerative diseases and the transition metal ions and its consequences. We have given a review on the coordination chemistry of zinc ions to Abeta, APP, alpha-synuclein and PrP. (Tõugu, V., P. Palumaa 2012 Coordination Chemistry Reviews, 256(19-20), 2219-2224.)

In a cooperation with Australian colleagues we found that redox-active Cu(II)-A beta causes pronounced axonal pathology in long-term neuronal cultures, including axonal fragmentation and the formation of hyperphosphorylated tau-immunoreactive axonal swellings. These dystrophic axonal manifestations resemble some of the characteristic neuritic pathology of the AD brain. Cu(II)-A beta directly caused formation of intra-axonal swellings via the generation of free radicals and subsequent efflux of K+ out of neurons. Thus, redox-active Cu(II)-A beta can induce substantial neurodegenerative changes in mature neurons, and may have an

important role to play in the slowly progressing pathogenesis of AD. (Howells, C., et al. 2012 Experimental Neurology 237(2), 499-506.)

Chair of Molecular Biology

Tõnis Timmusk: 1. We showed that KCNIP/DREAM family transcription factors (TFs) are not involved in membrane depolarization dependent (1) activation of BDNF gene transcription and (2) transcriptional activation mediated by CRE cis-elements in primary cortical neurons and thus do not support the results of some other labs about KCNIP/DREAM-mediated transcriptional activation of CRE-dependent genes (Pruunsild et al., 2012). 2. We have shown that the bHLH TF TCF4/ITF2/E2-2 is a potential regulator of BDNF transcription in neurons and that in Huntington's disease the expression levels and subcellular localization of this TF is changed. To study molecular mechanisms of TCF4 action we have re-evaluated the impact of mutations that cause Pitt-Hopkins syndrome, a rare form of mental redardation. TCF4 haploinsufficiency has been proposed as an underlying mechanism for PTHS. We showed that different mutations impair the functions of TCF4 by diverse mechanisms and to a varying extent, possibly contributing to the phenotypic variability of Pitt-Hopkins patients (Sepp et al., 2012). Our results could help to understand the dysfunction of TCF4, as a potent regulator of BDNF gene, in Huntington's disease.

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 a commentary article (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, submitted). In addition, we wrote a chapter about determination of transcription factor binding sites to the book Methods in Molecular Biology, Springer's Humana Press (in press).

Teet Velling group. 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 (α1β1, α2β1, and α11β1) 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 (Laht, P et al. 2012 Biochemica et Biophysica Acta, 888 – 893).

Chair of Molecular Diagnostics

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: In the marine sponge *Tethya aurantium* a novel endoribonuclease was found which specifically catalyzed the degradation of 2',5'-phosphodiester linkages. The enzyme did

not require the presence of metal ions for its activity. The novel enzyme exhibited the preference for 2',5'-oligoadenylates but hetero-oligomers and homodimers comprising guanylate and uridylate residues were cleaved as well. We suggest that the novel enzyme belongs to the group of specific 2',5'-specific ribonucleases that primarily control the cellular levels of 2-5A.

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.

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

2.5 Aruandeaasta tähtsamad T&A finantseerimise allikad.

Instituudi teadustöötajate T&A finantseerimisallikad on loetletud allpool.

2.6 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.

Ann Tiiman – Esimene preemia doktoritööde kategoorias riiklikul üliõpilaste teadustööde konkursil terviseuuringute valdkonnas.
Mari Sepp – preemia õpilaste teadustööde riiklikul konkursil gümnaasiumi astmes esimese koha saanud uurimistöö juhendamise eest (Jaan Toots, uurimistöö „Neurl1 valgu tuumatranspordi mehhanismide uurimine“ Tallinna Tehnikauülikool, Geenitehnoloogia instituut ja Tallinna Reaalkool, 2012).

Teadlasmobiilsus. Järeldoktorantuuri välismaale läksid Priit Pruunsild (Sakasamaa) ja Allan Olspert (Inglismaa). Järeldoktorantuuri Geenitehnoloogia Instituuti tuli Mari Palgi.

2.7 Instituudi teadus- ja arendustegevuse teemade ja projektide nimetused *(Eesti Teadusinfosüsteemi, edaspidi ETIS, andmetel)*

- Haridus- ja Teadusministeerium

– sihtfinantseeritavad teemad:

T108, Nukleotiidide metabolismis osalevad ensüümid - võrdlev biokeemia, molekulaarbioloogia ja evolutsioonilised aspektid, Kelve Merike (2008 – 2013)

T145A, Tuumorprogressiooni molekulaarbioloogia: molekulaarsed mehhanismid ja biomeditsiinilised rakendused, Kogerman Priit (2008 – 2013)

T055, Struktuurne ja meditsiiniline metalloproteoomika, Palumaa Peep (2008 – 2013)

T143,Geeniregulatsioon ja signaaliülekanne närvisüsteemis ,Timmusk Tõnis (2008-2013)

T106,Taim-patogeen molekulaarsed interaktsioonid ,Truve Erkki (2008-2013)

T066,Tsirkoviiruse bioloogia ja vaktsinoloogia, Rüütel Boudinot Sirje (2009 – 2014)

– baasfinantseerimise toetusfondist rahastatud projektid (sh TTÜ tippkeskused):

B12, Professor Peep Palumaa poolt juhitava uurimisgrupi toetamine (2011 – 2013)

– riiklikud programmid:

- Teiste ministeeriumide poolt rahastatavad riiklikud programmid:

556L, Põllumajanduskultuuride geneetilise ressursi kogumine ja säilitamine, Järve Kadri (1.01.2005- 31.12.2013)

RP9010, Sordiaretusprogramm aastatel 2009 - 2019, Järve Kadri (29.01.2009 - 1.12.2019)

- Uurija-professori rahastamine:

- SA Eesti Teadusfond/Eesti Teadusagentuur

– grandid:

ETF9185, Käsna ja mikroobi sümbioosi funktsionaalsed aspektid puriinide põhiainevahetuse näitel, Kelve Merike (2012 – 2015)

ETF8385, Amüloidsete peptiidide konformatsiooni ja agregatsiooni uurimine, Kumm Tiina (2010 – 2013)

ETF8811, Uudsed mass-spektroskoopial põhinevad metoodikad vase ja tsingi valkude metallide sidumisomaduste ja redoksregulatsiooni uurimiseks, Palumaa Peep (2011 – 2014)

ETF8604, Arabidopsis thaliana müosiinid, Paves Heiti (2011 – 2014)

ETF947, TrkB, Shh ja Notch signaaliradade funktsioon neuroblastoomides, Piirsoo Marko (2012 – 2015)

ETF8914, RGS16 osalus immuunregulatsioonis, Rüütel Boudinot Sirje (2011 – 2014)

ETF8381 , Transkriptsiooniline interferents – kompleksne regulatsioon geenide ja mobiilsete elementide vahel, Speek Mart (2010 – 2013)

ETF9415, Sobemoviiruste fülogenees ja seire Eestis, Sõmera Merike (2012 – 2015)

ETF8844, Neurotroofse teguri BDNF geeni regulatsioon ja selle häired närvisüsteemi haigustes, Timmusk Tõnis (2011- 2014)

ETF9318, Alheimeri amüloid-beeta fibrillide moodustumine, toksilisus ja võrdlus teiste amüloidsete peptiididega , Tõugu Vello (2012 – 2015)

ETF8116 , Kasvaja metastaseerumise molekulaarsed mehhanismid: Optilised kuivamismeetodid teadaolevate ja uudsete molekulide rollide selgitamiseks, Kogerman Priit (2009- 2012)

– ühisgrandid välisriigiga:
– järeldoktorite grandid (SA ETF ja Mobilitas):

MJD37, Kumm Tiina,High-throughput screening of inhibitors of A peptide aggregation (1.09.2009 - 31.08.2012)

JD155, Tomson Katrin, Cloning, overexpression and labelling of intracellular C-terminal domain of human Smoothened (a receptor protein synthesised from proto-oncogene) in Escherichia coli system for functional and structural studies (1.10.2009 - 30.09.2012)

MJD121, Toompuu Marina,Studies on function of human RNase L inhibitor (RLI) (1.11.2010 - 31.10.2013)

MJD341, Palgi Mari,Molecular studies of bHLH transcription factor daughterless and its mammalian homologue TCF4 in Drosophila (1.07.2012 - 30.06.2015)

– tippteadlase grandid (Mobilitas):

MTT4, Velling Teet,CO-OPERATION OF INTEGRINS AND RECEPTOR TYROSINE KINASES IN REGULATION OF CELL MOTILITY: ROLE OF FILAMIN A AND PKB/Akt (1.11.2009 - 31.10.2013)
MTT84, Urmas Arumäe, "MANF neurotrophic factor: novel mode of action and therapeutic potential" (01.08.2012-31.07.2015)

- <u>Ettevõtluse Arendamise SA</u>

– eeluuringud:
– arendustoetused:

- <u>SA Archimedesega sõlmitud lepingud</u>

– infrastruktuur (nn „mini-infra“, „asutuse infra“):

AP055A, Struktuurne ja meditsiiniline metalloproteoomika , Palumaa Peep (1.01.2012 - 31.12.2013)

AP143A, Geeniregulatsioon ja signaaliülekanne närvisüsteemis, Timmusk Tõnis (1.01.2012 - 31.12.2013)

ÜLTAP29-4, Makromolekulide struktuurianalüüs, Peep Palumaa (1.06.2012 - 18.01.2014)

ÜLTAP63A, Loodusteaduste Maja infrastruktuuri edasiarendus, Andres Veske (1.01.2010 - 19.04.2012)

– Eesti tippkeskused:

TAR11058, Keskkonnamuutustele kohanemise tippkeskus, Erkki Truve (1.01.2011 - 31.12.2015)

– riiklikud programmid:

AR11121, Biotehnoloogia,Põllukultuuride resistentsusaretus, Kadri Järve (1.06.2011 - 31.08.2015)

AR12030, Biotehnoloogia,Transgeensetel rottidel baseeruvate haigusmudelite loomine ja kuvamisplatvormid haigusmudelite elupuhuseks uurimiseks, Tõnis Timmusk (1.09.2011 - 31.08.2015)

AR12171, Biotehnoloogia,Development of Trk antagonists as drug candidates for the treatment of neuropathic pain, Tõnis Timmusk (1.09.2012 - 31.08.2015)

AR12098, Tervishoiutehnoloogia,Relevance of LSAMP in schizophrenia and comorbidities in diseases, Tõnis Timmusk (1.01.2012 - 31.08.2015)

– muud T&A lepingud:

- SA Keskkonnainvesteeringute Keskusega sõlmitud lepingud:
- Siseriiklikud lepingud:
- EL Raamprogrammi projektid:
- Välisriiklikud lepingud:

VA529, Taime müosiinide roll gravitropismis, Paves Heiti (1.09.2011 - 31.08.2014)

2.8 Struktuuriüksuse töötajate poolt avaldatud eelretsenseeritavad teaduspublikatsioonid *(ETIS klassifikaatori alusel 1.1, 1.2, 1.3, 2.1, 2.2, 3.1, 3.2, 3.3, 4.1 ja 5.1).*

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Lopp, Annika; Reintamm, Tõnu; Kuusksalu, Anne; de Rosa, Salvatore; Kelve, Merike (2012). A novel endoribonuclease from the marine sponge Tethya aurantium specific to 2',5'-phosphodiester bond. Biochimie, 94, 1635 - 1646.

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Otsus, Maarja; Uffert, Gabriela; Sõmera, Merike; Paves, Heiti; Olspert, Allan; Islamov, Bulat; Truve, Erkki (2012). Cocksfoot mottle sobemovirus establishes infection through the phloem. Virus Research, 166(1-2), 125 - 129.

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Katargina, Olga; Geller, Julia; Alekseev, Andrey; Dubinina, Helen; Efremova, Galina; Mishaeva, Nina; Vasilenko, Veera; Kuznetsova, Tatiana; Järvekülg, Lilian; Vene, Sirkka; Lundkvist, Åke; Golovljova, Irina. (2012). Identification of Anaplasma phagocytophilum in ticks populations in Estonia, European Part of Russia and Belarus. Clinical Microbiology and Infection, 18(1), 40 - 46.
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1.2

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3.2

3.3

4.1

5.1

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K. TAAL, G. RULLINKOV, M. PIIRSOO, M. SEPP, T. NEUMAN, R. TAMME, T. TIMMUSK. Neuralized-1 is an e3 ubiquitin ligase for cGMP-specific phosphodiesterase 9a. Society for Neuroscience of USA (SfN) Annual Meeting, New Orleans, USA, October 13-17.

K. JAANSON, A. PÄRN, M. SEPP, T. TIMMUSK. Bac based models for studying BDNF gene regulation. Society for Neuroscience of USA (SfN) Annual Meeting, New Orleans, USA, October 13-17.

2.9 Struktuuriüksuses kaitstud doktoriväitekirjade loetelu *(NB! struktuuriüksus lisab struktuuriüksuse töötaja juhendamisel mujal kaitstud doktoriväitekirjade loetelu)*

Kristel Kodar, geenitehnoloogia instituut
Teema: *Immunoglobulin G Glycosylation Profiling in Patients with Gastric Cancer* (Immuunglobuliin G glükosüleerituse profileerimine maovähihaigetel)
Juhendajad: PhD Oleg Kurtenkov ja prof Lilian Järvekülg
Kaitses: 28.06.2012
Omistatud kraad: filosoofiadoktor (geenitehnoloogia)

Ann Tiiman, geenitehnoloogia instituut
Teema: *Interactions of Alzheimer`s Amyloid-ß Peptides with Zn(II) and Cu(II) Ions* (Alzheimeri amüloid-ß peptiidide interaktsioonid Zn(II) ja Cu(II) ioonidega)
Juhendajad: prof Vello Tõugu ja prof Peep Palumaa
Kaitses: 30.08.2012
Omistatud kraad: filosoofiadoktor (geenitehnoloogia)

Olesja Bondarenko, geenitehnoloogia instituut
Teema: *Development of Bacterial Biosensors and Human Stem Cell-Based in Vitro Assays for the Toxicological Profiling of Synthetic Nanoparticles* (Rekombinantsetel sensorbakteritel ja inimese tüvirakkudel põhinevate in vitro testide väljatöötamine sünteetiliste nanoosakeste toksikoloogiliseks uurimiseks)
Juhendajad: vanemteadur Angela Ivask ja prof Erkki Truve
Kaitses: 24.10.2012
Omistatud kraad: filosoofiadoktor (geenitehnoloogia)

Mari Sepp, geenitehnoloogia instituut
Teema: *Functions of the Basic Helix-Loop-Helix Transcription Factor TCF4 in Health and Disease* (Aluselise heeliks-ling-heeliks transkriptsioonifaktori TCF4 funktsioonid ja seosed haigustega)
Juhendaja: prof Tõnis Timmusk
Kaitses: 21.12.2012
Omistatud kraad: filosoofiadoktor (geenitehnoloogia)

2.10 Struktuuriüksuses järeldoktorina T&A-s osalenud isikute loetelu *(ETIS-e kaudu esitatud taotluste alusel)*

Kumm Tiina, High-throughput screening of inhibitors of A peptide aggregation (1.09.2009 - 31.08.2012)

Tomson Katrin, Cloning, overexpression and labelling of intracellular C-terminal domain of human Smoothened (a receptor protein synthesised from proto-oncogene) in Escherichia coli system for functional and structural studies (1.10.2009 - 30.09.2012)

Toompuu Marina, Studies on function of human RNase L inhibitor (RLI) (1.11.2010 - 31.10.2013)

Palgi Mari, Molecular studies of bHLH transcription factor daughterless and its mammalian homologue TCF4 in Drosophila (1.07.2012 - 30.06.2015)

2.11 Struktuuriüksuses loodud tööstusomandi loetelu

US13/500902
Inhibition or activation of serine/threonine ULK3 kinase activity
Taotlus esitatud: 06.04.2012
Autorid: Torben Osterlund, Priit Kogerman, Alla Piirsoo, Piret Michelson, Marko Piirsoo
Omanik: TTÜ

EP2486130 (EP10765565.6)
Inhibition or activation of serine/threonine ULK3 kinase activity
Taotlus esitatud: 04.05.2012
Autorid: Torben Osterlund, Priit Kogerman, Alla Piirsoo, Piret Michelson, Marko Piirsoo
Omanik: TTÜ

US13/578703
Suppressors of RNA silencing as modulators of miRNA levels
Taotlus esitatud: 13.08.2012
Autorid: Maria Cecilia Sarmiento Guerin, Kairi Kärblane, Illar Pata, Pille Pata, Erkki Truve,
Omanikud: TTÜ, AS Vähiuuringute Tehnoloogia Arenduskeskus

EP2536423 (EP11708392.3)
Suppressors of RNA silencing as modulators of miRNA levels
Taotlus esitatud: 17.09.2012
Autorid: Maria Cecilia Sarmiento Guerin, Kairi Kärblane, Illar Pata, Pille Pata, Erkki Truve
Omanikud: TTÜ, AS Vähiuuringute Tehnoloogia Arenduskeskus

PCT/FI2012/050859
MANF/CDNF peptiides
Taotlus esitatud: 05.09.2012
Autorid/Omanikud: Urmas Arumäe, Pia Runeberg-Roos, Mart Saarma

US8192744B2
Drug for treating states related to the inhibition of angiogenesis and/or endothelial cell proliferation
Patent välja antud: 05.06.2012
Autorid: Priit Kogerman, Taavi Päll, Staffan Stromblad
Omanik: Celecure AS

3. Struktuuriüksuse infrastruktuuri uuendamise loetelu *(summa eurodes)*

PV007379, Sügavkülmik Innova U725 -80C, 5.06.2012 (9 940,00)

PV007462, Fluorestsents spektromeeter, 1.10.2012 (65 595,21)

PV007469, Spektrofotomeeter Biospec-nano, 10.10.2012 (6 620,00)

PV007476, Sülearvuti MacBook Pro i7, 30.10.2012 (2 273,00)

PV007517, Fotoaparaat NIKON D800E, 14.12.2012 (3 173,33)

PV005025, Konfokaalmikroskoobi täiendus: elus rakkude kuvamise funktsionaalne ühik, 16.11.2012 (64 895,00)