2nd

LIFESCIENCEMeeting

PROGRAM

Innsbruck Universities

CONGRESSPARK IGLS

September 24 – 25, 2010

Organizers: Lukas Huber & Jörg Striessnig
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BIOCENTER INNSBRUCK + CMBI Center for Molecular Biosciences Innsbruck

Friday, September 24, 2010

8:30 – 8:45	Opening remarks
8:45 – 9:30	Plenary lecture (Chairman: David Teis) **Maria Sibilia** Institute for Cancer Research, Medical University of Vienna, Austria ***EGFR signaling networks in cancer development***
9:30 – 9:45	Break
	Session 1: **Nucleic acids and interacting partners,** short talks Chairman: Alexander Hüttenhofer
9:45 – 10:00	*Folding of a transcriptionally acting PreQ1 riboswitch* **Ulrike Rieder** (Institute of Organic Chemistry)
10:00 – 10:15	*Non-coding RNAs in Epstein-Barr virus infection* **Roland Hutzinger** (Division of Genomics and RNomics)
10:15 – 10:30	*Novel insights into the functional role of three protein arginine methyltransferases in Aspergillus nidulans* **Ingo Bauer** (Division of Molecular Biology)
10:30 – 10:45	*Electron induced splitting of the cyclobutane pyrimidine dimer: an important step in the DNA damage repair via DNA photolyase* **Achim Edtbauer** (Institute of Ion Physics and Applied Physics)
10:45 – 11:15	Break
	Session 2: **Bioinformatics,** short talks Chairman: Reinhard Kofler
11:15 – 11:45	*Bioinformatics for cancer immunology* **Zlatko Trajanoski** (Division of Bioinformatics)

Friday, September 24, 2010

11:45 – 12:00	*Delineating the transcriptional response of acute lymphoblastic leukemia cells to glucocorticoid treatment* **Johannes Rainer** (Division of Molecular Pathophysiology)
12:00 – 12:15	*Backbone Flexibility Controls the Activity and Specificity of a Protein-Protein Interface – Specificity in Snake Venom Metalloproteases (SVMPs)* **Hannes G. Wallnoefer** (Institute of General, Inorganic and Theoretical Chemistry)
12:15 – 12:30	*A novel pipeline for automated assembly and analysis of high throughput cDNA libraries* **Marek Zywicki** (Division of Genomics and RNomics)
12:30 – 14:00	Lunch
	Session 3, **Cell proliferation in development and regeneration,** short talks Chairman: Dirk Meyer
14:00 – 14:15	*Stem cell-specific activation of multiple ancestral forms of the myc protooncogene in the early metazoan Hydra* **Markus Hartl** (Institute of Biochemistry)
14:15 – 14:30	*Flatworms as model systems to study stem cells, regeneration, and reproduction* **Peter Ladurner** (Institute of Zoology)
14:30 – 14:45	*Molecular and Cellular Studies of Islet Cell Regeneration in Zebrafish* **Robin Kimmel** (Institute of Molecular Biology)
14:45 – 15:00	*Spindly/CCDC99 Is Required for Efficient Chromosome Congression and Mitotic Checkpoint Regulation* **Marin Barisic** (Division of Molecular Pathophysiology)
15:00 – 16:30	Poster session (odd numbers – 1, 3, 5, ...)
	Session 4, **Signaling and gene regulation,** short talks Chairman: David Teis
16:30 – 16:45	*Late endosomal p14/MP1-MAP kinase signaling couples IQGAP1 and Rac1 to focal adhesion dynamics and cell migration* **Natalia Schiefermeier** (Division of Cell Biology)

Friday, September 24, 2010	
16:45 – 17:00 17:00 – 17:15	*Role of TIS7 and SKMc15 in lipid metabolism* **Domagoj Cikes** (Division of Cell Biology) *Investigating the Role of PIDD in the DNA Damage Response* **Florian Bock** (Division of Developmental Immunology)
17:15 – 17:30	Break
17:30 – 18:15	Plenary lecture (Chairman: Bernhard Kräutler) **Adriano Aguzzi** Institute of Neuropathology, University Hospital of Zürich, Switzerland ***Mammalian Prion Biology***
18:15	Barbecue dinner for registered participants

Saturday, September 25, 2010

8:30 – 9:15	Plenary lecture (Chairman: Peter Ladurner) **Rik Korswagen** Hubrecht Institute, Utrecht, Netherlands ***Wnt signaling and cell migration in C. elegans***
9:15 – 9:30	Break
	Session 5: **Drug discovery and disease targets,** short talks Chairman: Andreas Bernkop-Schnürch
9:30 – 9:45	*Gating properties of a human disease-causing mutation in a CaV1.3 L-type calcium channel splice variant* **Alexandra Koschak** (Institute of Pharmacy/Pharmacology and Toxicology)
9:45 – 10:00	*Identification of novel antiinfluenza constituents from Alpinia katsumadai* **Ulrike Grienke** (Institute of Pharmacy/Pharmacognosy)
10:00 – 10:15	*Coloured Pigments from Chlorophyll Breakdown* **Markus Ulrich** (Institute of Organic Chemistry)
10:15 – 10:30	*Development and in vivo evaluation of an oral drug delivery system for paclitaxel* **Javed Iqbal** (Institute of Pharmacy/Pharmaceutical Technology)
10:30 – 10:45	*Role of neuropeptides in fear, anxiety and depression* **Simone B. Sartori** (Institute of Pharmacy/Pharmacology and Toxicology)
10:45 – 12:15	Poster session (even numbers – 2, 4, 6, ...)
12:15 – 13:00	Plenary lecture (Chairman: Lukas Huber) **Reinhard Fässler** Max-Planck-Institute of Biochemistry, Martinsried, Germany ***Genetic analysis of integrin signalling***
13:00 – 13:15	Award session, poster prizes and closing of the symposium

Posters

P1	*Unexpected association of apoE2 genotype with polyglutamine expansions in the human androgen receptor (SBMA)* Köhler A, Achmüller C, Karagiannidis AI, Bösch S, Klocker H, Schoser B, Löscher WN, Schneider R
P2	*The Nogo Receptor 2 (NgR2) is Required for Proper Innervation of the Skin* Bastian E. Bäumer, Alesja Rjabokon, Michaela Kress and Christine E. Bandtlow
P3	*Investigating gene deregulation in the CNS of the NgR2 knockout mouse* Sarah C. Borrie, Simone Sartori, Nicolas Singewald, Christine E. Bandtlow
P4	*Identification and characterization of Nogo protein complexes* Levent Kaya, Barbara Meissner, Christine E. Bandtlow
P5	*Highly Flexible System based on Mammalian Cell Culture for Identification of new Protein-Protein Interactions* Florian Kern, Bettina Sarg, Herbert Lindner, Christine E. Bandtlow, Rüdiger Schweigreiter
P6	*The role of Nogo in the epileptic rat brain* Barbara Meissner, Meinrad Drexel, Günther Sperk, Christine Bandtlow
P7	*Lentivirus-based knockdown of Rtn1A in cerebellar granule cells in vitro* Sickinger, S., Schweigreiter, R., Kistner, S., Sigl, R., Geley, S., Bandtlow, C. E.
P8	*Thiolated polymers: development and in vivo evaluation of an oral delivery system for leuprolide* Javed Iqbal and Andreas Bernkop-Schnürch
P9	*Thiolated chitosans: in vitro comparison of mucoadhesive properties* Christiane Müller, Andrea Verroken and Andreas Bernkop-Schnürch
P10	*Enhanced transport of Rhodamine 123 in presence of thiolated hydroxyethylcellulose in Caco-2 cells* Deni Rahmat, Duangkamon Sakloetsakun, Gul Shahnaz, Federica Sarti, Andreas Bernkop Schnürch
P11	*NOVEL THIOLATED MUCOADHESIVE NANOPARTICLES: FOR SUSTAINED DELIVERY AND DRUG TARGETING* Gul Shahnaz, Deni Rahmat and Andreas Bernkop-Schnürch
P12	*Novel and conserved function of the regulatory subunit of protein kinase A* Stefan E, Malleshaiah M, Michnick S , Bister K

Posters

P13	*Role of arginine methylation in the control of nuclear factor-κB (NF-κB)* Valovka T, Reintjes A, Khan MI, Hartl M, Bister K
P14	*Vibrational spectroscopy as a Tool to monitor the microwave-assisted drying process of wood* L. Bittner, C. Lux, V. Huck-Pezzei, J. Pallua, C. Pezzei, S. Schönbichler, H. Pulker, G.K. Bonn, C.W. Huck
P15	*Fourier transform infrared imaging analysis in discrimination studies of prostate cancer and prostate cancer cell lines* J. D. Pallua, C. Pezzei, G. Schaefer, C. Seifarth, G. Dobler, V. Huck-Pezzei, L. K. Bittner, H. Hahn, H. Klocker, G. Bartsch, G. K. Bonn and C. W. Huck
P16	*Collisionally Activated Dissociation (CAD) and Electron Detachment Dissociation (EDD) of proteins in negative ion mode Electrospray Ionization (ESI)* Barbara Ganisl, Monika Taucher, Kathrin Breuker
P17	*Identification, localization, and relative quantitation of pseudouridine in RNA by tandem mass spectrometry of hydrolysis products* Taucher M, Ganisl B, Breuker K
P18	*Isolation and identification of novel substrate proteins of a protein arginine methyltransferase specific for filamentous fungi* Allipour Birgani Sh, Bauer I, Golderer G, Lindner H, Brosch G
P19	*STAT1 Deficiency Results in Resistance of Neu/ErbB2-induced Mammary Tumors to Chemotherapy* Lára Hannesdóttir, Nina Daschil, Sebak Datta, Sonja Philipp, Johann-Benedikt Koller, Nirmala Parajuli, Elisabeth Müller-Holzner, Wolfgang Doppler
P20	*The Role of Tumor-Infiltrating Myeloid Cells in Mammary Tumors of MMTV-neu Stat1 WT and KO Mice* Tymoszuk P, Hannesdóttir L, Daschil N, Datta S, Nogalo A, Philipp S, Doppler W
P21	*Investigation of Spindly domains* Friederike Finsterbusch, Marin Barisic,Veronika Rauch, and Stephan Geley
P22	*Molecular characterisation of the orphan cyclin dependent kinase PCTAIRE-1/CDK16* P. Mikolcevic, R. Sigl, Benedicte Sohm, K. Pfaller, M. Hess and S. Geley
P23	*Human chromokinesins hKid/Kif22 and Kif4A are required for chromosome arm movement and congression* Wandke C, Barisic M, Sigl R, Rauch V and Geley S
P24	*Investigating the role of vertebrate Fzr1, a master regulator of G1 phase* Reinhard Sigl, Giridhar Shivalingaiah, Anamika Dayal, Manfred Grabner, Dirk Meyer, Pia Aanstad, Stephan Geley

Posters

P25	*Phosphorylation of p27Kip1 by JAK2 directly connects cytokine signaling to cell cycle control* Heidelinde Jäkel, Christina Weinl, Dominik Wolf, Ludger Hengst
P26	*FHL2 is a negative regulator of the cell cycle inhibitor p57^{Kip2}* Michael Kullmann and Ludger Hengst
P27	*Generation of transgenic flatworms* Glashauser J, De Mulder K, Berezikov E, Ladurner
P28	*frizzled genes are expressed dynamically in Hydra axial patterning* Jenewein M. and B. Hobmayer
P29	*Advanced Electron Microscopical Methods for Hydra* Salvenmoser W , Hess MW , Eder MK , Hobmayer B
P30	*The life cycle of the acoel flatworm Isodiametra pulchra under laboratory culture* Schnegg R, Hobmayer B
P31	*Regulation of the endosomal p14/Mp1-MAP kinase scaffold complex by the proteasome* de Araujo M.E.G., Taub N., Teis D. and Huber L.A.
P32	*Conditional Gene Ablation of the MAP Kinase Adapter Protein p14 in Dendritic Cells leads to Severe Disturbance of Tissue Homeostasis* J.Scheffler, F. Sparber, B. Reizis, P. Stoitzner, N. Taub, N. Romani, L. A. Huber
P33	*Late endosomal p14/MP1-MAP kinase signaling couples IQGAP1 and Rac1 to focal adhesion dynamics and cell migration* Natalia Schiefermeier, Taras Stasyk, Mariana E.G. de Araujo, David Teis, Zhigang Li, Hannes L. Ebner, Julia Scheffler, Martin Offterdinger, Sebastian Munck, Michael W. Hess, Sara A. Wickström, Anika Lange, David B. Sacks, Reinhard Fässler and Lukas A. Huber
P34	*Screening for MAP kinase scaffold inhibitors for proliferative and inflammatory diseases* Simon Schnaiter, Winfried Wunderlich, Beatrix Fürst, Johannes Kirchmair, Waczek Frigyes, Thierry Langer, Gyorgy Keri, Lukas Huber
P35	*Proteomic analysis of endosomes from genetically modified p14/MP1 mouse embryonic fibroblasts* Taras Stasyk, Johann Holzmann, Sonja Stumberger, Hannes L. Ebner, Michael W. Hess, Guenther K. Bonn, Karl Mechtler, Lukas A. Huber
P36	*P14 – A Potential Novel Host Defense Factor* N. Taub, M. Nairz, D. Hilber, G. Weiss, L.A. Huber

Posters

P37	*The role of Myosin Vb in the pathogenesis of Microvilli inclusion disease* C.Thöni , N. Schiefermeier, N.Yannoutsos, P.Filipek, T.Mueller, A.Janecke , H.Ebner, M.Hess, K.Pfaller, S.Geley, L.A.Huber
P38	*Non-coding RNAs in Epstein-Barr virus infection* Hutzinger R, Feederle R, Mrazek J, Schiefermair N, Zavolan M, Polacek N, Delecluse HJ and Hüttenhofer A
P39	*Aptamers as diagnostic markers in Chronic Kidney Disease* Andreas Ploner, Herbert Schramek, Günter Mayer and Alexander Hüttenhofer
P40	*Identification of non-coding RNAs involved in neural differentiation* *Konstantinia Skreka, Irina Roxanna Nat, Marek Zywicki , Mathieu Rederstorff,Mathias Heiss, Ahmad Salti, Marcel Scheideler, Georg Dechant and Alexander Hüttenhofer*
P41	*"Bimbam", a novel glucocorticoid-regulated BH-3 containing transcript from the BCL2L11/Bim Locus* Trockenbacher A , Mansha M, Carlet M, Ploner C, Geley, S, Rainer J and Kofler R
P42	*A Blue Breakdown Product of Vitamin B_{12}* Markus Ruetz, Sergey Fedosov, Karl Gruber, Christoph Kratky and Bernhard Kräutler
P43	*Chlorophyll Breakdown in Senescent Leaves of the Lime Tree (Tilia cordata)* Mathias Scherl, Thomas Müller and Bernhard Kräutler
P44	*Chlorophyll Breakdown in the Tropical Evergreen Spathiphyllum wallisii* Clemens Vergeiner, Thomas Müller, Srinivas Banala, Simone Moser, Andreas Holzinger, Cornelius Lütz, Bernhard Kräutler
P45	*Shape and Dynamics of Transcription Factor Binding Sites* Julian E. Fuchs, Gudrun M. Spitzer, Ameera Javed, Hannes G. Wallnoefer, Roland Huber, Klaus R. Liedl
P46	*From Ensembles to Entropy – A Thermostatistical Approach* Roland G. Huber, Julian E. Fuchs, Monika Laner, Gudrun M. Spitzer, Hannes G. Wallnoefer, Romano T. Kroemer, Klaus R. Liedl
P47	*Searching for small-molecule interactions sites in DNA-binding proteins. Case study: FOXO3a* Kaserer T, Markt P, Ausserlechner MJ, Schuster D, Obexer P
P48	*Molecular modeling of telmisartan analogues as new PPARg agonists* Mauersberger R., Schuster D., Gust R.

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P49	*Development of a pharmacophore model for inhibitors of the NF-κB(p50)/DNA-interface* Noha SM, Grzywacz AM, Fakhrudin N, Schuster D, Heiss E, Atanasov AG, Rollinger JM, Dirsch, VM, Stuppner H, Wolber G
P50	*In silico toxicology predictions via pharmacophore-based virtual screening: The discovery of the UV filter benzophenone-1 as inhibitor of testosterone synthesis* Schuster D, Nashev LG, Laggner C, Sodha S, Langer T, Wolber G, Odermatt A
P51	*What Thermodynamic Profiles Tell Us About DNA Recognition Site Geometry* Spitzer GM, Fuchs JE, Biela A, Javed A, Kreutz C, Klebe G, Liedl KR
P52	*Trying to understand the complex dialogue of epigenethics: The role of mammalian chromatin remodeling factor CHD1* Paolo Piatti, Claudia Soratroi, Hildegard Wörle, Stephan Geley, Andreas Villunger, Nikos Yannoutsos and Alexandra Lusser
P53	*Purification of CHD1 protein complexes and characterization of potential interacting partners in Drosophila melanogaster* Zeilner A, Morettini S, Sarg B, Lindner H, Lusser A
P54	*Electron induced damage of DNA compounds* AnderLan L, Denifl S, Märk TD, Scheier P
P55	*Electron induced splitting of the cyclobutane pyrimidine dimmer: an important step in the DNA damage repair via DNA photolyase* Edtbauer A, Russell K, Feketeova L, Mitterdorfer C, O´Hair RAJ, Wille U, Denifl S, Märk TD and Scheier P
P56	*Low energy electron interactions with amino acids in the gas phase : effect of the isomers geometry* V.Vizcaino, S.Denifl, T.Märk and P.Scheier
P57	*Analysis of the beta-cell specific regulation of the zebrafish hb9 gene* *V.ARKHIPOVA, N DEVOS, B. WENDIK, B. PEERS AND D. MEYER.*
P58	*Characterization of a novel type of Retinoid-binding protein in zebrafish* Hao Chen, Sabrina Vorwerk, Valeryia Arkhipova, Dirk Meyer
P59	*A Transgenic approach to identify target genes of the Currarino syndrome related Hlxb9/Hb9 protein* Elisabeth Ott, Björn Wendik, Monika Srivastava, Dirk Meyer
P60	*Multiple roles for Sox17 in endoderm development in zebrafish* Claudia Ralser, Dirk Meyer and Pia Aanstad

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P61	*Hedgehog signalling controls proliferation in the early zebrafish endoderm* Tom Stückemann and Pia Aanstad
P62	*Chemical synthesis of 2'-azido modified RNA and its potential for bioconjugation and siRNA technologies* Aigner M, Hartl M, Fauster K, Steger J, Bister K, Micura R
P63	*Semisynthesis of a stable E.coli tRNA-3'-NH-Val-Val-Leu-Leu-Met conjugate containing genuine tRNA modifications* Geiermann A-S, Graber D, Steger J, Moroder H, Trappl K, Polacek N, Micura R
P64	*Screening method for cardiovascular diseases in zebrafish* Zipperle, J and Schwerte, T
P65	*Influence of hypoxia and the circadian clock on Hif-1α-protein in zebrafish* Köblitz L, Baus K, Kopp R, Egg M, Pelster B
P66	*Revealing the elusive molecular biology of the vault RNA* Amort M, Nauchbauer B, Nandy C, Polacek N
P67	*Does the "RNA world" still communicate with the translation machinery?* Kamilla Bąkowska-Żywicka, Marek Żywicki, Norbert Polacek
P68	*Atomic mutagenesis reveals a common mechanism for GTPase activation on translational G proteins* Nina Clementi, Ronald Micura and Norbert Polacek
P69	*Deciphering Translational Processes Using Chemically Engineered Ribosomes* Erlacher M.D., Micura R. and Polacek N.
P70	*Genomic screens for regulatory ncRNAs targeting the ribosomes in archaea and basal flatworms* Gebetsberger J, Ladurner P, Norbert Polacek
P71	*Probing the ribosomal exit tunnel with tRNA-peptide conjugates* Krista Trappl, Jessica Steger, Dagmar Graber, Holger Moroder, Ronald Micura, Norbert Polacek
P72	*A ligand-based 3D-pharmacophore model for the μ opioid receptor: application to the morphinan class of opioids* Asim MF, Wolber G, Spetea M, Schmidhammer H

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P73	*In vitro and in vivo pharmacological profile of 6-glycine substituted 14-phenylpropoxymorphinans, high affinity and potent opioid antinociceptive agents* Spetea M, Windisch P, Guo Y, Bileviciute-Ljungar I, Schütz J, Riba P, Kiraly K, Fürst S, Schmidhammer H
P74	*Role of I-II loop in the plasma membrane targeting of L-Type Voltage Gated Calcium Channels* Gurjot Kaur, Mathias Gebhart, Martina Sinnegger-Brauns, Gerald J. Obermair, Bernhard E. Flucher, Alexandra Koschak and Jörg Striessnig
P75	*Identification of biochemical pathways contributing to the aberrant behavioural phenotype induced by dietary-induced Mg-deficiency* Whittle N, Sartori SB, Li L,Chen W-Q, Yang J-W, Sinnegger-Brauns MJ, Lubec G, Singewald N
P76	*Treatment of the Metabolic Syndrome by Traditional Chinese Medicine (TCM)* Steinmann D, Baumgartner RR, Dirsch VM, Stuppner H, Ganzera M
P77	*In silico discovery of novel acidic microsomal prostaglandin E_2 synthase 1 inhibitors* Waltenberger B, Wiechmann K, Schuster D, Wolber G, Rollinger JM, Werz O, Stuppner H
P78	*Disassembly of the ESCRT-III complex* Manuel Alonso Y Adell, David Teis
P79	*Functional Genomic Characterization of a potential Membrane Stress Response pathway* Marietta Brunner, Daniel Bindreither, Johannes Rainer, Reinhard Kofler, David Teis
P80	*Identification of novel regulators in cell surface receptor degradation via the multivesicular body pathway* Martin Müller, Marcus Smolka, David Teis
P81	*TIS7 interacts with and regulates the methylosome* K. Patsch, T. Stasyk, M. Offterdinger, N. Schiefermeier, G. Brosch, J. Fürst, K. Pfaller, L.A. Huber, I. Vietor
P82	*Investigating the Role of PIDD in the DNA Damage Response* F. Bock, G. Krumschnabel, C. Manzl, A. Villunger
P83	*Investigating the Role of the BH3-only Proteins Bim and Bmf in Mammary Gland Development and Breast Cancer* F. Baumgartner, V. Labi, F. Grespi, G. Krumschnabel, E. Müller-Holzner, C. Marth, W. Doppler and A. Villunger
P84	*Elucidation of the Physiological Role of the Bcl-2 Pro-Survival Homologue A1* E. Ottina, F. Grespi, S. Geley, N. Yannoutsos, M. Herold and A. Villunger

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P85	*Investigating the role of the PIDDosome in B cell lymphomagenesis* *Peintner L.,Manzl C., Krumschnabel G. and Villunger A.*
P86	*Investigating the role of BH3-only proteins in B cell development* C Wöss, V Labi, P Schneider and A Villunger
P87	*The role of fibroblasts and macrophages in capsular fibrosis* Plank N, Pfaller K , Wolfram-Raunicher D, Wick G
P88	*HSP60 T cell epitopes and anti-HSP60 autoantibody are involved in the initiation and progression of atherosclerosis* Giovanni Almanzar, Cecilia Grundtman, Robert Öllinger, Ruurd van der Zee, Julianna Leuenberger, Elisabeth Onestingel, and Georg Wick
P89	*The activity of iron salophene complexes against tumor cells* Benjamin Ma and Ronald Gust
P90	*AKAP79/150 Palmitoylation is Required for Endosomal Targeting and Regulation of Neuronal Postsynaptic Structure and Function* Dove L. Keith, Emily S. Gibson, Jennifer L. Sanderson, Holly R. Robertson,Kyle Olszewski, Rujun Kang, Alaa El Husseini, Mark L. Dell'Acqua

EGFR signaling networks in cancer development

Maria Sibilia

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Mammalian Prion Biology

Adriano Aguzzi

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University Hospital of Zürich, Switzerland

Wnt signaling and cell migration in C. elegans

Rik Korswagen

Hubrecht Institute, Utrecht, Netherlands

Genetic analysis of integrin signalling

Reinhard Faessler

Max-Planck-Institute of Biochemistry, Martinsried, Germany

Folding of a transcriptionally acting PreQ1 riboswitch

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7-Aminomethyl-7-deazaguanine ($preQ_1$) sensitive mRNA domains belong to the smallest riboswitches known to date [1]. Although recent efforts have revealed the three-dimensional architecture of the ligand–aptamer complex less is known about the molecular details of the ligand-induced response mechanism that modulates gene expression [2,3]. We present an *in vitro* investigation on the ligand-induced folding process of the preQ1 responsive RNA element from *Fusobacterium nucleatum* using biophysical methods, including fluorescence and NMR spectroscopy of site-specifically labeled riboswitch variants [4,5]. We provide evidence that the full-length riboswitch domain adopts two different coexisting stem-loop structures in the expression platform. Upon addition of $preQ_1$, the equilibrium of the competing hairpins is significantly shifted. This system therefore, represents a finely tunable antiterminator/terminator interplay that impacts the *in vivo* cellular response mechanism. A model is presented how a riboswitch that provides no obvious overlap between aptamer and terminator stem-loop solves this communication problem by involving bistable sequence determinants.

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[1] Roth A, Winkler WC, Regulski EE, Lee BW, Lim J, Jona I, Barrick JE, Ritwik A, Kim JN, Welz R, Iwata-Reuyl D, Breaker RR, Nat Struct Mol Biol 2007, 14, 308-317.
[2] Klein DJ, Edwards TE, Ferré-D'Amaré AR, Nat Struct Mol Biol 2009, 16, 343-344.
[3] Kang M, Peterson R, Feigon, Mol Cell 2009, 33, 784-790.
[4] Rieder U, Lang K, Kreutz C, Polacek N, Micura R, Chembiochem 2009, 10, 1141-1144.
[5] Rieder U, Kreutz C, Micura R, Proc Natl Acad Sci USA 2010, doi: 10.1073/pnas.0914925107.

Non-coding RNAs in Epstein-Barr virus infection

Hutzinger R[1], Feederle R[2], Mrazek J[3], Schiefermair N[4], Zavolan M[5], Polacek N[1], Delecluse HJ[2] and Hüttenhofer A[1]
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Non-coding RNAs (ncRNAs) do not encode proteins but function on the level of the RNA itself. Members of a special ncRNA class, designated as small nucleolar RNAs (snoRNAs), are localized within the nucleolus, a sub-nuclear compartment, in which they guide ribosomal or spliceosomal RNA modifications, respectively. Until now, snoRNAs have only been identified in eukaryal and archaeal genomes, but are notably absent in bacteria. By screening of B lymphocytes for expression of ncRNAs induced by the Epstein-Barr virus (EBV), we here report, for the first time, the identification of a snoRNA gene within a viral genome, designated as v-snoRNA1. This genetic element displays all hallmark sequence motifs of a canonical C/D box snoRNA, namely C/C'- as well as D/D'-boxes. The nucleolar localization of v-snoRNA1 was verified by *in situ* hybridisation of EBV-infected cells. We also confirmed binding of the three canonical snoRNA proteins, fibrillarin, Nop56 and Nop58 to v-snoRNA1. The C-box motif of v-snoRNA1 was shown to be crucial for the stability of the viral snoRNA; its selective deletion in the viral genome led to a complete down-regulation of v-snoRNA1 expression levels within EBV-infected B cells. We further provide evidence, that v-snoRNA1 might serve as a miRNA-like precursor, which is processed into 24 nt sized RNA species, designated as v-snoRNA1^{24pp}. A potential target site of v-snoRNA1^{24pp} was identified within the 3'-UTR of BALF5 mRNA which encodes the viral DNA polymerase. v-snoRNA1 was found to be expressed in all investigated EBV-positive cell lines, including lymphoblastoid cell lines (LCL). Interestingly, induction of the lytic cycle markedly up-regulated expression levels of v-snoRNA1 up to 30-fold. By a computational approach, we identified a v-snoRNA1 homolog in the rhesus lymphocryptovirus genome. This evolutionary conservation suggests an important role of v-snoRNA1 during gamma-herpesvirus infection.

This work is done in cooperation with febit biomed gmbh and supported by the GEN-AU.

Novel insights into the functional role of three protein arginine methyltransferases in Aspergillus nidulans

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Protein arginine methylation has been implicated in different cellular processes including transcriptional regulation by the modification of histone proteins. Here we demonstrate significant *in vitro* activities and multifaceted specificities of *Aspergillus* protein arginine methyltransferases (PRMTs) and we provide evidence for a role of protein methylation in mechanisms of oxidative stress response. We have isolated all three *Aspergillus* PRMTs from fungal extracts and could assign significant histone specificity to RmtA and RmtC. In addition, both enzymes were able to methylate several non-histone proteins in chromatographic fractions. For endogenous RmtB a remarkable change in its substrate specificity compared to the recombinant enzyme form could be obtained. Phenotypic analysis of mutant strains revealed that growth of D*rmtA* and D*rmtC* strains was significantly reduced under conditions of oxidative stress. Moreover, mycelia of D*rmtC* mutants showed a significant retardation of growth under elevated temperatures.

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Electron induced splitting of the cyclobutane pyrimidine dimmer: an important step in the DNA damage repair via DNA photolyase

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A major DNA damage induced via UV radiation is the formation of cyclobutane pyrimidine dimers (CPD). These dimers represent a genotoxic lesion since they block the correct replication of DNA. CPDs play a major role in the formation of skin cancer [1], thus the repair of such damages is a process indispensable to life. A repair mechanism of nature (DNA photolyase) is capable of repairing these damages with the help of a sunlight activated electron transfer to the dimer. DNA photolyase was found in bacteria, fungi, plants invertebrates and many vertebrates but not in humans [1, 2]. In the marsupials CPD photolyase is present. It has been shown that mice provided with a transgene for marsupial DNA photolyase exhibit a significant increase of repair for CPD lesions [3].
Thus we carried out free electron attachment measurements to CPDs to gain a deeper insight into the reductive splitting of these dimers [4, 5]. For this study we employed a double focusing mass spectrometer. We observed an unusual rich fragmentation pattern upon electron attachment of electrons with energies close to 0 eV. The most abundant fragment was observed for the half split dimer. Additionally we were able to observe the parent anion which so far has not been observed.
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Bioinformatics for cancer immunology

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In this talk bioinformatics requirements and solutions for cancer immunology research are reviewed. First the various sources for generating data including large-scale approaches are highlighted. Then an overview on the bioinformatics tools necessary to analyze the data is given. In a case study on colorectal cancer we demonstrate the power of a combined computational-experimental approach.
We used data integration and biomolecular network reconstruction to generate hypotheses about the mechanisms underlying immune responses in colorectal cancer that are relevant to tumor recurrence. Mechanistic hypotheses were formulated on the basis of data from 108 colorectal carcinomas and tested with a combination of different assays (gene expression, phenome mapping, tissue-microarrays, TCR-repertoire). This integrative approach revealed chemoattraction and adhesion to play important roles in determining the density of intratumoral immune cells. The presence of specific chemokines and adhesion molecules correlated with different subsets of immune cells and with high densities of T cell subpopulation within specific tumor regions. High expression of these molecules correlated with prolonged disease-free survival.

Delineating the transcriptional response of acute lymphoblastic leukemia cells to glucocorticoid treatment

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Glucocorticoids (GCs) have a variety of physiological effects on different cell types, including regulation of glucose synthesis and suppression of inflammation in response to stress. In malignant cells of the lymphoid lineage GCs specifically induce cell death, which led to their inclusion in essentially all treatment protocols for lymphoid malignancies, especially acute lymphoblastic leukaemia (ALL). Almost all effects of GCs are mediated through their cognate receptor, the glucocorticoid receptor (GR), a ligand activated transcription factor (TF), and are thus of transcriptional nature.
The transcriptional response of leukaemia cells was studied both *in-vivo* and *in-vitro*, previously, however, with microarray technologies detecting only the 3' ends of transcripts and thus unable to identify gene variants that are regulated by GCs. We have now employed high density Exon microarrays and combined this with the ChIP-chip technology in two ALL systems, i.e., a precursor B-ALL and a T-ALL in vitro model. The former analysis was used to delineate the transcriptional response of all exons over the first 24 hours of treatment and the latter to reveal DNA binding sites of the GR.
To analyse data from the high density Exon arrays both for transcript expression as well as for differential splicing, we developed a new method based on a re-alignment and annotation of the oligonucleotide probes of the microarray. An increasing transcriptional response to GC treatment was identified in this analysis, especially in the T-ALL system, where it is accompanied with, and can be explained by, the strong auto-induction of the GR. In line with the results form the ChIP-chip analysis, that revealed both shared and lineage specific binding sites, the transcriptional response of the two ALL systems was only partially overlapping. For about 50% of the regulated genes (mostly induced genes after 2 and 6 hours) a GRE (glucocorticoid response element) was identified in the ChIP-chip analysis. The other genes might be under the control of another TF, or the GR might bind to the DNA outside of the promoter regions detectable on the microarray.
Several potentially new gene isoforms induced by GCs in these cells were identified by the differential splicing analysis of the data set. In depth verification was performed for one of these candidates, ZBTB16. The results from the splicing analysis were reconfirmed and a yet unknown exon, termed exon 1b, was identified by 5' RACE. Expression and regulation of this new exon and the new ZBTB16 isoform was verified by real time RT-PCR in 4 different B-ALL cell lines, and also in *in-vivo* GC treated B-ALL patients.
Taken together these results indicate a highly tissue specific response to GC treatment and the transcription of novel, potentially GC specific, gene isoforms. Whether these new transcript variants have an impact on cell death induction has to be further investigated.

Backbone Flexibility Controls the Activity and Specificity of a Protein-Protein Interface – Specificity in Snake Venom Metalloproteases (SVMPs)

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Protein-Protein interfaces have crucial functions in many biological processes[1]. The large interaction areas of such interfaces show complex interaction motifs. Even more challenging is the understanding of (multi-)specificity in protein-protein binding. Many proteins can bind several partners to mediate their function[2].
A perfect paradigm to study such multi-specific protein-protein interfaces are snake venom metalloproteases (SVMPs)[3]. Inherently, they bind to a variety of basement membrane proteins of capillaries, hydrolyze them, and induce profuse bleeding. However, despite having a high sequence homology, some SVMPs show a strong hemorrhagic activity, while others are (almost) inactive[4].
Our results indicate that the activity to induce hemorrhage, and thus the capability to bind the potential reaction partners, is related to the backbone flexibility in a certain surface region[5]. A subtle interplay between flexibility and rigidity of two loops seems to be the prerequisite for the proteins to carry out their damaging function. Presumably, a significant alteration in the backbone dynamics makes the difference between SVMPs that induce hemorrhage and the inactive ones.

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A novel pipeline for automated assembly and analysis of high throughput cDNA libraries

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In recent years increased interest in identification of novel functional transcripts can be observed. Fast development of high throughput DNA sequencing methods allowed the scientist for more complex investigation of cell's transcriptome dynamics. However analysis of huge amounts of data produced by such approaches requires highly specialized tools in order to fish out the interesting cases from hundreds of thousands of sequences of transcriptomic "noise".
We have developed a novel pipeline for automated assembly and analysis of cDNA libraries obtained with next generation sequencing methods. It is optimized for work with noncoding RNA derived libraries. The main advantages include dealing with non-unique reads, identification of RNA processing products and prediction of possible functions for intergenic transcripts. We have also proposed a novel method for estimation of the ncRNA expression levels which takes into consideration a possibility of processing of the primary transcripts. There is also possibility of using the pipeline in a fully automated way. In this mode, a set of optimized parameters for every step of the analysis is used. Thus, involvement of the user can be restricted to providing the raw sequencing data. Presentation of the results allows easy and convenient verification of the predictions and identification of sequences of interest.

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Stem cell-specific activation of multiple ancestral forms of the *myc* protooncogene in the early metazoan *Hydra*

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The *myc* oncogene was originally identified as a transduced allele (v-*myc*) in the genome of highly oncogenic retroviruses [1, 2]. The cellular c-*myc* protooncogene encodes the transcription factor Myc, a bHLH-Zip DNA binding protein controlling fundamental cellular processes [3]. Deregulation of *myc* leads to tumorigenesis and is a hallmark of many human cancers [3]. We have recently identified two ancestral forms of *myc* from the early diploblastic cnidarian *Hydra*, the most primitive metazoan organism employed so far for the structural, functional, and evolutionary analysis of this gene [4]. *Hydra myc1* is specifically activated in all rapidly proliferating cell types of the interstitial stem cell system and in proliferating gland cells. The paralogous gene *Hydra myc2* is more abundantly expressed and also detectable in regions where *myc1* mRNA is absent. The ancestral *Hydra* Myc1 and Myc2 proteins display the principal design of their vertebrate derivatives, with the highest degree of sequence identities confined to the bHLH-Zip domains. Furthermore, the *Hydra* Myc proteins contain basic forms of the essential Myc boxes I through III [4]. The promoter of the *myc2* gene contains the consensus sequence 5'-CACGTG-3' (E-box) to which recombinant *Hydra* Myc1 and Myc2 proteins bind with high affinity. The *myc2* gene is located immediately adjacent to the *Hydra CAD* gene. The human *CAD* gene encoding carbamoyl-phosphate synthetase, aspartate transcarbamoylase, dihydroorotase required for pyrimidine nucleotide biosynthesis represents a bona fide transcriptional Myc target [3]. The amino acid sequence of the 2170-amino acid *Hydra* CAD protein was deduced from the genomic DNA sequence of *Hydra magnipapillata* published recently [5]. *Hydra* CAD displays 59% sequence identity with the 2225-amino acid human ortholog. The promoter of the *Hydra CAD* gene was defined by transcriptional mapping using *Hydra* mRNA isolated from whole animals. Similar to the human *CAD* regulatory region, the *Hydra CAD* promoter contains two Myc binding sites in close proximity to the transcription start site, suggesting that *CAD* is an essential transcriptional Myc target from the base of animal evolution. Also, the oncogenic potential of Myc has evolved very early since hybrid proteins composed of segments from the retroviral v-Myc oncoprotein and of *Hydra* Myc1 or Myc2 display oncogenic potential in cell transformation assays. Our results suggest that the principal functions of the Myc master regulator arose very early in metazoan evolution, allowing their dissection in a simple model organism showing regenerative ability but no senescence.

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Flatworms as model systems to study stem cells, regeneration, and reproduction

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The extraordinary capacity of flatworms to regenerate a complete animal from any tiny piece of tissue of the body is based on the presence of a remarkable stem cell type - Neoblasts. They are undifferentiated cells that can self-renew and can differentiate into all cell types of the animal including germ cells. Neoblasts are responsible for cell renewal during regeneration, homeostasis, development, growth, and regrowth after starvation. The molecular regulation of these stem cells is of great interest not only for flatworms but also for stem cell systems of higher organisms and humans since the pathways appear to highly conserved.
In recent years, the simple flatworm *Macrostomum lignano* has emerged as a complementary model system to study development and regeneration. *M. lignano* is a small (1,5mm), marine free-living flatworm, a basal member of the Platyhelminthes. It can easily be cultured in the laboratory, is highly transparent, has a short generation time of 18 days, and a high regeneration capacity. *M. lignano* is an obligatory cross-fertilizing hermaphrodite that reproduces exclusively sexually and produces offspring all year round in the laboratory. The animal morphology is well known and its tissues and organs are organized in a simple way. The animals exhibit a high degree of plasticity and adapt to e.g. starvation by substantial degrowth or they can phenotypically adjust testis size to group size.
We have established this animal as a laboratory flatworm model system. We described this new species, adapted immunocytochemical and histological methods, and applied electron microscopy. We established in situ hybridization, RNA interference and generated monoclonal antibodies. We initiated an EST sequencing project and set up a large-scale whole mount in situ hybridization screening system. We are member of an international consortium headed by Dr. Eugene Berezikov (Hubrecht Institute, Utrecht, NL) to sequence the genome of *M. lignano* (http://www.macgenome.org/). As a breakthrough for the field, we succeeded in the generation of transgenic animals. In cooperation with other groups we studied embryonic development, the regeneration capacity, reproductive biology, neuropeptide families, and aging.
We currently address the function of boule, a member of the DAZ gene family. These genes pay critical roles during gametogenesis. In about 15% of sterile human males a mutation in DAZ genes accounts for the defect in sperm production. We show that Macrostomum boule can rescue fly spermatogenesis, and it in known that human boule can promote sperm production in boule mutant Drosophila. Therefore we aim to elucidate the underlying molecular mechanisms to understand flatworm spermatogenesis and human disease.

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Molecular and Cellular Studies of Islet Cell Regeneration in Zebrafish

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Type 1 and type 2 diabetes result from the loss or dysfunction of insulin-producing β-cells of the pancreas. Better understanding of pancreas formation and the mechanisms causing diabetes is vital for developing new therapeutic strategies to regenerate functional insulin-producing beta-cells. *Pdx1* and *Hb9* are genes of critical importance in beta-cell specification and islet formation, and have overlapping expression patterns, but little is known about their functional interactions. The zebrafish is a vertebrate model system well suited for studying pancreas development, combining rapid development, the possibility for direct observation of internal organogenesis, and ease of genetic manipulation. In zebrafish embryos, protein depletion via morpholino knockdown of *pdx1* or *hb9* individually transiently disrupts early β-cell development, with later recovery of some insulin-positive cells. By contrast, knockdown of both *hb9* and *pdx1* leads to irreversible beta-cell loss. Analysis of marker gene expression and proliferation within pancreatic precusor and differentiated cell types was used to clarify the localization and mechanism of regeneration of beta cells. Our data suggests that beta-cell regeneration may be mechanistically different following loss of *pdx1* versus *hb9* function. Furthermore, simultaneous knockdown of both *pdx1* and *hb9* leads to failure of beta cell differentiation from early endocrine progenitors and appears to deplete the precursor population for late-arising beta cells.

Spindly/CCDC99 Is Required for Efficient Chromosome Congression and Mitotic Checkpoint Regulation

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Spindly is required to recruit cytoplasmic dynein to kinetochores for poleward movement of chromosomes and control of mitotic checkpoint signaling. Here we show that human Spindly is a cell cycle–regulated mitotic phosphoprotein that interacts with the Rod/ZW10/Zwilch (RZZ) complex. The kinetochore levels of Spindly are regulated by microtubule attachment and biorientation induced tension. Deletion mutants lacking the N-terminal half of the protein (Ndel253), or the conserved Spindly box (delSB), strongly localized to kinetochores and failed to respond to attachment or tension. In addition, these mutants prevented the removal of the RZZ complex and that of MAD2 from bioriented chromosomes and caused cells to arrest at metaphase, showing that RZZ-Spindly has to be removed from kinetochores to terminate mitotic checkpoint signaling. Depletion of Spindly by RNAi, however, caused cells to arrest in prometaphase because of a delay in microtubule attachment. Surprisingly, this defect was alleviated by co-depletion of ZW10. We believe that Spindly regulates ZW10 function on KCs and that this process could favour the establishment of initial monopolar attachments over syntelic or merotelic ones to prevent chromosome segregation errors and aneuploidy. Thus, our conclusion is that Spindly is not only required for kinetochore localisation of dynein but is a functional component of a mechanism that couples dynein dependent poleward movement of chromosomes to their efficient attachment to microtubules.
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Late endosomal p14/MP1-MAP kinase signaling couples IQGAP1 and Rac1 to focal adhesion dynamics and cell migration

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Focal adhesions govern cell motility. Asymmetric distribution and dynamics of focal adhesions rely on a variety of signaling cascades and require polarized cellular distribution of molecular components. Enabled by scaffold complexes, endosomes can ensure optimal coupling of molecular effectors to compartmentalize cellular signaling. Here we show that late endosomes, carrying the p14/MP1 MAPK scaffold complex can move to the cell periphery where they specifically target focal adhesions. In the absence of the signaling complex on late endosomes we observed strongly impaired cell migration and a defect in focal adhesion remodeling. Our data suggest that binding of the signaling complex through MP1 to the IQ-domain of IQGAP1 is required for IQGAP1 localization to the plasma membrane, activation of Rac1 at the leading edge and proper focal adhesion remodeling. We propose a novel role for late endosomes carrying the p14/MP1 MAPK scaffold complex in signaling compartmentalization and cell migration.

Role of TIS7 and SKMc15 in lipid metabolism

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TPA induced sequence 7 (TIS7) and its homologue SKMc15 are members of a novel gene transcription regulators family. We have previously shown that TIS7 acts as a transcriptional co-repressor. TIS7 and SKMc15 double knockout mice have a significant decrease in overall body size, organ size, fat deposits and are resistant to high fat diet-induced obesity. Food consumption of TIS7 SKMc15 double knockout mice was normal but the entry of dietary fat into the circulation was reduced. Affymetrix analysis of the small intestines shoved among other regulated genes, a decrease in expression levels of CD36. This protein is crucial for the transport of very long chain fatty acids (VLCFA) in several organs involved in lipid metabolism e.g. intestines, liver, muscles and adipose tissue. Loss of CD36 results in decreased intestinal absorption of VLCFA [1] and resistance to diet induced obesity [2]. Independent experiments confirmed reduced of CD36 RNA and protein levels in several organs of TIS7 SKMc15 double knockout mice. Immunofluorescence analyses documented reduction in CD36 protein expression in enterocytes. At the moment we propose that decreased CD36 RNA and protein levels may be cause the decreased efficiency of intestinal fat absorption in TIS7 SKMc15 double knockout mice. Lack of CD36 may result in increased secretion rather than storage of dietary fat in white adipose tissue. The ultimate goal of our study is to prove that TIS7 and/or SKMc15 regulate CD36 expression on the transcriptional level and thereby affect the lipid metabolism. Our results may have an application in the treatment of obesity.

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Investigating the Role of PIDD in the DNA Damage Response

F. Bock, G. Krumschnabel, C. Manzl, A. Villunger

The p53-induced protein with a death domain (PIDD) is involved in cellular pathways which can induce programmed cell death (apoptosis), survival via the activation of NFkB and/or DNA-repair after DNA-damage. Depending on the duration, type and severity of genotoxic stress, PIDD, together with the adapter molecule RAIDD, can aid in the formation of an activating platform for Caspase-2, the so-called “PIDDosome”, or together with RIP1 assist in the activation of NFkB by promoting sumoylation of NEMO. Most recently, PIDD has also been shown to form complexes with DNA-PK, assisting DNA repair by enabling non-homologous end-joining. While the first complex induces apoptosis, the latter two prevent apoptosis by enabling the cell to repair the damage. PIDD therefore plays a role in the integration of at least two opposing signalling pathways. This dual role is based on the capacity of PIDD to auto-proteolytic cleavage, which leads to the generation of two different protein fragments, PIDD-C and PIDD-CC, each of which is involved in the induction of one of the aforementioned pathways.

In order to shed further light on these roles and to investigate the in vivo function of PIDD, we have generated a mouse model lacking the *pidd* gene. Using mouse embryonic fibroblasts (MEF) derived from those mice we performed survival assays and monitored NFkB activity in response to genotoxic stress.

Treatment with inflammatory cytokines did not reveal any differences in NFkB activation between wild type and PIDD deficient MEF. In contrast, there was a significant delay in activation of NFkB in response to DNA damage. Furthermore, various inhibitors of NFkB could sensitize wild type cells, but not PIDD deficient cells towards DNA damage induced apoptosis. Unexpectedly, we observed no differences of short and long term survival between wild type and PIDD deficient cells. However, PIDD deficient cells show a deficient cell cycle arrest in response to DNA-damage.

In conclusion, our results suggest that PIDD plays a rate-limiting role in NFkB activation following DNA damage. Surprisingly, this defect is not reflected in differences in cell survival.

Gating properties of a human disease-causing mutation in a $Ca_V1.3$ L-type calcium channel splice variant

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$Ca_v1.3$ voltage-gated L-type calcium channels (LTCCs) mediate window currents at negative membrane voltages allowing them to function as pacemaker channels in the sinoatrial node (SAN) and neurons and to trigger neurotransmitter release in cochlear inner hair cells (IHCs). Mice lacking $Ca_v1.3$ a1-subunits are congenitally deaf and show SAN dysfunction. We have identified families with deafness and SAN dysfunction resulting from a homozygous mutation in the splice variant exon 8B of the *CACNA1D* gene encoding $Ca_V1.3$ a1-subunits which, in mouse, are preferentially expressed in IHCs and SAN. The mutation leads to an amino acid insertion in a highly conserved region near the pore-forming transmembrane S6 helix.

To determine the functional consequences of the mutation we expressed wildtype (WT) and mutant (MUT) $Ca_v1.3$ channel complexes in tsA201 cells and analyzed their biophysical properties by whole-cell patch-clamp. Although expressed at protein levels comparable to WT, MUT channels did not conduct significant Ca^{2+} currents (I_{Ca}) (peak I_{Ca} 1.2 ± 0.14 pA/pF, n = 12) in contrast to WT (15.9 ± 1.7, n = 17). However, ON-gating currents were readily recorded in both WT and MUT channels during depolarization to the reversal potential where contaminating ionic current is absent during test pulses but can be quantified from the tail currents (I_{tail}) upon repolarization. In WT, integrated ON gating charge (Q_{ON}) increased with increasing ionic current whereas no I_{tail} was detectable in MUT channels even at Q_{ON} that resulted in robust I_{tail} in WT. MUT Q_{ON} exhibited a typical non-linear voltage-dependence of activation, and neither the size of Q_{ON} ($p = 0.2$, Mann Whitney) nor its voltage-dependence was affected by the Ca^{2+} channel activator BayK8644 (5µM). Amplitude and Q_{ON} were significantly smaller than in WT. MUT channel ON-gating current also showed pronounced kinetic differences from WT evident as a significantly shorter width, faster time-to-peak, and shorter time constant of decay ($p<0.05$, Mann-Whitney test).

The presence of intra-membrane charge movement in the absence of ionic currents implies that voltage-sensors in MUT channels move but either fail to trigger pore opening or opened MUT channels cannot conduct Ca^{2+}. Since the mutation is not located within voltage-sensing S4 helices the pronounced kinetic changes of gating currents suggest that the mutation in the pore indirectly alters gating by affecting the mechanistic coupling that exists between S4 voltage-sensor movements and the gate mediating pore opening. Our data also for the first time provide direct evidence that loss of $Ca_v1.3$ function causes deafness and SAN dysfunction in humans. The patients’ phenotype and our electrophysiological findings strongly suggest that the exon 8B splice variant is predominant in human IHC and SAN pacemaker cells.

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Identification of novel antiinfluenza constituents from *Alpinia katsumadai*

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Currently, neuraminidase (NA) inhibitors are the preferred class of antiviral drugs targeting the influenza virus.[1] There is a high medical need to find novel NA-inhibitors to overcome the emerging anti-influenza drug resistances.
In this study, the seed extract of *Alpinia katsumadai* Hayata (Zingiberaceae), which has been used in Traditional Chinese Medicine in antiviral formulas, was phytochemically investigated and tested for its potential to inhibit the influenza NA. Among the six isolated and identified constituents, four diarylheptanoids showed *in vitro* NA inhibitory activities in low micromolar ranges against human influenza virus A/PR/8/34 of subtype H1N1. Katsumadain A, the most effective compound, revealed an IC_{50} of 1.05 ± 0.42 µM. This secondary metabolite also inhibited the NA of four H1N1 swine influenza viruses (IC_{50} values between 0.9 and 1.64 µM) and demonstrated antiviral effects in plaque reduction assays.[2]
Taking into account the complex and flexible architecture of the macromolecule NA, binding interactions of the T-shaped Katsumadain A were investigated performing extensive molecular dynamics (MD) simulations. Well-established interactions between the protein and the core of this novel NA-inhibiting natural scaffold could be observed by computational docking. In addition, excellent surface complementarity to the simulated binding pocket and concordance with experimentally-derived SAR data could be confirmed.[2] These results have already provided a valuable tool for the identification of further novel natural leads to combat flu.

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Coloured Pigments from Chlorophyll Breakdown

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The appearance of the fall colours is commonly associated with chlorophyll breakdown in higher plants. The first chlorophyll catabolites from higher plants were structurally characterized as colourless linear tetrapyrroles (non-fluorescing chlorophyll catabolites, NCC) [1]. The colourless NCCs were commonly considered to be the 'final' tetrapyrrolic products of chlorophyll catabolism in higher plants, which are found in degreened leaves and in ripened fruit [2].
Our new findings indicate chlorophyll breakdown in senescent plants to proceed beyond the stage of the NCCs to give yellow [3] and other new brilliantly coloured tetrapyrroles. The same pigments were identified in freshly prepared extracts of deep yellow senescent leaves of *Cercidiphyllum japonicum* and in senescent leaves from other plants. This would suggest that part of the fall colours arise from chlorophyll breakdown products directly.

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Development and in vivo evaluation of an oral drug delivery system for paclitaxel

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Paclitaxel is one of the most important drugs widely used for the treatment of various types of cancers. Poor aqueous solubility, affinity for intestinal and liver cytochrome P450 metabolic enzymes and paclitaxel efflux are the major contributors for its low bioavailability. Due to these reasons paclitaxel is currently administered via i.v. infusion which is associated with certain complications. [1-2] Over the past few years thiolated polymers (thiomers) have appeared as a promising excipient for drug delivery and demonstrated improved mucoadhesive, controlled release, permeation enhancing and enzyme inhibitory properties.[3-4]
The aim of the present study was to investigate the effect of the poly(acrylic acid)-cysteine (PAA-cysteine) and reduced glutathione (GSH) on the absorption of P-glycoprotein (P-gp) substrate paclitaxel (taxol) in vitro and in vivo. In vitro transport studies were performed with Caco-2 monolayers. Furthermore, the delivery system based on PAA-cysteine, GSH and paclitaxel was evaluated in vivo in rats. In vitro, the formulation comprising 0.5 % (m/v) PAA-cyteine (100 kDa)/0.5 % (m/v) GSH (P_{app} = 8.7 ± 1.3 x 10^{-6} cm/s) improved the transport of paclitaxel 6.7-fold in comparison to paclitaxel served as a buffer control (P_{app} = 1.3 ± 0.4 x 10^{-6} cm/s). Moreover, in the presence of formulation containing 0.5 % (m/v) PAA-cyteine (250 kDa)/0.5 % (m/v) GSH (P_{app} = 9.7 ± 0.3 x 10^{-6} cm/s) paclitaxel absorption was even 7.4-fold in comparison to the buffer control. In vivo, the administration of oral formulations containing 1 mg of paclitaxel, 1 mg of GSH and 8 mg of PAA-cysteine conjugate (100 kDa or 250 kDa) resulted in an improved paclitaxel plasma concentration and bioavailability. The area under the plasma concentration time curve (AUC_{0-8}) of paclitaxel was 4.7-fold and 5.7-fold improved in comparison to the oral control, respectively. Moreover, c_{max} was improved by 6.3–fold and even 7.3-fold in comparison to the oral control, respectively.
Thus, according to the achieved results it is suggested that PAA-cysteine in combination with GSH would be a potentially valuable tool for improving the oral bioavailability of P-gp substrates such as paclitaxel.

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Role of neuropeptides in fear, anxiety and depression

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Current procedures to cure psychopathologies such as anxiety disorders and depression involve both psychotherapy and pharmacotherapies. Although a number of clinically effective drugs are available, an improvement of their therapeutic efficacy, treatment resistance and the number of side effects is desired. Neuropeptides, a group of neurotransmitters/modulators with distinct expression in the brain, are thought to represent novel drug targets for the treatment of these mental disorders. Anxiolytic and/or antidepressant effects of diverse neuropeptide ligands have been demonstrated in normal animals reflecting physiology rather than pathophysiology. We now used the rodent HAB lines selectively bred for a high anxiety-related phenotype to elucidate the role of selected neuropeptides including substance P or the recently discovered neuropeptides S in enhanced (pathological) fear, anxiety and depression. Control animals were either normal anxiety NAB or low anxiety LAB rodents. Application of selective neuropeptide receptor ligands such as a neurokinin-1 receptor antagonist attenuated exaggerated conditioned and unconditioned anxiety responses of HAB animals relative to their comparators. In addition, enhanced depression-related behavior could be reduced by this intervention. Using immediate-early gene mapping a distinct set of neuronal populations in defined cortical, limbic, hypothalamic and brainstem areas showed aberrant activation in HAB compared to NAB or LAB counterparts mimicking to a large extent observations in patients with anxiety disorders or depression. Modulation of neuropeptide receptor activity caused a normalization of many of these dysfunctions suggesting that these brain areas mediate the observed behavioural effects. Using real-time PCR, in situ hybridisation and microdialysis approaches, dysregulations of gene expression and neurotransmitter release were revealed in these brain areas. Taken together, HAB animals represent a clinically relevant model for studying neuropeptidergic drug targets with potential clinical benefit in pathological fear, anxiety and/or depression.

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P1

Unexpected association of *apoE2* genotype with polyglutamine expansions in the human androgen receptor (SBMA)

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Apolipoprotein E (APOE) is a carrier protein and directly involved in the uptake and distribution of plasma lipids and cholesterol. Maintaining normal levels of cholesterol is essential for prevention of cardiovascular disease, including strokes and heart attacks. The polymorphic ApoE gene is characterized by three major alleles, ApoE2, ApoE3 and ApoE4.
ApoE2 shows less than 2% of receptor binding activity and is associated with type III hyperlipoproteinemia and atherosclerosis. It is linked to lower serum cholesterol and higher ApoE concentration.
Spinal and bulbar muscular atrophy (SBMA) is a rare neurodegenerative disease of the lower motor neurons caused by a polyCAG expansion mutation in the androgen receptor. Besides the main neurodegenerative symptoms of SBMA, many patients are also affected by hypertension, hyperlipidemia, liver dysfunction, and glucose intolerance. Since androgens potentiate the severity of the disease and since cholesterol is the essential precursor of androgens, we hypothesised that the ApoE genotype could be a potential disease modifier.
In a pilot study we analyzed the apoE genotype of 20 diagnosed SBMA patients and found an increased frequency (P = 0.0218) of the apoE2/E3 genotype. Several hypotheses for a potential role of the ApoE genotype in the development and severity of Kennedy's disease are discussed.

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P2

The Nogo Receptor 2 (NgR2) is Required for Proper Innervation of the Skin

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While the first identified receptor of the Nogo Receptor family (NgR1) signals axonal growth inhibition via its coreceptors P75NTR and Lingo and the interactors Nogo, MAG or OMgp for NgR2 just MAG as binding partner was identified and its biological relevance is largely unknown. Therefore we obtained a mouseline deficient in NgR2 to investigate its function in more detail with respect to the molecular and histological effects of the receptor deletion. Surprisingly these mice show a significant increase of sensitivity to mechanical stimuli in behavioural experiments and *in vitro* single cell assays respectively but no other obvious abnormalities.
First we could show that the deletion of NgR2-/- results not in a shift or loss of neuron subpopulations in the dorsal root ganglia (DRG) what could led to the hypersensitivity phenotype. In this study we investigate weather the deletion of NgR2 effects the nerve innervation of the epidermis as the first source of tactile recognition. In the process the total number of free nerve endings in glabrous skin of the hindpaw from NgR2 deficient mice in comparison to the wildtype situation was significantly increased. Since the most dens innervation of the epidermis is observed at post natal day five and decreases drastically afterwards we addressed the question at which developmental stage a defect occurs and if this is restricted to a specific subpopulation of nerve endings namely $CGRP^+$ or $Mrgprd^+$ fibres.
Finally these data identify NgR2 as a receptor required for proper cutaneous innervation and could give a deeper insight in the largely unknown signal transduction and procession of mechanical stimuli. Additional cell culture based *in vitro* assays could show if the defect in axonal outgrowth is DRG-autonomous or dependent on factors secreted e.g. from keratinocytes of the epidermis.

P3

Investigating gene deregulation in the CNS of the NgR2 knockout mouse

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NgR2 is a member of the Nogo-receptor family, members of which are known to bind inhibitors of axonal regeneration in the CNS. Thus far the only known ligand of NgR2 is myelin associated glycoprotein (MAG). High expression of NgR2 mRNA has been reported in neurons of the rodent CNS, including hippocampal formation and amygdala. We have generated a NgR2 knockout mouse line to study NgR2 function. Behavioral studies show that under mild stress conditions, Ngr2-/- mice have a behavioral phenotype of reduced anxiety and depression compared to wildtype controls. An anxiolytic effect was seen in the increase in exploratory time in the open field test, and decreased depressive-like behavior was observed in decreased immobility time in the tail suspension test. To investigate the underlying molecular mechanism linking this receptor to behavioral changes, potential deregulated genes are being investigated in adult NgR2-/- CNS at the mRNA and protein level. The ATF/CREB family of transcription factors, particularly CREB, have been implied in the molecular changes associated with depression and anxiety. Investigation of brain structures implicated in depression and anxiety, such as the hippocampus and amygdala, showed increased levels of activating transcription factor 3 (ATF3), an adaptive response gene, in the hippocampus of NgR2-/- mice compared to wildtype controls. Preliminary results suggest there is no phosphorylation of c-Jun in the hippocampus, suggesting ATF3 does not heterodimerize with c-Jun to mediate its downstream effects. Increased levels of phosphorylated CREB were seen in the amygdala, supporting our behavioural data as the amygdala is reported to have decreased CREB activity under conditions of anxiety. Investigations into which particular intracellular pathways are activating CREB and ATF3 in this model are ongoing, and downstream targets of this transcription factos will also be examined.

P4

Identification and characterization of Nogo protein complexes

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RTN-4/Nogo proteins are myelin-associated proteins intimately implicated to impair axonal regeneration and structural plasticity in the adult mammalian nervous system. Apart from this well characterized function, recent *in vitro* studies have suggested that related to their subcellular localization to the endomembrane system, Nogo proteins may subserve additional roles, such as regulation of membrane curvature and protein trafficking [1-3].
To further investigate this hypothesis and to unravel possible molecular mechanisms of Nogo function in neurons, we aimed to identify interaction partners of Nogo in adult mouse brains.
For this purpose, we performed pull-down experiments using NiR, the N-terminal domain of Nogo-A and Nogo-B. Recombinant Nogo domain protein NiR (-GST) was used as bait to interact with proteins from mouse brain lysate. The pull-down products were analyzed by Mass Spectrometry (MS). This approach identified a number of proteins, majority of which had been classified as signal transduction, cytoskeleton dynamics related, and synaptic-vesicle and vesicle trafficking (endocytosis/exocytosis) associated.
We are currently in the progress of validation of these interactions for selected candidates, by performing co-immunoprecipitation, and colocalization studies by advanced microscopy techniques. Furthermore, in-detail biochemical characterization e.g. site/domain specificity of these potential interactions are being conducted.
As an outlook, we plan to address the functional importance and physiological significance of Nogo interactions. In brief, we envision to investigate the effect of these interactions in neurons, to close the gap of knowledge of how does Nogo function at a cellular level, for instance via the study of subcellular structures and related cellular mechanisms, such as endocytosis and exocytosis [4].

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P5

Highly Flexible System based on Mammalian Cell Culture for Identification of new Protein-Protein Interactions

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Protein production in mammalian cells offers great advantages for proteins that can not be expressed in bacteria or that have certain eukaryotic posttranslational modifications (PTMs), such as glycosylation. The establishing of stable cell lines and the screen for high producing clones is labour intense and time consuming. For getting good expression often gene amplification is needed, which requires additional selection rounds. Therefore at least several months are needed to get a high producing clone. But if the protein of interest is of any harm to the cell, the stability of the clones is an additional concern, which might lead to the complete loss of expression after some passages. A pull-down system that offers the advantages of mammalian protein production without the need for stable clones would be of great help in finding new protein-protein interaction.
We developed a system that uses Chinese Hamster Ovary (CHO) cells, grown in suspension and large scale transient transfection for protein production. In combination with the ONE-STrEP tag (IBA, Göttingen) and state of the art mass spectrometry we have a highly flexible system for pull down experiments in our hands. This technology will help us to identify new interaction partners and will lead to a better understanding of biological processes in the future.

P6

The role of Nogo in the epileptic rat brain

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The most common histopathological findings of mesial temporal lobe epilepsy (TLE) include neuronal cell loss as well as gliosis. Another characteristic phenomenon in TLE is termed mossy fiber sprouting – relating to the axons of granule cells - which is believed to enhance excitability. RTN-4A – better known as Nogo-A – is a possible candidate molecule which might be implicated in the regulation of activity-dependent plasticity. This protein is a member of the conserved family of endoplasmic reticulum-associated reticulon proteins and is best studied for its neurite outgrowth inhibitory effect when being expressed on the surface of myelinating oligodendrocytes after nerve injury.
High levels of Nogo-A can be detected in certain neurons of the CNS - not only during development, but also in the adult rodent brain. The intracellular localization of the protein – displaying a reticular pattern – suggests that Nogo-A might exert additional functions, such as a signaling molecule.(1) In studies of human(2) and experimental(3) temporal lobe epilepsy it was shown that Nogo-A mRNA and immunoreactivity were clearly up-regulated in hippocampal neurons and their processes. As it is believed that axonal and synaptic re-organizations in the hippocampal formation are promoting epileptogenesis, our group wants to test the hypothesis that knocking down Nogo-A leads to a change in the pathology of seizures in a rat model of temporal lobe epilepsy. Recombinant adeno-associated viral particles, containing shRNA targeted against Nogo-A, are injected into the rat hippocampus. After the system has reached peak expression, kainic acid is administered intra-peritoneally. This algae-derived acid is a specific agonist of the kainate receptor, mimicking the effects of glutamate and thereby eliciting seizures. The severity of the status epilepticus gets rated and continuous observation via video monitoring gives insight into seizure frequency and duration. Immunohistochemical techniques will be used to further characterize the plastic changes within the epileptic rat hippocampus.

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P7

Lentivirus-based knockdown of Rtn1A in cerebellar granule cells in vitro

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Rtn1A is a member of the reticulon protein family which also contains the neurite growth inhibitor Nogo (Rtn4). Reticulon proteins are associated with the ER membrane, and in particular Rtn1A is thought to be involved in trafficking and secretion of vesicles.

In preliminary experiments we observed enhanced neurite length following siRNA mediated downregulation of Rtn1A in cerebellar granular neurons (CGN). Interestingly, this effect was accompanied by a slight increase in BDNF secretion into the growth medium, implying that Rtn1A negatively modulates secretion of the neurite growth promoter BDNF. However, in those experiments only a small fraction of the CGN appeared to be transfected by siRNAs. Therefore we aimed to achieve high efficient down regulation of Rtn1A by taking advantage of lentivirus mediated gene transfer which has been reported to be superior to other viral mediated transfer systems because of its high infectivity for postmitotic cells including neurons.

We succeeded in the construction of vesicular stomatitis virus glycoprotein G pseudotyped lentiviruses expressing shRNAs targeting either all isoforms of Rtn1 or specifically Rtn1A, respectively. Two types of vectors were constructed: One set of vectors allowing constitutive bidirectional expression of both the shRNAs and red fluorescent protein (RFP), the other set allows tetracycline inducible expression of the shRNAs and eGFP.

We have achieved highly efficient infection of HeLa cells as well as of CGN isolated from P7 mice demonstrating the validity of our shRNA delivery approach. Furthermore, significant downregulation of Rtn1A in CGNs was observed by western blot analysis.

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P8

Thiolated polymers: development and in vivo evaluation of an oral delivery system for leuprolide

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Leuprolide acetate is a synthetic nanopeptide and a potent agonist of the luteinizing hormone-releasing hormone (GnRH or LH-RH) receptor. It is widely used for the non-surgical hormonal treatment of advanced prostate cancer, endometriosis, uterine fibroids and central precocious puberty in children.[1-2] Due to poor oral bioavailability leuprolide is currently administered via parenteral routes, which are often painful and inconvenient. Thus, development of non-invasive delivery systems is strongly on demand. Over the past few years, thiolated polymers (thiomers) have appeared as a promising candidate to improve the oral bioavailability of various hydrophilic macromolecules including peptide and protein drugs.[3]
The aim of the present study was to develop an oral delivery system for the peptide drug leuprolide. Gel formulations based on unmodified chitosan/reduced glutathione (GSH) and chitosan-thioglycolic acid (chitosan-TGA)/GSH were prepared and their effect on the absorption of leuprolide was evaluated in vitro and in vivo. Transport studies were performed with freshly excised rat intestinal mucosa mounted in Ussing-type chambers. Due to the addition of gel formulations comprising 0.5 % (m/v) unmodified chitosan/0.5 % (m/v) GSH and 0.5 % (m/v) chitosan-TGA/0.5 % (m/v) GSH, the transport of leuprolide across excised mucosa was improved up to 2.06-fold and 3.79-fold, respectively in comparison to leuprolide applied in buffer (Papp = 2.87 ± 0.77 x 10^{-6} cm/s). In vivo, the addition of oral gel formulation comprising 8 mg of unmodified chitosan, 1 mg of GSH and 1 mg of leuprolide increased the area under the plasma concentration time curve (AUC_{0-8}) of leuprolide 1.39-fold in comparison to leuprolide having been administered just in saline. Moreover, the administration of oral gel formulation comprising 8 mg of chitosan-TGA, 1 mg of GSH and 1 mg of leuprolide resulted in a further enhanced leuprolide plasma concentration and the area under the plasma concentration time curve (AUC_{0-8}) of leuprolide was increased 3.72-fold in comparison to the control. With the oral gel formulation comprising 8 mg of chitosan-TGA, a relative bioavailability (versus s.c. injection) of 4.5 % was achieved in contrast to the control displaying a relative bioavailability of 1.2 %.
Thus, according to the achieved results it is suggested that chitosan–TGA in combination with GSH is a valuable tool for improving the oral bioavailability of the peptide drug leuprolide.

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P9

Thiolated chitosans: *in vitro* comparison of mucoadhesive properties

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Mucoadhesive drug delivery systems enhance the contact to mucosal surfaces and prolong the residence time of dosage forms at the site of drug absorbtion [1]. Many attempts have been undertaken to improve the bioadhesive properties of various polymers. Thiolated polymers, also referred to as thiomers are capable of forming covalent bonds and thereby display much higher mucoadhesion. Due to thiol/disulfide exchange reactions and oxidation process, disulfide bonds serve as bridging system between thiol bearing side chains of these polymers and cysteine rich subdomains of glycoproteins in the mucus [2].
The purpose of the present study was to compare the mucoadhesive properties of thiolated chitosan with regard to the molecular mass and the chain length of various introduced thiol bearing ligands. For this purpose, thioglycolic acid, 2-iminothiolane HCl, N-acetyl-cysteine, glutathione, 4-mercaptobenzoic acid and 6-mercaptonicotinic acid were covalently attached to primary amino groups of chitosan under formation of amide bonds mediated by a carbodiimide. The chitosan conjugates obtained by this method displayed 320±50 µmol immobilized free thiol groups per gram polymer (mean±SD; n=5). In order to guarantee comparable mucoadhesive systems, all conjugates exhibited an amount of free thiol groups in this close range.
The mucoadhesion of chitosan conjugates adjusted to pH 4 was evaluated *in vitro* by using the rotating cylinder method and tensile studies on excised porcine intestinal mucosa. Results showed enhanced mucoadhesion of all thiolated chitosans ($P < 0.05$) compared to the unmodified polymer. The total work of adhesion (TWA) was in good correlation with the adhesion time.
Among all tested conjugates, a rank order of mucoadhesion of thiolated chitosans could be determined: Chitosan-TBA > Chitosan-4-MBA > Chitosan-GSH > Chitosan-6-MNA > Chitosan-NAC > Chitosan-TGA > unmodified Chitosan. According to this outcome, it could be claimed that aryl thiol bearing compounds and long-chain alkyl thiol groups displayed a stronger improvement of the residence time of chitosan compared to chitosan coupled with short aliphatic thiols.
In addition, the findings of this work give an extensive characterisation of a broad range of thiolated polymers, which were synthesized in the last years.

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P10

Enhanced transport of Rhodamine 123 in presence of thiolated hydroxyethylcellulose in Caco-2 cells

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Bioavailability is a very crucial factor in drug development. Chitosan and thiolated chitosan have shown to improve drug bioavailability (1) but the effect of hydroxyethylcellulose (HEC) and thiolated HEC on the bioavailability have not been investigated yet. After a drug has permeated into the cytosol of an enterocyte, it is often subject to efflux by apical membrane ATP-binding cassette (ABC) efflux transporters, for instance, P-glycoprotein (Pgp) (2). HEC is an inert cellulose and its properties such as biocompatibility, physiochemical stability and solubility in water make it suitable excipient for drug delivery (3). Thus, this study proposed to discover the effect of unmodified HEC and thiolated HEC on Pgp inhibition in Caco-2 cells using rhodamine 123 as a Pgp substrate. For thiolation of HEC we used cysteamine as ligand. The viability and cytotoxicity test of the polymers were performed using MTT and LDH assays in Caco-2 cells. HEC-cysteamine conjugate showed 536 ± 56 µmol free thiol groups and 850 ± 18 µmol disulfides bound to one gram of polymer. Unmodified HEC and HEC-cysteamine at concentration of 0.2% (m/v) did not effect viability of Caco-2 cells and resulted in significant by improved transport of rhodamine 123 compared to the control (medium treated only). These findings suggested that both unmodified HEC and HEC-cysteamine could attenuate the activity of Pgp in Caco-2 cells.

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P11

NOVEL THIOLATED MUCOADHESIVE NANOPARTICLES: FOR SUSTAINED DELIVERY AND DRUG TARGETING

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Prolonged residence time at the site of drug absorption maximizes not only drug concentration gradient but also influences the localization at a given target site. Accordingly, various approaches have been reported to prolong GI residence time, the use of bioadhesive polymers has been most investigated [1]. Thiolated polymers, or the so-called thiomers, are supposed to interact with cysteine rich subdomains of mucus glycoproteins via disulfide exchange reactions [2]. Based on thiol/disulfide exchange reactions (TDE) was formed, leading to even 6-fold improved viscosity of mucin [3]. Therefore, the overall objective of this study was to enhance the mucoadhesive property of a nanoparticulate delivery system which can be used in sustained delivery and targeting of drugs. Mediated by a carbodiimide, L-cysteine was covalently attached to the carboxymethyl dextran (CMD). The resulting carboxymethyl dextran–cysteine conjugate (CMD-Cys conjugate) displayed 273 ± 20 µmol thiol groups per gram of polymer (mean ± S.D.; *n*=3). CMD-Cys conjugate nanoparticles were prepared via a well-established high pressure homogenization technique. In the present study, nanoparticles were loaded with model compound fluorescein diacetate (FDA) instead of a therapeutic agent for analytical reasons. Zeta potential and mean particles size measurement of the unmodified nanoparticles and modified CMD-Cys conjugate nanoparticles in dialyzed water was performed using a PSS Nicomp 380 ZLS Particles Sizer. Transmission electron microscope (ZEISS LIBRA®120) was also used to examine size of nanoparticles. The mucoadhesive properties of $CMD-_{273}$ conjugate nanoparticles in comparison to unmodified nanoparticles were performed on porcine intestinal mucosa. Results showed that the CMD-Cys conjugate nanoparticles were to be a size of 275–300 nm. The zeta potential of the CMD-Cys conjugate nanoparticles was -24.69 ± 2 mV. The model compound FDA encapsulation efficiency ranged from 4.2 ± 0.98 to 10.6 ± 0.87% depending on the homogenization pressure and cycles. The pH dependent release of the entrapped model compound from these nanoparticles revealed about 83% release at pH 7.4. Mucoadhesive properties of CMD-Cys conjugate nanoparticles were eleven times as high as the mucoadhesive properties of unmodified CMD nanoparticles. According to these results, thiolated CMD-Cys conjugate nanoparticles described here might be a promising tool in transmucosal drug delivery targeting for poorly absorbed drugs such as class II and IV drugs according to the biopharmaceutical classification scheme.

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P12

Novel and conserved function of the regulatory subunit of protein kinase A

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The G-protein coupled receptor (GPCR) signal transduction pathways are regulated at several critical points via feedback loops and cross-talk. Elucidation of dynamic physical connections between pathways may help to understand how cells respond to environmental cues and integrate information through shaping of spatiotemporal signaling responses [1]. Using protein-fragment complementation assays [2, 3], we have identified an unforeseen active role of the cAMP-bound regulatory subunit (Reg) of protein kinase A (PKA) in feedback regulation and cross-talk between GPCR pathways. Classically, cAMP binds to Reg and induces the dissociation of the PKA holo-tetramer resulting in activation of kinase activity [4]. We have discovered that cAMP-bound Reg forms specific complexes with the inhibitory G protein ai (Gai), independent of PKA kinase activity. We demonstrate that these complexes affect conformation and activities of the trimeric G proteins ai:bg resulting in increased sensitivity, amplitude, and duration of GPCR-mediated MAP kinase activation in mammalian cells. We further demonstrate that this mechanism is conserved in a Gai-mediated pheromone response MAPK signalling pathway in budding yeast serving to control the sensitivity to pheromone and modulate the decision to mate, depending on nutrient availability. Similar tuning of Gai-coupled GPCR hormone responses through inputs of Gas-coupled signals may be a common event. Gas-coupled GPCRs are frequent targets for therapeutic intervention for a number of human diseases and thus, cAMP-mediated tuning of Gai-coupled GPCR signalling could result in many and varied indirect effects of Gas-coupled receptor targeted drugs.

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P13

Role of arginine methylation in the control of nuclear factor-κB (NF-κB)

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Nuclear factor-κB (NF-κB) is an inducible transcription factor which regulates the expression of a large number of genes critical for several processes, including apoptosis, viral replication, tumorigenesis, inflammation, and various autoimmune diseases. Transcriptional activity of NF-κB is regulated by complex mechanisms dependent on cellular localization, promoter specific exchange of NF-κB dimers, and modification of the transactivating p65 subunit of NF-κB by phosphorylation, acetylation, ubiquitination, or prolyl isomerization [1, 2]. Recently, protein arginine methyltransferases (PRMTs) have been identified as co-regulators of NF-κB dependent gene expression [3, 4]. However, the molecular mechanisms of PRMT mediated regulation of NF-κB remain elusive. Here we report that PRMT1 forms a constitutive complex with the p65 subunit of NF-κB. A deletion analysis of p65 indicated that the N-terminal Rel-homology domain (RHD) of p65 mediated direct interaction between p65 and PRMT1 *in vitro* and *in vivo.* Furthermore, we found that p65 was specifically methylated by PRMT1 *in vitro*. Several arginine residues located within the RHD were predicted as methylation sites for PRMT1 using bioinformatic tools. The methylation sites were experimentally confirmed by mutating the corresponding arginines to lysines or alanines. Sequence alignment of the RHD proteins from different organisms revealed that the identified methylation sites were evolutionary conserved from drosophila to man indicating their potential importance. We are currently investigating the regulatory role and the physiological significance of multiple arginine methylation of the p65 subunit of NF-κB.

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P14

Vibrational spectroscopy as a Tool to monitor the microwave-assisted drying process of wood

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Fourier transform mid-infrared spectroscopy with attenuated total reflection (FTIR-ATR), near infrared spectroscopy (NIRS) and infrared reflectance imaging spectroscopy (FTIRimaging) in combination with multivariate data analysis is used to determine wood moisture, as well as chemical changes in a microwave assisted drying process of timber. Being a fast, non invasive method with high automation potential vibrational spectroscopy is of high interest for industrial use especially regarding Process Analytical Technology (PAT). FTIR-imaging opens access to the areal distribution of spectra down to a resolution of 6.25 5m which is applied to show the changes in wood samples during the drying process. For future research it is planned to couple the NIRS and the microwave oven with a light fibre to allow online monitoring of the drying process. The potential of mid- and near-infrared spectroscopy for the characterization of physical and chemical parameters in the microwave assisted drying process of wood is demonstrated and discussed in the proposed poster.

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P15

Fourier transform infrared imaging analysis in discrimination studies of prostate cancer and prostate cancer cell lines

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Fourier transform infrared (FTIR) imaging is a vibrational spectroscopic technique that uses infrared radiation to image molecules of interest in thin tissue sections. Major advantage of this new technology is the acquisition of local molecular expression profiles, while maintaining the topographic integrity of the tissue. Therefore this technology has become an essential tool for the detection and characterization of the molecular components of biological processes, such as those responsible for the dynamic properties of tumor progression.
Using this method it is possible to investigate the spatial distribution of proteins and small molecules within biological systems by in-situ analysis. In this study, we have evaluated the potential of FTIR imaging to study biochemical changes during prostate cancer development. Selected tumor samples and prostate cancer cell lines were measured and analyzed by univariate and cluster analyses. For the interpretation and calibration of the system we correlated the FTIR-images with the histopathological information. With this new method it is possible to distinguish between tumor and non-tumor areas within a prostate cancer tissue with a resolution of 6.25 µm x 6.25 µm.
The possibilities, ad- and disadvantages and limits of this approach compared to other analytical techniques, including e.g. mass spectrometry, are discussed in detail within this proposed presentation.

P16

Collisionally Activated Dissociation (CAD) and Electron Detachment Dissociation (EDD) of proteins in negative ion mode Electrospray Ionization (ESI)

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Electron capture dissociation (ECD) and collisionally activated dissociation (CAD) give complementary sequence information in top-down mass spectrometry (MS) experiments using multiply protonated protein ions, $(M + nH)^{n+}$, from electrospray ionization (ESI) [1]. Acidic proteins, however, can be ionized more efficiently in negative ion mode ESI [2]. Here we investigate the use of collisionally activated dissociation (CAD) and electron detachment dissociation (EDD) [3] for top-down MS of multiply deprotonated protein ions, $(M - nH)^{n-}$. We anticipated that unimolecular protein dissociation in negative ion mode is affected by precursor ion charge, as in EDD of RNA [4]. We found that ESI additives of differing gas phase basicity can be used for efficient manipulation of protein ion charge in (-)ESI, and that high negative precursor ion charge is critical for high sequence coverage in EDD of proteins. Studies aimed at understanding the mechanism of EDD reveal that it involves deprotonation of the backbone amide, which is facilitated by ionic hydrogen bonds with side chains of T, S, Q and N. We demonstrate ~60% sequence coverage for melittin (2.8 kDa) and ubiquitin (8.6 kDa) in EDD experiments.

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P17

Identification, localization, and relative quantitation of pseudouridine in RNA by tandem mass spectrometry of hydrolysis products

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Among the numerous (>100) currently known post-transcriptional modifications of RNA, the conversion of uridine (U) into pseudouridine (Ψ) is the most common [1,2,3,4]. Clustering of Ψ residues in functionally relevant regions, and a high degree of conservation throughout phylogeny indicate an important role of this modification in cellular processes; nevertheless, the biochemical function of pseudouridylation is not yet understood [5]. A prerequisite for making out the meaning of pseudouridylation is to identify where, when, and to what extent the modification occurs.
Because the conversion of U into Ψ is a "mass-silent" post-transcriptional modification, the majority of mass spectrometry based approaches for the detection of Ψ in RNA uses chemical labeling reactions that alter the mass of Ψ residues. However, chemical labeling is generally laborious, and has limited selectivity for Ψ [6]. Here we propose a new mass spectrometry based method for identification, localization, and relative quantitation of Ψ in RNA consisting of ~20 nucleotides that does not require chemical labeling. Our straightforward approach combines RNA hydrolysis with MS/MS techniques, taking advantage of the different fragmentation behavior of uridine (N-glycosidic bond) and pseudouridine (C-glycosidic bond) residues in RNA upon collisionally activated dissociation [7].

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P18

Isolation and identification of novel substrate proteins of a protein arginine methyltransferase specific for filamentous fungi

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In the filamentous fungus *Aspergillus nidulans* three distinct protein arginine methyltransferases (PRMTs) are present which all possess *in vitro* and *in vivo* methyltransferase activity [1]. One of these enzymes, termed RmtB, has an exceptional position because it displays both enzymatic and structural properties in comparison to other known PRMTs [1]. Thus, our long term objective is to clarify the functional role of this fungal specific PRMT. For this purpose we want to separate RmtB protein complexes, to identify interacting proteins and to isolate novel substrate proteins, to finally get more information on the implication of this enzyme in fungal specific pathways. Recently, we have generated mutants of the corresponding PRMT genes by targeted gene replacement. Protein extracts of mutant mycelia were separated by ion exchange chromatography (IEX) and resulting fractions were used for the *in vitro* labeling of proteins with purified RmtB (IEX, gelfiltration) [2]. Labeled substrate proteins were further separated and analyzed by 2D gel electrophoresis and fluorography, respectively, and will be subsequently identified by mass spectrometry.
In an initial screen, we could identify several selective and non-selective proteins as putative targets of *Aspergillus* RmtB. The subsequent analysis of these protein fractions by 2D gel electrophoresis allowed the high resolution separation for the identification by mass spectrometry. Our results have confirmed our previous findings that demonstrated significant *in vitro* methylation potential of endogenous RmtB and the presence of a variety of yet unknown substrate proteins in *Aspergillus nidulans*. Importantly, our *in vitro* methylation assays revealed the presence of selective and non-selective substrate proteins in Aspergillus protein extracts depending on the initial methylation status of these proteins *in vivo*.

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P19

STAT1 Deficiency Results in Resistance of Neu/ErbB2-induced Mammary Tumors to Chemotherapy

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The signal transducer and activator of transcription STAT1 is a key mediator of cell cycle arrest and apoptosis (1). Furthermore, STAT1 is implicated in tumor immunosurveillance (2). By analysis of the STAT1 activation status in human primary tumors we have observed a correlation between loss of STAT1 activation and bad prognosis (3). One hypothesis to explain these findings is that impaired STAT1 function promotes resistance to chemotherapeutics, in particular to genotoxic drugs: by cooperation with p53 and participation in ATM DNA damage response STAT1 is postulated to induce tumor epithelial cell death via cell autonomous mechanisms (4). Another possible mechanism is the participation of STAT1 in the described enhancement of tumor immunosurveillance by genotoxic drugs (5).
To further resolve these issues MMTV-neu (N) mice are investigated in our laboratory as an animal model for erbB2 positive breast cancer. We have recently shown that the ex-vivo activation of the STAT1 target gene iNOS in tumor infiltrating myeloid cells from these mice can induce apoptosis (6).
We now have created STAT1 deficient FVB/N-MMTV-neu mice and have assessed the effect of STAT1 deficiency on tumor biology and response to chemotherapeutics. Our experiments revealed that STAT1 deficient MMTV-neu (N) mice exhibit shorter latency in the development and faster growth of erbB2 driven mammary adeno-carcinomas. Most interestingly, the response of the tumors to doxorubicin and/or lapatinib treatment *in vivo* is decreased in STAT1 deficient animals, demonstrating the important role of STAT1 for the response to chemotherapy. Surprisingly, STAT1 deficient mice are susceptible to a combined treatment with IFN-g and lapatinib and/or doxorubicin, implicating that stimulation of IFN-g triggered signaling pathways can compensate for STAT1 deficiency in tumor therapy.

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P20

The Role of Tumor-Infiltrating Myeloid Cells in Mammary Tumors of MMTV-neu Stat1 WT and KO Mice

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In the vast majority of human and murine malignancies tumor-infiltrating myeloid cells play important roles in creating an immunosuppressive environment [1] and supporting the growth of the tumor [2]. More specifically, CD11b+ CD11c+ myeloid cells in mouse mammary gland tumors of MMTV-neu origin were described to possess the ability to suppress antitumor adaptive immune response by expression of arginase-1 [3]. However, several cancer treatment protocols were reported, where myeloid cells can be activated in order to elicit potent antitumor T-cell response [4, 5].
Our previous results indicate that CD11b+ infiltrate cells may be responsible for mediating tumor cell death induced by IFN-γ [6]. We also found that tumors from STAT1 KO mice are less sensitive to therapy, and this could not be recapitulated *ex vivo* with explant cultures of pure tumor epithelial cells, which are devoid of CD11b cells (Hannesdóttir et al., in preparation).
In the current work we focus on the function and properties of infiltrating CD11b+ cells in MMTV-neu mammary adenocarcinomas under steady-state conditions. Our goal is (a) to elucidate their possible contribution in tumor interaction with the immune system, and (b) to test, whether an impaired function and/or abundance of these cells in Stat1 KO mice are responsible for the resistance of tumors to chemotherapy. The tumor-infiltrating CD11b+ cells were characterized with specific myeloid, macrophage and dendritic cell surface markers and their fractions in MMTV-neu Stat1 KO and WT tumors were determined. We also checked the expression of a panel of genes with known functions in immune response modulation, antigen presentation and immune cell trafficking.

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P21

Investigation of Spindly domains

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Human Spindly recruits a fraction of cytoplasmic dynein to kinetochores which is required for prometaphase poleward movement of chromosomes as well as for stripping off particular proteins from kinetochores. These processes are essential for proper chromosome congression and for control of mitotic checkpoint signalling. Besides known dynein-dependent functions, Spindly also regulates the function of ZW10, which promotes formation of stable end-on microtubule attachments and is required for mitotic checkpoint signalling. How Spindly interacts with ZW10 (or another member of the Rod/Zwilch/Zw10 complex), with the dynein motor complex and how it exerts its functions is still unclear.
Here, we have analysed a series of Spindly truncation and point mutants and investigated the effect of these alterations on protein localisation and function. These analyses revealed that the localisation of Spindly to kinetochores requires multiple domains. The N-terminal region of Spindly, encompassing one of the coiled-coil domains, is required for interaction with the dynein complex, while the C-terminal part seems to interact with ZW10. Although Spindly is phosphorylated at S515 in mitosis, this phosphorylation is not important for its localization. Interestingly, we showed that the C-terminal CPQQ sequence, which is suggested as a potential farnesylation site, plays an important role in kinetochore localization. Substitution of cystein to serine (C602S), depletion of the two last C-terminal amino acids and substitution to lysine and asparagine prevent Spindly from binding to the KCs. But an exchange of the whole motif to a consensus farnesylation site (CAAX) show kinetochore localisation. In further experiments we will investigate the potential farnesylation site and its role for dynein and ZW10 interaction in more details.
By using N-terminal truncation mutants as well as a mutant lacking the conserved 'Spindly box', we found that the dynamic behaviour of Spindly at kinetochores was abrogated and Spindly failed to translocate to the spindle poles during the process of chromosome bi-orientation. Interestingly these mutants exerted a dominant negative effect and blocked the onset of anaphase by maintaining the activity of the mitotic checkpoint.

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Molecular characterisation of the orphan cyclin dependent kinase PCTAIRE-1/CDK16

P22

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Cyclin-dependent kinases (CDKs) are well known for their function in cell cycle or transcriptional control, but the functions of several other members of this large serine/threonine kinase family are still elusive. CDK16, 17, and 18 (previously known as PCTAIRE kinases 1, 2 and 3) are highly conserved among metazoans but neither activator nor substrates of CDK16-18 are well established. CDK16 is expressed at high levels in the brain and in the testis but it is also found in several other tissues and cell lines. In the testis, CDK16 expression is highest in differentiated spermatids, suggesting a role in the differentiation of spermatozoa. Here we summarize our biochemical, cell biological as well as genetic analysis of CDK16. In gel filtration experiments using mouse testis cell lysate, Cdk16 eluted as a ~115 kDa complex and immunoprecipitated Cdk16 phosphorylated a ~116 kDa protein in an *in vitro* kinase assay using dephosphorylated testis cell lysate as substrate. These data suggested that active Cdk16 was associated with other proteins, which encouraged us to perform yeast two hybrid interaction screens. We used GAL4- and LexA-based systems in mating or conventional co-transformation approaches to identify potential proteins expressed in the testis for their ability to interact with Cdk16. Of 15 candidate proteins, only cyclin Y (CCNY) could be confirmed to interact with Cdk16 in co-immunoprecipitation and colocalisation experiments of epitope-tagged proteins expressed in human cells. In addition, when overexpressed Cdk16 is immunoprecipitated in the presence of overexpressed tagged CCNY, Cdk16 showed increased kinase activity towards myelin basic protein in an *in vitro* kinase assay. To determine the role of mouse Cdk16 in vivo, we created a mouse strain harbouring a conditional *Cdk16* allele. Cdk16 deficient male but not female mice were found to be infertile. Histological examination of testis sections, however, demonstrated robust spermatogenesis, but spermatozoa from *Cdk16* null mice were hypomotile and structurally abnormal. Transmission and scanning electron microscopy of epididymal sperm showed aberrantly formed sperm heads which contained an excess of residual cytosol. In addition, the annulus was disconnected from the neck region, causing spermatozoa to bend. In summary, our data suggest that Cdk16 is activated by a cyclin and is essential for the differentiation of spermatozoa.

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P23

Human chromokinesins hKid/Kif22 and Kif4A are required for chromosome arm movement and congression

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The human chromokinesins hKid/KIF22 and Kif4A are plus-end directed motor proteins which can bind to both microtubules and chromosomes during mitosis. While the frog proteins Xkid and Xklp1 were shown to be essential for establishing a bipolar spindle, the role of human chromokinesins is less well established. While hKid was described to generate the 'polar ejection force' pushing chromosomes towards the metaphase plate, Kif4A has mainly been implicated in late mitotic functions such as central spindle assembly and cytokinesis.
Here we used siRNA to deplete human HeLa cells for hKid, Kif4A or both and found that simultaneous knockdown of the two motors arrested cells in prometaphase while knockdown of either protein did hardly affect the mitotic timing. Arrested cells were characterized by prolonged spindles with multiple, disintegrated centrosomes. While hKid-depleted cells had shorter spindles, knockdown of Kif4A or both motors resulted in a broader interkinetochore distance, suggesting an excess of pulling forces. We could not detect any differences in kinetochore microtubule stability using cold treatment assays in Kif4A-depleted cells but we observed reduced microtubule flux, indicating that arm microtubules are less dynamic after knockdown of Kif4A. High resolution 4D time lapse imaging of histone H2B-GFP labeled HeLa cells revealed that chromosomes congressed with normal kinetics in Kid/Kif4A-knockdown cells with chromosome arms pointing towards the spindle poles, indicating improper attachment. After being retained in that state for a while, chromosomes became moved out of the plate until the metaphase plate finally fell apart.
We propose that hKid generates a pushing force which helps to move chromosome arms to, and keep them at, the metaphase plate, while Kif4A regulates microtubule dynamics allowing the rapid correction of attachment failures. Thus, human chromokinesins are required to establish and maintain a metaphase plate – a mechanism which provides robustness to the human spindle and might distinguish it from that of other species.

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P24

Investigating the role of vertebrate Fzr1, a master regulator of G1 phase

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The anaphase promoting complex / cyclosome (APC/C) is a cell cycle-regulated multisubunit ubiquitin ligase. In mitosis, the APC/C is activated by mitotic phosphorylation as well as association with CDC20, a seven WD40 repeat containing protein that serves as activator and substrate recruitment factor. The APC/C targets securin as well as the mitotic cyclins for ubiquitin-dependent proteolysis and is, thus, essential for the onset of sister chromatid separation (anaphase) as well as exit from mitosis. As CDK activity declines during anaphase, the CDC20-related protein FZR1 replaces CDC20 as APC/C activator and substrate recruitment factor to keep the APC/C active during the exit from mitosis and during the ensuing G1-phase. By using cellular model for FZR1 function, e.g., by RNAi-mediated depletion of FZR1 in human cells, we have found that FZR1 is not required for completion of mitosis but that it plays a crucial role in G1-phase. In the absence of FZR1, G1-phase is shortened and cells prematurely initiate DNA-synthesis. In addition, FZR1 knockdown cells exhibit a strong DNA damage response, which impairs proliferation. Thus, loss of FZR1 function appears to induce replication stress followed by p53 induction and proliferation arrest. To investigate the function of FZR1 in vivo, we have generated a conditional knockout mouse and used antisense morpholino-mediated suppression of FZR1 expression in the zebrafish. Deletion of Fzr1 in the mouse causes embryonic lethality due to a placenta failure. By restricting Fzr1 depletion to the epiblast (using a Sox2-Cre deleter strain), we could overcome this early embryonic lethality, which allowed Fzr1 deficient mice to develop to term. These mice are significantly smaller (~50%) and look much frailer than their wild-type littermates. In contrast, mice lacking Fzr1 in the hematopoietic system (Vav-Cre), the skin (K5-Cre) or the central nervous system (Nex2-Cre) did not show obvious phenotypes. We are currently analysing tissue homeostasis by investigating the expression of cell cycle markers and APC/C substrates in Fzr1 deficient tissues and primary cells. In developing fish, a small amount of Fzr1 can be detected in the zygote but Fzr1 becomes undetectable by immunoblotting until the mid-blastula transition, which coincides with the establishment of G1-phase during development. Injection of two independent morpholino antisense oligonucleotides targeting sequences in the Fzr1 mRNA surrounding the translation initiation codon delayed development beyond epiboly and arrested embryos in early segmentation stage. The embryos showed prominent extension but little or no conversion defects, as observed by in situ hybridization and antibody stainings. The morphant embryos displayed irregular somites and developed cardiovascular defects and defects in brain development during later stages of development, which depicts the importance of Fzr1 during development. We further investigate the regulation of Fzr1 during organogenesis using organ specific markers and transplantation experiments, which could gain an insight about the role of Fzr1 during development of an organism.
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P25

Phosphorylation of p27*Kip1* by JAK2 directly connects cytokine signaling to cell cycle control

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Janus kinase 2 (JAK2) couples ligand activation of cell surface cytokine receptors to the regulation of cellular functions including cell cycle progression, differentiation and apoptosis. It thereby coordinates biological programs including development and hematopoiesis. Unscheduled activation of JAK2 by point mutations or chromosomal translocations can lead to hyperproliferation and hematological malignancies[1]. The best-characterized signal transduction pathway by the JAK2 tyrosine kinase involves phosphorylation STAT transcription factors. Here we describe the identification of a novel JAK2 substrate, the cyclin dependent kinase (CDK) inhibitor p27^{Kip1}. JAK2 can directly bind and phosphorylate p27^{Kip1}. The JAK2 FERM and kinase domains are involved in complex formation with p27^{Kip1}. JAK2 mediated phosphorylation of p27^{Kip1} occurs at tyrosine 88. We previously reported that tyrosine 88 phosphorylation impairs p27^{Kip1} mediated CDK inhibition and initiates its ubiquitin-dependent degradation[2,3]. Accordingly, we now find that the activated JAK2V617F mutant reduces p27^{Kip1} expression in patient-derived cell lines harboring this mutation. p27^{Kip1} levels are restored upon JAK2 inactivation by shRNA or JAK inhibitor treatment. Tyrosine 88 phosphorylated p27^{Kip1} can be detected in primary erythroid progenitors cells and high levels of active JAK2 correlate with elevated tyrosine 88 phosphorylation of p27^{Kip1} in cells derived from polycythemia vera patients. Activation of JAK2 by interleukin 3 induces tyrosine 88 phosphorylation of p27^{Kip1}, suggesting a role of p27^{Kip1} tyrosine 88 phosphorylation in cytokine signaling of non-transformed cells. We propose that the interaction between p27^{Kip1} and JAK2 provides a novel and direct link between cytokine signaling and cell cycle control that may contribute to hyperproliferation of cells transformed with oncogenic JAK2V617F.

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P26

FHL2 is a negative regulator of the cell cycle inhibitor p57^{Kip2}

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The Cip-Kip-family of cyclin dependent kinase inhibitors (CKIs) consists of p21^{Cip1} p27^{Kip1} and p57^{KIP2}. The proteins are structurally and functionally related and bind to and usually inhibit cyclin-CDK-complexes thereby inhibiting proliferation. p57 is the least studied Cip/Kip-member and its regulation and function is not fully understood. p57-knock-out mice show a severe developmental phenotype, demonstrating that p57 is an essential gene. In order to gain more insight into the regulation of p57, we performed a yeast-two-hybrid screen. In addition to previously described binding proteins, we obtained several novel interaction partners for p57. One of those turned out to be FHL2, a member of the four-and-a-half-LIM-only protein family. FHL2 is a multifunctional protein and is involved in various cellular processes like transcriptional regulation, cell survival, cell motility and signal transduction. We found that FHL2 specifically binds to p57 and not to the structurally related CKIs p21 and p27. FHL2 and p57 are known to be expressed in a cell-type specific manner. Interestingly, both proteins are reported to be abundant in human cardiac tissue. We were able to detect p57-FHL2-complexes in the mouse cardiac myocyte cell line HL-1 and additionally in cervix and colon carcinoma cells, where p57- and FHL2 levels are lower. p57 binds and inhibits cyclin-CDK-complexes and high levels of p57 arrest cells in G1-phase of the cell cycle. We found that coexpression of FHL2 reverts p57-induced G1-arrest indicating an interference of FHL2 with the CDK-inhibitory function of p57. The CDK-inhibitory domain of p57 is located in the N-terminal part of the protein and harbors binding motifs for both, the CDK- and cyclin subunits of the holoenzyme. Preliminary results suggest that FHL2 binds to the CDK-inhibitory domain of p57 thereby preventing it from cyclin-CDK binding and inhibition. Based on our data we conclude that FHL2 is a negative regulator of p57 function. Further experiments are necessary to prove whether p57 also regulates FHL2. Molecular strategies to interfere with p57 inhibition by FHL2 might increase p57-CKI-activity and may be useful for cancer therapies.

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P27

Generation of transgenic flatworms

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Flatworms become an attractive new model system to address various biomedical questions. While many methodological approaches have been developed, the lack of the possibility of generate transgenic animals was a major hindrance for designing and performing particular research projects. Despite considerable effort of the community currently no transgenic flatworms can be produced.
We have succeeded in the generation of transgenic flatworm *Macrostomum lignano*. In a proof of principle we used a promoter for elongation factor alpha driving EGFP expression in a minos transposase vector system. Constructs injected into single cell eggs resulted in transient and stable EGFP expressing animals, i.e partially or completely green glowing worms. These animals will be used to study e.g. the differentiation potential of stem cells by transfer of EGFP expressing stem cells into irradiated host animals that completely lack stem cells - an approach comparable to bone marrow transplantation. We are currently generating vectors containing different promoters to obtain tissue or organ specific expression. The breakthrough accomplished here will put forward M. lignano as a flatworm model system. It will allow to address question related to flatworm biology and could also contribute to promote human health.

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P28

frizzled genes are expressed dynamically in *Hydra* axial patterning

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Seven-pass transmenbrane proteins of the Frizzled family act as receptors primarily for secreted Wnt proteins during early embryonic development throughout the eumetazoan clade. It is presently unclear, how many Frizzled receptors are encoded in the *Hydra* genome and in which functional context they may act.
We isolated three putative *frizzled* genes from the *Hydra* genome database[1]. One of these genes represents *frizzled1*, which has been previously published and which exhibits a uniform endodermal expression pattern[2]. The three *Hydra frizzled* genes cluster in three gene subfamilies that had evolved in the most basal metazoans. This confirms an earlier report about the phylogeny of *frizzled* genes in the hydrozoan *Clytia*[3]. For *frizzled2* and *frizzled3*, we have performed a detailed study of their spatial and temporal expression dynamics in adult polyps, evaginating buds, and head regenerates. *frizzled2* expression is co-localized with the non-canonical Wnt pathway genes *wnt8* and *dsh* under any experimental regime, suggesting that it encodes a non-canonical Wnt receptor[4]. *frizzled3* is co-expressed with *frizzled2* during tentacle formation, *frizzled2* in ectodermal epithelial cells and *frizzled3* in endodermal epithelial cells. In addition, *frizzled3* exhibits a gradual expression pattern during bud evagination similar to the *beta-catenin* and *tcf* genes. Hence, this *frizzled* gene may additionally act in the canonical Wnt pathway. Changes in transcriptional activation of *frizzled2* and *frizzled3* under the influence of small molecule inhibitors affecting GSK3, beta-Catenin-Tcf, and JNK indicate that Wnt/beta-Catenin signalling regulates the interaction between canonical and non-canonical Wnt pathways during tissue morphogenesis and axial patterning in *Hydra*.
For further investigation of protein function and localization we are currently working to establish transgenic *Hydra* lines expressing Frizzled proteins joined to a GFP reporter.

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P29

Advanced Electron Microscopical Methods for *Hydra*

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Electron microscopy of model organisms like *Hydra* has experienced renewed interest since transgenic animals and genome data became available[1]. Now, the presence or absence of genes can be compared with the appearance of structural components. New technical advancements like high-pressure freezing and cryo-substitution, as well as immunogold labelling of green fluorescence protein (GFP) localisation in transgenic animals give new insights into ultrastructure and biological processes. Different types of cell-cell and cell-matrix contacts can be studied in more detail. Fixation artefacts like the poor preservation of the cuticle layer in *Hydra* can be avoided by using cryo-preparation techniques[2]. Precise subcellular localisation of GFP can be achieved with immunogold labelling and these results can be then compared with regular TEM and fluorescence microscopic data to analyze biological processes.

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P30

The life cycle of the acoel flatworm *Isodiametra pulchra* under laboratory culture

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Acoel flatworms, a group of small marine worms, were considered to be a taxon within the phylum Platyhelminthes[1]. In the past decade, this phylogenetic position was put into question by several authors using modern molecular phylogenetic analyses. These results have placed the Acoela at the base of the Bilateria as the new phylum Acoelomorpha[2],[3],[4].
Bilaterians are thought to originate from animals of simpler, radially symmetric body organisation. Yet the transition from radially symmetric to bilaterally symmetric body organisation is still not well understood.The position of the Acoelomorpha at the base of the Bilateria will likely provide new insights into the evolutionary origin of bilateral symmetry.
The Institute of Zoology has kept a member of the acoel flatworms, *Isodiametra pulchra,* in laboratory culture for several years now. Standard molecular and histological techniques have been elaborated for this animal in our laboratory[5]. In our work, we took a closer look on basic aspects of life history of *I. pulchra* such as growth, lifespan, sexual reproduction and generation time. These results indicate that this acoel is a potent candidate for the use as a basal bilaterian model organism.

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P31

Regulation of the endosomal p14/Mp1-MAP kinase scaffold complex by the proteasome

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The endosomal adaptor p14 regulates EGF and G-CSF dependent ERK1/2 activation, EGFR degradation as well as biogenesis and subcellular distribution of late endosomes/MVBs. [1,2,3]. Reduced Mp1 protein levels were reproducibly found in all p14-deficient cellular and animal models generated up to date to study p14 functions (eg. after RNAi knockdown, MEF from $p14^{-/-}$ mice). Interestingly, B cells obtained from patients with a 3'UTR mutation on p14 show a similar reduction of MP1 protein levels [3]. We have, therefore, decided to investigate the mechanisms regulating Mp1 at the transcriptional, translational and posttranslational levels. In contrast to the protein levels, Mp1 mRNA levels are higher in p14-depleted MEFs when compared to controls, excluding altered transcription and indicating a possible compensatory mechanism. Experiments with cycloheximide and Velcade excluded translational impairment but indicated faster proteasomal degradation. We have also shown that Gst-Mp1 can be polyubiquitinated *in vitro* and that the rate of ubiquitination is higher in lysates of p14-depleted MEFs. In addition, a cytoplasmic mutant of p14 is sufficient to rescue Mp1 protein levels, confirming that heterodimer formation alone is critical for Mp1 stability. Mutational analysis indicated that the turnover of Mp1 is to a large extent dependent on ubiquitination of internal lysine residues. Most importantly, in contrast to the majority of proteins, Mp1 does not have a preferential ubiquitination site. More than one lysine in Mp1 can be used to efficiently promote a physiological turnover rate. The tight control exerted by the proteasome may be used to limit the abundance of Mp1 in a time dependent manner, therefore regulating its scaffolding function. Interestingly, we detected subunits of the proteasome co-immunoprecipitating with Mp1, namely PSMA3 and PSMC4. PSMC4, also known as S6, was shown to interact with polyubiquitinated chains in an ATP dependent manner and to be pivotal in the recognition of this signal for adequate substrate unfolding, translocation into the inner chamber of the proteasome and degradation [4]. This corroborates our mutational analysis indicating that lysine ubiquitination plays a role in Mp1 degradation.

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P32

Conditional Gene Ablation of the MAP Kinase Adapter Protein p14 in Dendritic Cells leads to Severe Disturbance of Tissue Homeostasis

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Background: Dendritic cells are key players of the immune system and link innate to adaptive immune response. Their major task is to take up pathogens, process them and present the antigen to T cells. These processes are strongly dependent on endosomal/lysosomal trafficking. Conditional gene disruption of the adapter protein p14 in mice demonstrates that the late endosomal p14/MP1-MEK1 signaling complex is required to control endosomal traffic and tissue homeostasis (Teis et al., J Cell Biol, 2006).
Methods: To address the molecular function of p14 in dendritic cells, we generated a conditional knock out mouse model which allowed us to specifically delete p14 in CD11c expressing cells. The effects were analyzed in tissue (histological methods) and primary cell culture (FACS-Analysis, Western Blot).
Results: The mice were viable and developed a severe pathological phenotype at the age of three months. The most obvious morphological characteristics included enlarged lymph nodes and splenomegaly. The structural integrity of these organs was disarranged and massive leukocyte infiltrates were observed. Furthermore, these mice developed infiltrates of monocytes and dendritic cells in skin and liver. The bone marrow of the CD11c-p14 knock out mice was hyperplastic, accompanied by an increase of hematopoetic stem cells. Furthermore a shift from the granulocytic towards the monocytic lineage, an increase in the T helper cell population and a decrease of the erythrocyte progenitors was observed. Bone marrow derived dendritic cells showed an increased expression of maturation markers after stimulation with Lipopolysaccharide. Immature dendritic cells were able to take up latex beads and yeast to late endosomal compartments but showed an impaired ability to migrate.
Conclusion: Taken together, p14 severely affects the tissue homeostasis of dendritic cells. Further immunological and cell biological investigations will help us to elucidate the role of p14 in dendritic cells.

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Late endosomal p14/MP1-MAP kinase signaling couples IQGAP1 and Rac1 to focal adhesion dynamics and cell migration

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Focal adhesions govern cell motility. Asymmetric distribution and dynamics of focal adhesions rely on a variety of signaling cascades and require polarized cellular distribution of molecular components. Enabled by scaffold complexes, endosomes can ensure optimal coupling of molecular effectors to compartmentalize cellular signaling. Here we show that late endosomes, carrying the p14/MP1 MAPK scaffold complex can move to the cell periphery where they specifically target focal adhesions. In the absence of the signaling complex on late endosomes we observed strongly impaired cell migration and a defect in focal adhesion remodeling. Our data suggest that binding of the signaling complex through MP1 to the IQ-domain of IQGAP1 is required for IQGAP1 localization to the plasma membrane, activation of Rac1 at the leading edge and proper focal adhesion remodeling. We propose a novel role for late endosomes carrying the p14/MP1 MAPK scaffold complex in signaling compartmentalization and cell migration.

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Screening for MAP kinase scaffold inhibitors for proliferative and inflammatory diseases

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Different scaffold proteins for the Ras-MAPK pathway have been found to reside at various subcellular compartments. The p14/MP1 complex, the target we are focusing our work on, functions as an endosomal scaffold protein. Genetic deletion experiments revealed a pronounced cell cycle progression defect, always combined with a severe reduction in ERK activation. Together with previous evidence demonstrating a role for the Ras-MAPK pathway in proliferation and apoptosis, these data emphasize scaffolds as potential targets for cancer therapy. We performed a screening project for allosteric inhibitors of the late endosomal p14/MP1 scaffold involving chemical screening and pharmacophore modeling.

P35

Proteomic analysis of endosomes from genetically modified p14/MP1 mouse embryonic fibroblasts

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Protein signaling complexes, which are organized by scaffold and adaptor proteins, in specific subcellular locations, coordinate cellular functions such as proliferation, differentiation, apoptosis and migration. We have shown previously that the Mitogen-activated protein kinase scaffold protein 1 (MP1) is localized to late endosomes by the adaptor protein p14 (MP1-interacting protein) [1]. The p14 protein forms a stable heterodimeric complex with the MP1 [2], which binds MEK1, ERK1 and ERK2, recruits the entire complex to the late endosome and thus facilitates signal transduction through the MAPK cascade on this specific subcellular location [3-6]. Recently, we described a novel human immunodeficiency syndrome caused by genetic deficiency of the p14 and, therefore, aberrant lysosomal signaling function [7].
By organelle proteomics we have compared the protein composition of endosomes purified from genetically modified p14-/- , p14+/- and p14rev mouse embryonic fibroblasts. The latter ones were reconstituted retrovirally from p14-/- mouse embryonic fibroblasts by reexpression of pEGFP-p14 at equimolar ratios with its physiological binding partner MP1, shown by absolute quantification of MP1 and p14 proteins on endosomes by quantitative mass spectrometry using the EtEP (Equimolarity through Equalizer Peptide) strategy [8]. A combination of subcellular fractionation, two-dimensional difference gel electrophoresis (DIGE) and MALDI-TOF/TOF mass spectrometry revealed 31 proteins differentially regulated in p14-/- organelles, which were rescued by reexpression of pEGFP-p14 in p14-/- endosomes. Down-regulation of p18, the recently identified p14-anchor protein on late endosomes, in p14-/- MEFs suggests that cells tightly control concentration of scaffold and adaptor proteins in order to regulate signal transduction intensity and duration in specific subcellular locations. Regulated proteins are known to be involved in actin remodeling, endosomal signal transduction and trafficking. Identified proteins and their *in silico* interaction networks suggested that endosomal signaling might regulate such major cellular functions as proliferation, differentiation, migration and survival.

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P36

P14 – A Potential Novel Host Defense Factor

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Signal specificity in the MAPK cascade is regulated by the assembly of different scaffold complexes at distinct subcellular locations. The scaffold protein MP1 (MEK1 partner) is localized to late endosomes by the adaptor protein p14 thereby regulating late endosomal traffic and cellular proliferation [1]. Interestingly, a novel human primary immunodeficiency syndrome was identified which is due to a homozygous single point mutation in the human p14 gene. Consequently, these patients suffer from severe neutropenia and defective lysosomal function in granulocytes and monocytes [2].
Therefore, we were specifically interested in addressing the molecular function of the late endosomal scaffold complex p14/MP1 in innate immunity *in vivo* and *in vitro*.
For this purpose, we generated conditional knock out mice expressing the Cre recombinase under the control of the lysozyme M promoter which allowed us to specifically delete p14 in the monocyte/macrophage cell lineage (LMCp14-/- mice). In an infection model LMCp14-/- mice were more susceptible to the pathogen *Salmonella tm* compared to their wild type littermates as could be judged by the higher bacterial load in spleen and liver. Furthermore, LMCp14-/- mice displayed fewer and less defined granulomas in these organs. Interestingly, an increased number of intracellular bacteria was observed in macrophages from LMCp14-/- animals *in vivo* and *in vitro*. Though, p14 is neither involved in early events of the phagocytic process nor does it affect the induction of caspase-1 induced apoptosis. However, p14 is needed for the efficient activation of ERK on late endosomes/phagosomes which enables the targeting of *Salmonella tm* into the phagolysosomal pathway for its degradation. Furthermore, p14 is involved in the induction of host microbicidal factors, including the recruitment of the NADPH oxidase subunit p47phox to late endosomes or the efficient induction of iNOS. As a consequence p14 deficient macrophages provide a better niche for the survival and replication of bacteria.
The late endosomal scaffold complex p14/MP1 governs the trafficking of *Salmonella tm* into the phagolysosomal system, thus ensuring the efficient clearance of intracellular bacteria in macrophages.

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P37

The role of Myosin Vb in the pathogenesis of Microvilli inclusion disease

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Microvilli inclusion disease (MVID) is an autosomal recessive disease of young children which is characterized by life – threatening malabsorption syndrome and watery diarrhea during infancy. The reason for these severe clinical manifestations of MVID is a disrupted brush border of the small intestine. The main pathological hallmarks of MVID patients are loss of microvilli on the surface of the small intestine and microvilli inclusions.
The pathogenesis of MVID is still not completely understood.
Recently different types of mutations (missense and nonsense mutations) were found in the MYO5B gene coding for the motor protein Myosin Vb. [1, 2]
Myosin Vb is a protein which is involved in intracellular trafficking processes in epithelial cells. This motor protein transport actively vesicles along actin filaments from basolateral to apical compartments of epithelial cells and therefore maybe controls epithelial polarity and play a role in brush border formation.
For in vitro studies transient and stable knock down experiments are performed in an human intestinal Coloncarcinoma cells line (CaCo 2 cells), an human intestinal cell line showing the potential for full polarization and formation of an abundant brush border.
In CaCo 2 cells with a transient Myosin Vb knock down the main pathological phenotypes of Microvilli inclusion disease, loss of microvilli and microvilli inclusions, were successfully demonstrated. [2]
To see long term effects of loss of Myosin Vb in human intestinal cells, a stable knock down in CaCo 2 cells will be established using retroviral or lentiviral shRNA against human Myosin Vb.
For in vivo studies of loss of Myosin Vb, a conditional Myosin Vb knock out mouse is designed in which the part of the protein which is essential for protein function will not be expressed anymore. The knock out mouse model offers the opportunity to investigate loss of Myosin Vb not only in the intestine, but also in other absorptive epithelial tissues like the liver, the kidney, the bile duct, or the lung to see other phenotypes which do not become manifest in MVID patients and to understand the function of this motorprotein in other tissues.

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Non-coding RNAs in Epstein-Barr virus infection

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Non-coding RNAs (ncRNAs) do not encode proteins but function on the level of the RNA itself. Members of a special ncRNA class, designated as small nucleolar RNAs (snoRNAs), are localized within the nucleolus, a sub-nuclear compartment, in which they guide ribosomal or spliceosomal RNA modifications, respectively. Until now, snoRNAs have only been identified in eukaryal and archaeal genomes, but are notably absent in bacteria. By screening of B lymphocytes for expression of ncRNAs induced by the Epstein-Barr virus (EBV), we here report, for the first time, the identification of a snoRNA gene within a viral genome, designated as v-snoRNA1. This genetic element displays all hallmark sequence motifs of a canonical C/D box snoRNA, namely C/C'- as well as D/D'-boxes. The nucleolar localization of v-snoRNA1 was verified by *in situ* hybridisation of EBV-infected cells. We also confirmed binding of the three canonical snoRNA proteins, fibrillarin, Nop56 and Nop58 to v-snoRNA1. The C-box motif of v-snoRNA1 was shown to be crucial for the stability of the viral snoRNA; its selective deletion in the viral genome led to a complete down-regulation of v-snoRNA1 expression levels within EBV-infected B cells. We further provide evidence, that v-snoRNA1 might serve as a miRNA-like precursor, which is processed into 24 nt sized RNA species, designated as v-snoRNA1^{24pp}. A potential target site of v-snoRNA1^{24pp} was identified within the 3'-UTR of BALF5 mRNA which encodes the viral DNA polymerase. v-snoRNA1 was found to be expressed in all investigated EBV-positive cell lines, including lymphoblastoid cell lines (LCL). Interestingly, induction of the lytic cycle markedly up-regulated expression levels of v-snoRNA1 up to 30-fold. By a computational approach, we identified a v-snoRNA1 homolog in the rhesus lymphocryptovirus genome. This evolutionary conservation suggests an important role of v-snoRNA1 during gamma-herpesvirus infection.

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Aptamers as diagnostic markers in Chronic Kidney Disease

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Aptamers are 3D-structured single-stranded DNA (or RNA) oligonucleotides that bind different classes of molecular targets with high affinity and specificity. Similar to antibodies, they can be used as analytical, diagnostic and therapeutic tools in various molecular biological and medical applications. Aptamers are typically selected from large libraries of random sequences in an approach termed Systemic Evolution of Ligands by EXponential Enrichment (SELEX). The selection process involves several rounds of two successive steps: (i) binding of the oligonucleotides to the target under stringent conditions and (ii) amplification of the target-bound nucleic acids by polymerase chain reaction.
Chronic kidney disease (CKD), also known as chronic renal disease, is a progressive loss of renal function over a period of months or years. It is primarily caused by diabetes and hypertension and affects approximately 10% of the population in western industrialized nations. In the course of the progression of the disease glomerular filtration rates significantly decrease leading to increased blood pressure, accumulation of urea and potassium in the blood and decreased erythropoietin synthesis. Thus, early detection can help to prevent the progression of kidney disease to kidney failure.
The aim of our work is the selection of high affinity aptamer sequences which serve as molecular detection tools for diseased kidney cells. As a target for the SELEX-procedure we employ IL-1β, TGF-β1 and OSM stimulated HK-2 and RPTEC/TERT1 cells, which mimic surface characteristics of CKD cells. After 14 selection cycles, a significant enrichment of cell line and stimulation-specific aptamers was observed. Further characterization of the individual sequences will identify aptamers which are able to discriminate between healthy and CKD cells. These aptamers can be employed as diagnostic tools in CKD and might facilitate early treatment and thus progression of CKD.

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Identification of non-coding RNAs involved in neural differentiation

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Non-coding RNAs are important for development, represent a significant portion of the transcriptome and have been shown to be involved in differentiation processes (Dinger, M.E. et al 2003). We aim to identify differentially regulated non-coding RNA species involved in neural cell differentiation, by combining an *in vitro* and an *in vivo* approach. An important *in vitro* model system not only to study development but also organ repair and stem cell therapy are embryonic stem cells, which have the ability to self renew and to differentiate into functional cell types. A significant portion of the stem cell transcriptome appears to correspond to non-coding transcripts, as both microRNAs and longer non-coding RNAs have been identified in those cells. An attractive *in vivo* system are dorsal root ganglia (DRGs), since they can be isolated in different developmental stages and are an important model system for axonal regeneration and pain.
Most, if not all biologically functional identified non-coding RNAs in Eukarya are known to be associated with proteins forming ribonucleo-protein (RNP) particles. In order to isolate non-coding RNAs that are likely to be functional, we have generated RNP libraries (Rederstorff, M. et al 2010) from various stages of mouse embryonic stem cells differentiating upon addition of FGF2 and different developmental stages of DRGs, derived from embryonic, postnatal and adult mice.
The RNP libraries raised were high-throughput sequence and revealed a significant amount of house keeping non-coding RNAs and candidates mapping to intergenic or intronic regions, which could potentially be novel functional non-coding RNAs, present in varying percentages between different libraries.
Candidates are verified by Northern blots and will be validated in a high-throughput manner by microarrays. The most interesting non-coding RNAs will be selected for further functional analysis, to investigate their roles in neural differentiation. A non-coding microarray chip will be developed as a diagnostic tool for disease and tissue profiling.

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P41

"Bimbam", a novel glucocorticoid-regulated BH-3 containing transcript from the BCL2L11/Bim Locus

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Glucocorticoids (GC) cause apoptosis and cell cycle arrest in lymphoid cells and are fundamental for acute lymphoblastic leukemia (ALL) therapy. We investigated a potential novel splice variant of human BCL2L11/Bim, termed "Bimbam",that was identified in an expression profiling screen of children with acute lymphoblastic leukemia (ALL) undergoing systemic GC monotherapy.
Bimbam encodes a putative protein consisting of the N-terminal region of Bim (including its BH3 domain) and 40 C-terminal amino acids derived from Bam, a previously postulated gene within the Bim locus, and not present in any known Bim transcript. Bimbam is a new splice variant of Bim that encodes a protein with almost the same MW but a different PI due to its markedly different C-terminus. Bimbam mRNA was verified by RT-PCR and, similar to Bim, 3 Bimbam variants were identified (Bimbam-EL, -L and -S). Using a Bam-specific antiserum, endogenous Bimbam protein was detected in ALL and PC3 prostate carcinoma cells. Using shRNA-mediated knock-down technology we are currently generating isoform-specific knock-down cell lines with reduced expression of Bim, Bimbam or both isoforms, respectively, to dissect the contribution of each isoform to apoptosis induced by different apoptosis inducers. Recombinant Bimbam induced apoptosis with similar potency as Bim despite their distinct C-termini. Contrary to Bim (which localized exclusively to mitochondria *via* its C-terminal domain), Bimbam localized both to mitochondria but also to other intracytoplasmic membranes. Whether this is due to interaction of its BH3-domain with other BCL2 proteins is currently being investigated using corresponding Bimbam mutants.
In conclusion, Bimbam may contribute to the anti-leukemic effects of GC and perhaps other apoptotic responses by a different mechanism than Bim.

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A Blue Breakdown Product of Vitamin B_{12}

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The biosynthesis of vitamin B_{12} in bacteria and its uptake in mammals are well understood today [1]. However, the decomposition of vitamin B_{12} in vivo is an unknown scientific territory.
In strongly acidic aqueous solution vitamin B_{12} is hydrolyzed to cobyric acid, cobinamide and the *neo* series of these compounds as the main products [2]. Vitamin B_{12} also decomposes under basic conditions, with *c*-lactams as main products [2]. In weakly basic carbonate buffer pH 9, vitamin B_{12} is decomposed to several new compounds, one of which shows a fascinating blue color. The UV/Vis spectrum of this blue corrinoid exhibits red shifts of the main bands compared to vitamin B_{12}.
Here we also report the structures of this new blue B_{12}-derivative elucidated first by spectroscopic studies (relying on mass spectrometry, homo- and heteronuclear 2D-NMR-spectroscopy). The results of a crystal structure analysis of this blue corrinoid will also be presented, which confirm the spectroscopic structure elucidation

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P43

Chlorophyll Breakdown in Senescent Leaves of the Lime Tree (*Tilia cordata*)

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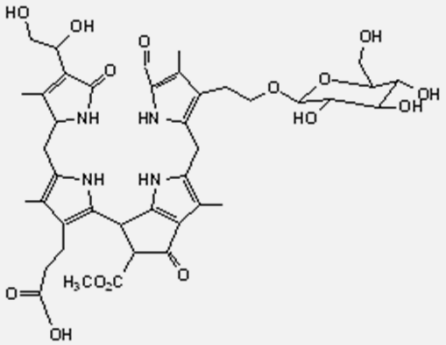
Chlorophyll breakdown is the visible symptom of leaf senescence. It is now known to occur in a thoroughly controlled and (basically) common path from the protein-bound chlorophyll in the membranes of the chloroplasts to the "nonfluorescent chlorophyll catabolites" (NCCs) in the vacuoles of a senescent plant leaf[1,2].



Structure of one "non-fluorescent" chlorophyll catabolite (called *Tc*-NCC-1) found in senescent leaves of the Lime Tree

Two colorless nonfluorescent chlorophyll catabolites (*Tc*-NCCs) were identified in extracts of senescent *Tilia cordata* leaves. They were isolated and characterized based on their HPL-chromatographic and spectroscopic data. Our latest results will be presented.

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Chlorophyll Breakdown in the Tropical Evergreen *Spathiphyllum wallisii*

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De-greening of leaves and the emergence of the fall colors are consequences of chlorophyll breakdown and are highly visible signs of leaf senescence. Until recently degradation of the green pigments in higher plants seemed to follow a largely common and well-controlled catabolic path providing colorless products [1]. When these chlorophyll catabolites were first identified in senescent leaves and ripening fruit [2], they turned out to be colorless tetrapyrroles (typified as "nonfluorescent" chlorophyll catabolites - NCCs), which appeared to be the "final" degradation products.
However, recent discoveries studying chlorophyll breakdown in leaves of the tropical evergreen Peace Lily (*Spathiphyllum wallisii*) revealed an unexpected divergence in the later stages of the chlorophyll degradation path suggesting chlorophyll breakdown to be more than a mere detoxification process. Our latest results will be discussed.

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Shape and Dynamics of Transcription Factor Binding Sites

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Sequence-specific transcription factor binding relies on two distinct mechanisms of sequence readout: base-wise readout based on sequence-specific hydrogen bonding patterns and a by now poorly understood process called indirect readout relying on shape and deformability of DNA. In case of Hox family transcription factors it was shown that subtle changes in DNA sequence distort local DNA shape and therefore disrupt transcription factor binding [1]. Three-dimensional shape of DNA was found to be even more conserved evolutionary than pure one-dimensional sequence information implying a fundamental function in DNA recognition [2].
B-DNA dodecamers containing sequence permutations of central AT and flanking GC base pairs were simulated as model systems for transcription factor binding sites using AMBER 10 to investigate the impact of small changes in DNA sequence on shape and dynamics. Molecular dynamics simulations of 10 ns show large differences in minor groove width between the individual sequences, an important factor for amino acid side chain insertion possibility. Pure A-tracts show a narrower minor groove than sequences containing TpA-steps, reproducing effects observed in NMR experiments [3]. Moreover, the latter sequences show additional conformational flexibility, which presumably causes the reduced affinity for groove binding [4] and side chain insertion of arginine and lysine residues [5].

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P46

From Ensembles to Entropy – A Thermostatistical Approach

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Entropy plays a crucial role in ligand binding, but is in general hard to quantify computationally. Several indirect methods have been proposed, mostly based on properties known to correlate with entropy, such as apolar surface area, number of rotatable bonds etc.
We demonstrate how to calculate entropy directly from state distributions. Unrestrained explicit-solvent Molecular Dynamics simulations using the AMBER ff99SB force field are applied to sample phase space. Consequently, a continuous state probability density function is derived by Kernel Density Estimation. To calculate an entropy measure we calculate the Kullback-Leibler Divergence, which is a generalization of the statistical thermodynamic formulation of entropy for continuous state distributions, over the density of states. After aligning the protein structures to a common reference, the site of interest is superposed with a grid. In vicinity to each probe point, solvent entropy is calculated from the rotational and translational state distributions of local water molecules. Translational states are considered either by clustering, or by calculation of radial density distributions. The cluster-based approach, though being computationally more expensive, offers additional insights into complex characteristics of the solvation shell, including networks of positionally restrained solvent molecules.
The local solvent entropy is derived from state distributions. As this approach is generally applicable, ligands and proteins entropy is accessible as well. By comparing local entropy measures for different ligands, differences in binding entropy are approachable, and therefore guide ligand optimization.

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Searching for small-molecule interactions sites in DNA-binding proteins. Case study: FOXO3a

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Pharmacological targeting of DNA binding sites is a relatively new and challenging approach, since protein-DNA interactions take place on big surfaces and not on small, well-defined binding sites. Molecular modeling techniques enable the detection of potential cavities for small molecule ligand binding and may thus be helpful in the discovery of drugs targeting the DNA-binding site.
We propose a combination of several techniques including site finder detection, molecular dynamics simulation, generation of grid maps, pharmacophore based virtual screening and docking in order to detect small, potentially active molecules.
As an application example, the DNA binding domain of forkhead boxO 3a (FOXO3a) was investigated using complementary *in silico* techniques.
Forkhead boxO 3a (FOXO3a) belongs to a highly conserved family of transcription factors which regulate expression of genes involved in apoptosis, cell cycle and longevity [1]. The protein is a downstream target of the PI3K/Akt pathway, which is known to be dysregulated in several malignancies including different types of cancer [1] and therefore modulation of FOXO function can have beneficial effects on the outcome of diseases. Currently, there are no ligands known that can influence FOXO3a activity.
Putative binding sites were elucidated using different sitefinder programs, an MD simulation of the related FOXO4 protein [2], information from mutational studies [3] and the generation of GRID maps. In a second step, small molecules were docked into the binding site and the proposed binding modes were then used to create pharmacophore models. Several databases were screened and the results will be tested in different in-vitro assays.

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P48

Molecular modeling of telmisartan analogues as new PPARg agonists

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Telmisartan, a well-known angiotensin II receptor antagonist used in the treatment of hypertension, revealed to be a partial agonist of the peroxisome proliferator-activated receptor g (PPARg), which is a pharmaceutical target of high interest due to its potential in the treatment of diabetes type 2 and other related diseases [1]. Clinically used PPARg agonists, such as members of the thiazolidindiones (glitazones) family, show extensive side effects like water retention leading to edema and an increased risk of cardiovascular complications [2], while telmisartan is well-tolerated [3]. Therefore, telmisartan is a promising starting point for the development of new PPARg agonists with a more advantageous pharmacological profile. Previous studies on telmisartan analogues determined a 4'-[(2-Propyl-1*H*-benzimidazole-1-yl)methyl]biphenyl-2-carboxylic acid core as the minimum requirement for activity on PPARg [4]. This lead structure was extended by creating a virtual database of multiple structural derivates using ilib:diverse software [5]. Based on X-ray crystal structures of the PPARg binding pocket with synthetic ligands, a pharmacophore model was developed and used for screening the virtual database employing LigandScout [6]. Potential PPARg agonists that returned as virtual hits will be synthesized and biologically evaluated.

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P49

Development of a pharmacophore model for inhibitors of the NF-κB(p50)/DNA-interface

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Nuclear factor-κB (NF-κB) transcription factors, a family of homo- and heterodimers that consist of five members including RELA (p65), RELB, c-REL, NF-κB1 (p105/p50), and NF-κB2 (p100/p52), are well accepted to regulate immune and inflammatory responses [1]. They have been identified to be required for the regulation of a variety of genes, of which many have a critical role in tumor genesis and tumor promotion [2].
We report here the development of a pharmacophore model for inhibitors interfering with the NF-κB(p50)/DNA-interface as well as pharmacophore-based virtual screening. The pharmacophore model development was based on a previous study reporting a modeled binding site for a chemical class of potent NF-κB inhibitors [3]. A ligand binding pose retrieved from docking a highly active compound with GOLD [4] into the suggested binding site of the p50 subunit was used to create a pharmacophore model using LigandScout 3.0 [5]. The final pharmacophore model was employed for a virtual screening campaign. Out of 13 virtual screening hits, ten were selected for biological evaluation. Four of these compounds showed activity, one of them with about 80% inhibition at a concentration of 30µM in a cell-based NF-κB transactivation assay.

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P50

In silico toxicology predictions via pharmacophore-based virtual screening: The discovery of the UV filter benzophenone-1 as inhibitor of testosterone synthesis

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The prevalence of male reproductive disorders and testicular cancer is steadily increasing [1]. Because the exposure to chemicals disrupting natural hormone action has been associated with these diseases [2], it is important to identify endocrine disrupting chemicals (EDCs) and their targets of action. Here, a 3D structural database that can be applied for virtual screening approaches to facilitate the identification of EDCs was constructed. As a frist application scenario, the database was screened using pharmacophores of 17b-hydroxysteroid dehydrogenase type 3 (17b-HSD3), which catalyzes the last step of testosterone synthesis in testicular Leydig cells and plays an essential role during male sexual development [3]. Among other chemicals, benzophenone (BP) UV-filters were predicted as potential 17b-HSD3 inhibitors. Biological analyses revealed (2,4-dihydroxyphenyl)-phenylmethanone (also known as benzophenone-1, BP-1) as an inhibitor of human 17b-HSD3 (IC_{50} 1.05 µM). BP-1 also efficiently blocked conversion of androstenedione to testosterone by mouse and rat 17b-HSD3 in whole-organ enzyme assays. Moreover, BP-1 antagonized the testosterone-dependent activation of androgen receptors (IC_{50} 5.7 µM), suggesting synergistic anti-androgenic effects of BP-1 by preventing testosterone formation and blocking receptor activation. According to these results, virtual screening of environmental chemical databases can facilitate the identification of compounds interfering with hormone action.

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P51

What Thermodynamic Profiles Tell Us About DNA Recognition Site Geometry

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The relevance of DNA shape steadily attracts attention and complements the picture of DNA as a chain of letters. Protein side chains inserting into the minor groove detect sequences with specific geometries instead of accomplishing base-wise readout. Although there exist some hypothesis on the molecular mechanisms that may underlie sequence specific readout, this step in the control of gene expression is still poorly understood[1].
Because of their small size minor groove binders are valuable probes for protein DNA interactions and respective contributions to binding free energy. Thermodynamic profiles were measured with isothermal titration calorimetry for Dickerson-Drew-Dodecamer and several closely related variants thereof. We found the profiles to correlate with minor groove width and thus DNA geometry. We searched for reasons for minor groove contraction and its relation to binding processes. We were not able to find support for the hypothesis that electrostatic focusing emerging from a narrow groove drives ligands into the groove[2]. Instead, our data confirm the importance of minor groove hydration and its relation to local DNA shape[3,4].
Minor groove binders are a valuable tool for further detection of sequence dependent DNA geometries and their influence on DNA recognition by transcription factors.

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P52

Trying to understand the complex dialogue of epigenethics: The role of mammalian chromatin remodeling factor CHD1

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The chromatin structure of metazoans is highly dynamic, allowing transcriptome changes during development and differentiation, and enforcing nuclear stability. This is possible through the highly coordinated cooperation of different epigenetic mechanisms. One of this is represented by ATP-utilizing chromatin remodelling factors and complexes that catalyze sliding and replacing of nucleosomes, and thus are involved in transcription regulation.
Currently, we are investigating the role of the mammalian ATP-dependent chromatin remodelling factor CHD1 in development by generating a knock-in mouse model and inducible cell systems.
Here, we report on the progress in generating the CHD1 knock-in mouse and the inducible cell systems. We will also show that CHD1 expression appears to be regulated by the Wnt pathway and by glucocorticoids.

P53

Purification of CHD1 protein complexes and characterization of potential interacting partners in *Drosophila melanogaster*

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Nuclear DNA is packed into a highly ordered nucleoprotein structure, which is termed chromatin. To render the nucleosomal DNA accessible for processes, like replication, transcription, repair and recombination chromatin remodeling factors are required for mobilizing and moving the nucleosomes along the DNA. The chromodomain protein CHD1 is one of these factors and was identified as an ATP-dependent chromatin assembly factor.
To elucidate further biological functions of CHD1 we aimed at identifying potential CHD1 interacting partners. To this end, a construct was designed, which encodes for CHD1 fused to a Flag-tag at the C-terminus. This allows the purification of CHD1 and potential associated proteins by affinity purification.
The CHD1 fusion protein was stably expressed in *Drosophila* S2 cells upon induction with $CuSO_4$. It was possible to purify CHD1-containing protein complexes from nucleoplasm and chromatin fractions by using the anti-Flag affinity agarose.
Potential CHD1 interacting partners, such as Antimeros (a Paf1 homologue), proteins involved in RNA processing, RNA translocation and RNA localisation and a FACT complex component could be identified by mass spectroscopy. Preliminary evidence is presented confirming the specificity of the interaction of selected proteins with CHD1.

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P54

Electron induced damage of DNA compounds

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Currently there are strong efforts in establishing new ion beam cancer therapy (IBCT) facilities in Europe as an alternative to conventional photon radiation therapy. For example, in Austria an IBCT facility is planned to be started for trial operation in Wiener Neustadt in 2013 (MedAustron). One of the biggest advantages of IBCT compared to photon therapy is the fact that the highest energy deposition occurs closer to the end of the ion track (Bragg Peak), i.e. deeply lying cancerous tissue can be selectively treated. However, the exact molecular and nanoscale mechanisms of IBCT are still under debate. The two major mechanisms of DNA damage by ion beams are indirect damage by neutral radicals and direct damage by secondary electrons formed along the ion track. In a laboratory experiment it was shown that low energy electrons can induce strand breaks in plasmid-DNA[1]. Thus the underlying effects of low energy electron damage of various DNA components need to be investigated thoroughly.
Our working group has started a series of investigations concerning low energy electron damage of small building blocks of DNA. Thereby an electron attaches resonantly and the transient negative ion formed decays via dissociation of the molecule. This dissociation can be very site specific[2], i.e. only certain bonds are cleaved in a molecule. Our attempt is to expand our mass spectrometric investigations in the gas phase to larger DNA compounds like nucleotides and oligonucleotides further elucidating damage of DNA by electrons. The big challenge is to transfer these complex biomolecules intact into the gas phase. We are currently testing the Laser-Induced-Accoustic-Desorption-Technique, where a titanium foil covered with thin layers of biomolecules is irradiated with green light and the formed acoustic waves in the foil induce the desorption of biomolecules. This method may allow the subsequent study of the damage of complex DNA compounds by low energy electrons under single collision conditions.
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P55

Electron induced splitting of the cyclobutane pyrimidine dimmer: an important step in the DNA damage repair via DNA photolyase

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A major DNA damage induced via UV radiation is the formation of cyclobutane pyrimidine dimers (CPD). These dimers represent a genotoxic lesion since they block the correct replication of DNA. CPDs play a major role in the formation of skin cancer [1], thus the repair of such damages is a process indispensable to life. A repair mechanism of nature (DNA photolyase) is capable of repairing these damages with the help of a sunlight activated electron transfer to the dimer. DNA photolyase was found in bacteria, fungi, plants invertebrates and many vertebrates but not in humans [1, 2]. In the marsupials CPD photolyase is present. It has been shown that mice provided with a transgene for marsupial DNA photolyase exhibit a significant increase of repair for CPD lesions [3].
Thus we carried out free electron attachment measurements to CPDs to gain a deeper insight into the reductive splitting of these dimers [4, 5]. For this study we employed a double focusing mass spectrometer. We observed an unusual rich fragmentation pattern upon electron attachment of electrons with energies close to 0 eV. The most abundant fragment was observed for the half split dimer. Additionally we were able to observe the parent anion which so far has not been observed.
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P56

Low energy electron interactions with amino acids in the gas phase : effect of the isomers geometry

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In recent years there has been a significant interest in processes induced by low-energy electrons to biological systems. It has been shown that a large number of low energy electrons, which are a by-product of high energy ionizing radiation, participate significantly in the damage of DNA, as they can induce single and double strand breaks in plasmid DNA through resonant scattering processes [1]. The mechanism that leads to this irreversible damage involves the capture of the incident low-energy electron by the molecule forming a transient negative ion which then dissociates into a (quasi-) stable negative ion and one or more neutral species. This process, dissociative electron attachment (DEA), often results in either the ion or neutral fragment being a reactive free radical. Since that discovery, many studies have been devoted to the understanding of this mechanism by looking at electron interactions with biologically relevant molecules such as DNA bases, constituents of the DNA backbone but also amino acids.
Dissociative electron attachment to amino acids [2]-[4] have been studied extensively and it is now well established that the main reaction, at low energy, corresponds to the loss of a hydrogen atom from the carboxyl group (-COOH). For all investigated amino acids, the $(M-H)^-$ ion yield presents similar features, a sharp peak at an energy of 1,2eV and a shoulder at around 1,5eV. However in this study we show that the shape of the $(M-H)^-$ ion yield changes significantly with the position of the amino group ($-NH_2$) in the molecule (α-, β- or γ- position). These new results complement previous work on α-amino acids and shed more light on the origin of this low energy resonance, which to date is still a matter of controversy.

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P57

Analysis of the beta-cell specific regulation of the zebrafish *hb9* gene

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The pancreas consists of two tissue types, the hormone secreting endocrine cell clusters termed islets and an exocrine component producing digestive enzyme. In most vertebrates the islets are formed by up to five cell types, which express Glucagon (a-cells), Insulin (b-cells), Somatostatin (d-cells) and Ghrelin (e-cells) and pancreatic polypetide (PP-cells).
We are interested in genes regulating beta-cells formation, specifically in the *Hb9/Hlxb9* homeobox factor, which it is one of the earliest beta-cells specific markers in all vertebrates. Loss of function studies in mouse and zebrafish showed that *hb9*-genes are required for pancreas morphogenesis and for beta-cell differentiation. In our approach to determine the regulatory mechanism underlying b-cells specific *hb9* expression, we have previously defines a 3 kb zebrafish promoter fragment, that is sufficient to drive GFP expression at various sites of hb9 expression including b-cells. The 3kb fragment contains two highly conserved non-coding regions, which have been suggested to regulate expression. In contrast, we found that beta-cell specific expression is regulated by a non-conserved 500bp element. Bioinformatics sequence analyse of this element suggested potential binding sites for pancreas specific transcription factors including Pax6b, NeuroD, Pdx1 and others. We now used EMSA studies to define which of them can directly bind to the enhancer DNA. Further, we generated a gain-of-function *in vivo* assay to determine which factors are sufficient for *hb9* expression, and we used Morpholino knock-down to define which of them is also required for beta-cell specific *hb9* expression. So far we could show that NeuroD and Pax6b both can bind to the different parts of *hb9* pancreas enhancer and that Pax6b is sufficient to induce *hb9/gfp* expression *in vivo*. Further we find that NeuroD, which in mouse is required for beta-cells differentiation, is neither required not sufficient for beta-cell specific *hb9* expression in zebrafish. Recent results will be presented.

P58

Characterization of a novel type of Retinoid-binding protein in zebrafish

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Retinoids are hydrophobic molecules with various important biological functions. Because of their low solubility in the aqueous medium and also because of their chemical instability, retinoids are bound to specific carrier proteins that facilitate plasma and inner cellular transports are that target them to downstream signaling receptors or enzymes for metabolism.
We previously identified a new member of the Retinol-binding proteins (Rbp) in zebrafish as a potential target of the Nodal-signaling pathway. Based on sequence similarity and conserved synteny between the mammalian *rbp7* genomic region with two zebrafish genomic regions we termed this genes as a duplication of *rbp7* in zebrafish that we named *rbp7b*. Consistent with a regulation by Nodal we now identified four binding sites for the Nodal signaling transcription factor FoxH1 close to the transcriptional start site and 3' terminal region of *rbp7b* and we also confirm *in vitro* interaction between these sites and FoxHI in EMSA studies. However, we also found that neither FoxH1 nor Nodal signaling is required for early regulation of *rbp7b*.
During our analyses we isolated a novel isoform of *rbp7b*, which is a fusion between *rbp7b* and the 5' localized gene *nmnat1 (nicotinamide mononucleotide adenylyltransferase1)*. The fusion mRNA appears to be generated by alternative splicing of single precursor RNA as it lacks the last exon of *nmnat1* and the first exon of *rbp7b*. RT-PCR analyses show that the levels of *nmnat1-rbp7b* fusion mRNA vary during development, suggesting that splicing is developmentally regulated. The function of this fusion protein is unknown and recent results of gain and loss of function experiments will be presented. Notably, the close proximity of *nmnat* and *rbp* genes is highly conserved in zebrafish and other species. As some key enzymes for catalysis retinoids to retinoic acid depend on NAD^+ which is synthesized by Nmnat proteins, these analyze may reveal new clues in retinoid metabolism.

P59

A Transgenic approach to identify target genes of the Currarino syndrome related Hlxb9/Hb9 protein.

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Hlxb9/hb9 is a homeobox gene found in cnidarians, worms, insects and vertebrates. In humans the heterozygous loss of HLXB9/HB9 is the main cause of sacral agenesis in patients suffering from the Currarino syndrome. Functional studies on *hb9* genes in C. elegans, Drosophila, zebrafish and mouse revealed additional conserved roles in motoneuron development and vertebrate specific functions in pancreas morphogenesis and beta-cell differentiation. How Hb9 is regulating these various activities is currently not known.
In order to identify Hb9 regulated genes we used a transgenic approach for constitutive activation or repression of Hb9 target genes in zebrafish. Previous studies suggest that Hb9 is mainly acting as a transcriptional repressor. To be able to block or activate Hb9 target genes we generated transgenic lines in which either Hb9 alone or Hb9 coupled to a viral transcriptional activator (Hb9-VP16) are set under the control of the heat-shock promoter hsp70, respectively. Potential target genes were identified by microarray based comparison of the transcriptome of Hb9-blocked or activated embryos and verified through in situ hybridization analysis and Real time PCR. Among other genes we found that a member of the Iroquois gene family is regulated by Hb9. Transplantation of cells from tg(hsp70:hb9-VP16) donors into wildtype host embryos and subsequent heatshock activation resulted in a cell autonomous activation of the potential Hb9 target mRNA in the transplanted cells. As treatment with a translation blocking agent does not prevent its transcriptional activation, our data suggest direct regulation by Hb9.

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P60

Multiple roles for Sox17 in endoderm development in zebrafish

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The endoderm is specified at the onset of gastrulation, and will give rise to the epithelial lining of the gut and organs such as the liver and the pancreas. The HMG domain Sox family member *sox17* is one of the earliest markers of endoderm in vertebrates, and in mouse and frog is essential for endoderm specification (1). In mouse and zebrafish, *sox17* is also expressed at later developmental stages in the foregut progenitor cells. A conditional *sox17* mouse knock-out showed that Sox17 is also required for the correct cell fate decisions in the endocrine pancreas and the gall bladder (2). In zebrafish, the role of *sox17* in endoderm development is currently unclear. The early expression of *sox17* is regulated by *casanova*, a closely related Sox factor which appears to be unique to zebrafish, and is absolutely required for endoderm specification (3,4). Morpholino knock-down of *sox17* has failed to reveal any function for Sox17 in zebrafish endoderm specification. These results suggest that in zebrafish, the role of Sox17 may have been taken over by Casanova.

To understand the role of Sox17 in zebrafish endoderm development, we have characterised a novel mutant for *sox17* identified in the Sanger Centre TILLING screen. *sox17* mutants do not appear to have any defects in endoderm specification or early patterning, as assessed by a panel of regional early endodermal markers. However, *sox17* mutants show a strong genetic interaction with *casanova* mutants, with double heterozygous embryos displaying a reduced number of endodermal cells. Surprisingly, overexpression of *sox17* mRNA in casanova heterozygous mutant embryos attenuates endodermal morphogenesis, raising new questions about the role of *sox17* in early endoderm development. We have also looked for later endodermal organ defects in the *sox17* mutants. Although the gut and associated organs appear grossly normal, we have found that loss of Sox17, in zebrafish as in mouse, causes an expansion of endocrine pancreatic cell fates. Suprisingly, we also found a reduction of hepatic marker gene expression in these mutants, suggesting that the liver differentiation programme requires Sox17 function. Our zebrafish *sox17* mutant is thus a unique tool to study the mechanism of cell fate decisions in the endodermal organs.

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P61

Hedgehog signalling controls proliferation in the early zebrafish endoderm

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Cell proliferation, patterning and migration are key mechanisms of animal development. The Hedgehog (Hh) signalling pathway has been implicated in the control of all these processes, and dysregulation of Hh signalling is correlated with cancer progression in basal cell carcinoma and pancreatic cancer. However, activation of Hh signalling does not cause uncontrolled proliferation in all cell types, although the basis for this cell type specific response to Hh is unknown. Moreover, the role of Hh signalling in controlling cell proliferation is controversial, with some reports suggesting Hh promotes cell proliferation, and other studies suggesting that Hh signalling promotes cell cycle exit.

We have found that Hh signalling cell-autonomously regulates endodermal cell proliferation in early zebrafish embryos. Loss of the Hh transducer protein Smoothened causes a reduction in the endodermal cell number, whereas activation of the pathway causes an increase in the number of endodermal cells. This response is specific to the endoderm: mesodermal cell proliferation is not affected by loss of Smoothened, and activation of Hh signalling in the mesoderm reduces proliferation in these cells. We are currently investigating whether Hh signalling affects cell cycle kinetics in the endoderm, using live cell cycle reporters, cell transplantation techniques and live imaging. The early zebrafish embryo offers an excellent and accessible model system to investigate the role of Hh signalling in regulating cell proliferation.

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Chemical synthesis of 2'-azido modified RNA and its potential for bioconjugation and siRNA technologies

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RNA with site-specific azide groups represents a promising candidate for applications in modern bioconjugation chemistry, such as Staudinger ligation or Click chemistry [1], but also for photochemically induced crosslinks to peptides and proteins [2]. Beside the advantage of high reaction selectivity and exceptional bioorthogonality, the azide group can be regarded as a non-invasive label with respect to RNA structure. Thus, potential biochemical applications are conceivable, e.g. in siRNA technologies.
Up to date, there is an obvious lack of methodologies for the efficient chemical synthesis of azide-modified RNA, most likely due to the widespread opinion that standard RNA solid-phase synthesis using phosphoramidite chemistry is incompatible with azide functionalities [3]. It is therefore not surprising, that most previous attempts to achieve azide-modified RNA either relied on enzymatic methods [2, 3] or on post-synthetic approaches which involved convertible nucleosides [4].
Here, we present a novel approach that allows for the direct and site-specific incorporation of 2'-azido nucleosides into RNA by using automated RNA solid-phase synthesis. Furthermore, we also demonstrate first potential applications for fluorescent labeling via Staudinger ligation and for gene silencing via siRNA technologies [5], using the *BASP1* gene encoding a potential tumor suppressor [6] as a model system.

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P63

Semisynthesis of a stable *E.coli* tRNA-3'-NH-Val-Val-Leu-Leu-Met conjugate containing genuine tRNA modifications

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The ribosomal elongation of the peptide chain takes place at the peptidyl transferase center (PTC) of the ribosome. Thereby, the growing peptide chain has to pass through the ribosomal exit tunnel before it can leave the ribosome. Macrolide antibiotics, such as erythromycin, clarythromycin, roxithromycin, and telithromycin, bind at the entrance of this tunnel and thus prevent elongation of the peptide chain [1]. However, bacteria can show resistance by translating short peptides that are highly conserved in their sequence. It is hypothesized that the macrolide interacts in a specific manner with the resistance peptide and thus is expelled from the PTC so that the ribosome is available for protein synthesis again. In this context, non-hydrolyzable 3'-peptidyl-tRNAs would represent valuable probes to study the interaction between macrolide antibiotic and resistance peptide in the PTC and the ribosomal exit tunnel.
Recently, we have presented a novel approach for the efficient synthesis of non-hydrolyzable 3'-peptidyl-tRNAs which contain all natural tRNA nucleoside modifications [2]. In short, we started from natural tRNAs that were cleaved within the TΨC loop by DNA enzymes to obtain a tRNA 5'-fragment containing all genuine modifications. After dephosphorylation of the 2',3'-cyclophosphate moiety, this fragment fulfills the structural requirements for enzymatic ligation to RNA-peptide conjugates which have been prepared according to a solid-phase synthesis approach elaborated previously in our laboratory [3]. Here, we present the synthesis of the *E.coli* $tRNA^{Val}$-3'-NH-VVLLM conjugate whose peptide moiety represents a sequence that confers resistance to the macrolide antibiotic clarythromycin.

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P64

Screening method for cardiovascular diseases in zebrafish

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Congenital heart disease, cardiomyopathy and heart arrhythmia are the most frequent myocardial diseases with strong genetic influence. Some forms of cardiomyopathy in particular are regarded as predominantly familial/genetic diseases. There are a number of genetic mouse and rat models engineered to address these disorders, but breeding and keeping of them is quite time-consuming and cost-intensive. It is definitely not possible to generate mice/rats for all disease-related mutations.
The zebrafish is still underutilized in modelling human diseases despite its quick generation cycle, short development times, and cost-efficient keeping of large numbers of animals. These animals are emerging as a valuable tool to identify novel genes that are specifically involved in cardiovascular pathology. Mutant zebrafish show phenotypes that closely correlate with those in human heart diseases.
In this study we used the power of zebrafish as a model, and advanced image analysis to verify the effects of suspected new disease genes and mutations for genetic heart disease. To increase screening speed a new mounting technique was evaluated to overcome the problem that cardiovascular malfunctions could be seen better compared to coarser methods. We used advanced high speed digital imaging and image data mining as a sensitive analytical tool to identify even slightest differences in cardiovascular performance.
Our combined innovative approach will establish a "pipeline" to evaluate novel variants and genes for congenital heart disease and cardiomyopathy with regard to their effects *in vivo*. This will give us new insights into the pathogenesis of these diseases and will hopefully show new options for therapeutic targets.

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Influence of hypoxia and the circadian clock on Hif-1α-protein in zebrafish

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Zebrafish is considered as one of the most suitable models for vertebrate embryology studies and extensive genetic analysis. The possibility of genetic manipulation and transgenesis, the optical transparency, low cost and small size of the larvae are of increasing benefit for biomedical researchers, who are looking for a model to investigate human disease mechanisms.[1][2]
Zebrafish are well adapted to hypoxia. Therefore we use zebrafish and zebrafish fibroblast cell lines for our research on hypoxic signalling, which is pivotally regulated by the hypoxia inducible factor-1α (HIF-1α). The concentration of HIF-1α in the cell is mainly regulated by oxygen dependent protein degradation. Analysis of Hif signalling thus requires Hif-1α specific antibodies. Unfortunately, specific antibodies available for mammals and other vertebrates show no cross reactivity with the zebrafish protein. We cloned the complete coding region of the Hif-1a protein of zebrafish and expressed it in a bacterial system. Using this protein as an antigen we obtained specific antibodies against zebrafish Hif-1a protein, as tested in Western-blots with protein obtained from cell culture systems as well as in protein extracts obtained from larval fish.
First experiments using a zebrafish fibroblast cell line revealed an accumulation of Hif-1a protein during hypoxic incubation (1% oxygen). Cells overexpressing the circadian Clock3 protein even showed a strongly elevated expression of Hif-protein under hypoxic conditions. These results indicate that circadian clock proteins interact with the hypoxic signalling pathway. Taken together with our previous results demonstrating that hypoxia interferes with the expression of clock proteins the results suggest a bidirectional crosslink between hypoxic signalling and the circadian clock.

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P66

Revealing the elusive molecular biology of the vault RNA

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In the recent past, the importance of the surprisingly diverse class of non-protein-coding RNA molecules (ncRNAs) has been widely recognized. The key feature of all ncRNAs is that they are not translated into proteins but rather exert their functions at the RNA level. They play key roles in a variety of fundamental processes in all three domains of life, that is Eukarya, Bacteria and Archaea. Their functions include DNA replication and chromosome maintenance, regulation of transcription and translation, RNA processing, protein synthesis and stability of mRNAs, and even regulation of stability and translocation of proteins. Many ncRNAs have been discovered fortuitously, suggesting they merely represent the tip of the iceberg [1].
In a recent genomic ncRNA screen we have identified the vault-associated RNAs to be significantly up-regulated in human B cells upon Epstein-Barr virus (EBV) infection [2]. Vault RNAs serve as integral parts of the so-called vault complex, a large hollow barrel-shaped ribonucleo-protein complex with a size of 13 MDa [3]. Very little is known about the function of this ncRNA class, mainly because the vault complex has been overlooked by cell biologists for many years.
As the up-regulation of vault RNA seems indeed to be causally linked to EBV [4], we would like to address the question which part of the virus genome is responsible for this event. Maybe then we will be able to unravel which function the vault RNAs might possess during virus propagation. Furthermore we want to identify the subcellular localization of vault RNAs in EBV infected cells. Cumulatively, the planned experimental strategy will eventually reveal the so far enigmatic molecular biology of this interesting ncRNA species.

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P67

Does the “RNA world” still communicate with the translation machinery?

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In the past years, it became evident that small non-protein-coding RNAs play key roles in regulatory networks, shaping cellular life. While the list of validated non-coding RNAs that regulate crucial cellular processes grows steadily, not a single ncRNA has been identified, with a notable exception of the signal recognition particle that directly interacts and regulates the ribosome during protein biosynthesis. All of the recently discovered regulatory ncRNAs (e.g. microRNAs, siRNAs or antisense RNAs) target the mRNA rather than the ribosome. This is unexpected, concerning the central position the ribosome plays during the gene expression. The fundamental question that we are asking is: are there ncRNAs that directly bind and possibly regulate the ribosome during translation? To address this question, we started genomic screens for novel regulatory ncRNAs that associate with *Saccharomyces cerevisiae* ribosomes under specific environmental conditions. We have constructed a specialized cDNA library and subjected it to high throughput deep sequencing analysis. Detailed computational analysis of 31.138 RNA sequences which were co-purified with yeast ribosomes was performed. Our data show that our cDNA library encodes for 107 putative ribosome-associated ncRNAs, including snoRNAs, highly abundant short ncRNAs derived from mRNA, ribosomal RNA and of other origin as well as antisense RNAs. We have also observed differential processing of tRNA and snoRNA molecules under specific environmental conditions. For a subset of the candidates expression tests proved their presence in the polysomes and monosomes generated from yeast as well their influence on translation *in vitro* and *in vivo*.

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Atomic mutagenesis reveals a common mechanism for GTPase activation on translational G proteins

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Following ribosomal peptide bond formation the deacylated and peptidyl tRNAs along with the mRNA need to be translocated from the P- and A- to the E- and P-sites, respectively. This process is an intrinsic property of the ribosome, but needs the assistance of the GTPase elongation factor G (EF-G) during *in vivo* protein synthesis. Subsequent to binding of EF-G, its G domain is activated by the ribosome, and GTP is hydrolyzed. Following the GTPase reaction the tRNAs and the mRNA are translocated. In order to activate the GTPase conformational changes within the switch regions and the P-loop in the G domain must take place. It is assumed that a catalytic histidine is reoriented by the opening of a hydrophobic gate, and can then activate a water molecule to initiate GTP hydrolysis. In principle there are two ribosomal regions, which could trigger GTP hydrolysis on EF-G, the GTPase-associated-center and the sarcin-ricin loop (SRL) of 23S rRNA. Several structural, biochemical and mutational studies have been performed to find the ribosomal trigger. However none of them could unambiguously identify the residue responsible for activation.
Using an atomic mutagenesis approach, which is based on the site-specific introduction of non-natural nucleoside analogs within any desired region of the 23S rRNA, it was possible to ascertain the nucleobase of A2660 within the SRL of the 23S rRNA to be the ribosomal element that stimulates EF-G GTP hydrolysis [1]. Mutagenesis at the atomic level revealed the N6 exocyclic amino group of this adenine to be the crucial factor for GTPase activation. The presence of this amine might be necessary to mediate π-stacking interactions of A2660 of the SRL with residues of EF-G, that promote conformational changes within the G domain, to open the hydrophobic gate and reposition the catalytic histidine. Due to the universal conservation of the SRL and the crucial elements within the G domain of all GTPases, the hypothesized model for GTPase activation was tested with other GTPases involved in ribosomal protein biosynthesis. Indeed it could be shown that GTPase activation on initiation factor 2 and LepA (EF4) as well, are strictly dependent on the presence of the amino group of A2660 of the SRL, suggesting a common mechanism for GTPase activation on translational GTPases [1], [2].

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P69

Deciphering Translational Processes Using Chemically Engineered Ribosomes

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Chemically modified macromolecules containing non-natural residues have been introduced in the past years into various biological systems in order to study the mechanism of enzyme catalysis. Recently the ribosome, the largest natural ribozyme known to date, has been added to the list of enzymes amenable to synthetic biology. Using an *in vitro* reconstitution approach, we were able to chemically engineer the ribosome and to introduce non-natural modifications at specific nucleotides of the 23S rRNA[1]. This experimental system, also referred to as "atomic mutagenesis", has already allowed us to identify crucial functional groups of the 23S rRNA for peptidyl transfer[2], peptidyl-tRNA hydrolysis[3] and EF-G GTPase activation[4]. However, all assays used so far covered only a single isolated step of the translation cycle. To overcome this obstacle and to gain more physiologically relevant insights into protein synthesis, we are developing *in vitro* translation assays using chemically engineered ribosomes for synthesizing peptides and proteins. We were already able to establish a poly(U) directed poly(Phe) synthesis assay, which gave first hints on the importance of specific nucleotides in the peptidyl transferase center for translocation[5]. However, the chemically peculiar characteristics of the synthesized poly(Phe) peptides could lead to poly(Phe) specific results considering protein synthesis. One known drawback of this assay is that the synthesized poly(Phe)-peptide most likely exits the ribosome through the intersubunit space instead via the exit tunnel. To circumvent this and to extend the biological authenticity of our assays we established an *in vitro* translation assay using natural messages. This should allow us to investigate interactions between the exit tunnel and various nascent peptides. Additionally, by performing "atomic mutagenesis" translational fidelity and the overall processivity of engineered ribosomes can be studied, leading to a more precise picture of the mechanism of translation.

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P70

Genomic screens for regulatory ncRNAs targeting the ribosomes in archaea and basal flatworms

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The functions of ribosomes in translation are complex and involve different types of activities critical for decoding the genetic code, linkage of amino acids via amide bonds to form polypeptide chains, as well as the release and proper targeting of the synthesized protein. During the elongation cycle the ribosome has to interact dynamically with various RNA (e.g. mRNA), RNP (e.g. aminoacyl-tRNA-EF-Tu) and protein ligands (e.g. EF-G, RF). Since ribosomes are so fundamental to life, understanding how they work and how they are regulated during protein biosynthesis is at the heart of molecular understanding of biology.
Non-protein-conding RNAs (ncRNAs) showed in the last years to be crucial in regulatory networks (e.g. chromosome remodelling; RNA polymerase activity; mRNA turnover; etc). However all of the recently discovered ncRNAs involved in translation regulation target the mRNA rather than the ribosome. This is unexpected given the central position the ribosome plays during gene expression and the assumption that the primordial translation system most likely received direct regulatory input from small molecules including ncRNA cofactors.
The main goal of this project is to identify potential novel ncRNAs that directly bind and possibly regulate the ribosome during protein biosynthesis. To address this question our group has already started genomic screens for novel regulatory ncRNAs that associate with *Saccharomyces cerevisiae* ribosomes under specific environmental conditions.
Recently we have expanded our genomic screens to two novel model organisms: the extremely halophilic archaea *Haloferax volcanii* and the basal flatworm *Macrostomum lignano*. These organisms can hopefully give us a better insight into the potential primordial regulation of translation. In order to select for ribosome-associated ncRNAs, we apply various stress conditions, isolate the ncRNA-ribosome-complexes and subsequently the small ncRNA candidates that co-purify with the translating ribosomes are used for cDNA library construction. The library will be then subjected to deep-sequencing analyses and potential ncRNAs will be tested for their presence and function during the ribosomal elongation cycle.

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Probing the ribosomal exit tunnel with tRNA-peptide conjugates

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The ribosome is playing the major role of protein synthesis in every living cell and its task is to decode the mRNA into the corresponding sequence of amino acids. By doing so each nascent peptide has to pass the ribosomal exit tunnel on it's way from the peptidyl transferase center, the catalytic center of the ribosome, to the surface of the particle. The task of the tunnel, which is mainly composed of 23S rRNA, and the interactions that are going on while the protein is passing through, is an integral part of ribosome research in the recent years. Nevertheless it's not jet fully elucidated how the interaction *in trans* between the ribosome and the protein which it is producing, works.
Other than that is the ribosomal exit tunnel a favored target of macrolide antibiotics. These macrolides, like erythromycin, clarithromycin, roxithromycin or telithromycin, are composed of a lacton ring and have a designated binding site in the exit tunnel near the peptidyl transferase center. They interfere with the growth of the nascent peptide, by stalling it after it reaches the length of about 5 amino acids. However, questions concerning the exact working mechanism of these antibiotics on the molecular level still await answers. The available data so far indicate, that it is not just a sterical interference with the growing peptide, which would lead to a stalling already after 2-3 amino acids. Additionally small peptides have been identified, which confer resistance to antibiotics sequence-specifically, indicating that the antibiotic must somehow sense the sequence of the peptide, while it is passing by, taking into account that the addition of the mature peptide alone is not leading to resistance.
Recent development in chemical synthesis enabled us the address this fundamental question on a new level. Stable RNA-peptide conjugates, which mimic peptidyl-tRNA, have been synthesized, carrying these so-called resistance peptides. By binding these substrates to ribosomes in the presence of macrolide antibiotics, the *in vivo* situation should be simulated. The peptidyl-tRNA/ribosome complexes were then subjected to chemical probing using DMS and CMCT, which modify flexible and accessible rRNA nucleobases. This experimental strategy allows studying potential conformational changes in the nascent peptide exit tunnel in the presents or absent of antibiotics.
The results will not only contribute to the elucidation of the working principle but could also be an important help in drug design and development of novel therapeutic strategies.

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A ligand-based 3D-pharmacophore model for the μ opioid receptor: application to the morphinan class of opioids

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Opioid receptors belong to the rhodopsin subclass within the superfamily of G-protein-coupled receptors (GPCR), which are characterized by the presence of seven transmembrane (7TM) helices. They interact with morphine and related opioid alkaloids as well as with various endogenous opioid peptides. It is widely accepted that there are three main types of opioid receptors, μ, δ and κ, which are differently implicated in opioid function. Opioid compounds specifically targeting each opioid receptor type are of high interest both as research tools and potential therapeutic agents [1]. Activation of the μ opioid receptor produces many other effects, besides its main involvement in pain control, including immunomodulation, respiratory depression, constipation, tolerance and physical dependence. With the lack of an experimental 3D structure of the μ opioid receptor, discovery of 3D pharmacophores for the receptor that can explain the activity of a series of ligands represent an important approach in drug discovery. In the present study, a new ligand-based pharmacophore model for the μ opioid receptor was generated using the LigandScout program [2]. A database consisting of morphinan derivatives was generated to provide the base for virtual screening and validation studies. Opioid receptor binding activity data earlier published by our group were included in this in-house opioid library and a general structure-activity relationship (SAR) for morphinan compounds was established. A merged feature ligand-based pharmacophore model for the μ opioid receptor was generated using a highly-active training set of morphinans (in-house opioid library). The model was optimized, validated and was shown to be able to identify highly active μ opioid ligands within a certain range, and excellent enrichments were achieved. The pharmacophore model indicated that the important features for the binding activity with the μ receptor are the presence of at least three hydrogen bond acceptors, one aromatic ring and one positive ionisable feature. The availability of the present pharmacophore model is expected to provide a more rational hypothetical picture of the primary chemical features responsible for activity, to be a valuable tool for 3D virtual screening and thus to facilitate the design of novel active candidates targeting the μ opioid receptor.

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P73

In vitro and in vivo pharmacological profile of 6-glycine substituted 14-phenylpropoxymorphinans, high affinity and potent opioid antinociceptive agents

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Being a disabling symptom of many medical conditions, effective pain control is one of the main therapeutic priorities. Among analgesic drugs, opioids (e.g. morphine, fentanyl and oxycodone), are the most effective analgesics for the management of moderate to severe pain, but they also share a number of adverse effects [1]. Our research in the morphinan class of opioid analgesics has led us to obtain several 14-alkoxymorphinan-6-ones that act as effective antinociceptive agents in various rodent pain models [2]. Herein, we describe the synthesis and pharmacological activities of a series of 14-phenylpropoxymorphinans containing a glycine residue in position 6. Binding affinities at opioid receptors (μ, δ and κ) were determined using in vitro competition binding assays in rodent brain membranes. Antinociceptive activities were assessed in rat models of nociceptive and inflammatory pain. In vitro binding studies showed that all compounds displayed high affinities at opioid receptors (μ, δ and κ), for several derivatives being in the subnanomolar range. The novel 14-phenylpropoxymorphinans exhibited dose-dependent antinociceptive effects in the tail-flick test in rats after subcutaneous (s.c.) administration, with considerably higher potency than morphine. Subcutaneous administration of the 14-phenylpropoxymorphinans significantly reduced mechanical hypersensitivity in rats with carrageenan-induced inflammatory pain with long-lasting effects. Analysis of the in vitro and in vivo opioid profile for this series of 14-phenylpropoxymorphinans leads to an improved understanding of the relationship between affinity and/or selectivity for the opioid receptors, antinociceptive potency and the nature of substituents in morphinans. On the basis of the structure-activity relationship that has emerged, certain modifications in the substitution pattern, e.g. introduction of a 14-phenylpropoxy group and/or a 6-glycine residue, results in interesting alterations in opioid activity by influencing the pharmacological properties of compounds interacting with μ, δ or κ opioid receptors that may emerge as novel analgesics.

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P74

Role of I-II loop in the plasma membrane targeting of L-Type Voltage Gated Calcium Channels

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Calcium influx through L-type voltage-gated calcium channels (LTCCs) underlies physiological processes like muscle contraction, neuronal excitability, modulation of gene expression and release of hormones [1]. In the multisubunit complex of a LTCC, the β-subunit binds with high affinity to the cytoplasmic I-II loop of the pore forming α1-subunit and thereby promotes plasma membrane targeting of the channel and modulates gating [2]. It is believed that β-subunit binding masks an endoplasmic reticulum retention signal on the I-II loop and promotes membrane targeting [3]. The aim of this study is to investigate if the I-II loops by themselves can support plasma membrane targeting of LTCCs. We produced FLAG-tagged I-II loops from different L-type ($Ca_v1.1$, $Ca_v1.2$, $Ca_v1.3$ and $Ca_v1.4$) and non L-type ($Ca_v2.1$, $Ca_v2.2$, $Ca_v2.3$) calcium channels, transiently expressed them in tsa-201 cells and monitored their subcellular localization by high resolution immunofluorescence microscopy in tsA-201 and GLT muscle cells. All L-type I-II loops were localized in the plasma membrane while non L-type I-II loops targeted to the nucleus. As expected, β-subunits (β_3, non-palmitoylated β_2) were evenly distributed in the cytoplasm but were targeted to the plasma membrane by co-expressed L-type I-II loops. Mutation of a single residue (W441A) in the β-subunit binding motif of the I-II loop disrupted β-subunit interaction but not plasma membrane targeting of the loop. This shows that independent structural motifs determine β-subunit binding and targeting of LTCC I-II loops. We hypothesize that LTCC I-II loops recognize a binding partner in the plasma membrane which mediates plasma membrane targeting even with the large (50-60 kDa) β-subunit bound. This interaction could be important for the plasma membrane targeting of the whole channel complex. We are currently identifying potential protein binding partners of the $Ca_v1.2$ I-II loop using co-immunoprecipitaion experiments. The nuclear targeting of Ca_v2 I-II loop (which cannot take place with the intact channel) is not studied further.

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P75

Identification of biochemical pathways contributing to the aberrant behavioural phenotype induced by dietary-induced Mg-deficiency

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An increasing number of clinical and preclinical studies proposes that changes in magnesium (Mg) homeostasis are involved in diverse psychopathologies including depression, but the underlying neurochemistry remains largely unknown. Here, mechanisms contributing to the enhanced depression- and anxiety-related phenotype of mice fed a Mg-restricted diet (10 % of the daily requirement) which is sensitive to chronic antidepressant treatment were investigated. Compared to control conditions, Mg-deficiency increased ACTH plasma levels which coincided with an elevated abundance of the corticotropin-releasing hormone mRNA in the hypothalamus indicating a hyperactive hypothalamic-pituitary-adrenal (HPA) axis. Furthermore, using an unbiased (proteomic) approach, the expression of four proteins (out of 302 identified), namely manganese-superoxide dismutase, voltage-dependent anion channel 1, glutamate dehydrogenase 1, and N(G),N(G)-dimethylarginine dimethylaminohydrolase 1 (DDAH1) were found to be altered in the amygdala/hypothalamus of Mg-deficient compared to control mice. Since DDAH1 is a signalling protein regulated by nitric oxide whose production can be regulated by NMDA receptor activity allosterically controlled by Mg, Mg-deficiency was induced in heterozygous nitric oxide synthase (nNOS) knockout mice. Indeed, Mg-deficient heterozygous nNOS knockout mice displayed an unaltered depression- but increased anxiety-like behaviour suggesting a role of the NO pathway in altered affective behaviour by Mg-deficiency. Chronic antidepressant treatment with either desipramine or paroxetine normalised the dysregulations in the identified systems strengthening the link between Mg-deficiency and anxiety/depression phenotypes. Collectively, the present data provide first evidence of deranged biochemical pathways, in response to dietary-induced hypomagnesaemia, differently contributing to mood and emotionality.

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Treatment of the Metabolic Syndrome by Traditional Chinese Medicine (TCM)

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Traditional Chinese Medicine (TCM) describes many herbal preparations that are used against various diseases associated with the metabolic syndrome. According to the International Diabetes Federation (IDF) this increasingly relevant disease pattern is characterized by type 2 diabetes mellitus (T2DM), malfunctioned glucose tolerance and pathologic insulin resistance [1]. The enzyme Protein Tyrosine Phosphatase – 1B (PTP-1B) is one of the major negative regulators of the insulin signaling pathway [2] and therefore its inhibition is of substantial interest for the treatment of T2DM and the metabolic syndrome [3].
Aim of our ongoing work is the isolation of PTP-1B inhibiting natural products from plants commonly used in TCM. Therefore, phytochemical and analytical investigations were performed in conjunction with parallel pharmacological screening (*in vitro* enzyme assay using human recombinant PTP-1B and a cell-based assay with human Hepatoma cells). Up to now we were able to show that *Pentagalloylglucose* (PGG) isolated from *Radix Paeoniae Rubra* [4] and *Oleic Acid* (OA) isolated from *Cortex Phellodendri* are significantly involved in the antidiabetic activity of these plant species. Ongoing research on other plant species used in TCM, like *Herba Agrimoniae*, also shows promising initial results.

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P77

In silico discovery of novel acidic microsomal prostaglandin E_2 synthase 1 inhibitors

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Microsomal prostaglandin E_2 synthase 1 (mPGES-1) is a terminal enzyme in the prostaglandin biosynthesis pathway that catalyzes the inflammation-induced conversion of cyclooxygenase (COX) -2-produced prostaglandin (PG) H_2 to PG E_2 [1], [2]. Specific inhibition of mPGES-1 is expected to leave the synthesis of constitutive PG E_2 as well as other COX-derived prostanoids unaffected [3], [4]. Thus, there is an increasing interest in this novel therapeutic strategy as an alternative to nonsteroidal anti-inflammatory drugs and coxibs. At present, some inhibitors of mPGES-1 are known, whereas none of them has reached clinical development.
The aim of our study was the discovery of novel mPGES-1 inhibitors. Therefore, a ligand-based pharmacophore model for acidic inhibitors of mPGES-1 was generated. The chemical database of the National Cancer Institute (NCI) was virtually screened using the model as search query. Pharmacological investigation of 19 selected compounds of chemically diverse structures led to the identification of three novel mPGES-1 inhibitors, i.e. NSC 152165, NSC 164958, and NSC 210627, showing IC_{50} values of 0.4, 3.7, and 0.5 µM, respectively.

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Disassembly of the ESCRT-III complex

P78

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Receptor down-regulation at the endosome requires the activity of the endosomal sorting complex required for transport (ESCRT) proteins. The ESCRT machinery is essential for the specific recognition and sorting of ubiquitin-modified cargo molecules (transmembrane proteins) into the intraluminal vesicles of multivesicular bodies (MVBs). The ESCRT system consists of five distinct protein complexes, ESCRT-0, -I, -II, -III and Vps4. While ESCRT-0, -I and –II collect ubiquitinated cargo, ESCRT-III functions late in the MVB sorting pathway. Assembling of ESCRT-III subunits on the endosomal surface probably drives membrane deformation and scission processes during MVB vesicle generation. ESCRT-III is composed of four proteins which oligomerize in an ordered manner on the endosomal membrane. The first ESCRT-III subunit Vps20, initiates the homo-oligomerization of Snf7 proteins; this filament is then capped by Vps24 which in turn recruits the last subunit Vps2.
Disassembly of the ESCRT-III polymer and release of its individual components from the endosome into the cytoplasm requires the activity of the AAA-ATPase Vps4. Interestingly, the recruitment of Vps4 to the site of MVB vesicle formation is regulated by ESCRT-III. However both, the molecular mechanisms of this recruitment and how Vps4 subsequently dissociates ESCRT-III subunits are poorly understood. We are investigating how Vps4 disassembles ESCRT-III and how ESCRT-III disassembly is involved in MVB vesicle generation. The disassembly of the ESCRT -III begins by the binding of Vps4 to the ESCRT-III subunit Vps2 and "starts" the disassembly process. Our data suggest that, once activated, Vps4 will begin to disassemble the entire ESCRT-III complex in an ordered and processive reaction. The ESCRT-III subunits are dissociated one after another, beginning with Vps2 and ending with Vps20. Our studies might help to explain how the ESCRT-III complex directs and times its own disassembly on endosomes and hence could provide understanding of the last step in MVB sorting.

P79

Functional Genomic Characterization of a potential Membrane Stress Response pathway

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The degradation of intracellular proteins regulates different processes, such as cell cycle and division, transcription factors, cellular quality control and cell surface remodelling. Defects in protein degradation systems have been implicated in the pathogenesis of various human diseases ranging from malignancies to neurodegenerative disorders. Four major pathways, the ubiqutin-proteasome system (UPS), the ER associated degradation (ERAD), the autophagy- and the multivesicular body (MVB) - pathway target proteins for degradation. The MVB pathway is, to the best of our knowledge, required for the degradation of most if not all transmembrane proteins which constitute approximately 30% of all cellular proteins. The pathway is mediated by the endosomal sorting complexes required for transport (ESCRT). The ESCRT machinery plays a critical role in cellular processes including the downregulation of cell surface receptors and formation of intra lumenal vesicles within the MVB, viral budding from the cell surface and cell abscission at the end of cytokinesis.
To understand how cells respond on the accumulation of transmembrane proteins we compared the gene expression profile of wild-type yeast cells with ESCRT deficient yeast mutants (*vps4D*) and whether this would trigger a specific stress response versus an ESCRT WT (*VPS4*) using microarray analyses. The analyses of the gene-expression profiles shows that 124 genes are differentially regulated. The mRNA levels of 66 % are upregulated in the ESCRT deficient cells and represent genes that are involved in stress response and water transport. 41 genes show a decrease in mRNA levels. 70% are direct targets of the yeast MAPK pathway. The canonical MAPK pathway transmits pheromone signals via a G protein coupled receptor and initiates mating between two haploid cells. Consistent with the results these results we detect a 3,5 fold decrease in mating efficiency of the ESCRT mutant (*vps4D*). Importantly, neither changes in the gene expression profile of any other ESCRT subunit nor changes of the other major degradation pathways were be detected. Taken together, it appears that cells might activate a specific membrane stress response when transmembrane proteins can no longer be degraded and accumulation in cells.

P80

Identification of novel regulators in cell surface receptor degradation via the multivesicular body pathway

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Cell surface receptors are important for sensing extracellular signals and in consequence triggering specific cellular reactions. Upon activation transmembrane proteins are selectively ubiquitinated and subsequently internalized into the endosomal membrane system. On endosomes, internalized receptors can either recycle back to the cell surface or selectively degrade by the MVB (multivesicular body) pathway. The MVB pathway is mediated by the highly conserved endosomal sorting complexes required for transport (ESCRT). The sequential action of 5 different ESCRT complexes sorts ubiquitinated membrane proteins into the inward budding MVB vesicles. Beside MVB vesicle formation ESCRT components are involved in the budding of retroviruses such as HIV and EBOLA and cytokinesis. Moreover dysfunction of the ESCRT contributes to diseases such as cancer and neurodegeneration.
While the assembly mechanisms of ESCRT are reasonably well understood in yeast, little is known about its regulation. Our aim is to identify regulatory mechanisms in transmembrane protein degradation and ESCRT function using genetic and proteomic approaches. Using a phosphoproteomic screen we have identified 25 phosphorylation sites in 7 ESCRT subunits. Some of which appear conserved in humans. We are currently testing mutated predicted phosphorylation sites, located on essential ESCRT subunits, to clarify their role in ESCRT regulation.
As yeast has only 133 kinases and 46 phosphatases, we are additionally conducting a genetic screen to identify regulatory kinases and phosphatases. First results will be presented on the poster.

P81

TIS7 interacts with and regulates the methylosome

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TPA Induced Sequence 7 (TIS7) was characterized by our laboratory as a transcriptional co-repressor [1]. The generation of TIS7 knockout mice showed that TIS7 is involved in muscle differentiation and regeneration [2]. Using MALDI-TOF/TOF in immunoprecipitates of TIS7, we detected all three known components of the methylosome complex [3, 4] protein arginine N-methyl transferase 5 (PRMT5), pICln and methylosome protein 50 (MEP50). The TIS7 complex contained methyl transferase activity *in vitro*. FRET experiments with NIH3T3 cells confirmed the co-localization of the methylosome complex with TIS7 *in vivo*. GST pull-down analyses showed that TIS7 directly interacts with all 3 methylosome subunits. Furthermore, we observed a down-regulation of the methylosome subunits expression on RNA and protein levels in TIS7 KO myoblasts.
As the methylation of the MyoD promoter is known to regulate muscle differentiation [5], and we found this muscle regulatory factor to be down-regulated in TIS7 KO myoblasts, we are testing whether TIS7 or methylosome subunits over-expression in TIS7 KO muscle satellite cells can rescue the expression of MyoD and thereby also the fusion deficiency phenotype.
Data shown here implicate that TIS7 regulates gene expression not only via its interaction with histone deacetylases, as we have previously shown [1], but also by affecting methylation processes. Clarification of the mechanisms directed by TIS7 during regeneration events, e.g. following muscle injury, may provide a possibility for therapeutical application.

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Investigating the Role of PIDD in the DNA Damage Response

P82

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The p53-induced protein with a death domain (PIDD) is involved in cellular pathways which can induce programmed cell death (apoptosis), survival via the activation of NFkB and/or DNA-repair after DNA-damage. Depending on the duration, type and severity of genotoxic stress, PIDD, together with the adapter molecule RAIDD, can aid in the formation of an activating platform for Caspase-2, the so-called “PIDDosome”, or together with RIP1 assist in the activation of NFkB by promoting sumoylation of NEMO. Most recently, PIDD has also been shown to form complexes with DNA-PK, assisting DNA repair by enabling non-homologous end-joining. While the first complex induces apoptosis, the latter two prevent apoptosis by enabling the cell to repair the damage. PIDD therefore plays a role in the integration of at least two opposing signalling pathways. This dual role is based on the capacity of PIDD to auto-proteolytic cleavage, which leads to the generation of two different protein fragments, PIDD-C and PIDD-CC, each of which is involved in the induction of one of the aforementioned pathways.
In order to shed further light on these roles and to investigate the in vivo function of PIDD, we have generated a mouse model lacking the *pidd* gene. Using mouse embryonic fibroblasts (MEF) derived from those mice we performed survival assays and monitored NFkB activity in response to genotoxic stress.
Treatment with inflammatory cytokines did not reveal any differences in NFkB activation between wild type and PIDD deficient MEF. In contrast, there was a significant delay in activation of NFkB in response to DNA damage. Furthermore, various inhibitors of NFkB could sensitize wild type cells, but not PIDD deficient cells towards DNA damage induced apoptosis. Unexpectedly, we observed no differences of short and long term survival between wild type and PIDD deficient cells. However, PIDD deficient cells show a deficient cell cycle arrest in response to DNA-damage.
In conclusion, our results suggest that PIDD plays a rate-limiting role in NFkB activation following DNA damage. Surprisingly, this defect is not reflected in differences in cell survival.

P83

Investigating the Role of the BH3-only Proteins Bim and Bmf in Mammary Gland Development and Breast Cancer

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The two BH3-only proteins Bim and Bmf were implicated in driving apoptosis upon various stresses, but also in developmental cell death during mammary gland formation. The aim of our study is to investigate the role of these proteins during development and tumourigenesis of the mammary gland.
We found that Bim and Bmf are differentially expressed during mammary gland development. As these two proteins have a proapoptotic function we investigated in detail tissue dynamics during involution in the absence of Bim or Bmf. Inducing the cessation of lactation by forced weaning caused delayed involution in mice lacking Bim or Bmf, when compared to wild type or *bad*$^{-/-}$ mice.
Assessing tumour development, using the MMTV/*neu* mouse model of breast cancer, we saw that mice lacking one or both alleles of *bmf* show the same tumour latency as wildtype mice, while, surprisingly, *bim*$^{+/-}$ mice develop the tumours significantly later and, so far, this is even more significant in *bim*$^{-/-}$ mouse.
We accredit both proteins to contribute to the remodelling of the mammary gland after lactation. In the MMTV/*neu* tumour model Bmf does not suppress breast cancer formation while, surprisingly, Bim seems to promote malignant disease.

P84

Elucidation of the Physiological Role of the Bcl-2 Pro-Survival Homologue A1

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The anti-apoptotic protein A1/Bfl-1 reportedly plays a role in lymphocyte and myeloid cell development and maturation. The physiological role of A1, however, is still unclear because conventional knockout techniques cannot be applied to generate a suitable mouse model.
In order to overcome this problem we have used an alternative strategy based on RNA interference (RNAi). We chose to generate an inducible as well as a tissue-specific transgenic mouse model to knock-down A1. Therefore, we designed an expression construct encoding a shRNA targeting A1 mRNA in the context of the miR30 micro RNA. In one model, this miR30-A1 sequence was embedded in the 3´UTR of a cDNA encoding the fluorescent marker gene *Venus* trancribed from a modified version of the *Vav*-gene promoter containing lac-repressor (lacI) binding sites (lacO), which is specific for the hematopoietic system and can be regulated by IPTG in the context of lacI. In a second approach the miR30-A1 sequence is expressed in the context of a Tet-*CMV*^{min} promoter followed by *EGFP* cDNA sequence driven by the *ubiquitin* promoter. These *Tet-miR30-A1* mice were crossed with *VavP-tTA* mice in order to drive the expression of the mir30-A1 in all hematopoietic cells. In addition, we generated Hoxb8-myeloid progenitor cell lines that can be differentiated into neutrophils or macrophages *in vitro* from *Tet-miR30-A1* mice to establish an *in vitro* system allowing manipulation of A1.
First results suggest that A1 may be important for thymocytes survival during negative selection and for B cell maturation. Furthermore, the differentiation potential into the granulocytic lineage also seems dependent on A1 availabilty.

P85

Investigating the role of the PIDDosome in *B cell lymphomagenesis*

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The "PIDDosome", a protein complex containing the death domain containing proteins PIDD and RAIDD is proposed to act as an activation-platform for the cell death-associated protease Caspase 2. Caspase-2 has been proposed to facilitate apoptosis and exhibits tumor suppressor potential in fibroblasts and B lymphocytes. In order to investigate the physiological role of the PIDDosome in B-cell lymphomagenesis, *caspase-2* and *pidd*-deficient mice were intercrossed with *Eμ-myc* transgenic mice that develop immature pre-B or IgM+ B-cell lymphomas within their first six months of life.
Consistent with published results, we observed a significantly accelerated lymphoma onset in mice lacking *caspase-2* when compared to wild-type mice, but no such effect was noted in mice lacking one or two alleles of *pidd*.
Notably, loss of *caspase-2,* but not that of *pidd*, favored the development of more mature IgM^+ B-cell over pre-B-cell tumors. Since it is well-known that a considerable number of Eμ-myc driven lymphomas show inactivation of the ARF/mdm2/p53 signaling-axis, we immunoblotted the arising B-cell-lymphomas for p53 and ARF expression. Our analysis revealed that the rate of p53 inactivation in *caspase-2* deficient tumors was significantly reduced.
Analysis of tumor cell apoptosis revealed that neither *caspase-2* nor *pidd* deficient cells show a survival advantage, when treated with different doses of anti-cancer drugs for 24 hours.
Premalignant mice lacking *caspase-2* or *pidd* showed increased populations of pre-B, T1 and mature-B-cells, similar to the phenotype known for *Eμ-myc* mice and survival assays using isolated B-cell subsets showed no difference in their survival rate upon c-myc-induced oncogenic stress.
Taken together, we could demonstrate that caspase-2 plays a potent role in tumor suppression but this tumor suppressive effect does not require the PIDDosome as an activation-platform for *caspase-2*.

Investigating the role of BH3-only proteins in B cell development

P86

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B cells undergo many selection processes before becoming mature and immunocompetent. The TNF family ligand B cell-activating factor (BAFF), which binds three receptors: BCMA, TACI and BAFF-R, plays an important role in B cell development and survival. Its absence causes the loss of most mature B cells including transitional 2, follicular and marginal zone B cells. This deficit can be partially rescued by overexpression of Bcl2, the founding member of the Bcl2 family of proteins. The prosurvival function of Bcl2 is antagonized by so-called BH3-only proteins, pro-apoptotic members within the same family, such as Bim or Bmf. Since loss of these BH3-only proteins results in an accumulation of mature B cells we crossed $bim^{-/-}$ and $bmf^{-/-}$ animals with mice that overexpress a TACI-Ig fusion protein, in which BAFF is sequestered and functionally inhibited.
Preliminary results suggest, that the deletion of Bim or Bmf can restore in part the survival of T2, FO and MZ B cells in TACI Ig transgenic mice, which is even more pronounced when both BH3-only proteins were knocked out.
We conclude that BAFF functions by modulating the expression and/or function of Bim and Bmf. It has to be determined if the rescue in the double-deficient mice is complete or if other BH3-only proteins or even a Bcl2-independent cell death pathway can also be targeted by BAFF-mediated signals.

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The role of fibroblasts and macrophages in capsular fibrosis

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Silicone is the most widely used inorganic biomaterial in medical practice applied in a large variety of active (cardiac pacemakers, cochlea implants, etc) and passive (drainage tubings, silicone mammary implants (SMIs), etc.) implants. For decades, SMIs have been accepted to be biologically inert, even though they induce a local inflammatory response resulting in capsular fibrosis [1]. This fibrotic consequence is characterized by an excessive deposition of extracellular matrix (ECM) components.
Within the peri- SMI connective tissue capsule, we found massive infiltrates of immune cells, predominantly T-lymphocytes and macrophages, but also dendritic cells as well as (myo)-fibroblasts. Moreover, the proteinaceous film deposited on the surface of silicone implants *in vivo* is presumed to be a key player in the activation of the innate and adaptive host defense mechanisms by promoting exposure of cryptic and/or formation of altered self epitopes. Therefore, we identified the most abundant proteins adhering to silicone surfaces with several biochemical modifications [2,3]. One of these, fibronectin (FN) plays a major role in cell adhesion, growth, migration and differentiation. FN is deposited during wound healing and is crucial for the differentiation of fibroblasts into myofibroblasts, induced by TGF-β1. On the other hand, FN is recognized by macrophages and frequently used in biomaterial modification to elicit greater cellular adhesion and tissue integration. Altered FN expression, degradation and organization has been associated with fibrosis.
In this project, we focused on the role of fibroblasts and macrophages in the fibrotic reaction to SMIs. Cell adhesion and proliferation of capsular and autologous dermal fibroblasts as well as monocyte-derived macrophages on textured *vs* smooth silicone surfaces pre-coated with different proteins were investigated *in vitro* using scanning electron microscopy (SEM), immunohistochemistry, FACS-analysis and cell adhesion assays. The impact of the adsorption of FN as well as heat shock protein 60 (Hsp60) on the morphology of fibroblasts and macrophages on the silicone surface was observed using SEM. After 24 hours of incubation, cells on silicone pre-coated with FN and Hsp60 already spread out, while controls retained a spherical morphology. Without proteinaceous pre-coating of the silicone surface, more apoptotic cells with characteristic membrane blebbing were found compared to controls. In addition, higher numbers of attaching cells on pre-coated silicone were verified with cell adhesion assays. This was also true for textured *vs* smooth silicone surfaces. SEM, immunohistochemical and FACS analysis showed the improved capability of monocyte-macrophage differentiation on pre-coated silicone surfaces compared to controls.
This study showed that both surface topography of the silicone implant and deposition of proteins on the silicone surface modulate cell morphology, differentiation and adhesion.
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HSP60 T cell epitopes and anti-HSP60 autoantibody are involved in the initiation and progression of atherosclerosis

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Atherosclerosis is a multifactorial chronic, inflammatory disease characterized by the appearance of intralesional T cells, macrophages, and dendritic cells. These cells contribute to the inflammatory process in the arterial wall by mechanisms that are not yet completely elucidated. Surface expression of HSP60 by arterial endothelial cells is believed to be a hallmark of the earliest stages of atherosclerosis. The induction of HSP60 and adhesion molecules is increased in endothelial cells subjected to classical atherosclerosis risk factors. These HSP60 positive endothelial cells can then become a target for pre-existent innate and adaptive cellular and/or humoral immunity. To characterize the proatherogenic HSP60 epitope/s leading to early atherosclerosis could therefore lead to new therapeutic interventions targeting the actual disease process in the arterial wall.
The aim of this study was to phenotypically and functionally characterize T cells isolated from **early** atherosclerotic lesions and to determine the potential reactivity to HSP60.
Iliac arteries and peripheral blood were collected from transplantation organ donors (n=6) and analyzed by immunohistochemistry and flow cytometry. The T cell immune-response was determined by proliferation assays against total recombinant human HSP60 protein (hHSP60) and HSP60 15mer overlapping peptides thereof.
Immunohistological analysis demonstrated the presence of $CD4^+$, $CD8^+$, dendritic cells ($CD1a^+$), and macrophages ($CD68^+$) within the intima in early atherosclerotic lesions. Surface expression of HSP60 was detected in endothelial cells and HLA class II cells. The predominant phenotype of intralesional T cells was defined as $CD4^+CD45RO^+CD28^+CD25^+FoxP3^+$ cells. Both, $CD4^+$ and $CD8^+$ T cells showed an increased production of IFNγ compared to IL-10 and TGFβ. Interestingly, a considerable percentage of $CD4^+$ T cells still produced IL-4 as well as IL-17. In vitro, isolated intra-lesional early atherosclerotic T cells proliferate in response to whole hHSP60 protein and derived peptides suggesting the presence of HSP60 atherogenic epitopes. Significantly increased levels of circulating anti-HSP60 autoantibodies were found in sera of patients with early lesions and even more pronounced in patients with late lesions as compared to normal donors.
In conclusion, this is the first demonstration in **early**, clinically still inapparent human atherosclerotic lesions that proves our previously proposed concept that HSP60 reactive T cells initiate the early inflammatory stage of atherosclerosis by recognition of atherogenic HSP60 epitopes and the anti-HSP60 antibodies accelerate and perpetuate the disease. These epitopes may be an attractive diagnostic and therapeutic target for atherosclerosis.

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The activity of iron salophene complexes against tumor cells

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Iron salophene complexes have outstanding biological activity in different types of tumor cells. The influence of the central atom and the role of the ligand on cytotoxic activity have been examined in previous studies for the lead substances [Fe^{II}(salophene)] and [Fe^{III}(salophene)Cl]. Iron as central atom is essential for growth inhibitory effects compared to manganese, cobalt, nickel, copper and zinc.
Iron complexes induce the generation of free radicals within the cells which leads to the release of superoxide dismutase and induces apoptosis via the mitochondrial pathway. Furthermore [Fe^{III}(salophene)Cl] shows the ability to overcome resistance in tumor cells which are resistant against chemotherapeutics like Daunorubicin, Doxorubicin and Vincristin. Contrariwise healthy leucocytes are not harmed by this compound. Cytotoxic effects are drastically reduced when 1,2-phenylenediamine (salophene) is replaced by 1,2-diaminocyclohexane (saldach). In vitro structure-activity-relationship studies with [Fe^{III}(salophene)Cl] complexes in MCF7-, MDA-MB231 breast cancer, and HT29 colon carcinoma cells demonstrated the regulation of antitumor activity by the position of electron donating groups (e.g. -OMe) in the salicylic moieties.[1]
Future research will involve synthesis and exploration of different complexes based on the salophene matrix since those complexes are proved as chelating agents with high cytotoxic activity. The focus will be on modification and enlargement of aromatic parts of the chelating agent in order to obtain even more active complexes. Another aim is the development of an in vitro method to show once more increased cytotoxic activity of those complexes under magnetic influence. Besides iron, cytotoxic potential of nickel- and platinum-complexes of novel chelating matrices will be investigated.

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AKAP79/150 Palmitoylation is Required for Endosomal Targeting and Regulation of Neuronal Postsynaptic Structure and Function

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AKAP79/150 is a postsynaptic scaffold protein for PKA, PKC, and Calcineurin (CaN) that is enriched in dendritic spines and is thought to play important roles in regulating AMPA receptor (AMPAR) activity and spine structure. AKAP spine targeting is controlled by a poly-basic N-terminal domain that binds acidic phospholipids, F-actin, and cadherin cell adhesion molecules. NMDA receptor-CaN signaling pathways implicated in AMPAR depression and spine shrinkage in LTD disrupt AKAP79/150 interactions with actin, MAGUKs and cadherins and lead to loss of the AKAP and anchored PKA from synapses. Recently, we identified AKAP79/150 as a palmitoylated protein in rat brain synaptasomes. We have now mapped two conserved Cysteine residues within the AKAP targeting domain (C36 and C129) as the sites of palmitoylation. In addition, we have found that endogenous AKAP79/150 in hippocampal neurons is subject to dynamic palmitoylation and depalmitoylation in response to stimuli that induce LTP and LTD, respectively. Importantly, in imaging experiments we observe palmitoylation dependent spine recruitment of AKAP79-GFP coincident with spine enlargement and increased GluR1-AMPAR surface expression during chemical induction of LTP (cLTP) in hippocampal neurons. A palmitoylation deficient 79C36,129S mutant is not recruited to spines and blocks both cLTP spine enlargement and GluR1 surface expression increases. We are currently further exploring the mechanism for the lack of spine recruitment and inappropriate AMPAR regulation seen following cLTP for 79C36,129S. One recent finding we are actively pursuing is that AKAP79WT assembles signaling complexes with PKA, CaN and the MAGUK SAP97 not only at the plasma membrane but also in endosomes including early and recycling compartments. However, while 79C36,129S and its associated binding partners are still targeted to the plasma membrane, they are largely absent from endosomes. Importantly, interfering with recycling endosome trafficking by overexpression of a Rab11 dominant-negative mutant blocks cLTP-induced recruitment of AKAP79WT to spines. These observations are potentially significant because AMPAR recycling is regulated by PKA and recycling endosomes provide the AMPARs necessary for synaptic potentiation and the membrane cargo required for spine enlargement during LTP. Thus, AKAP79 localization to endosomes controlled by palmitoylation may be required for regulating AMPAR endocytic recycling and spine enlargement in LTP.