

האגודה הישראלית לפיזיולוגיה ופרמקולוגיה

**Israel Society for Physiology and
Pharmacology**



**THE ISRAEL SOCIETY FOR
PHYSIOLOGY & PHARMACOLOGY**

Annual Meeting

הכנס השנתי

December 9th 2010 Ma'ale Hachamisha

PROGRAM & ABSTRACTS

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**THE ISRAEL SOCIETY FOR
PHYSIOLOGY & PHARMACOLOGY**

Annual Meeting

Ma'ale Hachamisha, Thursday, December 9, 2010

PROGRAM OUTLINE

08:30-09:30	Registration & Refreshments (posters hang up)
09:30-11:10	Morning Sessions A and B
11:10-11:30	Coffee Break
11:30-12:45	Student oral presentation competition
12:45-13:35	L U N C H
13:35-13:45	Business Meeting
13:45-14:45	The Magnes Plenary Lecture
14:45-15:30	Poster session
15:30-17:10	Afternoon Sessions C and D
17:10-17:20	Coffee Break
17:20-17:30	Ceremony Awarding the Winners of Poster and Student Lecture Competitions.

PROGRAM

9:30-11:10 Morning Sessions A and B

Session A: "Molecular Signaling Networks: Function and Disease" (Hall A)

Chairs: Nathan Dascal (Tel-Aviv University) and Mira Barda-Saad (Bar-Ilan University)

9:30–9:50 Varda Shoshan-Barmatz (Ben-Gurion University): Mitochondrial protein VDAC1: From apoptosis regulation to cancer therapy.

9:50–10:10 Nathan Dascal (Tel-Aviv University): Modes of coupling between GPCRs, G proteins and effectors: the case of G protein-coupled K⁺ channel (GIRK).

10:10–10:30 Mira Barda-Saad (Bar-Ilan University): Formation of molecular signaling complexes critical for the immune response.

10:30–10:50 Ronen Alon (Weizmann Institute): The function of endothelial stored chemokines in immune cell migration across inflamed blood vessels.

10:50–11:10 Yarden Opatowsky (Bar-Ilan University): Structure, domain organization and different conformational states of stem-cell-factor induced KIT dimers.

Session B: "Stem cells: from basic science to tissue engineering and therapeutic opportunities" (Hall B)

Chair: Lior Gepstein (Technion)

9:30–9:50 Lior Gepstein (Technion): Induced pluripotent stem cells: implications for basic and applied cardiovascular research.

9:50–10:10 Benny Dekel (Tel-Aviv University): Identification of novel human renal stem/progenitor cells: relevance to cancer and regeneration.

10:10–10:30 Yechiel Elkabetz (Tel-Aviv University): Standardized neural stem cells for regenerative medicine: How to harness the full potential of neural rosettes?

10:30–10:50 Dror Seliktar (Technion): Injectable biopolymers for regenerative medicine applications.

10:50–11:10 Yair Reisner (Weizmann Institute): Embryonic pig pancreatic tissue for the treatment of diabetes.

11:10-11:30 Coffee Break

11:30-12:45 Student oral presentation competition (Hall A)

Chairs: Bernard Attali (Tel Aviv University) and Yoav Paas (Bar-Ilan University)

11:30-11:42 Limor Cohen (Ben-Gurion University): ZnR signaling, mediated by GPR39, reduces butyrate-induced colonocytes cell death via upregulation of clusterin.

11:42-11:54 Hod Dana (Technion): Multi neuron activity monitoring using high-rate temporal focusing microscopy.

11:54-12:06 Yariv Kanfi (Bar-Ilan University): MOSES mice: The role of SIRT6 in the regulation of metabolism and aging.

12:06-12:18 Atara Novak (Technion): CPVT-specific cardiomyocytes generated from induced pluripotent stem cells are arrhythmogenic in response to β -adrenergic stimulation.

12:18-12:30 Maor Hazani-Pauker (Bar-Ilan University): The regulation of a multi-molecular complex formation leading to actin polymerization in T-cells.

12:30-12:42 Liat Ravid (Weizmann Institute): Direct and indirect roles of the mitochondria in caspase activation during *Drosophila* spermatogenesis.

12:45-13:45 LUNCH

13:35-13:45 Business Meeting (Hall A)

13:45-14:45

The Magnes Plenary Lecture (Hall A)

Prof. Ehud Isacoff

University of California, Berkeley

Title: "Lighting Up the Nervous System"

14:45-15:30 **Poster Session**

15:30-17:10 **Afternoon Sessions C and D**

Session C: “From synapse to brain” (Hall A)

Chairs: Israel Nelken (Hebrew University) and Ilan Lampl (Weizmann Institute)

15:30–15:50 **Noam Ziv** (*Technion*): Synaptic tenacity: Insights from live imaging experiments.

15:50–16:10 **Ilan Lampl** (*Weizmann Institute*): Correlation of spontaneous and sensory evoked synaptic inputs of cortical neurons.

16:10–16:30 **Sandro Romani** (*Weizmann Institute*): A unified network model of coexisting dynamical regimes in hippocampus.

16:30–16:50 **Israel Nelken** (*Hebrew University*): Cortical dynamics of sensory processing: stimulus-specific adaptation and novelty detection.

16:50–17:10 **Amir Amedi** (*Hebrew University*): What do sensory deprivation studies teach us about brain organization and reorganization?

Session D: “Cell death and proliferation” (Hall B)

Chair: Adi Kimchi (Weizmann Institute)

15:30–15:50 **David Wallach** (*Weizmann Institute*) Regulation of inflammation by the ‘apoptotic’ caspases.

15:50–16:10 **Eli Arama** (*Weizmann Institute*): A novel role for a caspase: the fly effector-like caspase Dcp-1 is an accelerator of apoptosis.

16:10–16:30 **Adi Kimchi** (*Weizmann Institute*): Studying the complexity of cell death by combining reductionist with systems levels approaches.

16:30–16:50 **Doron Ginsberg** (*Bar-Ilan University*): The transcription factor E2F: Balancing proliferation and apoptosis.

16:50–17:10 **Itamar Simon** (*Hebrew University*): Single cell analysis of cell cycle variability.

17:10-17:20 **Coffee Break**

17:20-17:30 **Ceremony Awarding the Winners of Poster and Student Lecture Competitions (Hall A).**

Abstracts of Invited Speakers
(Sessions A & B)

Session A

“Molecular Signaling Networks: Function and Disease”

The mitochondrial protein VDAC1: From structure to cancer therapy

Varda Shoshan-Barmatz

Department of Life Sciences, and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel.

The voltage-dependent anion channel (VDAC) located in the outer mitochondrial membrane, functions as gatekeeper for the entry and exit of mitochondrial metabolites and thus controls cross-talk between the mitochondria and the cytosol. VDAC also serves as a site for the docking of cytosolic proteins, such as hexokinase (HK) and Bcl-2. Accumulating evidence implicates the voltage-dependent anion channel (VDAC) as functioning in mitochondria-mediated apoptosis and as a critical player in the release of apoptogenic proteins, such as cytochrome c, triggering caspase activation and apoptosis. The mechanisms regulating cytochrome c release and the molecular architecture of the cytochrome c-conducting channel remain unknown.

We studied the relationship between VDAC oligomerization and apoptosis induction. We demonstrated that apoptosis induction by various stimuli, acting through different mechanisms but all involving mitochondria, was accompanied by highly increased VDAC oligomerization, as revealed by cross-linking and directly monitored in living cells using Bioluminescence Resonance Energy Transfer technology (BRET). Moreover, apoptosis inhibitors inhibited VDAC oligomerization and a correlation between the levels of VDAC oligomerization and apoptosis was observed. The VDAC interfaces in the oligomer were identified using site-directed mutagenesis and cysteine cross-linking. Thus we propose that cytochrome c release is mediated by a protein-conducting channel formed within a VDAC1 homo-oligomer or hetero-oligomer containing VDAC1 and pro-apoptotic proteins. A screen for small molecules, which can interact with VDAC and induce its oligomerization and apoptosis, was carried out. Targeting VDAC's oligomeric status and hence apoptosis, offers a therapeutic strategy for combating cancers and neurodegenerative diseases.

The role of VDAC in apoptosis has emerged also from various studies showing its interaction with apoptosis regulating proteins, including the mitochondria-bound isoforms of hexokinase (HK-I, HK-II) and Bcl2. Using site-directed mutagenesis, we defined the boundaries of VDAC1 sequences essential for Bcl2-, HK-I- and HK-II-binding. Based on these sequences, VDAC1-based synthetic and recombinant peptides were synthesized and found to bind to and detach HK bound to mitochondria. Moreover, expression of the VDAC1-based peptides in cells over-expressing Bcl2, HK-I or HK-II prevented their protection against apoptosis. We have developed a VDAC1-based cell penetrating peptide, LP4-Antp that efficiently and specifically induced apoptosis in several cancer cell lines but not in normal cells. When tested on lymphocytes derived from B-cell chronic lymphocytic leukemia (CLL) patients, the peptide induced dramatic and selective apoptotic cell death, whilst healthy lymphocytes were spared. These results suggest that HK and Bcl2 over-expression in cancer cells promote tumor cell survival through direct interaction with VDAC1, inhibiting cytochrome c release and thereby, apoptotic cell death. Targeting VDAC1-based peptides to tumor cells over-expressing anti-apoptotic proteins may prove an effective VDAC1-based cancer therapy, acting to minimize the self-defense mechanisms of cancer cells, thereby promoting apoptosis.

Modes of coupling between GPCRs, G proteins and effectors: the case of G protein-coupled K⁺ channel (GIRK)

Nathan Dascal

Department of Physiology and Pharmacology, Sackler School of Medicine, Tel Aviv University, Tel–Aviv, Israel.

G protein-coupled receptors (GPCRs) regulate a plethora of effectors, some of them directly (by binding and altering their activity) and some via elaborated cascades that involve the production of second messengers or depletion of cellular resources. G protein activated K⁺ channels (GIRKs) present a prototypical direct G protein effector, being activated by binding of Gβγ subunit to cytosolic segments of GIRK. GIRK channels mediate much of the actions of inhibitory neurotransmitters in heart and brain and are activated by a wide array of G_{i/o}-coupled GPCRs, and it has been proposed that speed and specificity of GIRK signaling rely upon preformed GPCR/Gαβγ/GIRK complexes. In view of their physiological role and their importance as a model signaling cascade, mechanisms of coupling between GPCRs, G_{i/o} and GIRK have been widely studied, yet both the general picture and the molecular details remain unclear and debatable. We have developed functional, biochemical and biophysical assays and mathematical modeling, to address the existence and molecular composition of the signaling complexes, and details of their functioning and of reciprocal regulation of the proteins within the complex. Using a functional kinetic approach based on quantitative titration of expressed components of the signaling cascade, combined with imaging, FRET and biochemical approaches, we demonstrate that the coupling of GPCR to the rest of the signaling complex is of the catalytic collision type; thus, GPCR is not a permanent part of the complex. In contrast, our data strongly indicate that Gβγ is permanently associated with GIRK channels (once the GIRK1 subunit is present), whereas Gα_i is dispensable but has strong regulatory effects when present, and it is necessary for the correct gating of the channels on whole cell level. We have developed a mathematical model that incorporates the GPCR activation by agonist, the ensuing G protein cycle, and the activation of GIRK channel described by a Markov scheme based on single channel measurements. Our GPCR-G protein-GIRK model satisfactorily describes basal, agonist and Gβγ- evoked activity of the channel and allows testable predictions which are currently being tested in the experiment.

Regulation of the actin cytoskeleton that is critical for effector functions of immune cells

Mira Barda-Saad

*The Mina and Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan
52900, Israel*

Actin polymerization plays a central role in multiple and crucial aspects of the immune response, including antigen recognition, T-cell proliferation, migration, and invasion through tissues. Dynamic rearrangements of the actin cytoskeleton following T-cell antigen receptor (TCR) engagement, provide the structural matrix and flexibility for signal transduction. The Wiskott-Aldrich syndrome (WAS) is a hematopoietic disorder characterized by thrombocytopenia, increased susceptibility to infections, autoimmunity, and lymphoid malignancies. The disease is caused by mutations of the gene encoding the Wiskott-Aldrich syndrome protein (WASp), the actin nucleation promoting factor, which is exclusively expressed in hematopoietic cells, where it plays a key regulatory role in cytoskeletal dynamics. Our studies demonstrated that following T-cell antigen-receptor (TCR) activation, actin polymerization is driven by the WASp and is dependent on its dynamic localization. Late in the activation process, vesicles containing WASp leave the periphery and move along microtubules to a central structure for internalization and, most probably, degradation. In the current study, we identify the molecular mechanisms governing the degradation of the active WASp molecular complex. Our data indicate that following TCR activation, WASp is ubiquitylated through the WASp homology 1 domain (WH1), which is the region with the vast majority of WAS mutations. We demonstrate that WAS protein levels are tightly regulated via proteasome-dependent degradation. Using a high-resolution molecular-imaging technique in combination with standard biochemical analysis, we explored the degradation mechanism of WASp in activated T cells *in vivo*. Special focus was put on exploring both the molecular mediators and the functional consequences of the ubiquitylation process. This knowledge explains the susceptibility of WASp to degradation in the hematopoietic cells of WAS patients, and provides novel insights into the pathogenesis of WAS immunodeficiency.

A novel mode of chemotaxis: transendothelial migration of effector lymphocytes by contact-guided consumption of vesicle-stored endothelial chemokines

Ronen Alon¹, Ziv Shulman¹, Valentin Grabovsky¹, Shmuel J. Cohen¹, Eugenia Klein², Vera Shinder², Sara W. Feigelson¹, Itamar Goldstein³, Amos Etzioni⁴ and Adit Ben-Baruch⁵

¹Department of Immunology, The Weizmann Institute of Science, Rehovot, Israel; ²The Irving and Cherna Moskowitz Center for Nano and Bio Nano Imaging, The Weizmann Institute of Science, Rehovot, Israel; ³Immunology Program, Cancer Research Center, The Chaim Sheba Medical Center, Tel-Hashomer, Ramt-Gan, Israel; ⁴Department of Pediatrics, Meyer Children Hospital, Rambam Medical Center and the B. Rappaport School of Medicine, Technion, Haifa, Israel; ⁵Department of Cell Research and Immunology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

Endothelial-presented chemokines play a major role in lymphocyte adhesion and transendothelial migration (TEM). In the present study, we explored in vitro how inflammatory chemokines coordinate effector T cell TEM across inflamed endothelial barriers. Effector Th/Tc1 lymphocytes express adhesive LFA-1 and VLA-4 integrins which supported their arrest, spreading and crawling over high density ligands induced on cytokine-stimulated endothelial cells under shear flow. These integrin activities did not require inside-out activation signals from chemokines or endothelial selectins. Nevertheless, endothelial crossing required chemokine signaling to lymphocyte Gi-coupled receptors. Although none of the major endothelial-transcribed chemokines was deposited on the apical or basolateral endothelial surfaces, Gi signals triggered by these chemokines were necessary for formation of invasive protrusions and their merging into a subluminal leading edge. Strikingly, these two processes were promoted by chemokines stored in endothelial vesicles docked on cortical actin fibers directly beneath the apical and basolateral endothelial membranes. These vesicles released their content within integrin-stabilized lymphocyte-endothelial synapses resistant to chemokine blocking antibodies. We propose a new multistep cascade that is used by spontaneously adherent effector lymphocytes to sense and extravasate endothelial barriers in response to intra-endothelial chemokines.

Structure, domain organization and different conformational states of stem cell factor induced KIT dimers

Yarden Opatowsky^{1,2}, Irit Lax², Francisco Tomé², Franziska Bleichert³, Vinzenz Unger³ and Joseph Schlessinger²

¹The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan 52900 Israel; ²Department of Pharmacology; ³Department of Molecular Biology and Biophysics, Yale University School of Medicine, New Haven, CT 06520, USA

Activation of receptor tyrosine kinases (RTKs) is one of the most important molecular events in oncogenesis. Over the past three decades, much progress has been made in understanding the general mechanisms by which RTK function and stimulates their intracellular effectors. Yet, important questions concerning RTK activation and signaling remain unanswered due to a lack of information about the structural arrangements of intact RTKs. Here, we describe the three dimensional (3D) structure of the type III RTK - KIT in an intact, ligand-stimulated form, and show that analysis of the 3D structure of a whole receptor molecule provides valuable insights about receptor activation, signal transduction and pharmacological targeting.

The RTK KIT and its ligand stem cell factor (SCF) play an important role in the control of many cellular processes. Oncogenic gain of function mutations in KIT were identified in a variety of cancers including gastrointestinal stromal tumors (GIST), acute myeloid leukemia (AML), melanoma and mast cell malignancies.

We have used a combination of single-particle electron microscopy (EM), x-ray crystallography and biochemical characterization of KIT mutants to describe the structure of intact KIT receptor dimers induced by ligand stimulation. Visualization of the overall structural organization of the extracellular, TM and cytoplasmic regions of SCF induced intact KIT dimers reveals close contacts between each KIT protomer throughout the entire dimeric structure. Moreover, several forms of dimeric KIT molecules with different asymmetric arrangements of the two tyrosine kinase domains are identified. We propose that cooperative interactions mediated by multiple weak homotypic inter receptor contacts play a critical role in ligand induced KIT activation, cell signaling and oncogenesis.

Session B

”Stem Cells: From Basic Science to Tissue Engineering and Therapeutic Opportunities”

Human Pluripotent Stem Cells: Potential Implications for Basic and Applied Cardiovascular Research

Lior Gepstein

The Bruce Rappaport Faculty of Medicine. Technion - Israel Institute of technology, Haifa, Israel.

Myocardial cell replacement strategies are emerging as novel therapeutic paradigms for cardiac repair but are hampered by the paucity of sources for human cardiomyocytes. Human embryonic stem cells (hESC) are pluripotent stem cell lines derived from human blastocysts that can be propagated, in culture, in the undifferentiated state and coaxed to differentiate into cell derivatives of all three germ layers, including cardiomyocytes. In the current presentation, the derivation and properties of the hESC lines will be described as well as the spontaneous and directed cardiomyocyte differentiation system established so far using these cells. The possible applications of this unique differentiation system in several cardiovascular research areas such as developmental biology, functional genomics, (patho)physiological modeling, drug testing, and regenerative medicine will be discussed. Specific emphasis will be placed on the descriptions of the efforts performed to date to assess the feasibility of this emerging technology in the fields of cardiac cell replacement therapy and tissue engineering. We will next present the recently described induced pluripotent stem cell (iPS) technology and detail its potential impact on cardiac regenerative medicine, drug development, and patient/disease specific *in-vitro* modeling of cardiac disorders. Finally, the obstacles remaining on the road to clinical translation will be described as well as the steps required to fully harness the potential of these new technologies.

Identification of novel human renal stem/progenitor cells: relevance to cancer and regeneration

Benny Dekel

Pediatric Stem Cell Research Institute, Sheba Medical Center, Tel Aviv University.

The prospective isolation of several tissue-specific stem and progenitor cells has recently promoted the initial delineation of their properties and the beginnings of their utility in regenerative medicine. Nevertheless, in contrast with other organs, such as the hematopoietic system, in which the identification of surface markers enabled purification of tissue-specific stem cells, the lack of such in the kidney has hampered progress in identifying and isolating stem cells. Recent molecular advances have much contributed to our understanding of the cell lineages and the renal progenitor pool in the developing murine kidney. Utilizing this information and applying genomic profiling and a wide array of functional *in vitro* and *in vivo* assays on human cells selected from normal and cancerous kidney tissue has now afforded insights into relevant markers and enabled us the isolation of normal and malignant human renal stem/progenitor populations.

These novel human progenitor cell types are ideal targets for genetic manipulation, cell therapy and tissue engineering aimed at kidney repair and the treatment of kidney cancers.

Standardized Neural Stem Cells for Regenerative Medicine: How to Harness the Full Potential of Neural Rosettes?

Daria Bloch, Reuven Edri, Yoni Bhonker, Gil Manor and Yechiel Elkabetz

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Neural Stem cells (NSCs) are considered to hold therapeutic promise for modeling neural diseases, drug discovery and future cell replacement therapies. This is due to their ability to propagate extensively in vitro while retaining ability to provide an unlimited source of neurons, astrocytes and oligodendrocytes (multipotency). However, two less appreciated facts about isolated NSCs are their highly heterogenic nature and their narrow repertoire for generating specific neuron types.

In contrast, human embryonic (pluripotent) stem cells (hESCs) can extensively propagate in vitro while continuously retaining information to yield all neuronal and glial cell types. Nevertheless, also NSCs derived from such pluripotent stem cells rapidly lose their broad differentiation potential during in vitro culturing, and progress into restricted NSCs.

These findings suggest that, although remaining multipotent in vitro, NSCs follow a program of cell fate restriction that ultimately renders them with a very limited spectrum of neuronal cell potential. Importantly, this restriction occurs regardless of the neural tissue of origin, developmental stage, or currently available NSC culture techniques. Therefore, there is an enormous need to standardize NSCs for research and for future applications.

Our lab has a strong interest in translating developmental principles in vivo into differentiation paradigms in vitro using human embryonic stem cells. Our primary goal is to map the earliest developmental events from neural induction onset that lead to heterogeneity, and by that uncover mechanisms of self-renewal of primitive neuroepithelial cells – a key step for standardizing heterogenic NSC populations.

With this aim in mind, we have recently isolated a novel and early NSC type derived from human embryonic stem cells, termed Rosette-NSCs (**R-NSCs**). At their early stages R-NSCs exhibit considerable self-renewal and broad differentiation potential along CNS and PNS lineages, and are able to readily yield a wide range of neuron types that are inaccessible to later-stage, restricted NSCs such as midbrain dopamine neurons and spinal cord motoneurons.

We have started to map subpopulations within R-NSCs and characterize their potential and genetic/epigenetic signature. We have a set of R-NSC specific genes with functional roles in R-NSC biology, a first step in developing strategies to induce and maintain R-NSC self-renewal. We hope to gain fundamental insights into genetic/epigenetic mechanisms of neural potency and ultimately result in novel conditions for the continued in vitro expansion of fully potent R-NSCs - a key step towards establishing a stable expandable universal NSC population.

Injectable biopolymers for regenerative medicine applications

Keren Shapira-Schweitzer¹, Cesare Gargioli², Giulio Cossu², and Dror Seliktar¹

¹Faculty of Biomedical Engineering, Technion-IIT, Haifa, Israel; ²Fondazione Parco Biomedico San Raffaele, Rome, Italy.

Regenerative medicine aims to replace lost, damaged or failing tissues and organs, starting with proliferating cells and ending with reconstituted and functional in vivo cellular structures. In the context of in vivo muscle regeneration, the successful implementation of cell transplantation for treating ailments such as muscle atrophy will likely require not only a suitable cell source, but also a biocompatible and injectable biomaterial that can localize their effectiveness without compromising survival and host integration. The extracellular matrix (ECM) plays a pivotal role in determining cell behavior during the initial steps of graft integration, and also the longer term tissue remodeling processes leading to mature functional reconstituted tissues. Therefore, it is desirable to control in vivo cell engraftment and fate during tissue regeneration by mimicking the ECM using engineered biomaterials. Ideally, modifying the biophysical and mechanical properties of these biomaterials can be used as a strategy to elicit specific cellular responses. Moreover, an injectable hydrogel biomaterial, having such capabilities, will have an added advantage that it is easily engrafted in vivo in order to carry stem cells and/or bioactive molecules to promote tissue renewal. Our research is thus focused on the use of an injectable hydrogel made from polyethylene glycol (PEG) conjugated to Fibrinogen, and used as a cell delivery vehicle for muscle regenerative medicine. The PEG-fibrinogen (PF) is a highly biocompatible cell carrier when used in conjunction with a number of muscle-associated cell types, including mesoangioblasts (vessel associated progenitor cells), and freshly isolated myoblasts (i.e., muscle satellite cells). We have assessed the in vitro ability of mesoangioblasts and myoblasts to survive and generate functional myotubes and myofibers in the three-dimensional PF injectable hydrogel microenvironment. Each cell type was cultured in a PF hydrogel microenvironment for up to two weeks while maturation and de novo muscle function were documented in terms of contractile behavior and biomolecular organization. In vivo experiments using PF as a cell carrier were performed with an anterior tibialis crush injury model in the mouse. The in vivo results showed increased graft cell survival, improved in situ cellular retention and an overall improvement in cell engraftment as measured by newly regenerating skeletal muscle fibers. These findings indicate that the PF hydrogel biomaterial can be used as an injectable carrier for stem cells and their myoblast derivatives in regenerative medicine applications for treating muscle injury.

Embryonic pig pancreatic tissue for the treatment of diabetes

Yair Reisner

Department of Immunology, Weizmann Institute of Science, Rehovot, Israel.

Transplantation of pancreas islets has been used increasingly in the treatment of diabetes. Unfortunately, this curative modality is available for a limited number of patients only, due to the shortage of organs for transplantation.

During the past few years we have evaluated the potential of fetal pig precursor tissues as a new source for organ transplantation and have defined the optimal gestational 'window' for organogenesis of several tissues – the kidney (E28), pancreas (E42), liver (E28), spleen (E42), heart (E28) and lung (E56). The 'window of opportunity' was defined by three main parameters: tumorigenicity, growth and differentiation potential and immunogenicity. While no risk for teratoma could be detected following implantation of fetal pig pancreas, even when harvested at the earliest possible time point (E24), our analysis in SCID mice revealed optimal outcome following implantation of E42 pancreatic tissue. Most importantly, this appropriately timed fetal pancreatic tissue displays reduced immunogenicity, compared to later gestational time points and to adult tissue. In particular, we demonstrated that the E42 tissue is completely devoid of CD11c positive dendritic cells and therefore exhibits greatly reduced ability to serve as a target for direct immune rejection. However, the growing implants can still be rejected through the indirect pathway.

Further studies in the mouse model revealed that co-stimulatory blockade with anti-CD40L and CTLA4-Ig could overcome this rejection. However, as CD40L was shown to be thrombotic in humans, we searched for alternative agents that have already been approved for human use. Thus, very recently, we demonstrated that transient administration of anti-CD48 and CTLA4-Ig, in conjunction with FTY720 maintenance, is effective in this model.

A major concern in translating these results to primates is the potential of hyperacute or acute rejection mediated by pre-existing antibodies against antigens predominantly expressed on endothelial cells. However, our results in non-human primates (NHP) showed that islet-like structures, as well as other cells within the stroma, were primarily vascularized by host type (primate) endothelium. Thus, the ability of the growing implant to use host vasculature might be critical for graft survival and growth in the NHP recipient.

Indeed, one year follow-up of two NHP recipients demonstrated the ability of the grafts to cure diabetes. Both animals exhibited a very similar pattern of recovery of glucose control, parallel to the growth and development of the implant. Similarly to our results in the mouse model, a marked reduction of exogenous insulin requirement (i.e. less than 10% of the daily insulin dose used prior to transplantation) was noted by the fourth month after transplantation, reaching complete exogenous insulin independence during the fifth month post-transplantation, with full physiological control of blood glucose levels. A radioimmunoassay (RIA) specific for porcine C-peptide (i.e. non cross reactive with primate C-peptide) confirmed the presence of basal porcine C-peptide levels as early as 3 months following transplantation and marked elevation of porcine C-peptide blood levels was demonstrated in both by the 5th month.

Collectively, our studies in the NHP model demonstrate the proof of concept for correction of diabetes by transplantation of E42 pig pancreatic tissue. Further studies are now directed at defining the minimal immune suppression required for long term maintenance of this curative mode of transplantation.

*Abstracts of Student Lecture
Competition*

ZnR signaling, mediated by GPR39, reduces butyrate-induced colonocytes cell death via upregulation of clusterin

Limor Cohen¹, Israel Sekler² and Michal Hershinkel¹

Department of Morphology¹ and Physiology², Ben Gurion University, Israel.

The ZnR was suggested to serve as a link between extracellular zinc and cellular signaling pathways. The orphan receptor GPR39, recently found to be activated by zinc, may serve as the ZnR. We previously demonstrated that ZnR activation in colonocytes enhances MAP and PI3 kinase phosphorylation and upregulates the activity of the Na/H exchanger, NHE1. Colonocytes are exposed to the short chain fatty acid butyrate, which reduces survival and proliferation of human colon cancer cells. Here we show that zinc, acting through ZnR, reduces PARP1 cleavage and protects HT29 colonocytes from butyrate induced cell death. Silencing of GPR39 abolished zinc-dependent signaling and survival of butyrate treated colonocytes, indicating that GPR39 is mediating ZnR activity. Similarly, inhibition of MEK1/2 and PI3 kinases reversed the increased zinc-dependent survival of butyrate exposed cells. While NHE1 is an important regulator of intracellular pH, inhibition of NHE1 by cariporide did not decrease the survival of butyrate-treated HT29 colonocytes, suggesting that NHE1 does not mediate the zinc-dependent colonocyte survival. Instead, we show that zinc, via GPR39, increases the expression level of the anti-apoptotic protein clusterin in butyrate-treated cells. Furthermore, silencing of clusterin abolished the zinc-dependent survival in HT29 colonocytes. Our results elucidate a novel role for extracellular zinc, acting through ZnR/GPR39 to regulate clusterin expression thereby to enhance colonocyte survival.

Will also be presented as a poster (Poster #42)

Multi neuron activity monitoring using high-rate temporal focusing microscopy

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Two-photon dynamic calcium imaging of neural populations enables optical monitoring of spiking activity *in-vivo* and *in-vitro*. However, standard laser scanners are too slow for monitoring large neural population with sufficient temporal resolution. Temporal focusing is a simple approach for achieving optically sectioned wide-field excitation in nonlinear microscopy and multiphoton photo-manipulation. Temporal focusing based systems enhance data acquisition rate significantly and are also used for photo-stimulation of neural cells. By combining a detailed new geometrical optics model with Monte-Carlo scattering simulations, we theoretically analyze the dependence of the temporal focus and its broadening on the microscope's and the media's parameters. Theoretical predictions are validated using light-scattering phantoms and *ex-vivo* brain tissue.

By combining an amplified laser source with temporal focusing based imaging system we constructed a fast imaging system, with frame rates of up to 200 Hz, which is suitable for two and three dimensional imaging of large neural population. Initial physiological results from *in-vitro* rat retinas and neural cell cultures will be presented, and the potential advantages this approach could have for *in-vivo* imaging.

Will also be presented as a poster (Poster #43)

MOSES mice: The role of SIRT6 in the regulation of metabolism and aging

Yariv Kanfi, Victoria Peshti, Shoshana Naiman, Reuven Gil, Liat Nachum and Haim Y. Cohen

The Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel

The Sir2-related NAD-dependent deacetylases (sirtuins) are implicated in diverse biological processes, including DNA regulation, metabolism and longevity. Sirtuins are thought to be the underlying mechanism of the beneficial effects of calorie restriction (CR), a dietary regimen that extends the lifespan of various organisms. The aim of this study was to evaluate the potential role of SIRT6 in the regulation of metabolism and aging. We found that SIRT6 protein levels increase in response to CR. This finding suggests that SIRT6 overexpression might mimic, at least in part, the effect of CR on an organism's health and life span. To answer this question, a number of mice overexpressing exogenous SIRT6 (MOSES) were generated. While MOSES showed almost no apparent metabolic phenotype when fed a standard diet, they were notably protected from diet-induced obesity and associated metabolic complications. MOSES fed a high-fat diet accumulated significantly less visceral fat, and LDL-cholesterol and triglyceride levels were lower in comparison to control littermates. Moreover, MOSES displayed improved glucose tolerance. Gene expression analysis of adipose tissue revealed that SIRT6 overexpression is associated with down-regulation of a selective set of PPAR-responsive genes, and genes associated with lipid storage. The protective role of SIRT6 against metabolic disorders suggests that it might positively affect age-associated metabolic diseases and increase longevity. Indeed, lifespan study revealed an extension in the lifespan of SIRT6 transgenic male mice, while no difference was found between control and transgenic female mice. Taken together, this study shows a protective role for SIRT6 against the metabolic consequences of diet-induced obesity and demonstrates, for the first time, a pro-longevity role for a mammalian sirtuin.

Will also be presented as a poster (Poster #44)

CPVT-specific cardiomyocytes generated from induced pluripotent stem cells are arrhythmogenic in response to β -adrenergic stimulation

Atara Novak, Lili Barad, Naama Zeevi-Levin, Ronit Shtreichman, Avraham Lorber, Joseph Itskovitz-Eldor, Ofer Binah

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Aim: Sudden cardiac death caused by ventricular arrhythmias is a disastrous event, especially when it occurs in young individuals. Among the 5 major arrhythmogenic disorders occurring in the absence of a structural heart disease is Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT), which is a highly lethal form of inherited arrhythmias. Our study focuses on the autosomal recessive form of the disease caused by the missense mutation D307H in the cardiac calsequestrin gene, CASQ2. Since CASQ2 is a key player in excitation contraction coupling, the derangements in intracellular Ca^{2+} handling may cause delayed afterdepolarizations (DADs) which constitute the mechanism underlying CPVT. To investigate catecholamine-induced arrhythmogenesis in the actual CASQ2 mutated cells, we generated CPVT-specific cardiomyocytes differentiated from induced pluripotent stem cells.

Methods and Results: We generated for the first time CPVT-derived iPSCs by reprogramming fibroblasts from a skin biopsy, and demonstrated that the iPSCs carry the CASQ2 mutation. Next, iPSCs were differentiated to cardiomyocytes, which generated DADs and after-contractions in response to isoproterenol.

Conclusions: Our results show that the mutated cardiomyocytes can be used to study the electrophysiological mechanisms underlying CPVT and for tailoring patient-specific anti-arrhythmic therapy.

Will also be presented as a poster (Poster #45)

The regulation of a multi-molecular complex formation leading to actin polymerization in T-cells

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Following T-cell antigen receptor (TCR) engagement, multiprotein signaling complexes essential for regulating T-cell functions are formed. A key multi-molecular complex consisting of SLP-76, Nck and VAV is recruited to the T-cell/antigen presenting cell (APC) site during initial T cell activation and is crucial for regulation of the actin machinery. Most of the studies performed to date have concentrated on the role of the signaling molecules that comprise this molecular complex. However, the regulation of the complex formation and the cooperation and competition among its components during formation have not yet been explored. In this study, a multidisciplinary approach including molecular imaging, biochemical and biophysical techniques was utilized in order to define the composition, stoichiometry, and specificity of the SLP-76:Nck:VAV complex. High-resolution imaging techniques, such as fluorescence resonance energy transfer (FRET) analysis, were performed in live activated T cells in order to focus on the binding properties within the trimolecular complex. Our data reveal a direct interaction between Nck and VAV which is mediated by the binding of the C-terminal SH3 domain of Nck to VAV's N-terminal SH3 domain. Disruption of the VAV:Nck interaction significantly impaired actin polymerization. This research provides novel insights into the regulation of a signaling complex formation critical for immune response.

Will also be presented as a poster (Poster #46)

Direct and indirect roles of the mitochondria in caspase activation during *Drosophila* spermatogenesis

Liat Ravid

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Being at the core of the intrinsic apoptosis pathway, mitochondria have emerged as the central regulators of the apoptotic program in mammalian cells, providing a reservoir for protein factors, such as cytochrome C, which induce caspase activation upon their release to the cytosol. Recent studies suggest that the release of pro-apoptotic factors from the mitochondria is a two-step process, regulated by mitochondrial dynamics and cristae structures. Whereas a role for cytoplasmic cytochrome C in the activation of the apoptosome complex and apoptosis has been well established, the mechanisms by which mitochondrial dynamics promote apoptosis are still poorly understood.

The *Drosophila* sperm system is an ideal model to study the significance of the mitochondria for caspase activation/apoptosis, as we have previously shown that spermatids are the only known cells in *Drosophila* that absolutely require the mitochondrial pathway (i.e. cytochrome C) for caspase activation^{1,2}. Furthermore, we have identified eight different mutants in a genetic screen, which in addition to blocking caspase activation, also display severe defects in late mitochondrial structural organizations. Finally, using bioinformatic analysis we revealed duplication of 50 out of 500 mitochondrial genes where one of the duplicates is somatic and the other is testis-specific. Although the reason for this gene duplication phenomenon is unclear, it allows us to investigate possible additional functions of essential proteins, such as those involved in oxidative phosphorylation.

We mapped three of the mitochondrial mutants, one of which corresponds to one of the two *Drosophila cytochrome c1 (cyc1)* genes. Cyc1 is a component of the respiratory chain complex III, which passes electrons to cytochrome C, and from there to complex IV. As opposed to *cytochrome c* mutant spermatids, which block caspase activation in spermatids, but still display an overall normal mitochondrial structural organization², fluorescent and electron microscopy analyses of *cyc1* mutants revealed severe defects in the exit from the fused state, and hence the abnormal unfurling and elongation of the mitochondrial derivatives. Since a role for Cyc1 in the regulation of caspase activation/apoptosis has never been demonstrated, we started investigating possible mechanisms. For this, we set up three major goals: (1) Determine whether Cyc1 is required for mitochondrial localization of cytochrome C and/or its release to the cytosol. (2) Investigate whether the roles of Cyc1 and/or cytochrome C in caspase activation are independent of their major roles in respiration. (3) Investigate the role of Cyc1 in mitochondrial dynamics during spermatogenesis. We have made major progress in each of these aims. Collectively, our findings thus far suggest that Cyc1 indirectly regulates caspase activation, independently of cytochrome C, by affecting energy metabolism and the structural organization of the mitochondria.

1. Arama,E., Agapite,J., and Steller,H. (2003). Caspase activity and a specific cytochrome C are required for sperm differentiation in *Drosophila*. *Dev. Cell* 4, 687-697.
2. Arama,E., Bader,M., Srivastava,M., Bergmann,A., and Steller,H. (2006). The two *Drosophila* cytochrome C proteins can function in both respiration and caspase activation. *EMBO J.* 25, 232-243.

Will also be presented as a poster (Poster #47)

*Abstract of the
Magnes Plenary Lecture*

Lighting up the nervous system

Ehud Isacoff

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The ability to selectively stimulate specific signaling proteins in select neurons can provide a powerful approach for investigating their role in circuits and behavior. We have synthesized a family of tethered glutamate photoswitches that covalently bind to glutamate receptors and modulate their activity in response to millisecond long pulses of light. Key photophysical and chemical properties of the photoswitches will be examined, including: a) “Photolithography” to constrain switch attachment spatially; b) Analog control of photocurrent magnitude; c) Bi-stable photoswitching that enables sustained activation in the dark to minimize photodamage and interference with behavioral assays; d) Agonist and antagonist switches; e) Altered receptors that are light-gated but minimally sensitive to native transmitter; g) Different attachment sites that flip the wavelength dependence of activation, yielding a pull-push control over activation; h) Protein engineering to change excitatory light-gated receptors into inhibitory ones; i) Light-gated NMDA receptors; and j) Light-gated GPCRs: our first light-gated metabotropic glutamate receptor, which we use to remote control excitability and transmitter release. Experiments will be described that employ light-gated receptors, channels and pumps to probe neural circuit development and function in behaving zebrafish.

Abstracts of Invited Speakers
(Sessions C & D)

Session C

“From Synapse to Brain”

Synaptic tenacity: Insights from live imaging experiments

Noam E. Ziv

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The human brain consists of a vast number of neurons interconnected by specialized communication devices known as synapses. It is widely believed that activity-dependent modifications to synaptic connections – synaptic plasticity - represents a fundamental mechanism for altering network function, giving rise to emergent phenomena commonly referred to as learning and memory. This belief also implies, however, that synapses, when not driven to change their properties by physiologically relevant stimuli, should retain these properties over time. Otherwise, physiologically relevant modifications would be gradually lost amidst spurious changes and spontaneous drift. We refer to the expected tendency of synapses to hold onto their properties as "synaptic tenacity".

We have begun to examine the degree to which synaptic structures are indeed tenacious. To that end we have developed unique, long-term imaging technologies that allow us to record the remodeling of individual synaptic specializations in networks of dissociated cortical neurons over many days and even weeks at temporal resolutions of 10-30 minutes, and at the same time record and manipulate activity levels in the same networks. These approaches have allowed us to uncover intriguing relationships between network activity, synaptic tenacity and synaptic remodeling.

Correlation of spontaneous and evoked synaptic inputs of cortical neurons

Ilan Lampl

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Only about a fifth of the neurons in the cortex are inhibitory cells and yet they are central in the regulation of network activities. In recent years in-vivo intracellular recording techniques were developed to measure the relations between excitation and inhibition in different sensory areas. Yet, little is known on the mechanisms that regulate their balance during stimulation or during ongoing activity. At first, we asked whether excitation and inhibition adapt equally to sensory stimulation. To address this question, we patched layer 4 neurons in lightly anesthetized rats while applying repetitive whisker stimulation. We found that inhibition adapts more than excitation during repetitive whisker stimulation, causing the balance between these inputs to shift towards excitation. Because the lag of evoked inhibition relative to the onset of excitation during repetitive stimulation remains constant despite the large reduction in inhibition, we suggest that adaptation of inhibition occurs at the inhibitory synapses rather than by reduced firing of inhibitory neurons. The more pronounced adaptation of inhibition may act as a gain mechanism which enhances the subthreshold response during sustained stimulation, despite a large reduction in the strength of excitatory input. In the second part of my talk I will present experiments that addressed a long lasting debate on the instantaneous relations between excitation and inhibition. Some studies suggested that these opposing inputs balance each other only on average, when measured at time scales of many milliseconds, while others proposed a correlation on the fine millisecond scale. We developed a new approach that is based on simultaneous intracellular recordings from pairs of nearby cortical cells. Our studies suggest that the balance between excitation and inhibition depends on past history of stimulation and that inhibitory control of excitation has high temporal precision.

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A unified network model of coexisting dynamical regimes in hippocampus

Sandro Romani and Misha Tsodyks

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Hippocampus exhibits different regimes of activity in different behavioral states of the animal. During locomotion, hippocampal activity oscillates at theta frequency and cells fire at specific locations in the environment (place fields). As the animal runs through a place field, spikes are emitted at progressively earlier phases of the theta cycles. During immobility, hippocampus exhibits irregular bursts of activity, with occasional rapid orderly activation of place cells expressing a possible trajectory of the animal. It is not known how these different regimes emerge and what causes the switch between them. We propose an attractor network model which encodes a map of the environment in its recurrent connections endowed with short-term synaptic depression that accounts for such a diverse range of behaviors. Network behavior can be rapidly controlled via modulation of the external inputs. Thus attractor networks with short-term plasticity are flexible enough to subservise different behavioral states of the animal. "

Cortical dynamics of sensory processing: stimulus-specific adaptation and novelty detection

Israel Nelken

Department of Neurobiology, Alexander Silberman Institute of Life Sciences, Hebrew University of Jerusalem, Israel.

The responses of neurons in auditory cortex to sounds depend very strongly on the recent past, going back many seconds. Thus, the responses of a neuron to a tone is reduced when the tone is presented often, but this reduction in response does not generalize, or generalizes only partially, to other frequencies, a phenomenon called stimulus-specific adaptation (SSA). I will discuss a number of recent findings which show that the responses to both common and rare sounds are in fact sensitive to more than just their probability. Thus, the responses to a common tone depend on whether the stimulation sequence is fixed or random; and the responses to the rare tone decreases if instead of a single common frequency, the common tone is roved by tiny amounts. These results suggest that the responses in auditory cortex are in fact determined by the novelty of the stimuli and the change they represent with respect to a detailed representation of the past stimulation sequence.

The plastic multisensory human brain: insights from brain plasticity and sight restoration efforts in the blind

Amir Amedi

Dept. of Medical Neurobiology, IMRIC, Faculty of Medicine & Program of Cognitive Science, The Hebrew University of Jerusalem (HUJI), Jerusalem, Israel. Inter-disciplinary center for neuronal computation (ICNC), HUJI.

In the talk I will cover novel spectral analysis approaches to study the human brain using fMRI. These methodologies were applied to study central questions in perception, brain plasticity, large-scale brain dynamics and multisensory integration. In unisensory perception experiments we used it to reveal many novel topographical maps of the body (somatosensory, motor, visual). For brain plasticity and brain dynamics we used it to reveal the large scale re-organization in the blind for language, memory and perception (but also in sighted, i.e. developmental vs. adult plasticity). I will focus on new recent results imaging blind's brain after learning to use visual-to-auditory sensory substitution algorithms to 'see' using a webcam and soundscapes (aka artificial vision). Finally, I will present how such methods can be used to start looking into the binding problem: how we integrate information into a coherent percept, an old question in neuroscience which has relatively poor answers, especially in humans.

Acknowledgment: This work was supported by the Human Frontiers Science Program Career Development Award, an EU-FP7 MC International Reintegration Grant, an Israel Science Foundation grant and a German Israeli Foundation grant (to AA). Lab website: <http://brain.huji.ac.il/> contact: amir.amed@ekmd.huji.ac.il

Session D

“Cell Death and Proliferation”

The ‘apoptotic’ caspases as regulators of inflammation: New lessons from the study of caspase-8 function

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While the ‘inflammatory’ caspases promote inflammation by mediating the generation of cytokines such as IL1- β , the apoptotic caspases serve to prevent inflammation by safeguarding against release of alarmins from the dying cells. Findings will be presented to demonstrate that caspase-8, which is mainly known for its key role in immune-mediated induction of apoptotic death, also serves to restrict inflammation in other, more direct, manners. One of the ways in which it does so is by associating with the RIG-I helicase complex and suppressing its signaling for activation of IRF3, a transcriptional factor dictating the expression of interferon. As in the case of death induction by caspase-8, its effect in the RIG-I complex depends on the proteolytic activity of this caspase. However, rather than prompting massive cleavage of a large set of cellular substrates through activation of a cascade of other caspases this function of caspase-8 leads to disassembly of the specific protein complex with which caspase-8 associates and thus to arrest of its signaling activity. The action of caspase-8 within the RIG-I complex is temporally restricted. It is coordinated with RIG-I signaling in a way that links maximal activation of the signaling to its pursuant termination. Specific deletion of *caspase-8* in the epidermis — a tissue where massive cell death constantly occurs — results in chronic skin inflammation associated with IRF3 activation. Detailed characterization of this process suggests that, besides restricting the activation of this transcription factor by viral stimulators of the RIG-I pathway, caspase-8 also serves to withhold the activation of this pathway by nucleic acids released from epidermal cells that disintegrate during cornification.

Vital roles of deadly apoptotic caspases: Lessons from flies

Eli Arama

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The cysteine-aspartate proteases called Caspases are key executioners of apoptosis. However, caspases are also involved in a number of vital cellular processes, including cell differentiation, cell signaling, and cellular remodeling. To date, more than two dozen vital cellular processes have been described in both insects and mammals (1). Nevertheless, very little is known about how caspases promote these vital processes and how some cells avoid death in the presence of these deadly proteases. In *Drosophila*, spermatids terminally differentiate by removing their bulk cytoplasmic contents in a process that requires active caspases (2). Recently, we discovered a ubiquitin-pathway mechanism for spatial regulation of caspase activation in spermatids, which permits caspase-dependent differentiation while preventing full-blown apoptosis (3). Based on these studies and others, we propose that transient and low activation of caspases may promote vital processes, whereas their high and prolonged activity induces apoptosis. I will discuss our recent findings using the sperm system, dendritic pruning (4), and other *Drosophila* systems.

1. Feinstein-Rotkopf Y and Arama E (2009) Can't live without them, can live with them: roles of caspases during vital cellular processes. *Apoptosis*. 14:980-995.
2. Arama E, Agapite J and Steller H (2003) Caspase activity and a specific cytochrome C are required for sperm differentiation in *Drosophila*. *Dev. Cell* 4:687-697.
3. Kaplan Y, Gibbs-Bar L, Kalifa Y, Feinstein-Rotkopf Y and Arama E (2010) Gradients of a Ubiquitin E3 Ligase Inhibitor and a Caspase Inhibitor Determine Differentiation or Death in Spermatids. *Dev. Cell* 19:160-173.
4. Schoenmann Z, Assa-Kunik E, Tiomny S, Minis A, Haklai-Topper L, Arama E and Yaron A (2010) Axonal degeneration is regulated by the apoptotic machinery or a NAD⁺-sensitive pathway in insects and mammals. *J Neurosci*. 30:6375-6386.

Studying the complexity of cell death by combining reductionist with systems level approaches

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The mammalian cell death network comprises three distinct functional modules, apoptosis, autophagy and programmed necrosis. Together they constitute a network of approximately 200 proteins connected to each other in a non linear fashion via different types of post translational modifications. To establish new pathways within the PCD map and further analyze the global network's structure/function organization we currently undertake in the lab two main approaches. One is the classical bottom up approach in which we focus on a group of death associated genes previously isolated in our lab by performing genetic screens (named the DAP genes). The biochemical pathways which link these individual proteins to the particular cell death phenotype which they drive is then identified. One example refers to the DAPk/Beclin-1 connection. We found that DAPk phosphorylates Beclin-1 on T119 located at a critical position within its BH3 domain, and thus promotes Beclin-1 dissociation from Bcl-X_L and autophagy induction. These results reveal a substrate for DAPk that serves as one of the core proteins of the autophagic machinery. Another recent example refers to the link of DAP1, a novel substrate of mTOR, to autophagic regulation. This small scaffold protein suppresses autophagy and prevents its over activation by introducing specific brakes into the system. For studying the systems level organization of PCD we assessed the extent to which the inter-modular connectivity affects cell death performance. To this end, we developed a platform which is based on single and double sets of RNAi-mediated perturbations targeting combinations of apoptotic and autophagic genes. We measured the outcome of perturbations on the overall cell death responses, on the output of each module, and on the molecular responses in the proximity of the knocked-down genes and at distal sites. By running this platform in etoposide treated cells we have recently identified an autophagic gene which is recruited to the apoptotic machinery and further discovered its molecular mode of action. This platform potentially has a wide, general scope of applicability and serves as a basis for future modeling of the cell death system including all its branches.

The transcription factor E2F: Balancing proliferation and apoptosis

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The retinoblastoma tumor suppressor, Rb, restricts cell cycle progression mainly by regulating members of the E2F transcription factor family. The Rb/E2F pathway is often inactivated in human tumors, resulting in deregulated E2F activity that promotes proliferation or cell death, depending on the cellular context. Alterations in cell proliferation and cell death pathways are key features of transformed cells and, therefore, understanding the variables that determine the outcome of E2F activation is pivotal for cancer research and treatment.

MicroRNAs (miRs) are small non-coding RNA molecules that have recently emerged as critical regulators of gene expression and are often deregulated in cancer. In particular, miRs encoded by the miR-15a, miR-16-1 cluster appear to act as tumor suppressors while miR-21 is overexpressed in a wide variety of human tumors and exhibits oncogenic activity.

Our recent studies demonstrate that expression of miR-15, 16 and 21 is regulated by E2F and their levels are elevated upon activation of E2F. Importantly, miR-15 and miR-16 inhibit expression of cyclin E, the latter a key direct transcriptional target of E2F pivotal for the G1/S transition. Additionally, miR-21 inhibits expression of the tumor suppressor maspin, which is a transcriptional target of E2F mediating E2F-induced apoptosis. These data raise the possibility that E2F, E2F-regulated miRs and their target genes constitute feed forward loops that modulates E2F activity. In support of this, inhibition of miR-15 expression enhances E2F-induced up regulation of cyclin E1 levels as well as E2F-induced G1/S transition. Furthermore, inhibition of miR-21 expression enhances E2F-induced up regulation of maspin as well as E2F-mediated apoptosis.

In summary, our data identify the cancer-related microRNAs, miR-15, miR-16 and miR-21, as novel transcriptional targets of E2F that, in turn, modulate E2F activity and in particular affect the balance between E2F-induced proliferation and apoptosis.

Single Cell Analysis of Cell Cycle Variability

Itamar Simon

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One of the recent focuses of systems biology studies is to distinguish between mechanisms that are defined solely by the genetic sequence of organisms and those resulting from non-genetic factors. Analyses of clonal populations at the single-cell level have revealed a wealth of phenomena that were previously masked by population level measurements. Even in uniform environmental and genetic backgrounds, cells may adopt different fates. Of particular interest is the variability in basic cell cycle features of a genetically identical population of cancer cells since it may explain the variability in drug response of such populations. Recent advances in microscopy techniques and cell cycle markers allow tracking the cell cycle in the single cell level thereby enabling the accurate description of the cell cycle variability and the identification of its sources in a systematic manner. Live cell imaging followed by automatic image analysis, allowed us to study the basic cell cycle properties of mouse L1210 lymphoblastic cell line. We have characterized both the variability of basic cell cycle parameters and the changes in those parameters along cell lineages. We found that i) a significant portion of the variability resides in the S/G2 phase; ii) there is no memory of those features between generations and iii) cell division is symmetric therefore sister cells are identical.

*Abstracts of Posters**

** Arranged alphabetically according to the family name of the presenting (underlined) author.*

Effect of c-Jun on HTLV-1 LTR activation by TPA

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Adult T-cell leukemia (ATL) is an aggressive lethal malignancy with short life survival that does not respond to current anti cancer medications. However, after infection the virus enters into latency and its carriers remain asymptomatic. Development of ATL is thus dependent on activation of the latent virus. Therefore, strategies avoiding the possibility of virus re-activation could be of particular importance for ATL prevention in HTLV-1 carriers. Establishing such strategies requires deep understanding of the factors that can re-activate the virus and the mechanism of their action. In a parallel study we have shown that TPA activates HTLV-1 LTR in Jurkat T-cells through PKC α - and PKC ϵ -antagonized mechanism and that this mechanism involves binding of an Sp1-p53 complex to an Sp1 site residing in the Ets responsive region 1 (ERR-1) of the LTR. However in H9 T-cells TPA was found to activate the LTR in two consecutive phases, the first is induced by PKC η through a mechanism requiring the three 21bp repeats of the LTR. The second phase involves a mechanism resembling that observed in the Jurkat cells, except that in H9 cells it is antagonized by PKC δ . The present study was undertaken to explore the downstream molecular events mediating these two mechanisms. We found that in H9 cells PKC η elevated the level of non-phosphorylated c-Jun which promotes the first LTR activation phase by binding to the 21bp TRE repeats. No such 21bp-dependent activation was observed in Jurkat cells because there was no elevation of non-phosphorylated c-Jun in these cells. However, PKC α and PKC ϵ in Jurkat cells, and PKC η and δ in H9 cells, elevated phosphorylated c-Jun, which, by interacting with the Sp1-p53 complex, prevented its binding to the Sp1 site. This binding of the phosphorylated c-Jun proved to block the ERR-1-associated LTR activation. Therefore, this PKC-antagonized LTR activation started in both cell types only when the relevant antagonizing PKCs were downregulated during the extending exposure to TPA.

HTLV-1 LTR activation by factors participating in the mitochondrial apoptotic pathway

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In a parallel study we have shown that HTLV-1 LTR can be activated by DNA-damaging and other stress-inducing environmental factors and that this activation can be blocked by Bcl-2. This finding indicates that the LTR activation by such factors is mechanistically linked to the mitochondrial apoptotic pathway. It should be noted that besides their apoptotic functions each of the factors participating in this pathway also interacts with many cellular proteins and induces, thereby, a wide range of non-apoptotic biological effects. LTR activation seems very likely to be one of them, In order to identify the exact apoptotic factor that directly mediates the LTR activation we examined in the present study the LTR activating capacity of each of the factors participating in the mitochondrial pathway. First, we found that perturbation of the mitochondrial outer membrane, by ectopic expression of either caspase 2 or Bax, activated reporter gene driven by the LTR. This activation was inhibited by Bcl-2 which is known to inactivate Bax. These findings indicate that caspase 2 exerted its effect through Bax activation. Next we found that shRNA against cytochrome C inhibited the LTR activation by both Caspase 2 and by Bax, indicating that cytochrome C, secreted from the perturbed mitochondria, was involved in their LTR activating effect. This was further confirmed by showing that ectopic expression of cytochrome C activated the LTR. Then we found that the pan-caspase peptide inhibitor z-VAD-fmk prevented the activation of the LTR by cytochrome C, proving that the effect of cytochrome C was mediated by one or more of the downstream activated caspases. To identify the exact activating caspase we re-examined the effect of cytochrome C in the presence of specific peptide inhibitors of caspase 9, caspase 3, caspase 6 and 7 and found that only the anti caspase-9 inhibitor blocked the LTR activation. This finding was re-confirmed by showing that shRNA against caspase 9, but not of caspase 3 caspase 6 and caspase 7, inhibited the LTR activation. These findings suggest that caspase 9 is the ultimate factor of the mitochondrial apoptotic pathway that mediates the LTR activation by the stress-inducing agents. A further support to this conclusion emerged from the next experiment showing that ectopic expression of caspase 9, but not of caspase 3 and caspase 7, stimulated the LTR expression.

Bcl-xL mediates its anti-apoptotic effect through direct interaction with the mitochondrial protein, VDAC

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The anti-apoptotic proteins of the Bcl2 family are expressed at high levels in many types of cancer. However, the mechanism by which Bcl2 family proteins regulate apoptosis is not fully understood. Here, we demonstrate the interaction of both Bcl2 and Bcl-xL with the outer mitochondrial membrane protein, voltage-dependent anion channel 1 (VDAC1).

Direct interaction between VDAC1 and the Bcl2 proteins was reflected in Bcl2 and Bcl-xL reducing the channel conductance of VDAC reconstituted into a planar lipid bilayer. The VDAC domains interacting with these anti-apoptotic proteins were defined using site-directed mutagenesis. Synthetic peptides corresponding to the VDAC1 N-terminal region and selected cytosolic loops bound specifically, in a concentration and time-dependent manner, to immobilized Bcl-xL, as revealed by real time surface plasmon resonance (SPR) technology. Expression of the VDAC1-based peptides in cells over-expressing Bcl-xL prevented Bcl-xL-mediated protection against staurosporine induced release of cytochrome *c* and subsequent cell death. These results point to Bcl-xL as promoting tumor cell survival through binding to VDAC1, thereby inhibiting cytochrome *c* release and apoptotic cell death. Moreover, these findings suggest that using VDAC1-based peptides to interfere with the binding of Bcl2 anti-apoptotic proteins to VDAC may represent a practical modality by which to potentiate the efficacy of conventional chemotherapeutic agents.

An interaction between the enzyme cyclooxygenase-2 and the EP1 prostaglandin receptor mediates a new mechanism of reciprocal regulation

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The enzyme cyclooxygenase (COX) catalyzes the rate-limiting step in the reduction of arachidonic acid into prostanoids-bioactive lipids that regulate diverse cellular and pathophysiological function. Of the five different prostanoids formed by the actions of COX and subsequent synthases, the most abundant, prostaglandin E₂ (PGE₂) exerts its effects through four subtypes of G protein-coupled receptors (GPCRs) called prostaglandin E receptors (EP₁₋₄). Unlike "typical" GPCRs that signal mostly on the plasma membrane or its vicinity, EP receptors reside in nuclear membranes and several intracellular structures (*e.g.* Golgi, endoplasmic reticulum), where they engage in functional signaling that is not fully understood. Despite the proximity in their location and functional relationship, COXs and EP receptors are currently viewed as independent entities, with no direct connection between them. Contrary to this belief, we found that the COX-2 isoform and the EP₁ receptor interact with each other *in vitro* and *in vivo* and that this interaction is dynamic and depends on the conformation of enzyme. Furthermore, we show that elevation in the cellular levels of COX-2, is followed by an increase the levels of the EP₁ receptor, but at the same time an attenuation in certain EP-mediated downstream signaling. Finally, we show that elevation in EP₁ receptor levels causes COX-2 levels and activity to significantly decrease. Based on these data we propose a new model whereby a previously unknown interaction between COX-2 and the EP₁ receptor modulates functional aspects of both. Specifically, we suggest that an induction of COX-2 expression upregulates EP₁ receptor levels, an event that in turn lowers COX-2 levels in a negative feedback mechanism. This new model ascribes a previously unknown role for EP₁, and possibly other GPCRs, in regulation of synthesis and/or degradation of COX. Furthermore, it suggests a new role for COX-2 in regulation of GPCR signaling pathways. Identifying a direct link between cellular EP receptors and the enzymes that generate their ligands provides an additional regulatory mechanism for both, and is likely to generate new targets of intervention in many diseases such as inflammation and cardiovascular pathologies.

Effects of deletions in the extra- and intracellular domains of a chimeric acetylcholine-glutamate Cys-loop receptor on channel activity

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Eukaryotic Cys-loop receptors are pentameric ligand-gated ion channels (pLGICs) that respond to neurotransmitters such as acetylcholine, serotonin, γ -aminobutyric acid, glycine, glutamate or histamine by opening (and closing) of an intrinsic transmembrane channel. Each subunit has a long extracellular N-terminal segment, four transmembrane helices (M1 to M4) and a long intracellular M3-M4 connecting segment (“M3-M4 loop”). Recently, the X-ray crystal structure of a prokaryotic pLGIC from the cyanobacterium *Gloeobacter violaceus* (termed “GLIC”) was determined at atomic resolution. This prokaryotic receptor does not respond to any of the aforementioned neurotransmitters, but it is activated by protons. In addition, compared to eukaryotic Cys-loop receptors, GLIC lacks a short N-terminal helical segment in its ligand-binding domain, and has a much shorter M3-M4 loop. To determine whether (or not) these segments are crucial for the function of a eukaryotic acetylcholine-glutamate Cys-loop chimeric receptor ($\alpha 7$ -GluCl β R), we deleted those segments of the chimera that are missing in GLIC. Ligand binding assays performed on transfected live cells indicate that chimeras lacking most of the M3-M4 loop can readily bind ^3H - α -bungarotoxin (a competitive antagonist) and nicotine (an agonist). These deletion chimeras were visualized on the cell surface by confocal microscopy using rhodaminylated α -bungarotoxin and specific antibodies. In addition, chimeras lacking the M3-M4 loop display ACh-induced currents with unchanged EC_{50} , Hill coefficient and ionic selectivity. In contrast, chimeras lacking the N-terminal helical segment do not bind ^3H - α -bungarotoxin, but they do migrate as non-degraded proteins in SDS-PAGE and are readily visualized on the surface of transfected cells with specific anti-HA tag antibodies. Electrophysiological experiments show however that acetylcholine, nicotine or protons do not activate the N-terminus truncated chimeras, indicating that the putative N-terminal helix of eukaryotic pLGICs is crucial for the functional integrity of their ligand-binding domain.

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Modulation of Kv7.1 (I_{KS}) channels by intracellular Ca^{2+} , Ca^{2+} -calmodulin - dependent processes and by PIP₂

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Kv7.1 is a member of a subfamily of voltage-gated potassium channels (Kv7) which plays important functions in various tissues including heart, kidney, stomach, pancreas and inner ear. The assembly of Kv7.1 with KCNE1 produces the I_{KS} potassium current that is crucial for the repolarization of the cardiac action potential. Both PIP₂ and Ca^{2+} -calmodulin (Ca^{2+} -CaM) were previously shown to modulate Kv7 channels. Here we investigated the regulation of Kv7.1 and I_{KS} channel activity by intracellular Ca^{2+} , Ca^{2+} -CaM -dependent processes and PIP₂ using the whole-cell and inside-out configurations of the patch-clamp technique in transfected CHO cells. Increasing intracellular free Ca^{2+} concentrations markedly depressed I_{KS} currents recorded in inside-out macropatches, with an IC₅₀ of 72 nM and a 91% inhibition at 300 nM free Ca^{2+} . In the presence of 10 μ M PIP₂, the inhibition of I_{KS} currents by intracellular Ca^{2+} was noticeably attenuated with an IC₅₀ of 375 nM and a 59% maximal inhibition at 10 μ M free Ca^{2+} , suggesting competition between PIP₂ and intracellular free Ca^{2+} . Interestingly, Ca^{2+} -CaM pull down experiments of purified His-tagged Kv7.1 C-terminal constructs with increasing PIP₂ concentrations revealed the existence of a competition between PIP₂ and Ca^{2+} -CaM binding to Kv7.1 C-terminus. In the presence of K⁺ pyrophosphate, a nonspecific phosphatase inhibitor, increasing intracellular free Ca^{2+} did not inhibit I_{KS} currents. Purified Ca^{2+} -CaM kinase II stimulated by more than 2.5-fold I_{KS} current amplitude, while purified calcineurin induced run-down of the currents. Thus, intracellular Ca^{2+} may trigger pleiotropic signals converging to I_{KS} channels and involving Ca^{2+} -CaM-dependent protein kinase and phosphatase and possibly lipid phosphatases.

Deficient regulation of G $\beta\gamma$ effectors by fluorescently labeled G α_{i3} subunits reveals two distinct aspects of coupling to GIRK channel

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G-protein Coupled Receptor (GPCR) signaling is one of the most important cellular signaling cascades, judging by the hundreds (~800) of genes in the human genome, their ubiquitous expression. Some cells express over a hundred different types. A common trait shared by ligand-bound GPCRs involves the activation of the heterotrimeric G $\alpha\beta\gamma$ complex. Subsequently, activated G α (G α^{GTP}) and free G $\beta\gamma$ bind and modulate their effectors. In the nervous system, activation of the pertussis-toxin (PTX) sensitive G $\alpha_{i/o}$ subunits, via inhibitory-neurotransmitter binding GPCRs, activates the G-protein Inward Rectifying K⁺ channel (GIRK). Prior to its activation via GPCRs, the GIRK channel participates in the control of the resting membrane potential. Moreover, GIRK enhances hyperpolarization when activated by GPCRs. It is becoming more and more apparent that the GIRK channel serves as a multi-protein scaffold (signaling complex) for various proteins, such as G $\beta\gamma$, G α and even RGS. The exact role of each protein in the complex, if any, is still unknown and remains under great debate. Both GDP and GTP bound G α were suggested to interact and regulate GIRK's activity. Our results, in conjunction with the reports of others, suggested to us that G α_{i3} and GIRK are in a stable or dynamic complex. We were interested in assessing the direct interaction between G α_{i3} and GIRK and have therefore labeled them using fluorescent proteins (FP) for the assessment of Förster Resonance Energy Transfer (FRET) signals. We have created and tested three different xFP-G α_{i3} subunits: N and C-terminally labeled (G α_{i3} NT, G α_{i3} CT, respectively) and internally labeled (G α_{i3} 117).

We have rigorously tested the functionality of our constructs and found that all G α_{i3} xFPs and *wt*-G α_{i3} bound purified G $\beta\gamma$ and GIRK1. However, G $\beta\gamma$ failed to enhance the G α_{i3} xFP-GIRK1 interaction, as observed for *wt*-G α_{i3} . Only G α_{i3} 117 and G α_{i3} CT, but not G α_{i3} NT, regulated GIRK's basal activity (I_{basal}), despite their comparable ability to bind G $\beta\gamma$. The muscarinic 2 receptor (m2R) activated the GIRK channel via PTX insensitive *wt*-G α_{i3} , G α_{i3} 117 and G α_{i3} NT, but not G α_{i3} CT. However, only G α_{i3} NT produced fast activation kinetics comparable to *wt*-G α_{i3} , whereas G α_{i3} 117 greatly slowed the activation kinetics. Expectedly, activation of m2R inhibited the neuronal N-type Ca²⁺ channel Ca_v2.2 in a voltage-dependent manner but only via *wt*-G α_{i3} and G α_{i3} NT, and not via G α_{i3} 117 or G α_{i3} CT. Only G α_{i3} 117 and G α_{i3} CT were able to restrict G $\beta\gamma$ -binding by Ca_v2.2, judging by reduction of the voltage dependent facilitation (VDF) prior m2R's activation. Only xFP-G $\beta\gamma$, but none of the xFP-labeled G α_{i3} subunits, showed FRET with GIRK1-CT-xFP.

Our results suggest that xFP labeling of G α , even at positions widely regarded as producing functional G α proteins, distort some or all of G α -effector interactions and regulations. Incidentally, the differential regulation of basal and evoked GIRK activity by G α_{i3} NT and G α_{i3} 117 reveal that control of GIRK's basal activity and the provision of "free" G $\beta\gamma$ for GIRK activation (following GPCR activation) are distinct functions of the G α subunit. Neither mechanism is a prerequisite for the other.

Method for a quantitative assessment of heart functions in Zebrafish embryo utilizing an analysis of high-speed video microscopic images

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The zebrafish (*Danio rerio*) possesses a host of advantages that have established it as an excellent model of vertebrate development. These include ease of genetic manipulation and transgenesis, optical clarity, and small size and cost. Biomedical researchers are increasingly exploiting these advantages to model human disease mechanisms. This model system has been extensively used to study the influences of gene mutation and manipulation on cardiac development and structure but has scarcely being used for functional analyses of the cardiovascular system because of lack of appropriate methodologies. In the present study we describe a method for a continuous measurement of heart function in zebrafish embryo. The method is based on a suite of software tools for the analysis of high-speed video microscopic images. It allows the measurements of ventricular heart diameter and perimeter during both diastole and systole periods, from which ejection fraction (EF) and fractional area changes (FAC) are calculated, serving two independent parameters of heart contractility. The method was validated by testing the classical effects of cholinergic and adrenergic agonists as well as the effects of cardiac steroids on heart contractility and rhythm in the zebrafish model: The addition of adrenalin to embryo standard media for 90 minutes increased, dose dependently, the heart force of contraction while the addition of Acetylcholine and Carbachol reduced it. The addition of the cardiac steroids Digoxin (1 μ M) and Bufalin (1 nM) dramatically increased both EF and FAC. The Bufalin-induced increase in contractility was inhibited by the synthetic cardiac steroids antagonist, 4-(3' α 15' β -Dihydroxy-5' β -estran-17' β -yl) furan-2-methyl alcohol. Our methodology for quantifying cardiac function in the zebrafish, together with the available genetic and molecular tools for this species, should provide an exceptional powerful combination for studying physiological processes and pharmacological mechanisms of the cardiovascular system.

Inhibition of BRCA1 expression and function by HTLV-1 Tax oncoprotein

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BRCA1 is multifunctional protein whose malfunction in breast epithelial cells accounts for 30-45% of all breast cancer cases. HTLV-1 is etiologically implicated with many different severe clinical disorders. The viral Tax protein, which is the key factor in the HTLV-1 pathogenic potential, is also multifunctional, but most of its functions are just opposing those of BRCA1. Therefore, we hypothesize that Tax expression within breast epithelial cells may likely antagonize BRCA1. This hypothesis is supported by our present study which shows, for the first time, that introducing Tax into breast epithelial cells inhibits the basal expression BRCA1 and its stimulation by estrogen in cells expressing the estrogen receptor-alpha (ER α) such as MCF-7 and MCF-10A. Ectopic expression of the transcriptional co-activators, CBP or p300, abolished Tax-mediated inhibition of both the basal and the estrogen-stimulated BRCA1 expression. This finding indicates that Tax exerts its inhibitory effect by competition for these co-activators which are required for activation of BRCA1 expression. In other experiments we found that BRCA-1 activates reporter genes driven by p53-targeted promoters. shRNA against p53 abolished this reporter activation, indicating that BRCA1 exerted this transcriptional activity by activating the p53 transcriptional function. Introducing Tax into these cells abolished this BRCA1 transcriptional effect by functional p53 inactivation. In view of these finding we speculate that introducing Tax into breast epithelial tissues of HTLV-1 infected women during their lactation, may inhibit BRCA1 expression and functions and create in these cells a BRCA1-deficiency-like micro-environment that leads to breast cancer development. This can likely occur in HTLV-1 infected women practicing prolonged breastfeeding, since breast milk of such women is loaded with HTLV-1 producing T-cells that can transmit the virus to neighboring breast epithelial cells via cell-to-cell contacts.

Activation of Human T-cell leukemia virus type 1 in human T-cells by TPA

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Human T-cell leukemia virus type 1 (HTLV-1) is the etiological agent of adult T-cell leukemia (ATL) and of tropical spastic paraparesis/HTLV-1 associated myelopathy. However, the vast majority of the infected people never develop an HTLV-1 related disease, since shortly after infection the virus enters into a dormant state, in which viral gene expression is very low. Thus generating such a disease requires activation of the dormant virus. Previous studies from our laboratory have indicated that the LTR of the virus can be activated by various environmental and intrinsic stress-inducing agents. In present study we investigated the effect of TPA on the LTR activation. We demonstrate here that TPA activates the LTR expression in Jurkat human T-cells by a mechanism that is antagonized by PKC α - and PKC ζ . Due to this antagonism the onset of this activation occurs after depletion of these PKCs by extended exposure to TPA or after blocking their function by a metabolic inhibitor. This mechanism operates through the Sp1 binding site located in the Est responsive region 1 (ERR-1) of the LTR. However, in H9 T-cell line TPA activates the LTR by two consecutive mechanisms. The first one can be blocked by PKC inhibitor, thus providing an initial indication that it depends on PKC activity. Subsequent experiments aimed to identify the effective PKC isoform have shown that this activation can be selectively abrogated by PKC α -specific shRNA, indication that it is mediated by this PKC isoform. Furthermore, using LTRs with various mutations, we have noted that this activation is hampered by mutations inserted in any of the 21bp Tax responsive element (TRE) repeats of the LTR, indicating that maximal LTR activation by this mechanism required all the three intact TRE repeats. The second mechanism of the LTR activation in these cells was found to resemble that of Jurkat cells, except that it is antagonized by PKC ζ . Since infected T-lymphocytes of latent HTLV-1 carriers may, individually, vary from each other with respect to the regulation of the viral LTR expression, these data indicate that physiological and pathological PKC-activating factors may activate the latent virus in this cell population by more than one mechanism.

A Pharmacological Approach for Carcinoma Tumor Imaging Using Near Infra-Red Labeled EGF

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Major clinical applications for molecular imaging are improvement of disease detection through increased image contrast between normal and diseased tissue and providing a cue for degree of response to specific therapies. The biological concentration of target for imaging is a critical point for successful development of novel diagnostic tools. Over-expression of epidermal growth factor receptor (EGFR) and its oncogenic forms is associated with many types of cancers including colorectal carcinoma (CRC) thus serving as a promising target for imaging purposes.

In this study, we used EGF labeled with a NIR dye (IRDye800CW), which has several relative advantages over visible fluorescence emission in terms of optical molecular imaging: less auto fluorescence and deeper penetration of the tissue for whole body imaging. Using EGF-NIR we imaged EGFR by three different approaches: i) *in vitro* - using a panel of CRC cell culture novel models specifically designed for imaging purposes; ii) *in vivo* - using mouse orthotopic CRC tumor models as well as iii) *ex-vivo* – investigating CRC human biopsies. The *in vitro* results indicated that EGF-NIR preserved its pharmacological properties and that the signal obtained from EGRF-positive cells was 15-fold higher compared with normal enterocytes. *In vivo* imaging showed a time dependent tumor accumulation of the EGF-NIR probe. The A431, CRC cell line, orthotopic tumors were observed after 4 hr post-injection and a significant optical signal was monitored up to 72 hrs. Images of isolated organs demonstrated a relative accumulation of EGF-NIR, in addition to the tumor, in the kidneys, liver, testis or ovaries (known to express EGFR). In human *ex-vivo* CRC biopsies EGF-NIR probe identified specifically the tumors overexpressing EGFR, findings further validated using Western blotting of tumor extracts.

These findings propose EGF-NIR as a suitable agent for bioimaging of EGFR-positive CRC tumors. Moreover, the novel pharmacological *in vitro* and *in vivo* models may serve as robust enabling platform technology for development and characterization of additional biomarkers and targeting agents.

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Reduction in endogenous brain ouabain elicits changes in catecholamines level and attenuates depressive behavior in the rat

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Depressive disorders are among the world's greatest public health problems. Na^+ , K^+ -ATPase is the established receptor for the steroidal digitalis-like hormones, one of which is ouabain. Alteration in brain Na^+ , K^+ -ATPase and endogenous ouabain have been detected in depressive disorders, raising the hypothesis of their involvement in these pathology. The present study was designed to further elaborate this hypothesis by investigating the behavioral and biochemical consequences of reduction in brain ouabain attained by the administration of anti-ouabain antibodies. SD Rat's behavior was evaluated by the forced swimming test (FST) an animal model of depression. Catecholamines and their metabolites in selected brain regions were measured by high performance liquid chromatography. Intracerebroventricular injection (i.c.v.) of ouabain-antibodies reduced depression-like symptoms in the forced swimming test in the rats and significantly changed the levels of catecholamines and their metabolites in brain areas associated with depression: In the ventral tegmentum, anti-ouabain antibody administration caused a significant reduction in DOPAC/DA and 5-hydroxyindoleacetic acid (5-HIAA) and a significant elevation in the DA levels vs that in the control. In the hippocampus there was a marked reduction in the levels of DA, epinephrine, norepinephrine and homovanillic acid, 5-HIAA and in the ratio 5-HIAA/5-HT in the anti-ouabain antibody-treated group. A significant increase in the ratio of NE/DA was found in this region in rats that received anti-ouabain antibodies. In the nucleus accumbens there was a significant increase in the ratio of DOPAC/DA following anti-ouabain antibody administration. These results are in accordance with the notion that malfunctioning of the Na^+ , K^+ -ATPase/ouabain system may be involved in the manifestation of depressive disorders and identify Na^+ , K^+ -ATPase as a potential new target for drugs for the treatment of these diseases.

UTP Reduces Infarct Size and Improves Mice Heart Function After Myocardial Infarct via P2Y₂ Receptor

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Pyrimidine nucleotides are signaling molecules which activate G protein-coupled membrane receptors of the P2Y family. P2Y₂ and P2Y₄ receptors are part of the P2Y family which is composed of 8 subtypes that have been cloned and functionally defined. We have previously found that Uridine-5'-triphosphate (UTP) reduces infarct size and improves cardiac function following myocardial infarct (MI). The aim of the present study was to determine the role of these receptors in cardiac protection following MI using knock out (KO) mice, *in vivo* and wild type (WT) for control. In each of the 3 experimental groups used (WT, P2Y₂ KO and P2Y₄ KO) there were 3 subgroups: sham, MI, and MI+UTP. 24 h post MI we performed echocardiography and measured infarct size using TTC staining on all mice. Fractional shortening (FS) was higher in WT and P2Y₄ KO UTP-treated mice (44.7±4.08% and 42.2±5.6% respectively) than the MI group (33.5±2.7% and 29±5.5%, respectively, p<0.01). However, the FS of P2Y₂ KO mice were not affected by UTP treatment (34.7 ±5.3% vs 35.9±2.9%). Similar results were obtained with TTC and Hematoxylin & Eosin staining. Moreover, Troponin T measurements demonstrated reduced myocardial damage in WT mice and P2Y₄ KO pretreated with UTP (8.8±4.6 and 7.2±2, respectively) vs untreated mice (12±3.1 and 9.8±3, respectively, p<0.05). In contrast, P2Y₂ KO mice pretreated with UTP did not demonstrate reduced myocardial damage. These results indicate that the P2Y₂ receptor mediates UTP cardioprotection, *in vivo*.

G-protein coupled K⁺ channel type 2, GIRK2, trisomy affects neuronal depotentiation, cognitive functions and reward mechanisms in mice

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G-protein coupled K⁺ (GIRK) channels generate slow inhibitory postsynaptic potential in the brain, via G_{i/o}-protein-coupled receptors (G_{i/o} PCR). GIRK channels consist of 4 types of subunits (GIRK1-4), of which the GIRK2 subunit is widely abundant in the brain and was implicated in various functions including memory, reward and motor coordination. In addition, the GIRK2 channels were found critical for depotentiation, a form of excitatory synaptic plasticity that involves activation of NMDAR to elevate GIRK channels on the plasma membrane. Interestingly, *kcnj6* gene, that encodes for GIRK2 was found to localize in the region triplicated in Down Syndrome (DS). DS is the most prevalent cause of mental retardation that results from the presence of an extra maternal chromosome 21 (trisomy 21).

The current study assessed the cognitive manifestation of GIRK2 trisomy in mice utilizing a mouse line that harbors three copies of the *kcnj6* gene (Smith et al., 1995). Using a set of behavioral and locomotor tests, we found that GIRK2 trisomy did not change mice motility, anxiety-like behavior and the circadian cycle dependent activity. However, these mice exhibited deficits in hippocampal-dependent learning and memory, i.e. the fear conditioning context test, and an alteration in the reward system function, as indicated by an increase in sucrose consumption in the sucrose preference task.

Electrophysiological measurements in hippocampal-primary cultures show elevation in basal GIRK current in triploid neurons. Channel current depotentiation was assessed following the removal of long term exposure to APV, an NMDAR blocker, indicated that basal GIRK currents did not increase in response to APV wash in triploid neurons, suggesting of depotentiation deficits. Indeed greater expression of GIRK1 and GIRK2, but not GIRK3, was evident in triploid mice hippocampus coinciding with the rise in GIRK currents.

To conclude, GIRK2 trisomy was found to affect neuronal activity, cognitive functions and reward mechanisms in mice, underlying the importance of genes dosage that encode for proteins with high potency to affect electrical activity. Better understanding the neurological manifestations related to abnormal *kcnj6* gene dosage may shed light on both GIRK2 functional roles in neural activity and DS pathologies.

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Is Endogenous Ouabain a Nerve Growth Factor?

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Ouabain is a steroid hormone synthesized in and released from the adrenal gland. The only established receptor for this hormone is the Na⁺, K⁺-ATPase, a major plasma membrane transporter. The interaction of ouabain with the Na⁺, K⁺-ATPase inhibits the enzymatic activity and induces cell specific activation of several signaling pathways. Among the various signal transduction cascades activated by ouabain is the pathway affecting cell growth. Accordingly, numerous studies have shown that ouabain induces proliferation of several cell types. Ouabain and the Na⁺, K⁺-ATPase are important players in the nervous system, nevertheless, their effect on neural cell viability and growth has been scarcely studied. We raised the hypothesis that endogenous ouabain and its receptor, the Na⁺, K⁺-ATPase, have a role in regulating neural cells viability, proliferation and differentiation. This hypothesis was tested by investigating the biological and physiological effects of ouabain on neural cell lines and embryonic mice dorsal root ganglia (DRG). In the present study we show that 1. ouabain-like compound is present at nM concentrations in human CSF and in fresh and commercial available sera. 2. Reduction of endogenous ouabain in the medium, by incubating it with anti-ouabain antibodies, resulted in attenuation of NT2 but not PC12 cells viability. This growth inhibition effect was partially rescued by the addition of 10 nM ouabain to the anti-ouabain antibodies treated cells. The addition of anti-ouabain antibodies to NT2 cells incubated in serum-free medium did not affect cell viability. 3. The addition of 1 nM ouabain to the incubation media stimulated NT2 cells viability as determined by MTT and Bradford protein assays as well as cell proliferation as assayed by Thymidine incorporation. 4. The addition of ouabain to mice DRG explants, expressing $\alpha 1$ and $\alpha 2$ isoforms of the Na⁺, K⁺-ATPase, dramatically and does-dependently enhanced neurite elongation, an indication for cell differentiation. These results strongly suggest that ouabain is a nerve growth factor and may participate in the regulation of neural cell viability and neurite outgrowth in normal development and pathological conditions.

The role of NGF and its receptors in skeletal muscle

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Disruption of membrane-associated proteins results in progressive muscle wasting and weakness in Duchene and congenital muscular dystrophies. Following sarcolemma damage during contraction, muscle injury produces dysregulation of structural and regulatory proteins, which accompany muscle fiber death. NGF transgenic knock out mice and anti-NGF transgenic mice result in severe skeletal muscle damage resembling myopathy and muscular dystrophy, implying that NGF plays an important role in muscle physiology. Our aim is to evaluate the role of NGF and its receptors on muscle proliferation, differentiation and regeneration. The pharmacological models employed include C₂C₁₂ skeletal muscle cell cultures as well as wild type and homozygote dystrophic *dy^{2J}* mice muscles. We characterized mRNA expression of NGF and its receptors p75^{NTR} and alpha9 integrin in both WT and *dy^{2J}* mice. In C₂C₁₂ cell cultures a similar expression of mRNA for NGF and p75^{NTR}/alpha9 integrin was found. Serum starvation induces C₂C₁₂ differentiation reflected by increased expression of the myogenic markers myoD and myogenin. In undifferentiated C₂C₁₂ cultures, the level of betaNGF secreted to the media is about 60pg/ml/24h while this growth factor was not secreted by differentiated cultures. The NGF tyrosine kinase receptor trkA was not detected in undifferentiated or differentiated muscle cultures or in the mice muscles. NGF was found to induce PGE₂ release in C₂C₁₂ cultures, a process most probably mediated by p75^{NTR}/alpha9 integrin NGF receptors. Clarification of the role of NGF in skeletal muscle and muscular dystrophy models will provide novel targets for drug development.

Coupling of mitochondria to store-operated Ca²⁺-signaling sustains constitutive activation of protein kinase B/Akt and augments survival of malignant melanoma cells.

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Mitochondria are emerging as a major hub for cellular Ca²⁺-signaling, though their contribution to Ca²⁺-driven growth- and survival-promoting events in cancer is poorly understood. Here employing flow cytometry to monitor mitochondrial and cytosolic Ca²⁺, we assessed trans-mitochondrial Ca²⁺-transport and store-operated Ca²⁺-influx (SOC) in malignant vs. non-malignant B16BL6 melanoma clones. Remarkably, mitochondrial Ca²⁺-fluxes measured in whole cells or in isolated mitochondria were accelerated in the malignant clones compared to their non-malignant counterpart clones. This coincided with enhanced SOC and high levels of constitutively active Protein Kinase B/Akt (PKB). Interruption of trans-mitochondrial Ca²⁺-transport in the malignant cells with an antagonist of the mitochondrial Na⁺/Ca²⁺ exchanger, CGP-37157, abolished SOC, inactivated PKB, retarded cell growth and increased vulnerability to apoptosis. The direct SOC blockade by silencing Stim1 produced a similar PKB inhibition, indicating that the crosstalk between SOC and mitochondria is essential to preserve PKB in constitutively active state. Finally, the retraction of mitochondria from sub-plasmalemmal micro-domains triggered by Fis1 over-expression inhibited SOC-coupled trans-mitochondrial Ca²⁺-flux, Ca²⁺-entry via SOC and PKB activity. Taken together, our data show that in the malignant melanoma cells, the functional and spatial relationship of up-regulated mitochondrial Ca²⁺-transport to the SOC sustains the robust Ca²⁺-responses and down-stream signaling critical for apoptosis-resistance and proliferation.

Cardiac steroids induce the formation of high density glycogen-microtubules granules in human NT2 cells

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The Na⁺, K⁺-ATPase enzyme is essential for cellular homeostasis maintaining Na⁺ and K⁺ gradients across the plasma membrane and indirectly affecting intracellular pH and Ca⁺⁺ and numerous transport systems. In addition, Na⁺, K⁺-ATPase is engaged in the assembly of protein complexes into functional micro-domains that transmit intracellular signals. A group of steroids, collectively termed cardiac steroids (CS), interact with Na⁺, K⁺-ATPase and inhibit its activity. Such compounds have been identified in mammalian tissues and are considered a hormone family involved in numerous physiological roles and pathological states. We have previously demonstrated that the interaction of low concentration of CS with Na⁺, K⁺-ATPase elicit the inhibition of endosomal recycling and the accumulation of endosomal vesicles in the cells. The mechanism for this process was elucidated and found to involve CS-induced inhibition of endosomal Na⁺, K⁺-ATPase. In the course of these studies we have discovered that treatment of human NT2 cells with CS resulted in the formation of protein-glycogen granules which join to form a large cluster of matter adjacent to the nucleus. These clusters were characterized by specific sugar staining and identification of glycogen granules using electron microscopy (EM). Microtubules associated with these clusters were identified by specific monoclonal anti-tubulin antibodies utilizing confocal microscopy and recognized as microtubule-like structures using EM. Dose- and time- dependent analysis revealed that while ouabain and digoxin are extremely potent in forming these granules, their aglycons, ouabagenin and digoxigenin, are not. This study shed light on a novel aspect of the biology of Na⁺, K⁺-ATPase and may have significant implications regarding the possible mechanisms of action of endogenous CS and pathological states associated with glycogen metabolism such as diabetes and glycogen storage diseases.

A possible role for GIRK4 in B cell migration

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GIRK4 is a potassium channel activated by G proteins from the Gi/o family. It is expressed mostly in atrial myocytes, where it is responsible for vagal response that decreases heart rate. There are also evidence of mild expression in other tissues such as the brain, pancreas, lung, kidney and spleen, where its role remains unclear. In order to explore GIRK4's expression pattern in the different tissues, we used transgenic mice expressing GFP under a GIRK4 promoter. Our observations revealed novel expression of GIRK4 in immune organs such as the spleen, bone marrow, and lymph nodes. Analysis of cell types expressing GIRK4, using FACS, resulted in exclusive expression of the channel in several B but not T lymphocytes. GIRK4's presence in GFP cells was confirmed by patch clamp and Real-time PCR. In B lymphocytes, the channel is expressed in a few mature lineages including the natural antibody secreting B1 cells.

FACS analysis of the cell content of inguinal lymph nodes and the peritoneum of GIRK4^{-/-} mice, revealed an increase in B cell portions in the lymph node, and a decrease in the peritoneal cavity. In vitro CXCL13-dependnet chemotactic migration assays, a chemokine specializing in B cell homing to the lymph nodes, revealed an increase in migration of B cells in knockout mice compared to cell from wt mice. Similar results were obtained in the presence Tertiapin-Q, a GIRK-specific channel blocker. These results confirm the involvement of GIRK4 in the CXCL13-dependent chemotactic migration assay.

We are currently exploring GIRK4's involvement in cell migration using 2-photon imaging in wt and knockout mice and elucidating the molecular mechanism where GIRK4 is involved. These finding are the first example of the involvement of GPCR-mediated ion channels modulation in B cell, and thus serves as a novel physiological path that translates chemical information into electrical activity in the immune system.

Identification of the binding site for the anthelmintic drug ivermectin in pentameric ligand-gated ion channels

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Ivermectin (IVM) is an antiparasitic drug widely used in veterinary medicine, mainly in cattle, to kill intestinal worms. Additionally, it is used to treat human parasitic diseases like Onchocerciasis (river blindness). The major target of IVM is a heteropentameric glutamate-gated chloride channel (GluCl α / β receptor), which is unique to invertebrates and belongs to the Cys-loop receptor superfamily. Nanomolar concentrations of IVM irreversibly activate the native GluCl α / β R, thereby causing sustained hyperpolarization, suppression of nervous impulses, paralysis and death of the nematode. Little is known about the IVM binding site(s). We first studied a chimeric α 7-GluCl β receptor that consists of the ligand-binding domain (LigBD) of the α 7 nicotinic acetylcholine receptor (α 7-nAChR) and the channel domain of a homopentameric GluCl β R. IVM was expected to increase the chimera's activity since the activity of the α 7-nAChR is enhanced by IVM and the homopentameric GluCl β R is unresponsive to IVM. Surprisingly, IVM strongly inhibited the chimeric α 7-GluCl β R suggesting that IVM does not bind exclusively to either of the domains, but binds somewhere at the interface between the LigBD and the pore domain. Next, we explored the natural target of IVM, the GluCl α / β R. Since the α -subunit is known to be responsible for IVM binding by the native GluCl α / β R, we replaced the β 8 β 9 and Cys loops of the β -subunit by the homologous loops of the α -subunit. These two loops reach the interface between the LigBD and the pore domain. Our results show that the aforementioned loop swapping significantly enhances receptor activation by IVM. Furthermore, site-specific mutagenesis suggests that IVM binds at the interface between the LigBD and the pore domain, in a crevice between the β 8 β 9 loop and the Cys loop of two adjacent subunits.

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The mode of coupling between GABA_B receptor, G protein and GIRK channel

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The GABA_B-heteromeric G protein-GIRK (G-coupled inward-rectifier K⁺) channel signaling cascade is an important pathway in the neuronal network, mediating inhibitory post-synaptic currents (IPSC's) and controlling neuronal excitability. However, the way specificity is achieved in this pathway is not well understood. The mode in which the GABA_B receptor and its effectors are coupled can affect the specificity, but is highly debated. Two suggested modes of coupling are pre-coupled complex, in which all of the signaling components sit in a preformed macromolecular complex, and collision coupling, in which all of the components freely diffuse in the plasma membrane. Recently, there has been increasing body of evidence that point to a macromolecular complex that comprises of GABA_B, G protein and the GIRK channel. We sought to investigate this by expressing GABA_B receptors with in *Xenopus laevis* oocytes, and studying various aspects of this signal cascade using electrophysiological and optical means.

We titrated the amount of the GABA_B receptor by injecting increasing amounts of its RNA into the oocytes. According to our model, increasing the amount of the receptor will cause faster activation kinetics, since each receptor could interact with more G proteins. However, if the proposed model of pre-coupled complex is correct, than increasing the amount of the receptor will not cause faster activation kinetics, since every receptor can interact with only a pre-determined number of proteins. We found out that increasing the amount of the receptor, causes faster activation kinetics. In contrast to the evidence that GABA_B and GIRK reside in a macromolecular complex, this data implies that the GABA_B receptor and the GIRK channel are dissociated before or during activation.

We also found that GABA_B affects the basal activity of the GIRK channel. High doses of injected GABA_B RNA cause a sharp decrease in the size of basal GIRK activity. There is a stronger effect on the basal activity when co-expressing G α of the i/o subfamily. Another surprising result is that very low doses of GABA_B RNA injected causes a significant increase in the basal activity of the GIRK2 homotetramer, and to a lesser extent of the GIRK1/2 heterotetramer. There was no significant change in the levels of expressed G α that affects the basal activity of GIRK. In an optical-based research, we also found out that increasing the amount of GABA_B causes also a decrease in the surface expression of fluorescent-tagged GIRK1/2. Understanding of molecular mechanisms of these novel effects is an important challenge for future studies.

Gradients of a Ubiquitin E3 Ligase Inhibitor and a Caspase Inhibitor Determine Differentiation or Death in Spermatids

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Caspases are executioners of apoptosis, but also participate in a variety of vital cellular processes. Here, we identified Soti, an inhibitor of the Cullin-3–based E3 ubiquitin ligase complex required for caspase activation during *Drosophila* spermatid terminal differentiation (individualization). We further provide genetic and biochemical evidence that the giant inhibitor of apoptosis-like protein dBruce is a target for the Cullin-3–based complex, and that Soti competes with dBruce for binding to Khlh10, the E3 substrate recruitment subunit. We then demonstrate that Soti is expressed in a subcellular gradient within spermatids, and in turn promotes proper formation of a similar dBruce gradient. Consequently, caspase activation occurs in an inverse graded fashion, such that the regions of the developing spermatid that are the last to individualize experience the lowest levels of activated caspases. These findings elucidate how the spatial regulation of caspase activation can permit caspase-dependent differentiation while preventing full-blown apoptosis.

Neurotoxins' Pharmacological Domains Undergo Rapid Evolution

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A diverse range of organisms utilize toxins for both capture of prey and defense. Those include mollusks, arthropods and vertebrates. Venom components might play a role in immobilizing, paralyzing, liquefying and killing a prey as well as deterring competitors and predators. Many of these peptides are neurotoxins that target specific types of ion channels and modulate their activity in a variety of mechanisms. Typically, a large array of peptides, clustered into several multigene families, is produced by each venom gland which enables the venomous animal to have the upper hand in the continued predator-prey arms race. The evolution of these large multigene families and the evolution of the individual toxins fit the 'birth-and-death' model of evolution.

Diversifying selection is thought to cause changes in toxins amino acid composition thus promoting its adaptation to specific targets and even enabling toxin discrimination between orthologous ion-channels. However, the way these evolutionary forces are distributed along the toxin molecule is still uncertain and it is yet to be determined whether the same forces dictate active-surface evolution within paralogs and of neurotoxins across the different life phyla and whether the same mechanism promote toxins' diversification and neo-functionalism. In order to address these questions, we utilized a newly developed strict model, Mechanistic Empirical Combination Model (MEC), to analyze the different selective pressures affecting the mature peptide within a variety of toxin families, species and venomous animals. We have analyzed various toxin families including: alpha- beta- and depressant scorpion toxins, hanatoxin-like spider toxins as well as alpha- and PLA₂ snake toxins. We then mapped residues under selection pressure on the protein surface and looked for correlation with known functional surfaces.

We were able to detect a clear correlation between toxin's pharmacological surface and rapidly evolving domains, rich in positively selected residues. On the other hand, patches of negatively selected residues were restricted to the non-toxic face of the molecule and most likely help in stabilizing the tertiary structure of the toxin. Our results provide clear evidence for a mutual evolutionary strategy of venomous animals in which adaptive molecular evolution is directed towards toxin regions that are implicated in protein-protein interactions, *i.e.* the active site, enabling both their diverse activity and selectivity. Furthermore, we propose that the binding domains of unstudied toxins could be readily predicted using evolutionary considerations, enabling the design of novel pharmacological tools with desired activity.

Linking NFAT to Ca²⁺-driven Growth- and Survival-promoting Signaling in Malignant Melanoma Cells.

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Our previous studies demonstrated that robust Ca²⁺-responses promote aggressive growth of malignant melanoma cells and their resistance to apoptosis-inducing stimuli [1, 2]. Proteins belonging to the Nuclear Factor of Activated T cells (NFAT)-family are classical targets of Ca²⁺-signaling, but their role in melanoma malignancy is largely unknown. Here, employing malignant B16BL6-8 (murine), JB/RH1 (murine) and GA (human) melanoma lineages we show that inhibition of NFAT using its blocker 9,10-dihydro-9,10[1',2']-benzoanthracene-1,4-dione (INCA6) dose- and time-dependently arrested *in vitro* growth of these cells, rendered cells vulnerable to apoptosis triggered by serum starvation and attenuated their migration capacities. This was associated with down-regulation of high basal NFAT-driven reporter gene activity, compromised nuclear localization of NFAT1c protein ubiquitously expressed in melanoma cells and reduced levels of cyclooxygenase 2 (COX2), mediator of NFAT signaling [3], indicating that effects of INCA6 were specifically related to the NFAT blockade. Remarkably, inhibition of NFAT transcriptional function, diminished nuclear NFATc1 trafficking and down-regulation of COX2 expression also followed blockade of protein kinase B/Akt (PKB), the constitutive activation of which in malignant melanoma cells is promoted by accelerated Ca²⁺-influx via store-operated channels (SOC) and crucial for their growth and survival [1, 2]. Indeed, sustained activation of NFAT in B16BL6-8 cells was coupled to robust SOC-mediated Ca²⁺-permeation, as opposed to the non-malignant Kb30 counterpart clone exhibiting low NFAT, PKB and SOC activities. Taken together, our findings implicate NFAT in Ca²⁺-driven tumor-promoting signaling events in melanoma and imply potential significance of its targeting for eradication of this frequently fatal cancer type.

[1]. Fedida-Metula et al. (2008), Carcinogenesis 29, 1546 -1554

[2]. Feldman et al. (2010), Cell Calcium 47, 525 – 537

[3]. Yiu et al. (2006), J Biol Chem 281, 12210 – 12217

Ischemic Post-Conditioning by Bilateral Carotid Occlusion Maintains Tissue Vitality following Focal Cerebral Ischemia

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Ischemic post-conditioning is a phenomenon in which interruptions to cerebral blood flow (CBF) during the post ischemic phase protect the brain from reperfusion injury and reduce infarct size.

The present study investigated the effects of transient bilateral carotid occlusion (BCO) prior to reperfusion of focal ischemia on mitochondrial metabolism and CBF.

Male Wistar rats underwent middle cerebral artery occlusion (MCAO) for 60 minutes, with or without BCO, which was induced 15 minutes before reperfusion for a period of 45 minutes. Monitoring in the core and the penumbra of the ischemic brain was performed using a unique Multi-Site – Multi-Parametric (MSMP) system, which measures mitochondrial NADH using the fluorometric technique and CBF using laser Doppler flowmetry. Short anoxia and spreading depression (SD) waves were induced in order to test the ability of the tissue to cope with oxygen deficiency and metabolic challenges following reperfusion.

MCAO lead to a decrease in CBF and an increase in NADH in both core and penumbra, a trend which was enhanced following BCO occlusion. After the removal of both occlusions all parameters returned to baseline. No metabolic disruptions were visible during short anoxia and SD waves following reperfusion.

These results imply that ischemic post-conditioning by BCO assists in maintaining tissue metabolic state following cerebral focal ischemia and may serve as a protective mechanism against mitochondrial injury.

The effect of acute hemorrhage on the Blood Supply and Mitochondrial Metabolic State in Vital and Less Vital Organs in vivo

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Severe body stress induced by hypoxemia and hypotension, may lead to total body energy state deterioration. The perfusion of the most vital organs is maintained at the expense of "less vital" organs.

In the present study, we used a multi-site multi-parametric (MSMP) monitoring system for real time evaluation of Tissue Blood Flow (TBF) and mitochondrial NADH fluorescence of the brain and the small intestine following hemorrhage. In group 1, uncontrolled hemorrhage, mean arterial pressure (MAP) was decreased to 40 mmHg within 2 minutes and shed blood was reinfused after 30 minutes. In group 2, controlled hemorrhage, during the 30 minutes MAP was kept at 40 mmHg. Then after, both groups were monitored for additional 2 hours.

During hemorrhage, in both groups, the intestinal TBF and NADH deteriorated, while the brain remained relatively well protected. In group 1, all parameters partly recovered within the hemorrhage phase, while in group 2 complete recovery occurred only after resuscitation. At the end of the experiment, both models showed a decrease in intestinal vitality (TBF decreased, NADH increased), while the brain metabolic state in group 2 slightly declined.

Our unique multi-parametric monitoring device demonstrated that, under hemorrhage, the small intestine responded entirely differently from the brain. This may suggest the potential usefulness of the monitoring of less vital organs, as proxy organs, in critical conditions such as massive hemorrhage. The present study also highlights the importance of mitochondrial function monitoring in similar conditions in the clinical environment.

Evidence for crosstalk between atrial natriuretic peptide and digitalis-like hormone families

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Natriuretic Peptides (NP) and Digitalis-Like Compound (DLC) are hormone families that act on the renal and cardiovascular systems in response to changes in salt and volume load. The aim of the present study was to test whether there is crosstalk between the two hormonal systems on cardiovascular function using pharmacological and biochemical methods. Reduction in circulating ouabain following active or passive immunization of rats, resulted in an increase in the sensitivity of rat aorta to atrial natriuretic peptide (ANP)-induced vasodilatation. Furthermore, ANP modulated ouabain-induced contraction in isolated guinea pig aorta and papillary muscle preparations: ANP at 1 nM significantly decreased ouabain (5 μ M)-induced contraction of aortic smooth muscle. In papillary muscle preparations, ouabain (1 μ M) caused a positive inotropic effect, manifested by an increase in pulse amplitude, +dT/dt, -dT/dt and resting twitch values. The addition of ANP (1 nM) prior to ouabain resulted in a significant decrease in the rise of +dT/dt and the resting twitch values, without affecting pulse amplitude and -dT/dt. At the biochemical level, ANP and ouabain at physiological concentrations caused an increase in Na⁺, K⁺-ATPase activity in rat heart microsomal preparations. Surprisingly, the ouabain-induced increase in activity was significantly inhibited by ANP. A similar result was obtained in ouabain- and ANP-induced ERK phosphorylation in primary adult mouse cardiomyocytes, in which ANP inhibited ouabain-induced ERK phosphorylation. These results point to the existence of a physiological crosstalk between NP and DLC actions on the cardiovascular system, which may be mediated through regulation of the Na⁺, K⁺-ATPase activity and/or signal transduction mechanisms.

Disrupted Wnt signaling affects neurogenesis under amyloid β -peptide

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The neuronal loss associated with Alzheimer's disease (AD) affects areas of the brain that are vital to cognition. Adult Neurogenesis in the subgranular zone (SGZ) is thought to play a role in learning and memory and impaired neurogenesis is associated to memory dysfunctions. Previous studies have shown that Wnt signaling has a major role in neural stem cells proliferation and differentiation. Moreover, Wnt signaling major components are found to be reduced in AD.

In this study, we investigated the efficacy of Wnt signaling to promote neurogenesis in *in vitro* model of AD. We cultured embryonic hippocampal progenitors (HP) and evaluated the effect of Wnt3a on A β ₄₂ treated cells differentiation. Changes in Wnt signaling components were analyzed by using immunocytochemistry, real-time PCR and In-cell western analysis. We demonstrated that oligomeric A β ₄₂ reduced neuronal differentiation *in vitro* accompanied with reduction of active β -catenin levels and proneural gene expression. Wnt3a was able to increase neuronal differentiation at the expense of astrocyte differentiation from HP treated with A β ₄₂ and its effect was also mimicked by treatment with the GSK-3 inhibitor L803-mts. We demonstrated that the effect of Wnt signaling was not due to increased proliferation or the rescue of neurons, but by committing HP differentiation to the neuronal lineage.

Our data show that Wnt signaling interruption induced by oligomeric A β ₄₂ may contribute to the impairment of neurogenesis in HP. We propose that activation of Wnt signaling or inhibition of GSK-3 may enhance neurogenesis and improve cognitive state in AD patients.

Arginine deprotonation underlies membrane voltage sensing

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Sensing of voltage across membranes is fundamental in many cell functions. S4-based voltage sensor modules (VSMs) are well known proteins that sense changes in the electric potential across a cell membrane. A stretch of several positively charged arginines on the fourth transmembrane segment (S4) that translates along the membrane electric field is the principal sensing component of the VSM. However, the energy cost for the movement of these charged arginines, which are paired with negatively charged residues on S1-S3 in a low-dielectric cavity, is incompatible with experimental data.

As channel conductance and voltage-dependent activation are affected by low external pH, we examined whether extracellular titration of arginines on S4 affects channel gating. We show that titration of the three outermost arginines on S4 stabilizes different states of the VSM of the bacterial voltage gated sodium channel (NaChBac) during its activation. Accordingly, we propose that the voltage sensor machinery of channel activation operates via a voltage dependent pK_a shift that controls the ionization of arginines and directs the movement of S4 while generating the capacitive gating current by protons relocation.

Postnatal treatment with Rimonabant is associated with ADHD-like symptoms in adulthood

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Attention Deficit Hyperactivity Disorder (ADHD) is characterized by inattention, impulsivity and hyperactivity. ADHD is a condition that becomes apparent in some children in preschool or early school years. It is estimated that 3-5% of children in USA and 5-10% in the Israel have ADHD. Although ADHD was identified over 80 years ago, the etiological and risk factors associated with ADHD are still unclear. Recently, low birth weight was found to be one of the most important predictive factors of ADHD. The CB₁ receptor antagonist Rimonabant (SR141716A) was developed for obesity to effectively reduce body weight. We hypothesised that treatment with Rimonabant might lead to ADHD-like symptoms. We have demonstrated (Rabichev et al., ICRS meeting 2009, USA) that a single injection of Rimonabant within 24 hours after birth leads to hyperactivity ADHD-like behaviour in adult mice (model 1).

In this study, we explore the effects of Rimonabant given orally to mothers (0.06 mg/ml) while feeding their offspring between postnatal days 1 to 15 (model 2). In another model we treated pregnant mothers orally (0.06 mg/ml) 12 hours before and 24 hours after giving birth (model 3). At 8 weeks of age, offspring mice were tested for pre-pulse inhibition (PPI) of the acoustic startle response. At the age of 9-10 weeks the mice were tested for motor activity in the open field and for anxiety in the 'plus-maze' tests.

Both male and female offspring mice of mothers that had been treated with Rimonabant at the end of their pregnancy (model 3) showed a significant increase in acoustic response in adulthood. In contrast, the response to acoustic stimuli in male offspring of mothers that had been treated with Rimonabant (model 2) was unchanged, while female offspring showed a significantly reduced response to acoustic stimuli compared with their vehicle control littermates.

In addition, both female and male offspring mice from each model showed a significantly reduced response in the PPI test, suggesting that their sensorimotor-gating system was affected. Both males and females displayed a significant hyperactivity in rearing and a significant increase in exploration behaviour in the open field test. In the plus-maze test, both males and females spent more time in the open arms than in the closed arms, suggesting a decreased vulnerability to anxiety-provoking situations. Taken together, these results suggest that a direct inhibition of the endocannabinoid system in offspring after birth or indirectly via the mother contributes to the development of ADHD-like behaviour in adult offspring.

Rapid desensitization of GIRK channel activity by a novel nonenzymatic mechanism of the G protein coupled receptor kinase

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G-protein coupled receptors (GPCRs) respond to agonists to activate downstream enzymatic pathways or to gate ion channel function. Turning off GPCR signaling is known to involve phosphorylation of the GPCR by GPCR kinases (GRKs) to initiate their internalization. The process, however, is relatively slow and cannot account for the faster desensitization responses required to regulate channel gating. Here we show that GRKs enable rapid desensitization of the G protein coupled potassium channel (GIRK/Kir.3x) through a mechanism independent of their kinase activity. Upon GPCR activation, GRKs translocate to the membrane and quench channel activation by competitively binding and titrating G protein $\beta\gamma$ subunits away from the channel. Interestingly, the ability of GRKs to effect this rapid desensitization depends on the receptor type. The findings thus reveal a stimulus-specific, phosphorylation-independent mechanism for rapidly downregulating GPCR activity at the effector level.

**Regulation of the actin cytoskeleton that is critical
for effector functions of immune cells**

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Actin polymerization plays a central role in multiple and crucial aspects of the immune response, including antigen recognition, T-cell proliferation, migration, and invasion through tissues. Dynamic rearrangements of the actin cytoskeleton following T-cell antigen receptor (TCR) engagement, provide the structural matrix and flexibility for signal transduction. The Wiskott-Aldrich syndrome (WAS) is a hematopoietic disorder characterized by thrombocytopenia, increased susceptibility to infections, autoimmunity, and lymphoid malignancies. The disease is caused by mutations of the gene encoding the Wiskott-Aldrich syndrome protein (WASp), the actin nucleation promoting factor, which is exclusively expressed in hematopoietic cells, where it plays a key regulatory role in cytoskeletal dynamics. Our studies demonstrated that following T-cell antigen-receptor (TCR) activation, actin polymerization is driven by the WASp and is dependent on its dynamic localization. Late in the activation process, vesicles containing WASp leave the periphery and move along microtubules to a central structure for internalization and, most probably, degradation. In the current study, we identify the molecular mechanisms governing the degradation of the active WASp molecular complex. Our data indicate that following TCR activation, WASp is ubiquitylated through the WASp homology 1 domain (WH1), which is the region with the vast majority of WAS mutations. We demonstrate that WAS protein levels are tightly regulated via proteasome-dependent degradation. Using a high-resolution molecular-imaging technique in combination with standard biochemical analysis, we explored the degradation mechanism of WASp in activated T cells *in vivo*. Special focus was put on exploring both the molecular mediators and the functional consequences of the ubiquitylation process. This knowledge explains the susceptibility of WASp to degradation in the hematopoietic cells of WAS patients, and provides novel insights into the pathogenesis of WAS immunodeficiency.

Molecular mechanisms involved in the cardiac steroids-induced aggregation of glycogen-microtubules granules in human NT2 cells

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Cardenolides such as ouabain and digoxin and bufadienolides such as bufalin, collectively termed cardiac steroids (CS), were widely used in the Western and Eastern clinical practices for the treatment of atrial fibrillation and heart failure. However, induction of arrhythmia and a narrow therapeutic window limits their therapeutic application. CS bind to and inhibit the ubiquitous transmembrane protein Na⁺, K⁺-ATPase which transports three Na⁺ out of the cell and two K⁺ into the cell, utilizing ATP hydrolysis as the driving force. In addition, the interaction of CS with the Na⁺, K⁺-ATPase elicits cell-specific activation of several intracellular signaling mechanisms. These include phosphorylation of Src-kinase/MAP-kinase and PKC, Ca⁺⁺ oscillations and changes in intracellular membrane traffic. We have discovered that treatment of human NT2 cells with CS resulted in the formation of glycogen-microtubules granules. The involvement of Na⁺, K⁺-ATPase in this effect was proven by demonstrating that modulation of Na⁺, K⁺-ATPase $\alpha 1$ isoform gene expression by SiRNA specifically affects the CS-induced granules formation. We also demonstrate that CS-induced ERK1/2 phosphorylation is essential but not sufficient for the CS-induced glycogen-microtubules granules formation. Furthermore, results of the effect of CS on the granules formation in the absence of glycogen and in the presence of nocodazole showed that the CS-induced glycogen redistribution and the microtubules deformation processes are independent of each other. These results prove that the signal for the CS-induced glycogen-microtubules granules formation is mediated by the Na⁺, K⁺-ATPase, ERK1/2 phosphorylation and additional yet unknown factor.

The Role of Cytosolic Phospholipase A₂ alpha in Neuronal Degeneration

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Alzheimer's disease (AD) is clinically characterized by dementia and pathologically by a progressive loss of neurons, senile plaques, neurofibrillary tangles and vascular deposits of amyloid-beta peptide (A β). Recent evidence indicates that inflammation occurs in pathologically vulnerable regions of the AD brain. Recent studies show that aggregated A β may cause direct neuronal damage and death. Cytosolic Phospholipase A₂ alpha (cPLA₂ α) is a requisite component in the cascade of events leading to the production of eicosanoids during acute and chronic inflammation.

The aim of the present study is to examine whether cPLA₂ α participates in cortical neuron damage induced by aggregated A β ₁₋₄₂. Addition of 1 μ M A β ₁₋₄₂ to primary cortical neurons caused a decrease in neuronal viability determined by cell count and MTT reduction, and induced immediate activation of cPLA₂ α detected by its phosphorylation of serine 505 and upregulation detected after 24 h. The presence of specific antisense against cPLA₂ α (AS) that prevented its upregulation also prevented neuron death induced by A β ₁₋₄₂. Like wise, A β ₁₋₄₂ induced activation of NADPH oxidase releasing internal superoxides that was inhibited in the presence of diphenyliodonium or Apocyanin. The presence of NADPH oxidase inhibitors prevented cPLA₂ α activation, indicating that cPLA₂ α is down stream to the oxidase. Neuronal cell death is not a result of necrosis as determined by LDH release, but probably of apoptosis indicated by caspases activation, Dapi and Tunel staining. Active caspase 8 was detected prior to cleaved caspase 3 and both were prevented by the presence of AS. Further more, exposure of neurons to A β ₁₋₄₂ for 24 h induced cPLA₂ α -dependet production of amyloid precursor protein (APP) that may result with higher accumulation of A β and enhance its toxic effect. Our results suggest that cPLA₂ α is involved in A β ₁₋₄₂ induction of neuron apoptosis via activation of caspases 8 and 3, and in the production of APP and thus may serve as a therapeutic target of AD.

Analysis of the contribution of domain swapping interactions to *Shaker* Kv channel gating reveals a dynamic upper voltage-sensor pore domain interaction surface

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Recent structural information on the Kv 1.2 potassium channel revealed two interaction surfaces between the voltage sensor and pore domains. While the lower interaction surface is intra-subunit and its contribution to the electro-mechanical coupling underlying channel opening is relatively understood, the upper (domain-swapped) interaction surface is inter-subunit and its contribution to the mechanism of Kv channel gating is not yet clear. Evolutionary information, mutagenesis and covalent cross linking analyses indicated that residues spanning the upper interface play an important role in Kv channel function. These analyses, however, did not provide mechanistic information regarding possible rearrangements associated with this interface during Kv channel gating. To further address the nature of the upper interaction surface, whether dynamic or static, we assessed the contribution of such inter-subunit domain swapping interactions to Kv channel gating by combining electrophysiology recordings of wild type and (upper interface) mutant proteins, introduced in the context of a tandem-dimer channel construct, with thermodynamic coupling analysis by means of double-mutant cycles formalism. Our results reveal that: (1) point mutations of pore and voltage-sensor residues at the upper interaction surface stabilize the closed channel state, (2) that pore-voltage sensor residue pairs across the upper inter-subunit interaction surface are coupled and (3) that the coupling is state-dependent and is stronger in the open channel state as compared to the closed state. Overall, our results suggest that the upper interaction surface is dynamic in nature and further support the assertion that the structure of the Kv channel solved is indeed that of the open channel state.

Yes I can: mechanisms underlying long-lasting sensory responses of accessory olfactory bulb mitral cells

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The ability to detect and process social information is essential for all social species and usually mediated, at least partially, by olfactory cues. In mammals, two of the sensory systems that deal with this kind of information are the main olfactory system (MOS) and the vomeronasal system (VNS), which are associated with reproduction, aggression, and parental behavior. Sensory neurons at the main olfactory epithelium project to the main olfactory (MOB), while sensory neurons at the vomeronasal organ project to the accessory olfactory bulb (AOB) where they synapse on AOB mitral cells. We previously showed that mitral cells of these two systems are characterized by different intrinsic properties and response patterns. Previous studies that used extracellular recordings from behaving mice showed that responses of AOB mitral cells to natural stimuli are long-lasting. Here we use whole-cell recordings from acute mouse olfactory bulb slices to show that a brief (0.1 ms) stimulation of the sensory afferents elicits a long-lasting firing response in AOB mitral cells, response that lasts between 10-60 s. Synaptic-like current injections into the cell's soma (SL) generated a similar long-lasting response, despite the presence of glutamate and GABA receptors antagonists. These results suggest a postsynaptic mechanism underlying these prolonged responses, rather than a network activity. We hypothesized that this property of AOB neurons is mediated by a calcium-activated non-selective cationic current (I_{can}). Indeed, the intracellular presence of BAPTA, a calcium chelator, reduces firing duration response of AOB mitral cells to SL stimulation by 45.7% and to synaptic stimulation by 89.5%. Application of the I_{can} blocker flufenamic acid to the recording chamber reduces the duration of AOB mitral cells firing response to SL stimulation by 43.9% and to synaptic stimulation by 79.8%. These results suggest that I_{can} activity is essential for AOB mitral cells prolonged responses.

Effect of propolis and caffeic acid phenethyl ester (CAPE) on NFκB activation by HTLV-1 Tax

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HTLV-1 is the etiological agent of an aggressive malignancy of the CD4⁺ T-cells, called adult T-cell leukemia (ATL) and of certain other severe clinical disorders (1). The viral Tax protein is a key factor in HTLV-1 pathogenicity. A major part of Tax oncogenic potential is accounted for by its capacity of inducing the transcriptional activity of the NF-κB factors, which regulate the expression of numerous cellular genes (2). Persistent activity of NF-κB factors has been proved to play a central role in the pathophysiology of ATL and other clinical disorders. PE, a natural product produced by honeybees, has been used for long time in folk medicine. One of its active components, caffeic acid phenethyl ester (CAPE), was found to be a potent inhibitor of NF-κB activation. The main aim of this project is to pursue the possibility of blocking all Tax oncogenic effects in the cytoplasm and the nucleus, by treatment with these products. The cells were transfected with a plasmid expressing Tax protein and plasmids containing the examined promoters. Our results showed that both PE and CAPE substantially inhibited the activation of NF-κB-dependent promoter by Tax. However, only PE could efficiently inhibit also the activation of SRF- and CREB- dependent promoters by Tax. Also, both tested materials strongly inhibited Tax binding to IκBα and β and prevented their induced phosphorylation and degradation by Tax. However, they were not able to prevent Tax or of NF-κB transport to the nucleus.

Mechanism of Stargazin modulation of Ca_v2.2 channel is Gβγ dependent

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The neuronal protein stargazin ($\gamma 2$), which belongs to a family of AMPA receptor regulatory proteins (TARPs), is associated with recurrent epileptic seizures and ataxic gait in mice. When first identified, stargazin and its other family members (termed $\gamma 3-8$) were classified as γ subunits of neuronal calcium channels due to structural and functional similarities to the skeletal muscle calcium channel $\gamma 1$ subunit. Detailed studies revealed a major effect of stargazin on AMPA receptor as well as inhibition of neuronal Ca²⁺ channel expression, whereas its effects on the biophysical properties of Ca²⁺ channels have yielded controversial results, casting doubt on stargazin role as neuronal γ subunit of calcium channels. Neuronal voltage dependent Ca²⁺ channels (class Ca_v2.1-3) are localized in synaptic terminals where they control transmitter release and synaptic communication. An important regulation of Ca_v2 channels is their negative control by activation of G protein coupled receptors, resulting in binding of the G protein $\beta\gamma$ subunit to the pore forming subunit of the calcium channel ($\alpha 1$). The role of TARPs in G protein mediated modulation of Ca_v2 type channels has not been investigated previously. We studied the mechanism of Ca_v2.2 regulation by stargazin and Gβγ in *Xenopus* oocytes expression system and *in vitro* protein interaction assays. Electrophysiological data showed that stargazin counteracts all Gβγ mediated inhibitory effects on Ca_v2.2 channel elicited either by coexpression of the Gβγ subunit or by activation of G protein coupled receptor (GPCR). These effects include: voltage-dependent facilitation, rightward shift of the conductance-voltage relation, changes in activation and inactivation kinetics, and inhibition by a G_i-coupled neurotransmitter. The effects of stargazin resembled those of high affinity Gβγ scavenger proteins m-cβARK or m-phosducin, but were somewhat milder. The effects of m-cβARK and stargazin were non-additive and mutually exclusive. Coexpression of Ca_vβ3 subunit diminished some of the inhibitory effect of Gβγ modulation by either stargazin or m-cβARK. The effect of stargazin is not unique to Ca_v2.2 as it also inhibits Gβγ mediated activation of the GIRK1/2 channel. In addition, the effect of stargazin on Gβγ mediated modulation of the channel is independent from its effect on channel surface expression and current amplitude. Biochemical data shows that stargazin binds Gβγ *in vitro*. In summary our results suggest that stargazin acts on the Ca_v2.2 indirectly, but via Gβγ dependent mechanism. We propose that stargazin is not a neuronal γ subunit of Ca_v2.2 channels, instead having a general role in opposing Gβγ mediated effects.

Different pacemaker mechanisms in human embryonic stem cell-derived cardiomyocytes

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Embryonic stem cells differentiate *in vitro* into spontaneously beating multicellular cardiomyocyte clusters, while recapitulating developmental stages of embryonic cardiomyogenesis. Human embryonic stem cell-derived cardiomyocytes (hESC-CMs) express specific cardiac markers and exhibit spontaneous action potentials (AP), $[Ca^{2+}]_i$ transients, and contractile activity. However, the mechanisms underlying the pacemaker activity in developing hESC-CMs are not well understood. In this work, we succeeded to record in the same cell spontaneous action potentials and the I_f current (HCN), using alternatively, the current-clamp and voltage clamp modes of the patch-clamp technique, in young isolated beating cardiomyocytes (d11-d21 after EB formation). Although I_f was expressed in all cells at variable amplitudes, no correlation was found between I_f current density and a specific cardiac subpopulation, based on APD₅₀ values. This heterogeneity of hESC-CMs was also confirmed by the lack of correlation between the APD₅₀ and the slope of diastolic depolarization (mV/ms). Blocking I_f (HCN) with zatebradine (10 μ M) or ZD7288 (25 μ M) as checked by voltage-clamp, and monitoring the consequences of I_f inhibition in the current-clamp mode, revealed two distinct hESC-CM phenotypes. One cell population showed a bradycardic pacing, followed by a progressive depolarization and vanishing of APs, suggesting an I_f (HCN)-dependent pacing mechanism. The pacing in these cells was insensitive to the application of NCX1 blockers such as KB-R7943 (3 μ M). The other cell population exhibited no modification of its pacing, despite the clear I_f inhibition, suggesting an I_f (HCN)-independent pacing mechanism. Adding NCX1 blockers like KB-R7943 (5 μ M) or the FRCRCF peptide (1 μ M) in this latter cell population led to bradycardia, followed by a progressive depolarization and vanishing of APs, suggesting an NCX1-dependent pacing mechanism. Thus, despite the ubiquitous expression of I_f , the hESC-CM cell population is heterogeneous and exhibits different pacemaker mechanisms, one depending on I_f (HCN) and another depending on NCX1.

Novel, caspase-independent, noncanonical cell death pathway in *Drosophila*

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Apoptosis, also referred to as type I cell death, is the major form of programmed cell death (PCD). Apoptosis depends on the action of proteases called caspases and is characterized by distinct morphological features. However, alternative cell death pathways have been also reported. Type II death displays the molecular and morphological features of autophagy, while necroptosis (type III death) exhibits a necrotic morphology manifested by a pronounced swelling of cytoplasmic organelles. Cell death pathways that exhibit mix morphologies were also reported and recent analyses of molecular networks point to many cross-talks among the different types of death pathways. Nevertheless, relatively little is known about the molecular mechanisms, constituents and physiological significance of these alternative death pathways. One reason for the slow progress in this field of research is the extremely few physiological paradigms available for studying alternative death pathways. We discovered and characterized a new noncanonical cell death pathway acting to normally execute about 20% of premeiotic germ cells (spermatogonia) in the adult *Drosophila* male. Using mutants in several key components of the apoptotic pathway, including caspases, we demonstrate that spermatogonial death is independent of the core apoptotic machinery. On the other hand, dying spermatogonia display high lysosomal activity, and specific mutations in the lysosomal pathway attenuate this death. Additional mechanistic studies and ultrastructural analysis are consistent with the idea that spermatogonial death is highly similar to type III death pathway. Importantly, the mitochondrial serine protease HtrA2/Omi was identified in a genetic screen as a positive regulator of this death pathway, providing a first physiological paradigm for the involvement of this protease in any type of cell death.

Single channel analysis of GIRK1/2 gating by G $\beta\gamma$

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In the present work we conducted kinetic analysis of single channel recordings of GIRK1/2 activity. GIRK1/2 channels were expressed in *Xenopus laevis* oocytes and channels activity was recorded in excised patches in presence of G $\beta\gamma$ in bathing solution. Dwell times histograms of open and closed times distributions were generated and subsequently fitted with 2 exponential and 5 exponential probability density functions respectively. 2 G $\beta\gamma$ concentrations were included in the analysis.

Comparisons between various parameters of open times distribution between 2 and 20 nM G $\beta\gamma$ demonstrated no statistically significant differences. The major changes in 4th and 5th exponential components (the longest closed times) took place between 2 and 20 nM G $\beta\gamma$. Moreover, it seems that the fast 3 states (1-3) are much more populated when the channel is activated by 20 nM G $\beta\gamma$ compared to 2 nM.

Two populations of bursts were defined based on the analysis of closed times distribution. Imposition of shorter critical time rendered distribution of relatively short bursts while the longer critical time segregated the channel activity to ~20 fold larger time scale phenomena which were defined as clusters. G $\beta\gamma$ concentration influenced mainly the burst frequency and the interburst interval, while cluster analysis renders more complex relationship showing G $\beta\gamma$ dependent changes in intercluster interval, number of events per cluster and cluster density.

To our knowledge this is the first attempt to analyze GIRK1/2 activation by G $\beta\gamma$ under true single channel patch clamp recording conditions. According to kinetic analysis conducted here it seems that G $\beta\gamma$ functions as an allosteric modulator which shifts the channel from reluctant to willing mode of gating, without an influence of fast channel gating events, namely, cluster and interburst gating is G $\beta\gamma$ dependent while intraburst gating is G $\beta\gamma$ independent. More single channel recordings containing GIRK1/2 activity in presence of a single G $\beta\gamma$ concentration will be required in order to generate detailed Markov model of channel gating.