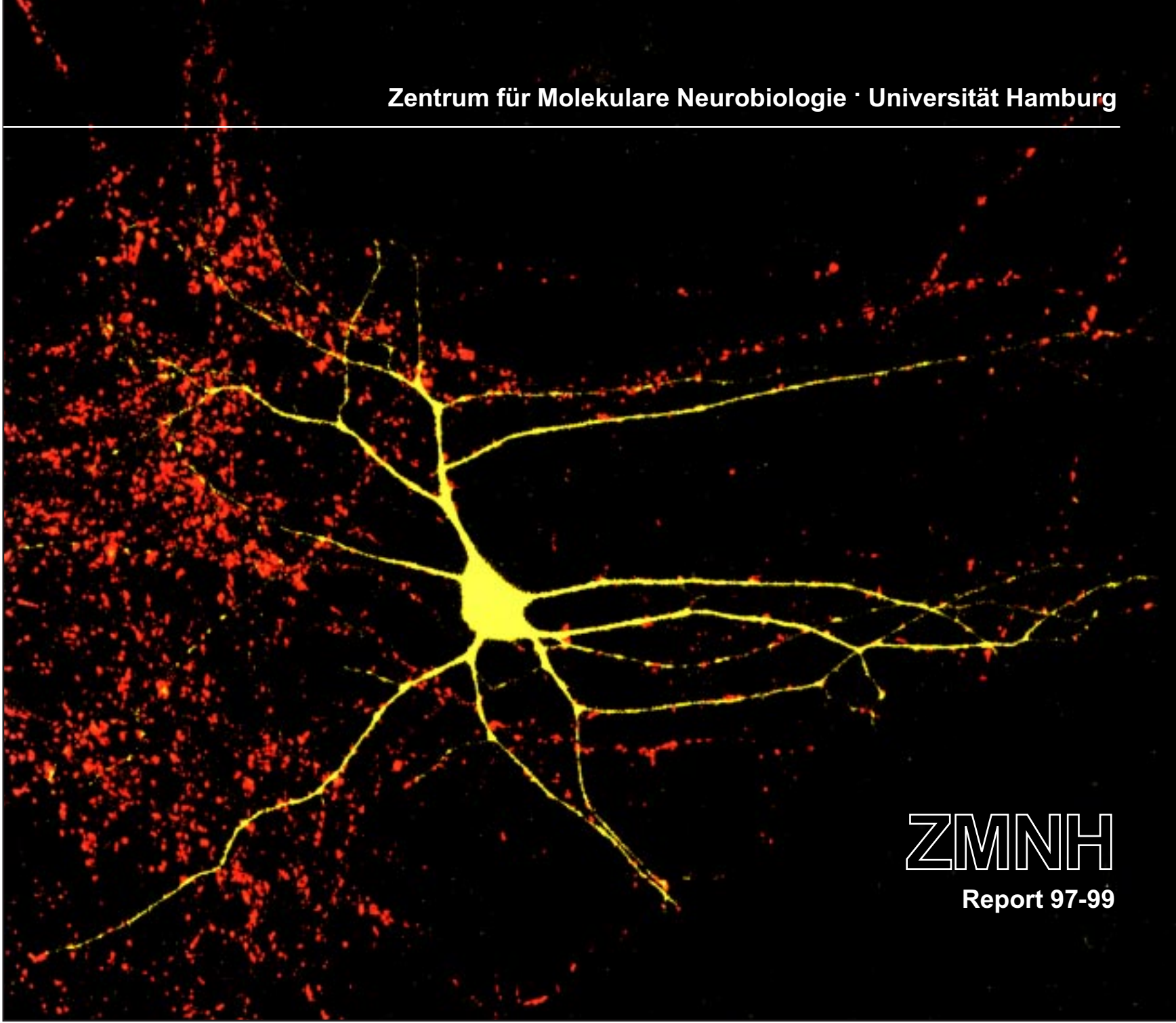
Zentrum für Molekulare Neurobiologie · Universität Hamburg



ZMNH

Report 97-99

Front cover: Pyramidal cells from dissociated rat hippocampi after 12 days in culture were stained with an antibody against synaptophysin (red). One neuron was filled with Lucifer Yellow, see page 103.

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INTRODUCTION

The ZMNH is a basic research institute of the University of Hamburg. It is affiliated to the Faculty of Medicine and is part of the UKE, the University Hospital of Eppendorf. The ZMNH was founded with the aims to promote innovative research in molecular neurobiology, to establish a teaching program for advanced graduate and postgraduate students, and to initiate and intensify cooperations with clinical departments of the UKE. An international Scientific Advisory Board assesses progress and future directions of research every two years.

Current research and future goals of the ZMNH

Molecular biology has contributed considerably to scientific progress during the past four decades. Sequencing of the human genome is scheduled to be completed within the next two years. This means that all human genes will be available in a database and that their chromosomal locations will be known. Thus correlations with inherited diseases may be established more easily. While it is clear that genetic analysis will continue to play an important role, future research will increasingly focus on understanding structure, function, and regulation of proteins. In neurobiology molecular and cell biological methods will be supplemented by neuroinformatics to decipher the circuits that finally result in higher brain functions such as learning, memory, and behavior.

In the past we have been successful in characterizing new genes. Starting from isolated proteins in my institute genes for morphogens and morphogen receptors from hydra and in Olaf Pongs' group for β subunits of potassium channels were cloned. In another approach Thomas Jentsch applied expression cloning to identify the first chloride channel gene from the electric organ of the ray Torpedo. Human and mouse homologues were found by homology screening which is facilitated by the rapid growth of sequence databases. Homology screening led to the characterization of the human cell adhesion molecule CHL1 by Melitta Schachner's group. Human Sox10, a transcription factor important for neural development and involved in Hirschsprung disease and Waardenburg's syndrome, was discovered by Michael Wegner. Similarly, the group of Thomas Jentsch identified the potassium channel genes KCNQ2, KCNQ3, and KCNQ4, mutations of which cause epilepsy and deafness, respectively.

Differentially regulated genes can be isolated by a subtractive approach. Dietmar Kuhl's group found several genes, the transcription of which is upregulated by synaptic activity. Of special interest is arg3.1, because its mRNA is transported to the dendrites.

As a first step to analyze the function of a new gene, mRNA and protein expression are monitored during embryonal development and in the adult. Another approach was pursued by the group of Olaf Pongs, where potassium channels are being purified and crystallized to determine their three-dimensional structure by X-ray crystallography.

Protein function can be studied by heterologous expression in frog oocytes, mammalian cell lines, primary neurons, and brain slices. Analysis of the electrophysiological properties

of mutant channel proteins allowed many breakthroughs in ion channel physiology by the groups of Olaf Pongs and Thomas Jentsch. For large, neuron-specific proteins like cell adhesion molecules and a new family of neuropeptide receptors, transfection of the respective genes by viral vectors seems necessary to allow full-length expression, proper processing, compartment shuttling, and membrane insertion.

Molecules interacting with the protein of interest can be recognized by using specific antibodies or by the yeast two-hybrid method. In the group of Dietmar Kuhl a tri-hybrid system was developed which allowed identification of proteins involved in dendritic transport of arg3.1-mRNA. Interactions between defined partners can be further characterized by Biacore analysis, a technique recently established at the ZMNH.

To analyze gene function *in vivo,* several groups at the ZMNH have utilized the mouse knock-out or the transgene technology. Knock-out mice for L1 and for NCAM/MAG were generated in Melitta Schachner's group and are excellent models for the human CRASH syndrome and for demyelinating diseases, respectively. Such mice can now be used to study molecular and therapeutic approaches for medical application.

A very promising new technology is based on the discovery that embryonic and neural stem cells can be engineered to express genes of interest. Implantation of such cells has opened up the possibility of treating neurodegenerative diseases such as Alzheimer's dementia and Parkinson's disease. In a mouse model dysmyelination could be repaired by implanting stem cells, and Melitta Schachner's group has initiated collaborations with clinical departments at the UKE to exploit this technology for patients.

One aim for future neuroscience research is to understand how genetic differences translate into different personal traits, behavior, and susceptibility to disease. We are confident that the ZMNH will contribute towards this goal.

Events and highlights during the past two years

With the completion of the new building, all of the ZMNH groups are now located under one roof which has facilitated communication and led to joint publications. Interaction and collaboration with UKE groups has been intensified via three SFBs, one on molecular medicine, a second on neural communication, and a third on glycobiology. Ties were also strengthened by two research programs, one on Alzheimer's dementia and another on RNA transport, as well as by a graduate program on neural signal transduction and its pathology.

The quality of research at the ZMNH is documented by its publications, by the rating within the UKE, and by the award of several prizes to its members, especially the Gerhard-Hess prize for Michael Wegner. It is also evidenced by job offers to ZMNH members, for example that of the Max-Planck society to Thomas Jentsch.

The term for our second generation of junior group leaders is coming to an end. Roger Nitsch has already started as full professor of Molecular Psychiatry in Zürich, and Dietmar

Kuhl and Michael Wegner are in negotiations for their future jobs. This opened up space for new junior groups. We are very grateful that the UKE provided DM 1.5 million per year to fund three new group leaders. Dieter Riethmacher and Maike Sander started early this year, and Thomas Schimmang will join them at the end of 1999. In addition to these three groups, Ingolf Bach, holder of a habilitation fellowship from the DFG, began to work at the ZMNH at the end of 1998. All new groups contribute new expertise to their respective fields: Dieter Riethmacher to peripheral nervous system development, Maike Sander to hindbrain, motor neuron, and pancreas development, Ingolf Bach to transcriptional control early in neuronal development, and Thomas Schimmang to molecular aspects important for ear development. The newcomers will strengthen the area of developmental neurobiology, which currently has a great impact on neuroscience.

Molecular neurobiology is a rapidly expanding field, requiring new technologies and equipment at fast rates. Therefore, budget increases for new investments are needed to ensure up-to-date research facilities. We hope that generous support of the ZMNH will help us to maintain our success and productivity in the neurosciences.

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(Director)

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Research Projects

Institutes

Institut für Biosynthese Neuraler Strukturen

Melitta Schachner Camartin

Formation of the appropriate connections among nerve cells is essential for the correct and efficient functioning of the nervous system. It is through very specialized interactions between the different neural cell types that such connections are formed during development, maintained or modified in the adult, and reformed or even prevented after trauma. Cell surface and extracellular matrix molecules that have been recognized to mediate such interactions are now being implicated in such diverse phenomena as neural induction, neural cell proliferation, neuronal migration, neurite outgrowth, synaptogenesis, signal transduction between neurons and glia, and finally, the capacity of neurons to regenerate or not. For instance, how does a neuron sense where to position its cell body, into which direction to send out its neurites, and when to engage in stable connections or to destabilize such connections under conditions requiring plasticity, such as learning and memory. Thus, not only recognition between interacting cells is called for, but mechanisms must be implemented that transduce cell surface triggers - resulting from recognition - into sensible and sensitive intracellular responses that guide a cell's ultimate behavior in the intricate context of network activities. The aim of our research is to understand the molecular events that mediate communication among cells in the nervous system not only during the ontogenetic formation of connections, but also in the adult nervous system under conditions of synaptic plasticity and trauma. This report is subdivided into several thematically interconnected projects which relate to the communication between neural cells on the basis of such recognition phenomena. Several research areas are being investigated.

1. The L1 family of neural recognition molecules

Udo Bartsch, Reiner Czaniera, Birgit Hertlein, Michael Kutsche, Janice Law, Alan Lee, Melanie Richter, Bettina Rolf, Birte Rossol, Annette Rünker, Sandra Schmidt, Birthe Schnegelsberg

The neural cell adhesion molecule L1 is a multifunctional molecule that has been implicated in neuronal migration, neurite extension and fasciculation, myelination in the peripheral nervous system and synaptic plasticity. It is the founding member of a family comprising several L1-like molecules, all of which enhance neurite outgrowth (Fig. 1).

The L1-like molecules are present in overlapping and distinct subpopulations of neurons at different stages of development and may be important determinators of specific neurite outgrowth patterns during development. Structure-function-relationships of the different domains of L1 have been characterized and the molecular associations of L1 with other neural recognition molecules, including N-CAM, CD24 and laminin have been investigated. As a prominent glia-associated neurite outgrowth promoting molecule in the peripheral nervous system - being absent in the central nervous system on glial cells after trauma - its neurite outgrowth pro-

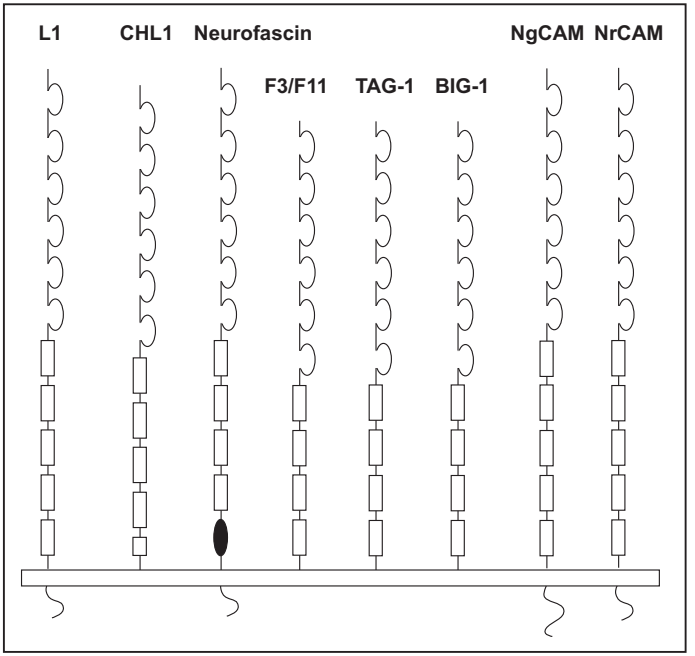

Figure 1. Schematic representation of the structure and the structural motifs of L1-like molecules in vertebrates. The NH2-terminal ends of the molecules comprise 6 immunoglobulin-like domains followed by fibronectin type III homologous repeats. L1-like molecules can be, like L1, transmembrane glycoproteins, while others, such as TAG-1, are linked to the cell surface via a GPI anchor.

moting role has been evaluated in the central nervous system using transgenic mice that overexpress L1 in glial cells during crucial stages of regeneration after a lesion. In this transgenic mouse, neuronal differentiation and survival (see, also, Fig. 2) and the learning performance in the Morris water maze test are enhanced. Analysis of an L1-deficient mouse mutant generated by homologous recombination has revealed this mutant to be a very good animal model for the inherited human diseases carrying mutations in the L1 gene that are now summarized under the name of CRASH syndrome (implicating mental retardation, aphasia, shuffling gate, adducted thumbs, spastic paraplegia type Ib, and hydrocephalus). A knock-out mouse mutant deficient in the close homologue of L1 (CHL1) shows a much less severe phenotype. Conditional neural knock-out mutants are being generated and double knock-out mutants carrying defects in genes of the L1 family are being analyzed to further probe into the functions of these molecules. Other members of the L1 family have been searched for to obtain a more complete picture of the range of diversity of L1-like molecules implicated in the fine-tuning of neuron cell type-specific interactions.

2. Neural recognition molecules and signal transduction

Suzhen Chen, Judith Clees, Markus Delling, Ling Dong, Silke Gorissen, Jens Lütjohann

The intracellular consequences of homophilic (self binding) and heterophilic (binding to other molecules) interactions of L1 are of central importance for the understanding why L1-like molecules (for instance F3/F11) engage, on the one hand, in stable interactions and, on the other, in repulsion of growth cones and neuronal cell bodies. The signaling cascades involving tyrosine and serine/threonine kinases and phosphatases, calcium, G-proteins and the cascade of ras and raf signaling mechanisms are being investigated.

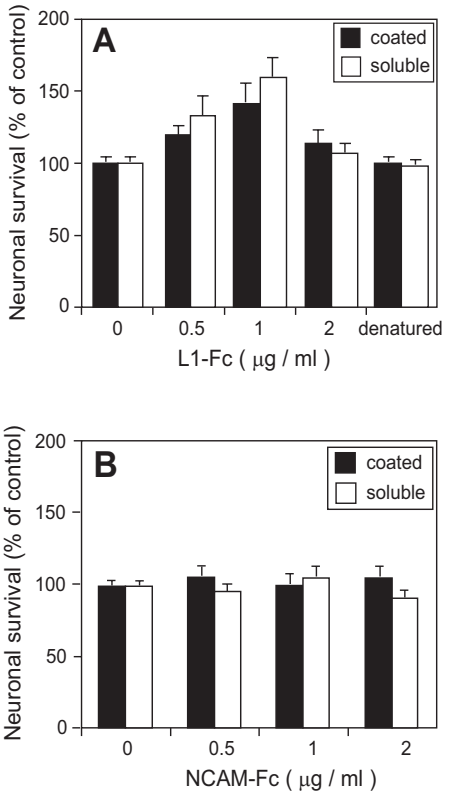

Figure 2. Influence of L1 on survival of hippocampal neurons in culture (A). A fusion protein of the extracellular domain of L1 with the constant region of immunoglobulins (Fc) is offered either as substrate-coated or as soluble molecule to primary cultures. Survival of neurons is assayed by the number of cells undergoing oxidative phospholyration (MTT test). At 2 μg/ml, L1-Fc fusion proteins undergo homophilic interaction and are no more available for L1-L1 interaction with neurons. Cultures were treated in a similar way with the NCAM-Fc fusion protein, not resulting in enhanced survival (B).(Suzhen Chen and Melitta Schachner, unpublished observations).

Furthermore, the interactions of adhesion molecules of the L1 family and the isoforms of the neural cell adhesion molecule N-CAM with the cytoskeleton are investigated by using the yeast-two-hybrid system, by direct binding assays with identified cytoskeletal elements and by co-localization studies using immunocytochemistry. Signal transduction mechanisms evoked by different domains of the extracellular matrix molecule tenascin-C and -R are studied by analyzing the patterns of proteins undergoing changes in expression and phosphorylation by high resolution 2D gel electrophoresis. Furthermore, the consequences of triggering of recognition molecules at the cell surface resulting in either repellent or adhesive cell responses will be studied at the transcriptional level.

Conventional and conditional knock-out mutants are being generated for recognition molecules involved in cell adhesive and/or cell repellent functions to study their involvement in development, regeneration and synaptic plasticity (see below).

3. Activity dependent regulation of neural recognition molecule expression

Alexander Dityatev, Thomas Schuster, Vladimir Sytnyk, Thomas Tilling

An intriguing feature of the regulation of neural recognition molecule expression is the observation that this expression is regulated by neural activity. For instance, stimulation of cultured cerebellar neurons by elevation of extracellular K^+ concentrations and application of NMDA receptor agonists upregulate the expression of L1, but not that of the neural

cell adhesion molecule N-CAM. In other cell types, namely dorsal root ganglion neurons and Schwann cells, expression of L1, but again not of N-CAM, is downregulated by a very precise frequency of electrical stimulation: at 0.1 Hz, but not at 0, 0.5 and 1.0 Hz both mRNA and protein levels of L1, but not of N-CAM are downregulated by approximately a factor of 2. A challenging question is now how changes in membrane potential and/or neuronal activity translate into changes in the expression of recognition molecules. Also, we are looking at correlates of these phenomena in vivo, where changes in neuronal activity may enhance or destabilize cell contacts resulting from alterations in such activity.

4. The role of the amyloid precursor protein as a cell adhesion molecule

Tanya Odenthal, Frank Plöger

The amyloid precursor protein which accumulates in its abnormally cleaved form as amyloid plaques in Alzheimer's disease is a recognition molecule that is exposed at the cell surface and carries the HNK-1 glycan, a carbohydrate ligand shared by all neural adhesion molecules investigated so far (see below). The roles of the amyloid precursor protein in cell interactions are being studied in view of its association with other recognition molecules and with regard to signal transduction mechanisms leading to normal and abnormal cleavage of the extracellular domain of this molecule.

5. Recognition molecules in neural degeneration and regeneration

Marius Ader, Udo Bartsch, Reiner Czaniera, Stephan Grau, Jinhong Meng, Bettina Rolf, Sandra Schmidt, Emanuela Szpotowicz

The importance of neurite outgrowth conducive and inhibitory recognition molecules has been studied in the central and peripheral nervous system of mammals. In the peripheral nervous system, neurite outgrowth promoting molecules prevail over those that inhibit neurite outgrowth. Conversely, in the central nervous system inhibitory molecules predominate over those that promote neurite outgrowth. We have attempted to tip the balance towards neurite outgrowth promoting molecules in the central nervous system to enhance neurite outgrowth after a lesion. The myelin-associated glycoprotein MAG has not been recognized to be involved in inhibitory functions in the central nervous system, but is inhibitory in the peripheral nervous system, as seen in knock-out mice deficient in expression of MAG. The major peripheral nervous system myelin protein P0 has been shown to be neurite outgrowth promoting in mammals. In contrast to mammals, it occurs in the central nervous system of fish which are able to regenerate in adult. The functions of P0 in neurite outgrowth in the central nervous system of fish are under study.

Knock-out mutants have been generated that are defective in neural recognition molecules. Tenascin-R and tenascin-C which have been recognized as inhibitory molecules when presented as molecular barriers are being investigated in these knock-out animals with regard to degeneration of neurons and regeneration of neuronal processes. Similarly, con-

ventional and conditional knock-out mutants for the neural cell adhesion molecules L1 and the close homologue of L1 (CHL1), both of which are enhancing neurite outgrowth even in a generally inhibitory environment, such as the central nervous system, are under study. These experiments are designed to advance our understanding of the cellular and molecular mechanisms that underlie the regulation of proliferation, cell death and axon regrowth.

Recognition molecules, such as L1 and CHL1 not only promote neurite outgrowth, but enhance survival of neurons in culture. Particularly interesting with regard to neurodegenerative diseases in the human is the observation that L1 promotes survival of dopaminergic neurons of the substantia nigra. These neurons die in the human in Parkinson's disease. Neural stem cells that have been transfected to express L1 are being injected stereotactically into the substantia nigra in animal models of Parkinson's disease in the hope to reconstitute the ablated neurons and enhance their chances of survival and neurite outgrowth by expression of the neurite outgrowth and cell survival promoting adhesion molecule L1.

6. Functional roles of adhesion molecule-associated glycans in development and regeneration

Martine Albert, Meliha Karsak, Jens Lütjohann, Maren von der Ohe, Claudia Senn

We are engaged in studies on different glycans that are expressed by partially overlapping sets of glycoproteins, many of which have been shown to be recognition molecules.

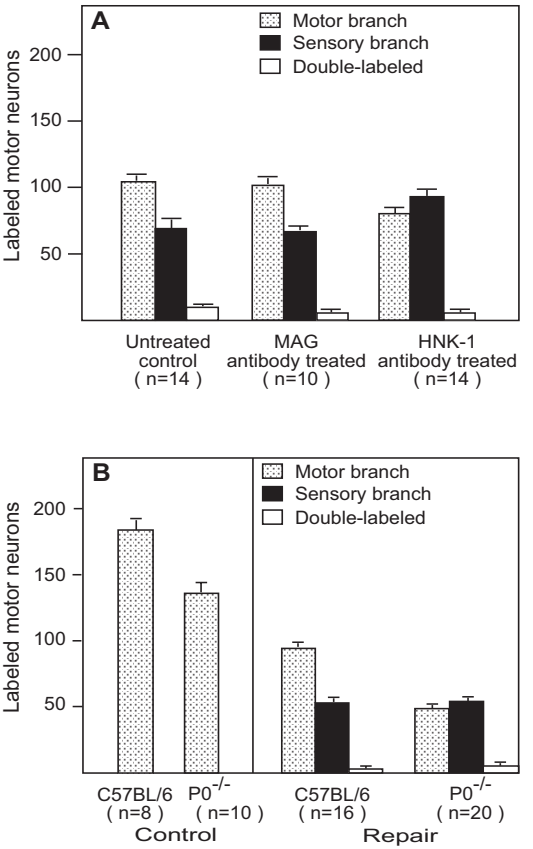

Figure 3. Influence of HNK-1 antibodies (A) on preferred motor axon outgrowth into the quadriceps branch of transected femoral nerves of adult rats. After 14 days, retrograde double labelling identifies the number of motor neurons regrown and labeled from either the motor or sensory branches (or double labeled neurons). (B) Regrowth of motor axons of the femoral nerve in P0-/- knock-out mice which do not express the HNK-1 carbohydrate (Tom Brushart, Rudolf Martini and Melitta Schachner, unpublished observations).

In vitro assays have shown that glycans themselves are involved in different aspects of cell adhesion and migration and in outgrowth of neuritic and astrocytic processes. Several glycans have been identified, among them the HNK-1 carbohydrate, oligomannosidic carbohydrates recognized by monoclonal antibodies L3 and L4, the unusual alpha 2,8-linked polysialic acid, and the Lewis^x antigen recognized by monoclonal antibody L5. All of these carbohydrates are involved in the modulation of cell interactions. The role of the motorneuron-associated HNK-1 carbohydrate in motoraxon-specific regeneration in the peripheral nervous system of adult mice is an important focus of this research (Fig. 3). We are presently looking for receptors of these molecules by using immunological, biochemical and molecular biological techniques. Furthermore, the regulatory mechanisms underlying the synthesis and degradation of these functionally important carbohydrates are investigated.

7. Myelin formation and maintenance as studied by knock-out mutants for myelin genes and somatic gene therapy

Udo Bartsch, Malte Raether

We have generated several knock-out mutants deficient in myelin proteins in the central and peripheral nervous system. The knock-out mutant for the recognition molecule P0, the major glycoprotein of peripheral nervous system myelin, is an animal model for a severe form of peripheral neuropathy, the Déjérine-Sottas disease in its homozygous state, and for a less severe demyelinating neuropathy called Charcot-Marie-Tooth disease in the heterozygous state. The knock-out mutant for the myelin-associated glycoprotein MAG, a minor myelin constituent in the central and peripheral nervous system, shows surprisingly normal formation of myelin in the peripheral nervous system and relatively minor disturbances of myelin formation in the central nervous system. However, both in the central and peripheral nervous systems myelin maintenance is disturbed in aged animals. Double knock-out mutants which are deficient both in MAG and the neural cell adhesion molecule NCAM, the latter of which appears to cooperate functionally with MAG, have a more aggravated and earlier onset of myelin degeneration than the single knock-out mutants. (The NCAM single knock-out mutant does not show any deficiency in myelin formation or maintenance.) The onset and course of myelin degeneration in the double knock-out animals is reminiscent of the pathology of human patients afflicted with multiple sclerosis.

We are planning to re-introduce P0 and MAG into these knock-out mutants. By expression of the molecules that have been ablated in the knock-out mutants, we envision a somatic gene therapeutic approach with the hope to revert the abnormal phenotype into a normal one. Adeno-virus and adeno-associated virus constructs containing the cDNA for expression of wild type P0 and MAG have been constructed and will be injected into the peripheral and central nervous system of the knock-out mice. The time course of remyelination is being studied by immunohistochemical, electron microscopic and electrophysiological techniques.

8. Neural recognition molecules and synaptic plasticity

Helen Bukalo, Alexander Dityatev, Galina Dityateva, Nikolas Fentrop, Constanze Rehbehn, Thomas Schuster, Tatyana Strekalova, Vladimir Sytnyk, Jianrong Tang, Greg Williams, Carsten Wotjak

An important aspect of recognition molecule function is the question whether recognition molecule-dependent interactions between pre- and post-synaptic membranes and glial cells can influence synaptic plasticity *in vitro* and *in vivo*. We have used immunochemical, immunocytochemical, and electrophysiological methods, and behavioural paradigms to investigate the function of L1, N-CAM, and tenascin-R in synaptic plasticity. We could show that L1 and N-CAM influence synaptic efficacy, while other recognition molecules do not. We have also shown that the percentage of synapses expressing the isoform of N-CAM with the longest cytoplasmic domain (N-CAM 180) increases by almost a factor of two 24 hours after induction of long-term potentiation in vivo. Furthermore, we have observed that the unusual polysialic acid specifically associated with N-CAM mediates synaptic plasticity both *in vitro* and *in vivo*. We are now extending these studies to analyze the molecular mechanisms underlying the roles of L1 and N-CAM in synaptic efficacy with the hope that we can distinguish morphological changes from intracellular signaling cascades. Also, we are trying to dissect the sites of action of recognition molecules, that is, for instance, whether they are implicated pre- or postsynaptically. The binding partners for the intracellular domains of L1 and N-CAM, in particular N-CAM 180, are being looked for in the postsynaptic density and in the synaptic cleft.

The functions of neural recognition molecules are not only studied *in vitro* by electrophysiological methods with the hope to identify the molecular mechanisms by which neural recognition molecules modify the strength of synaptic interactions, but also *in vivo* using behavioural paradigms, such as spatial learning tasks, fear conditioning, object recognition and others. These have been particularly adapted for the mouse because of the possibility to investigate the knock-out and transgenic mutants deficient for overexpressing neural recognition molecules that have been implicated in synaptic plasticity in *in vitro* experiments.

Interestingly, carbohydrates have turned out to be involved in synaptic plasticity, such as oligomannosidic glycans which mediate the interactions between L1 and N-CAM within the cell surface membrane. The HNK-1 carbohydrate has also been shown to be involved in synaptic transmission and plasticity in the hippocampus. The carrier molecule for this carbohydrate and its receptor are now being studied by using genetic manipulation of the synthesis of the HNK-1 carbohydrate (the sulfotransferase) and the tenascin-R knock-out mutant which is deficient in the extracellular matrix component carrying the HNK-1 carbohydrate.

9. Development, regeneration and learning and memory in zebrafish

Robert Bernhardt, Catherina Becker, Thomas Becker

The zebrafish is a favoured vertebrate for neurobiological studies since its nervous system is relatively simple and easily accessible because of its transparency during development. Adult zebrafish have been used as model systems,

since they have the capacity for nerve regeneration in the central nervous system and for learning and memory. Furthermore, efforts in the zebrafish community offer considerable hope for the targeted generation of mutants. In order to develop the necessary analytical tools for the study of recognition molecules in zebrafish, we have identified and characterized several zebrafish homologues of the mammalian genes for L1, N-CAM, adhesion molecule on glia (AMOG), tenascin-C, another tenascin homologue, and semaphorin-D. cDNA clones have been obtained and are expressed by recombinant technology for the isolation of the proteins and their molecular fragments, and for the generation of antibodies against these proteins. These reagents and overexpression of these molecules by injection of mRNAs into the fertilized egg will be used to manipulate neuron-target interactions in the visual and motor systems. Furthermore, we have established an experimental paradigm to investigate learning and memory in zebrafish. Antibodies against a zebrafish homologue of L1 could be shown to interfere with learning in an active avoidance paradigm. Also, we have shown that functional regeneration after spinal cord lesion and optic nerve crush in the adult zebrafish leads to upregulation of neurite outgrowth conducive molecules, such as L1, both in neurons and glia. There is a good correlation between neurons that promote neurite outgrowth after a lesion and L1 homologue expression. These investigations show that the zebrafish is an excellent animal model to investigate development, regeneration and synaptic plasticity in the context of adhesion molecule function.

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Awards

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Warner-Lambert-Prize of the US Society for Neuroscience to Melitta Schachner Camartin

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In the early development of the nervous system the neuropeptide head activator (HA) plays an important role. HA was first discovered in the coelenterate hydra, later also in other animals. The structure of the undecapeptide HA was found to be identical from hydra to humans. In hydra HA stimulates head-specific growth and regeneration, acting as growth factor for cell proliferation and as signal for cell determination. In mammals HA has several functions, the most prominent being stimulation of proliferation of neural precursor and of neuroendocrine cells, stabilization of nerve-cell survival, and enhancement of neurite outgrowth. HA also plays a role in abnormal development by acting as growth factor in tumors of neuroectodermal or neuroendocrine origins. In the adult brain HA is involved in memory consolidation and acts as positive factor in the arousal system. In neurodegenerative diseases like Alzheimer's HA has neuroprotective functions.

As main research project during the report period the biochemical isolation and partial sequencing of a HA binding protein and cloning of the respective gene from hydra were accomplished. Expression studies with a subsequently cloned mouse homologue confirm a very early function of HA and its binding protein for central and peripheral nervous system development .

1. HA Signal transduction

Determination in hydra occurs, like in other organisms, in S phase. In the presence of high concentrations of HA (10 picomolar) in early S-phase epithelial cells become determined to head-specific hypostomal or tentacle cells, and interstitial stem cells enter the nerve-cell pathway. This determination and differentiation to nerve cells by HA is mediated by cAMP as second messenger, characterizing the responsible signaling receptor as a member of the family of G protein coupled receptors.

HA promotes cell proliferation both in hydra and in mammals by stimulating entry into mitosis. HA signal transduction at the G2/mitosis transition was studied in mammalian cell lines of neuroectodermal or neuroendocrine origin. For signal transduction we found that stimulation of mitosis by HA is mediated by an inhibitory G protein, requires calcium influx, inhibition of the cAMP pathway, and hyperpolarization of cells. These data implied that the respective HA receptor should belong to the G protein coupled receptor family.

Cell cycle progression and proliferation of cells were most efficiently inhibited with specific inhibitors of the calcium-activated potassium channel, which by pharmacology and RNA analysis we identified as a Gardos-type potassium channel. Two imidazole derivatives, clotrimazole and SK&F 96365, which specifically target this channel, were found to be potent inhibitors of HA-triggered and basal cell proliferation. Because of its physiological compatibility with the human organism, clotrimazole may be a suitable lead structure to design specific drugs for in vivo therapy of neural and neuroendocrine tumors.

2. Head-activator binding protein HAB

To identify HA-binding proteins we synthesized photolabeled HA ligands which cross-linked to a 200 kDa protein both from hydra membrane and from soluble fractions. For isolation of the 200 kDa protein a multiheaded mutant of *Chlorohydra viridissima* was used, which not only contained more HA than normal hydra, but also overexpressed the 200 kDa protein. This protein was purified by HA-affinity chromatography, and sequence information was obtained for the N-terminus and, after protease digestion, for several internal peptides by Edman degradation. Using these partial sequences for designing oligonucleotides, the cDNA of the HA-binding protein (HAB) was cloned. Hydrophobicity analysis revealed that HAB, in addition to the amino-terminal signal peptide, contains only a single transmembrane segment, located near the carboxy terminus. We found that HAB is synthesized as a proprotein which is cleaved posttranslationally behind amino acid 84 to yield the mature protein. HAB is a novel type of mosaic receptor consisting of domains unique in their combination and alignment. The amino-terminal half of hydra HAB shares homology with the lumenal domain of human sortilin, a neurotensin receptor, and with the yeast sorting protein VPS10. The VPS10-like domain is followed by repeats with homology to members of the low-density lipoprotein (LDL) receptor family. The transmembrane domain with a short 55 residues long intracellular carboxy-terminus is preceded by two consecutive fibronectin type III domains, as found in many transmembrane receptors, neural cell adhesion molecules, and extracellular matrix proteins. The intracellular domain is devoid of known catalytic functions, but contains motifs for internalization, G-protein coupling, acidic clusters, and putative casein kinase II phosphorylation sites.

To study function and expression we raised an antiserum against hydra HAB, which on Western blots recognized HAB from hydra membranes and from soluble fractions. Likewise, the HAB antiserum was able to precipitate the HA-binding activity from soluble and membrane fractions thus proving that membrane and soluble HAB are identical and that HA binds HAB. Hydroxylamine cleavage yielded differential carboxy-terminal proteins differing in molecular weight by 15 kDa. This showed that soluble HAB is created by cleaving membrane-anchored HAB outside the transmembrane domain.

3. Mammalian HAB

While sequencing of hydra HAB was completed, homologues were discovered in chicken, rabbit, and human and named SorLA or LR11. These HAB homologues were isolated by homology to LDL receptors or by binding to the LDL receptor-associated protein RAP. Combination and alignment of domains are identical between hydra and mammals, but the number of repeats differs. The homology between each domain of hydra HAB and its vertebrate counterparts is higher than to any other protein. Since no other homologues were found by PCR analysis or by searching the EST database, we assume that hydra HAB and the vertebrate homologues are orthologues. The fact that HA binds to all cells expressing the mammalian homologue, and that SorLA binds to HA sepharose, supports this notion.

To get insight into the function of this new type of protein we isolated the mouse HAB homologue and used it to study the expression pattern in the adult organism and during embryonial development. In situ hybridization revealed that

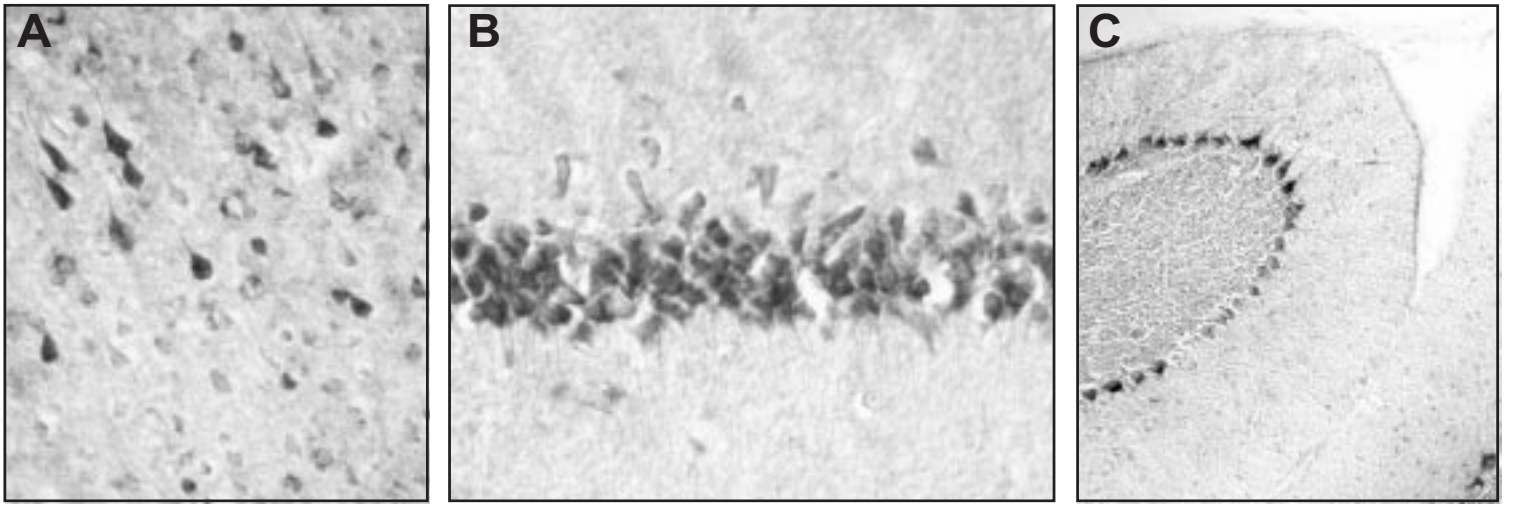

Figure 1: Expression of HAB in pyramidal cells of the cortex (A), in pyramidal cells of the hippocampus (B), and in Purkinje cells of the cerebellum (C). Note that both cell body and processes are stained.

in the brain HAB expression is restricted to specific neuronal cell populations. A unique pattern of expression was observed early in the developing telencephalon, where HAB transcripts were detected in the cortical area, but were absent from the striatum. This suggests that HAB may be involved in establishing the borders between cortical and basal zones.

We meanwhile raised a polyclonal antiserum against fibronectin domains of recombinant mammalian HAB. Immuncytochemistry confirmed the in situ studies showing strong expression in the adult brain in pyramidal cells of the cortex and of the hippocampus and in Purkinje cells of the cerebellum (Fig. 1).

4. Identification and expression of new HAB-like receptors in brain development

When searching for HAB homologues with a VPS10-like domain we discovered two other proteins, sortilin which was recently described as a third neurotensin receptor, and a new orphan member which we called SorCS. We isolated a cDNA for SorCS from a murine brain library. SorCS is an interesting new receptor containing two furin cleavage sites in front of the VPS-10 domain, which is followed by a leucin-rich repeat, suitable for protein interaction, and by a transmembrane domain, preceding an intracellular carboxy terminal tail with internalization and sorting signals. A splice variant without these signals offers the opportunity for dif-

ferential regulation. Expression of sortilin and SorCS were studied in early brain development and found to be highly specific.

5. Structure and function of peptides stimulating foot differentiation

The structure of two peptides, pedin and pedibin, was elucidated, which stimulate foot-specific differentiation during regeneration in hydra. Pedin acts as a mitogen on big interstitial cells, and it stimulates terminal differentiation of nerve cells. In collaboration with A. Grens, H. Shimizu, H. R. Bode and T. Fujisawa, the role of pedibin was examined. When adult animals were preincubated with pedibin prior to foot excision, foot regeneration occurred faster. Pedibin also influenced patterning. As a marker for positional value the homeobox gene *CnNK-2* was used. Pedibin treatment caused a substantial displacement of the apical border of expression. Likewise, in transplantation experiments treatment of tissue with pedibin increased the probability of foot formation in host animals. After dissociation of hydra tissue the obtained cell suspension can reaggregate and develop into intact hydra. During this process the positional value gradient and all other patterning processes are reestablished de novo. Due to preincubation of the animals with pedibin prior to dissociation, there was an eight-fold reduction in the number of heads and a 45-fold increase in that of feet in the aggregates after three days. These data show that pedibin significantly alters the patterning processes in favor of foot formation.

By use of 3'RACE PCR a cDNA clone for pedibin was obtained which contains the full-length message for the peptide. Preliminary results obtained by in situ hybridization studies show that the mRNA is expressed predominantly in the endoderm of the foot and in the endoderm at the base of tentacles.

6. Nuclear receptors

Nuclear receptors are studied to understand molecular processes underlying differentiation and development in the early nervous system. A novel receptor termed Germ Cell Nuclear Factor (GCNF) was cloned from mouse and human cDNA libraries. This orphan receptor is expressed during germ cell differentiation and during early embryonic development. Main expression of GCNF is found in the neuroectoderm. Two transcripts were found in the testis, only one in embryos. The *in vitro* translated receptor binds as a homodimer with high affinity to the direct repeat of the sequence -AGGTCA-. The mouse and human embryonal carcinoma cell lines P19 and NT2/D1 express GCNF. Treatment of these cells with retinoic acid triggers neuronal differentiation. Following the induction of differentiation, the GCNF message and protein levels are first up-regulated and later down-regulated. The receptor isolated from these cells binds in concert with an as yet unidentified cellular factor. Functions of GCNF for undifferentiated cells and for neuronal determination and differentiation and interaction with other factors are under investigation.

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Contributions to Books

(1) Schaller, H.C., Hoffmeister, S.A.H. (1999). Hydra, nervous system . In: Encyclopedia of Neuroscience (Elsevier Science BV, Amsterdam), Adelman, G., ed., 917-919.

(2) Hampe, W., Hermans-Borgmeyer, I., Schaller, H.C. (1999). Function of the neuropeptide head activator for early neural and neuroendocrine development. In: Regulatory Peptides and their Cognate Receptors (Springer Verlag, Heidelberg), Richter, D., ed., 323-337.

Theses

Diploma

Urny, Jens (1998). Expression des Kopfaktivatorrezeptors aus Hydra in eukaryontischen Zellinien. Universität Hamburg, Fachbereich Chemie.

Dissertations

Köster, Frank (1997). Klonierung und Charakterisierung von *shyk8*, einem Gen mit entwicklungsspezifischer Expression in der Maus. Universität Hamburg, Fachbereich Biologie.

Hermey, Guido (1998). Identifizierung und Charakterisierung neurospezifischer Rezeptoren aus Mus musculus (L). Universität Hamburg, Fachbereich Biologie.

Collaborations

Drs. Claus M. Petersen, Jörgen Gliemann, and Anders Nykjaer, University of Aarhus, Denmark.

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Institut für Molekulare Neuropathobiologie

Thomas J. Jentsch

The research of our group is concerned with ion transport mechanisms, in particular ion channels. Our main focus is on chloride channels of the CLC gene family, and recently we have also done some work on potassium channels of the KCNQ subfamily. We are interested in their structure and function, their biophysical properties, and the roles these channels play for the cell and the entire organism. A major aspect concerns their role in human genetic diseases.

Our work on CLC chloride channels started several years ago with the expression cloning of ClC-0, the voltage-gated chloride channel from the Torpedo electric organ. It defined a new family of chloride channels that is present from bacteria to man. In mammals, there are at least nine different CLC genes. Their physiological importance is best illustrated by the fact that mutations in three of these are known to cause human inherited disease: mutations in the skeletal muscle chloride channel cause myotonia congenita (characterized by muscle stiffness), mutations in the renal ClC-Kb channel cause a form of Bartter's syndrome (characterized by a massive salt loss), and mutations in the ClC-5 kidney chloride channel cause Dent's disease (associated with proteinuria, hypercalciuria, and kidney stones). We are investigating in detail the pathophysiology of these diseases, and are generating mouse models to understand the physiology of these and other CLC channels. Additionally, we are using a combination of site-directed mutagenesis and electrophysiology to elucidate their structure-function relationship. Finally, we have been studying CLC channels in the model systems S. cerevisiae and C. elegans, and have cloned plant CLC channels from Arabidopsis thaliana.

In the past few years we have extended our interest in ion channel diseases to KCNQ potassium channels. Beginning with the functional analysis of KCNQ1 mutations found in the long QT syndrome (associated with cardiac arrhythmias), we cloned the two novel KCNQ2 and KCNQ3 potassium channels and demonstrated that they are involved in Benign Familial Neonatal Convulsions (BFNC), a neonatal, dominantly inherited epilepsy. We also cloned the novel KCNQ4 potassium channel that is expressed in sensory hair cells of the cochlea. We showed that dominant negative mutations in KCNQ4 cause dominant deafness of the DFNA2 type.

1. ClC-0: Structure and function of the prototype CLC chloride channel

We have continued to use ClC-0 as a prototype of CLC channels since it is well characterized and allows for single channel analysis. We have shown previously that ClC-0 is a dimer that has two identical pores (a 'double-barreled' channel). One pore is probably formed by one ClC-0 subunit (Nature 383: 340 (1996)). Its mechanism of gating does probably not involve a voltage-sensor as in cation channels, but is accomplished by the permeant anion (Nature 373: 527 (1995)). The pore region in CLC chloride channels is not yet clearly defined, and several regions of the protein influence pore properties. We have now confirmed and extended these observations (1, 7) and have shown that mutations at several different positions render the channel inwardly rectifying (9). The 'slow' gating of ClC-0, which acts on both pores of the channel simultaneously, has a very steep temperature dependence, suggesting a complex

conformational change of the corresponding gating transition (2). Somewhat similar to the chloride-dependent individual 'fast' gating of the single pores, also 'slow' gating depends on chloride (26).

2. ClC-1: Analysis of the channel that underlies myotonia congenita

We used ClC-1 to investigate the transmembrane topology of CLC channels in general. A combination of glycosylation scanning, protease protection assays, and cysteine modification supports a model of 10-12 transmembrane domains with intracellular amino- and carboxyl-termini (6). Using 'split' channels, we demonstrated that several parts of the protein can fold independently and assemble to functional chloride channels (8). Interestingly, the channel is non-functional when the last cytoplasmic CBS domain is deleted. Co-injection of the missing cytoplasmic part containing CBS2 restores channel activity. This suggests that this segment binds to the truncated channel protein.

We also investigated the anion-dependence of ClC-1 gating (18,16), and found and analysed new ClC-1 mutations of patients with myotonia congenita (10,17,21). Some recessive mutations decrease the single channel conductance of ClC-1 (10). Nearly all mutations found in patients with dominant myotonia (Thomsen type) exert their dominant negative effect by shifting the voltage-dependence of the heteromeric WT/mutant channel. Some mutations that shift the voltage dependence in homomultimeric mutant channels, however, have only a moderate effect on the voltage-dependence of WT/mutant channels that would predominate in heterozygous patients. Interestingly, these mutations were found in pedigrees that were at the border between a dominant and a recessive pattern of inheritance (17,21).

3. ClC-2: gating of a swelling-activated chloride channel

ClC-2 is a broadly expressed chloride channel that can be activated by hyperpolarization and cell swelling. We had previously identified an amino-terminal cytoplasmic domain that is necessary for these gating processes, and suggested a 'ball and chain'-type gating mechanism (Nature 360: 759 (1992)). Using a chimeric approach, we now identified an intracellular loop between ClC-2 transmembrane domains that may be part of a receptor for the amino-terminal 'ball', and showed that both this loop and the 'ball' are also involved in the gating of ClC-2 by pH (3). Further, the gating of ClC-2 also depends on extracellular chloride (26). We also compared ClC-2 to a hyperpolarization-activated chloride current present in rat sympathetic neurons (13).

4. ClC-3, ClC-4 and ClC-5: role of ClC-5 in Dent's disease

We have shown previously that ClC-5, a member of the ClC-3/4/5 branch of the CLC gene family, is mutated in Dent's disease (Nature 379: 445 (1996)). We have now analyzed several new ClC-5 mutations found in Dent's disease and showed that these either abolish or greatly reduce the associated currents (4,11,22). A disconcerting finding is that the currents elicited by its expression are strongly outwardly rectifying and measurable only in a very positive, seemingly unphysiological voltage range. Further, mutations found so far in Dent's disease, while reducing these currents quantitatively, did not change their characteristics. It was therefore

important to show that certain mutations in ClC-5 change characteristics like rectification, kinetics of activation, or ion selectivity (24). This proves that these currents are directly mediated by ClC-5. We could also express ClC-4, which has characteristics that are very similar to those of ClC-5. However, we could not reproduce data by another group who suggested that ClC-3 is a swelling-activated chloride channel.

Dent's disease is characterized by low molecular weight proteinuria and hypercalciuria, which in turn leads to kidney stones, nephrocalcinosis, and renal failure. Small proteins that pass the glomerular filter are normally reabsorbed by the proximal tubule via endocytosis. Indeed, immunocytochemistry reveals the presence of ClC-5 in a subapical region of the rat proximal tubule where it co-localizes with the H^+-ATPase and endocytosed proteins (20). In transfected cells, ClC-5 is present in numerous small vesicles in the cytoplasm (and to some extent in the plasma membrane). ClC-5 again co-localizes with endocytosed proteins, and is present in the enlarged early endosomes created by co-expressing a GTPase -deficient rab5 mutant (20). Thus, ClC-5 is present in the endocytotic pathway and probably provides an electrical shunt for the efficient pumping of the vesicular H^+-ATPase.

Since acidification is known to be important for vesicle trafficking, including endoytosis, this explains the proteinuria in Dent's disease. We expect other chloride channels to have similar roles in intracellular organelles.

5. ClC-K channels and Bartter's syndrome

In a collaboration with Alain Vandewalle and using a polyclonal antibody that recognizes both isoforms of these

Figure 1. Expression of the ClC-5 chloride channel (which is mutated in Dent's disease) in the rat kidney (20). Left, confocal microscopy shows that ClC-5 is expressed in a subapical region below the brush border of proximal tubule cells. Right, electron microscopy reveals the presence of ClC-5 in intercalated cells of the distal nephron. It is present in vesicles that are known to contain proton pumps that are inserted into the plasma membrane upon changes in acid-base status.

highly related kidney chloride channels (ClC-K1 and ClC-K2), we localized these proteins to the basolateral membranes of the nephron (5). This includes the thick ascending limb of the loop of Henle, an important site of chloride reabsorption. Interestingly, Richard Lifton (Yale) showed that the human isoform hClC-Kb is mutated in patients with Bartter's syndrome. This strongly suggests that ClC-Kb plays a role in transepithelial transport in the thick ascending limb.

6. CLC channels in model organisms

Disruption of the single yeast CLC gene leads to an iron-suppressible phenotype, as first shown by Greene et al. (Mol. Gen. Genet. 241: 542 (1993)). Interestingly, another gene identified in the same genetic screen encodes a subunit of the H^+-ATPase. This suggests a role in intravesicular acidification as with ClC-5. Indeed, the yeast CLC knock-out strain is sensitive to alkaline pH (19). The yeast CLC protein is expressed in a late Golgi compartment (19). Using alanine

scanning of sequences in the amino- and carboxyl-termini, we identified regions that are important for proper intracellular localization and for the functional complementation of the knock-out strain. This includes the two CBS domains at the carboxyl-terminus (19).

We also isolated cDNAs for five CLC channels from the nematode *C. elegans.* We studied their localization using reporter gene constructs in transgenic animals or by immunocytochemistry. This revealed highly specialized expression patterns. Some of these channels yield functional chloride channels when expressed in *Xenopus* oocytes and other cells, increasing the repertoire of CLC channels that are suitable for structure-function analysis.

7. KCNQ1 in the long QT syndrome

We identified a new mutation in the KCNQ1 potassium channel in a large family with the dominant long QT syndrome (Romano-Ward type) and inserted it into the functional cDNA (12). We compared its functional effects to those of other mutations found by other groups, including recessive mutations found in the Jervell and Lange-Nielsen (JLN) syndrome. Mutations found in Romano-Ward patients had dominant negative effects on co-expressed WT subunits both in the presence or absence of the β-subunit minK, while JLN mutations lacked such an effect (12).

8. KCNQ2 and KCNQ3: channels involved in neonatal epilepsy

By homology to KCNQ1, we cloned the novel potassium channels KCNQ2 and KCNQ3 (14, 23). These were localized to chromosomal loci (20q13 and 8q24) that were known to harbour genes for Benign Familial Neonatal Convulsions (BFNC). This rare, autosomal dominant form of neonatal epilepsy begins at about three days after birth and disappears after several weeks. In collaboration with Ortrud Steinlein we determined the exon-intron structure of KCNQ2 and identified a mutation in a large Australian BFNC family (14). This is the first gene identified in idiopathic, generalized epilepsy. KCNQ2 yields outwardly rectifying currents that were abolished by the mutation. In contrast to dominant KCNQ1 mutations in the Romano-Ward syndrome, the KCNQ2 mutation from the BFNC family did not have a dominant negative effect (14).

KCNQ2 and KCNQ3 have overlapping expression patterns and can form heteromeric channels whose currents are much larger than those from homooligomers (23). Currents of KCNQ2/3 heteromers can be increased by raising intracellular cAMP. This effect is due to a phosphorylation of an amino-terminal consensus site for PKA (23). The increase in current (30-60%) by cAMP is similar to the loss of channel function with BFNC mutations, all of which lack a dominant negative effect (23). This suggests novel approaches to treat epilepsies.

9. KCNQ4 and dominant deafness

The novel KCNQ4 potassium channel was cloned by homology to KCNQ3 and was localized to human chromosome 1p34 (25), a region encompassing the DFNA2 locus for dominant progressive hearing loss. In a collaboration with Christine Petit, we identified a French family with dominant progressive hearing loss that had a mutation in the pore region of KCNQ4. The mutation abolished the associated outwardly rectifying potassium currents and exerted a strong

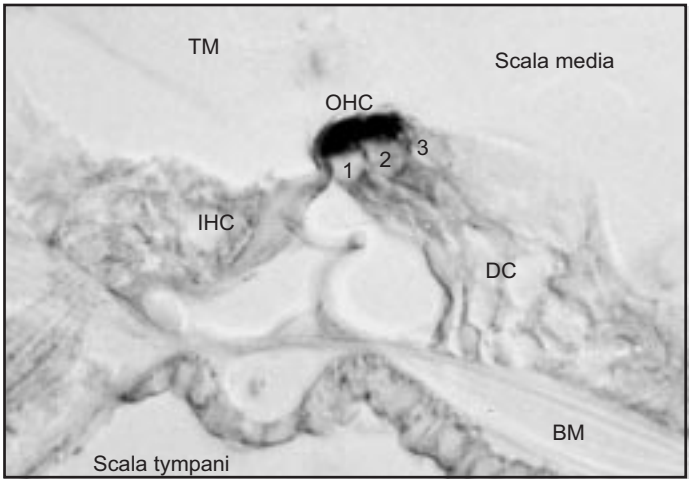

Figure 2. In situ hybridization of a mouse cochlea section using a mouse KCNQ4 antisense probe. The KCNQ4 message is just present in the three outer hair cells (OHC), labeled 1 to 3, but not in the inner hair cell (IHC). BM = basilar membrane; DC = Deiters cells; TM = tectorial membrane.

dominant negative effect on co-expressed WT subunits (25). In contrast to KCNQ1, which is localized to the stria vascularis of the scala media where it is involved in potassium secretion, KCNQ4 is expressed in sensory outer hair cells. We are currently generating mouse models to investigate how a loss of this potassium channel leads to progressive hearing loss.

Support

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Teuscher, Marc (1998). Lokalisation der Chloridkanäle ClC-6 und ClC-7 in transfizierten Zellen. Universität Hamburg.

Schaffer, Sven (1998). Herstellung und Charakterisierung polyklonaler Antiseren gegen ClC-Proteine aus *Caenorhabditis elegans*. Universität Hamburg.

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Schmidt-Rose, Thomas (1997). Struktur-Funktionsuntersuchungen an CLC-Chloridkanälen am Beispiel des Humanen Skelettmuskelkanals hClC-1. Universität Hannover.

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Schriever, Antje (1998). Klonierung und Charakterisierung von CLC-Kanälen des Nematoden *Caenorhabditis elegans*. Universität zu Köln.

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Habilitation

Pusch, Michael (1997). Struktur-Funktions-Analyse spannungsabhängiger klonierter Chloridkanäle. Universität Hamburg.

Awards

Alfred Hauptmann Preis für Epilepsieforschung to Thomas Jentsch, 1998

Franz Volhard Preis für Nephrologie to Thomas Jentsch, 1998

Carl-Ludwig-Preis für Nephrologie to Siegfried Waldegger, 1998

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Institut für Neurale Signalverarbeitung

Olaf Pongs

A fundamental property of neurons is the generation and propagation of eletrical signals. They are generated by flow of ions across the neural membrane upon excitation. Since the classical work of Hodgkin and Huxley it is known how different voltage-dependent conductances contribute to the propagated action potential. The molecular approach to neurobiology revealed that different membrane proteins forming voltage-gated ion channels selective for potassium, sodium, or other ions are the basic units of biological excitability and that the concerted opening and closing of these channels determines the waveform of the generated action potential. Travelling along the axonal cable, the impulse possesses a stereotypic pattern but when finally invading the presynaptic terminal of a synapse, the locus of electro-chemical coupling, this situation changes dramatically. The synapse is able to modify action potential width as well as to respond to changing frequencies of incoming action potentials. This modulatory behaviour of the synapse subserves the translation of electrical signal into a quantized chemical signal, i.e. neurotransmitter release. Consequently, the modulation of incoming action potentials in the synapse is an important molecular basis of synaptic plasticity, i.e. of learning and of acquired behaviour. Voltage-gated ion channels play a fundamental role in the modulatory abilities not only of the presynaptic terminal but also in integration of postsynaptic potentials mediated by ligand gated ion channels. The activities of potassium channels determine action potential duration as well as the setting of firing frequencies of neurons. Since calcium influx into neurons is mediated by voltage-gated calcium channels that open upon depolarization, potassium channel activity is related to the accumulation of calcium in the synapse and thereby to neurotransmitter release. Increase of synaptic calcium concentration may be directly correlated to the amount of neurotransmitter released by the synapse. The persistence of accumulated calcium in the synapse over time is a further important factor in translating electrical signal intensity into neurotransmitter quanta released. Our interest is to understand the molecular basis underlying synaptic plasticity and to characterize functionally and structurally the molecules involved in modulating synaptic activity. The aim of these studies is to further the molecular understanding of learning and behaviour.

1. Structure and function of voltage-gated potassium channels

T. Leicher, C. Lorra, S. Plüger, F. Reimann, J. Roeper, C. Schmidt, S. Sewing, K. Weber, Y. Zhang

Potassium channels are both, ubiquitously occuring membrane proteins and highly diverse. The diversity of potassium channels reflects the special needs and fine tuning of a given excitable cell to fulfill its role and function in signal transduction. We are interested in the molecular basis of this diversity and in the characterization of the structural determinants which impose the properties on potassium channels. The cloning and functional expression of many potassium channel cDNAs has shown that most voltage-gated and ligand-gated (e.g. Ca) potassium channels are members of a superfamily of ion channels. This result has

greatly aided ongoing *in vitro* mutagenesis experiments for structure function studies. We have cloned and extensively characterized the members of three potassium channel subfamilies expressed in the rat nervous system (in collaboration with W. Stühmer´s group at Max-Planck-Institut für Experimentelle Medizin, Göttingen). Altogether this comprises 23 distinct potassium-channel cDNAs which encode voltage-gated potassium-channels with distinct activation, deactivation and inactivation kinetics, different voltage sensitivities, different pore structures and distinct pharmacologies.

Among these many different channels, we have concentrated mainly on the study of one voltage-activated, rapidly inactivating potassium channel Kv1.4 in order to understand as thoroughly as possible the properties of this protein. Kv1.4 channels are expressed in the central nervous system, e.g. hippocampus, corpus striatum and cortical areas. In hippocampal slice preparations Kv1.4 channels have been detected and shown to determine action potential profiles and firing rates. Extensive *in vitro* mutagenesis of Kv1.4 channels has so far helped to identify domains which determine the gating and opening of this channel, its conductance for inward and outward potassium currents, its permeability for various ion sthe kinetics of inactivation and recovery from inactivation. The results of these experiments have yielded a quite detailed working hypothesis about the strucutre of Kv1.4 channel pore and the gate which opens and closes the channel from inside. The activity of Kv1.4 channels is regulated by extracellular potassium and by intracellular calcium. The molecular and biophysical basis of these ion effects on the activity of Kv1.4 channels has been characterized. With this studies we have learned not only structure-function relationships for Kv1.4 channels, but also have got some insight in the molecular, structural and biophysical basis which underlies potassium channel diversity.

The detailed study of heteromultimeric assembly of Kv1 channels has lead to the discovery of a new functional domain in certain Kv1a-subunits. This domain prevents N-type inactivation in a dominant negative manner. Thus, rapidly-inactivating Kv channels may only be expressed in heteromultimers in the absence, but not in the presence of this preventive domain. The results of these studies redefine the assembly of heteromultimeric Kv channels and imply a novel hierarchy in assembly of non-inactivating and rapidly-inactivating Kv channels.

2. Eag-type potassium channels

B. Engeland, D. Isbrandt, J. Ludwig, A. Neu, C. Stansfeld, R. Weseloh

Voltage-gated potassium channels share sequence and structural similarities with cyclic nucleotide-gated unselective cation channels and form part of a large superfamily of ion channels. This similarity is especially pronounced between cyclic nucleotide-gated channels and eag-type potassium channels, which might be a link between these ion channel families. The *ether-à-go-go* family comprises three subfamilies: *ether-à-go-go* itself (*eag*), *ether-à-go-go related* (*erg*) and *ether-à-go-go* like (*elk*). The human *erg* channel was recently shown to be implicated in the LQT syndrome, but physiological roles for *eag* and *elk* are not yet known.

We have cloned two eag channels from the rat brain (rat *eag* 1 and rat *eag* 2) which apparently arise from different genes. In contrast to most membrane-proteins and espe-

cially to the voltage-gated *Shaker* like (Kv) family of potassium channels, where subunit-assembly domains have been identified in the amino-terminus, the subunit assembly of rat *eag* channels seems to be mediated by a carboxy-terminal domain. Electrophysiological examination of heterologously expressed rat *eag* channels revealed that they are regulated in a complex and quite unusual way. Their activation rate strongly depends on the cell´s resting potential, channel opening being much slower from hyperpolarized membrane potentials (e.g. in the after hyperpolarization of an action potential).

Localization of rat eag 1 and rat eag 2 is being investigated using *in situ* hybridization and immunocytochemistry. Despite the electrophysiological similarity between both ion channel isoforms, their expression pattern within the brain is distinct, with few overlapping regions. A detailed analysis of the physiological role of rat eag channels will be possible by examination of „knockout" mice that lack functional *eag* channels.

Recently, we have cloned and functionally expressed other members of the eag-K channel family. Screening of rat cortex cDNA resulted in cloning of two complete and one partial orthologue of the *Drosophila ether-à-go-go* like K channel (*elk*). Northern Blot and RT-PCR analysis revealed predominant expression of rat *elk* mRNAs in brain. Each rat *elk* mRNA showed a distinct, but overlapping expression pattern in different rat brain areas. Transient transfection of CHO cells with rat *elk*1 or rat *elk*2 cDNA gave rise to voltage-activated K channels with novel properties. RELK1 channels mediated slowly activating sustained potassium currents. The threshold for activation was at -90 mV. Currents were insensitive to TEA and 4-AP, but were blocked by µM concentrations of Ba^{2+}. RELK1 activation kinetics were not dependent on prepulse potential like REAG mediated currents. RELK2 channels produced currents with a fast inactivation component and HERG like tail currents. Presently, the expression of elk channels and their subunit composition in the central nervous system is being characterized further.

In another screen, we have isolated ether-à-go-go related K channel subunits, which extend the HERG-family of Kv a-subunits. The HERG2 and HERG3 genes have been mapped and are presently being further investigated for screening DNA of patients with potential mutations in these genes.

3. Auxiliary subunits of potassium channels

M. Berger, R. Bähring, T. Leicher, J. Röper, K. Schöder, S. Sewing, R. Waldschütz, J. Wolfart, Y. Zhang

We have recently cloned a family of auxiliary (Kvß-) subunits of voltage-gated potassium (Kv) channels with distinct expression patterns in the rat brain. The different Kvß proteins are encoded by 3 different genes. Due to alternative splicing the Kvß1 gene gives rise to 3 different gene products. We have determined the exon-intron structure of the Kvß1 gene to the telomere of chromosome arm 3q by FISH analyses. The human Kvß1 gene is unusually large and complex, having 16 exons and a size of about 350kb. The human Kvß2 gene is relatively small and gives rise to two gene products by alternative splicing. Nevertheless, the exon-intron structure is similar to the one of the Kvß1 gene. The Kvß2 gene has been localized to chromosome 1p36:3. Also, the human Kvß3 gene has a comparable exon-intron struc-

ture, but is only about 20kb large. The ß-subunits share sequence homology with members of the aldo-keto reductase superfamily. Heterologous expression studies in *Xenopus* oocytes or cell lines revealed that coexpression of the pore forming a and ß subunits dramatically alters the kinetic properties of the channels. In particular, ß1 and ß3 subunits are capable of changing the inactivation behaviour of potassium channels; conferring rapid inactivation or delayed rectifier channels in the extreme case. The structural basis of a-ß interaction was investigated with protein overlay assays in combination with heterologous a-ß coexpression studies. Our results show that Kvß1-subunit binding is restricted only to Kv1a-subunits which contain a specific binding domain for Kvß1-subunits at their cytoplasmic localised amino termini. We could define a region of up to 90 amino acids within the Kv1.5 amino terminus that is sufficient for Kvß1 interaction. This region overlaps with the amino terminal T1 domain of Shaker related Kv1a-subunits, which specifies subfamily-specific assembly of functional channels.

During the course of these studies we discovered a new domain in Kv1.6 subunits. Its presence in Kv channels may prevent an effective rapid inactivation of Shaker-related Kv channels. However, Kvß3 mediated rapid inactivation is not influenced by this domain.

The importance of auxiliary subunits for Kv channel function was addressed by generating a knock-out (k.o.) mouse, which does not synthesize Kvß1. The mutant mice are healthy and do not have any gross abnormalities. However, pyramidal neurons in the hippocampal CA1 field have acquired abnormal firing properties. Action-potentials do not broaden during bursts of action-potential firing as normally observed. This defect apparently reduces Ca^{2+}-influx leading to a reduced Ca^{2+}-activated K channel activity. The result is a reduction in the amplitude of slow afterhyperpolarization (sAHP). Previously, it has been described that sAHP amplitude durations increase during ageing. This may be correlated with reduced cognitive capabilities in old rodents. The sAHP amplitudes in Kvß1 k.o. mice do not increase comparably. There is a marked difference between wild-type litter mates and Kvß1 k.o. mice. Interestingly, aged Kvß1 k.o. have retained their cognitive capabilities in standard spatiotemporal learning paradigms. This gain in cognitive function is being investigated further. In particular, more mouse mutants of other rapidly-inactivating Kv channels are being generated to study the effects of mutant Kv channels on cognitive functions.

4. Studies on cardiac potassium channels

K. Böhlke, Q. Liu, R. Netzer, K. Sauter, N. Schmitt, M. Schwarz, X. Zhu

Voltage-gated potassium (Kv) channels in cardiac and skeletal muscle, and in the central nervous system, are partly responsible for determining the frequency and duration of action potentials. Several distinct classes of K^+ channels have been identified in the mammalian cardiac muscle by molecular, electrophysiological and pharmacological approaches. We have recently cloned a new a-subunit, Kv6.2 with preferential expression in mouse heart. This expression pattern may imply an important physiological function in repolarization of cardiac-cell membranes. Indeed, our expression studies in heterologous systems revealed that the Kv6.2 a-subunit is able to interact specifically with a well-known cardiac a-subunit, Kv2.1, *in vitro*. The heteromultimeric Kv2.1 / Kv6.2 channel mediates K^+ cur-

rents with distinct voltage sensitivity and deactivation kinetics in comparison with the homomultimeric Kv2.1 channel. Currents mediated by Kv2.1 / Kv6.2 heteromultimers can be blocked by some antiarrhythmic drugs, and this in combination with its coassembly with Kv2.1, support a role for Kv6.2 / Kv2.1 in heart arrhythmia. We are using a new gene-targeting strategy to generate a mouse strain that has a tissue and stage specific loss of the Kv6.2 gene product. Morphological and physiological consequences of this mutation will be investigated further for elucidation of exact functions of the Kv6.2 a-subunits *in vivo*.

5. Bacterial potassium channels

A. Farrell, C. Legros, V. Pollmann, O. Pongs, M. Wolters

We have cloned the *Bacillus stearothermophilus lctB* gene which encodes a small K^+ channel subunit of 134 amino acids. The LctB protein reveals the typical M1-P-M2 topology of simple K^+ channel subunits, M1 and M2 being hydrophobic transmembrane segments flanking the pore forming P-domain, which has a characteristic K^+ channel signature motif. LctB channels could be expressed in *E.coli*. Their properties have been compared with those of the *Streptomyces lividans* KcsA channels, which was recently crystallized. Surprisingly, both K channels have different properties. KcsA channels are targeted in *E.coli* to inner membranes. LctB channels have a final destination in the outer membrane. Thus, LctB channels are translocated through the inner membrane (and periplasm), whereas KcsA channels are not. The latter are gated by pH, the former are not. KcsA channels have a relatively large single-channel conductance in lipid bilayers, LctB channels have a relatively small conductance. Finally, KcsA channels cannot be functionally expressed in eukaryotic cells. In contrast, injection of *lctB* cRNA in *Xenopus* oocytes produced genuine K^+ channels mediating a novel type of K^+ current. The most salient features were a biphasic I-V relation, a slope conductance that did not increase linearly with external K^+ concentration, and a block by external Ba^{2+} or Cs^+. Mutant LctB channels containing a cysteine in the P-domain could be reversibly blocked by the sulfhydryl reagent MTSEA. Thus, LctB K^+ channels can mediate distinct currents in the *Xenopus* oocyte expression system. The contrasting properties of KcsA and LctB channels may allow us to study in detail in the future the transport systems in eukaryotic cells which may be involved to translocate and target K channels to their final destinations in the plasma membrane.

The ease of expressing and purifying KcsA channels from *E.coli* has lead us to produce KcsA-Kv channel chimaeras and to overexpress these in *E.coli*. The chimaeras allow us to characterize in molecular (and atomic) detail the structure of toxin-binding sites near and at the outer K channel pore entrance. We have produced chimaeras which bind toxin in the pM range similar to native mammalian Kv channels. The chimaeras can be fixed to small chip surfaces to study on-line toxin binding parameters in biosensor-based analyzers. This new technology is also being used to search for new toxins.

6. Calcium-activated BK channels

R. Behrens, S. Plüger, O. Pongs, F. Reimann, O. Steinmetz, M. Schwarz, R. Waldschütz

BK channels are assembled from membrane-integrated α-subunits and auxiliary ß-subunits. The α-subunits probably have at least 7 membrane-spanning segments designated S0 to S6. The ß subunits have most-likely two membrane spanning segments. The α-subunits are usually very large proteins consisting of ~1200 amino acids, whereas the ß subunits are relatively small peptides of ~200 amino acids. The mammalian sloα-subunit genes contain a relatively large number of alternative exons. Transcription of the sloα-gene could potentially give rise to many different sloα transcripts and subunits. We have cloned and characterized the mouse sloα gene and have extensively investigated sloα transcript diversity. The results showed a distinct occurence of specific splice variants in different brain regions and neurons. Furthermore, the number of splice variants detected was considerably less complex than might be inferred from gene structural analysis. Also, we have cloned sloß cDNAs. They were coexpressed in *in vitro* heterologous expression studies to study their modulatory role on BK channel activities. The most salient feature of sloß1 is to increase the voltage/Ca^{2+}-sensitivity of BK channels. In the presence of Ca^{2+} at much more negative membrane potentials than BK channels consisting only of α subunits. Surprisingly, this effect strongly depends on the extracellular K^+ concentration and cannot be observed at low K^+ concentrations. We have generated sloß1 k.o. mice in order to characterize further the physiological role of BK channels in vasodilation and in hearing.

7. Synaptic modulation of facilitation

A. Hauenschild, J. Dannenberg

Probably, the most salient feature of the nervous system is its plasticity in signal transduction. Underlying short and long term facilitation of neurotransmitter release is the ability of neurons to modulate the translation of electrical activity into neurotransmitter signalling intensity. This activity strongly depends on intracellular calcium concentrations. Molecular mechanisms which regulate synaptic calcium in- and efflux, are the major determinants of neural plasticity. Glutamate receptors play a prominent role in controlling and modulating calcium influx. Their structure and function is therefore being intensely studied in many laboratories. We have cloned a calcium binding protein which apparently is involved in the modulation of calcium efflux. As the activity of this protein only becomes apparent in paired pulse stimulations of nerve terminals as well as in high frequency stimulations, we have dubbed this new synaptic protein frequenin. Frequenin appears to be a member of an emerging new family of calcium binding proteins. The sequence of these membrane proteins is conserved between *Drosophila* and vertebrates. Therefore, frequenin must be a very old protein which evolved very early in the evolution of eucaryotic organisms. Electrophysiological analysis of signal transduction at the neuromuscular junction of third instar larvae prepared from wild type *Drosophila*, frequenin mutants or *Drosophila*, transgenic for a frequenin minigene, has indicated that frequenin may regulate the Ca^{2+}-dependent phosphatase/protein kinase cascade involved in the regulation of synaptic efficacy. Presently, the biochemical and physiological properties of frequenin are being studied in more detail in order to characterize the molecular basis of the observed

frequenin dependent facilitation of neurotransmitter release in synaptic terminals. We have been able to produce sufficient quantities of purified human frequenin for a detailed structural analysis. The purified protein has been crystallized. The crystals were of sufficient quality for X-ray diffraction analysis, up to a resolution of 2.4A. These data are being used to develop a detailed high-resolution crystal structure of human frequenin (in collaboration with W. Saenger, Fu Berlin).

Using a two-hybrid screen, we have searched for possible Frequenin-binding partners. We have isolated several clones which corresponded to MAP kinase kinase kinases 1 and 2 (MLK1 and MLK2). *In vitro* Frequenin interacted with a specific MLK domain present in MLK1 and MLK2, but not in MLK3. Antibodies directed against MLK2 coimmunoprecipitated Frequenin/MLK-complexes from lysates of cotransfected CHO-cells. Collectively, the results indicated that Frequenin may bind to MLK-type kinases in the central nervous system. Presently, we investigate the regulation of MLK activity by Frequenin and its implications for facilitated neurotransmitter release.

8. Generation of a knockout mouse model for guanidinoacetate N-methyltransferase deficiency

D. Isbrandt, A. Schmidt, J. Röper, A. Neu, S. Fehr, K. Ullrich

Guanidinoacetate methyltransferase (GAMT) deficiency is a disease of creatine biosynthesis and manifests during the first months of life as developmental delay or arrest. Neurological symptoms are heterogeneous, including muscular hypotonia and weakness, poor head control, involuntary extrapyramidal movements, epilepsy and in older patients autistic behaviour.

GAMT deficiency is an autosomal recessive disorder. Two GAMT deficiency alleles have been identified, one of which accounts for five of the six alleles in three patients analyzed so far. The two alleles give rise to alternatively spliced transcripts, which encode truncated or elongated, presumably non-functional polypeptides.

The neurological abnormalities observed in GAMT-deficiency might be explained partially by the deficiency of high energy phosphates in cells with high and fluctuating energy demand, while others appear to be related to the accumulation of guanidinoacetate (GAA), which is a known epileptogen.

In order to study the pathophysiology of the disease by generating an animal model, the murine GAMT gene will be inactivated by targeted disruption of exon 1. The resulting homozygous knockout mice will be analyzed biochemically and neurophysiologically.

While creatine synthesis is mainly attributed to liver, kidney, pancreas and testis, the exact distribution of GAMT in other tissues , especially in brain, is largely unknown. To study the distribution of GAMT mRNA and protein in different murine tissues and particularly in brain, the murine GAMT cDNA was isolated and used in Northern blot experiments and compared to the expression of creatine kinase B and the creatine transporter. In addition, polyclonal antisera were raised against recombinant murine GAMT purified from E. coli and used in Western blotting and immunohistochemical analyses. These experiments do not only confirm the high expression of GAMT in liver, kidney and testis but also show considerable amounts of both GAMT mRNA and protein in

spleen and all brain areas tested. No expression was found in skeletal and smooth muscle. In situ hybridization and immunohistochemical staining of murine coronal brain sections revealed an ubiquitous and overlapping distribution but particularly high expression of GAMT mRNA and protein in cortex and hippocampus, which is comparable to the expression of creatine kinase B.

In order to analyze the role of GAA in the pathophysiology of GAMT deficiency, pathophysiological concentrations of GAA were applied to cultured neonatal cortical rat neurons, which resulted in a dramatic increase in the network activity and induced strong inward currents. This GAA-induced current could be blocked by selective $GABA_A$ receptor antagonists. The reversal potential of these GAA-induced inward currents is compatible with the reversal potential of Cl^--currents under the experimental conditions chosen. In contrast, creatine and creatinine had no effect on the neuronal activity of these neurons. In summary, these data prove and localize intracerebral creatine synthesis in the murine brain. In addition, a possible GABA-mimetic action of GAA in the basal ganglia may be an attractive candidate mechanism to explain some of the extrapyramidal symptoms in patients with GAMT deficiency which will further be studied in the animal model.

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Research Groups

Synaptic Plasticity: Learning about Activity-dependent Genes

Dietmar Kuhl

Activity dependent remodeling of synaptic efficacy and neuronal connectivity is a remarkable property of synaptic transmission and characteristic of plastic events in the nervous system. To understand the brain, both as the organ of mental function and as a target for disease, we need to understand synaptic plasticity on the cellular and molecular level. The main goal of our research is to bring to bear molecular biological approaches on the identification and study of genes contributing to synaptic plasticity in the mammalian brain. A large body of work indicates a broad role for activity-dependent gene products in neuronal plasticity, including cellular processes underlying learning and memory, epileptogenesis, drug abuse, and neurological diseases. Several of the genes recently identified in our laboratory code for proteins that can directly modify neuronal function. Consequently, they represent promising targets for therapeutic intervention.

Neuronal plasticity is associated with critical physiological processes in the developing and adult brain. Particularly fascinating examples of naturally occurring neuronal plasticity are seen in studies of learning and memory. Learning is the process by which we acquire knowledge and memory is the process by which we retain that knowledge over time. In both invertebrates and vertebrates long-term memory differs from short-term memory in that it requires RNA and protein synthesis. This suggests that retention mechanisms should depend on changes in transcriptional state (see Figure 1). This idea is supported by the demonstration that in invertebrates behavioral training elicits changes in the levels of specific mRNAs in cells involved in learning and memory critically depends on induced gene expression (see e.g. Kuhl, D., Kennedy, T.E., Barzilai, A., and Kandel, E.R. (1992). J. Cell Biol. 119, 1069-1076. Kennedy, T.E., Kuhl, D., Barzilai, A., Sweatt, J.D., and Kandel, E.R. (1992). Neuron 9, 1013-1024).

Our attention has been focused on identifying activity-induced genes in the mammalian hippocamus and cortex. Both brain regions are subject to plastic alterations during physiological processes and neuropathological states, such as epilepsy. Seizure episodes set in motion a cascade of events that include gene expression, sprouting of fibers and the establishment of new synaptic contacts. These long-lasting alterations are remarkably reminiscent of changes that occur during long-term potentiation (LTP) of synaptic transmission in the mammalian brain. LTP is an activity-dependent and persistent enhancement of synaptic efficacy that may underlie certain forms of explicit learning. In contrast to implicit memory, explicit memory requires attention and conscious participation, and involves to an important degree the hippocampus as well as the cerebral cortex. As is the case for memory in the intact animal, LTP is blocked by inhibitors of RNA and protein synthesis, suggesting that neuronal activity resulting in LTP initiates a cascade of changes in gene expression. To understand the underlying genetic program it will be necessary to identify the specific genes that are induced during learning.

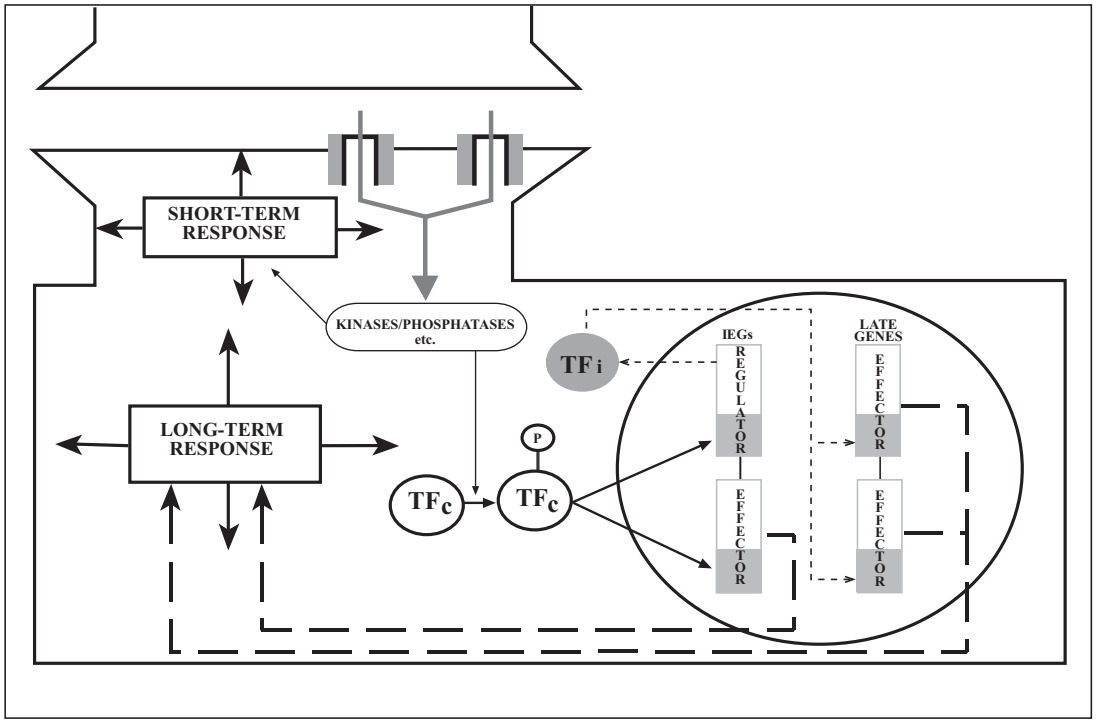
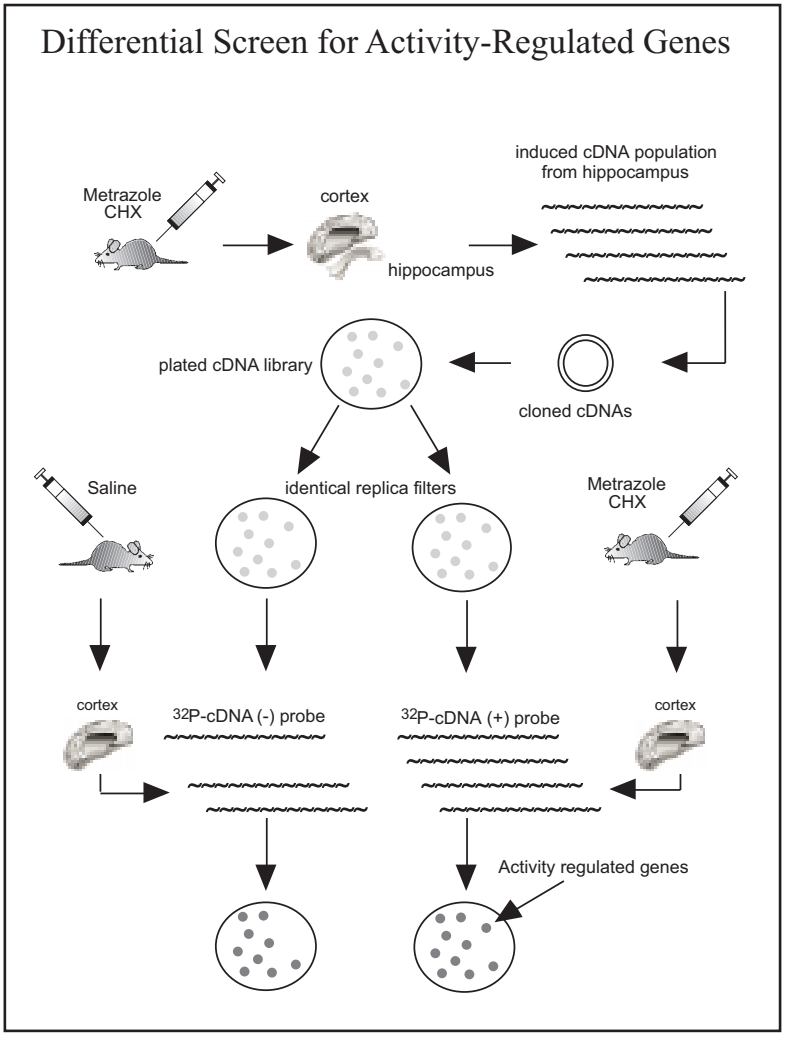


Figure 1. Trans-synaptic activation of memory systems with different time courses. In this model a synaptic signal acts on the neuron to initiate separate memory processes with various durations. Short-term memory has a time course of minutes to hours and depends on the covalent modification of preexisting proteins. The duration of these covalent modifications determines the retention of short-term memory (short-term response). Unlike these short-term processes, long-term memory requires new RNA and protein synthesis. The same signaling systems activated during the short-term response are used to modify constitutively expressed transcription factors (TFc). These transcription factors mediate the induced expression of immediate early genes (IEGs). Some of these IEGs might have effector functions like t-PA or arg3.1 (see text), others represent regulatory genes encoding inducible transcription factors (TFi). These inducible transcription factors trigger the expression of late genes. Both early effector and late genes participate in the maintenance of the long-term response.

1. How can we identify activity-regulated genes in the mammalian brain?

Until recently, insights into the molecular basis of plasticity in the mammalian brain have been largely dependent on tools originally generated in studies of non-neuronal cells. In this way the protooncogenes c-fos and zif268 have been found to be activated by neuronal activity, as have a number of other genes for which probes are available. To explore the possibility that novel immediate early and late genes are induced during plastic events in the brain we started to differentially screen cDNA libraries generated from hippocampi of rats in which seizures had been induced (see Fig. 2). More recently we developed and implemented subtractive cloning methodologies that further improve the sensitivity of these screens (Konietzko, U. and Kuhl, D. (1998). Nucleic Acids Res. 26, 1359-1361.). Do these methods allow us to identify the molecules that define the neuronal response to synaptic activity in the brain?

Figure 2. Differential screen for activity regulated immediate early genes. Immediate early genes are typically induced only transiently and unlike late genes their transcripts are stabilized and accumulate in the presence of protein synthesis inhibitor cycloheximide (CHX). We took advantage of this property known as superinduction to generate a cDNA library enriched for immediate early genes from hippocampal RNA isolated from rats that had undergone metrazole induced seizures. Duplicate filters of this library were then screened with two cDNA probes, one derived from seizure-induced animals (+ probe), the other probe is derived from the brain of control animals (- probe). Clones that hybridized preferentially to the + probe, represent putative activity regulated genes and were subjected to further analysis.



2. Tissue Plasminogen activator is induced as an immediate early gene by synaptic activity

Differential screening revealed that expression of the gene for tissue plasminogen activator (t-PA), an extracellular serine protease, is activated during several forms of synaptic plasticity, including LTP. t-PA might contribute to structural changes that can be observed during activity-dependent plasticity (Qian, Z., Gilbert, M.E., Colicos, M.A., Kandel, E.R., and Kuhl, D. (1993). Nature 361, 453-457). In continuation of this work we asked in a collaborative study how the absence of t-PA gene expression affects the establishment and maintenance of LTP (Frey, U., Mueller, M., and Kuhl, D. (1996). J. Neurosci. 16, 2057-2063.). We analyzed long-lasting LTP (L-LTP, >4 hours) in CA1 hippocampal slices of mice homozygous for disrupted t-PA genes. The genetically engineered mutant mice develop normally, are fertile and have a normal life span. Our histochemical analysis did not reveal any gross anatomical abnormalities in hippocampus or other regions of the brain. In contrast, important indices of synaptic transmission are altered dramatically. Although mutant mice appear to exhibit long-term potentiation,

we find that they are completely devoid of conventional, homosynaptic L-LTP at the Schaffer collateral - CA1 pyramidal cell synapse. Most remarkably, t-PA deficient mice exhibit a different form of potentiation that is characterized by N-methyl-D-aspartate-(NMDA)-receptor dependent down-regulation of g-amino-butyric-acid (GABA) transmission in the CA1 region. This form of potentiation provides t-PA deficient mice with an output of CA1 neurons identical to that seen in wildtype mice during conventional L-LTP and, functionally, might fully compensate for L-LTP. Compensation of conventional LTP by a GABA-dependent potentiation could explain why spatial memory is unaffected in the mutant mice. During control stimulation, t-PA deficient mice are characterized by a stronger Gabaergic transmission in the hippocampal CA1 region. Interestingly, in line with these results are recent observations by Tsirka, Strickland and colleagues that t-PA-deficient animals are less susceptible to experimentally induced seizures, ischemia, and neuronal degeneration. Various human pathologies involve excitotoxic damage to the brain. The contribution of t-PA to the degeneration pathway suggests that inhibitors of t-PA might have therapeutic potential for targeting these diseases.

Although these experiments establish a link between gene expression and physiological and pathological neuronal plasticity, it remains an open question how transcriptional activation taking place in the nucleus can selectively modify stimulated synaptic sites in the distant dendritic compartment of the neuron. Such selective modifications of synapses that have experienced coincident activity are required by the Hebbian rule and might be a prerequisite for the input specificity of LTP. The analysis of the novel immediate early gene arg3.1/arc/bad-1 might guide our thinking and provide insights into this problem (Kuhl, D. and Skehel, P. (1998). Curr. Opin. Neurobiol. 8, 600-606).

3. Somato-dendritic expression of arg3.1 is regulated by synaptic activity

We cloned and characterized the novel immediate early gene arg3.1 (Link, W., Konietzko, U., Kauselmann, G., Krug, M., Schwanke, B., Frey, U., and Kuhl, D. (1995). Proc. Natl. Acad. Sci. USA 92, 5734-5738). Our studies provide evidence of expression and regulation of arg3.1 mRNA in the brain, where synaptic activity markedly increased mRNA levels in discrete populations of neurons. Within the hippocampus constitutive expression was low. Basal expression of arg3.1 RNA was high in cortical areas, particularly in the visual cortex. In cortex NMDA-receptors make a major contribution to normal excitatory synaptic transmission. We found that blocking the NMDA receptor led to a marked reduction in the basal level of expression of arg3.1 mRNA and suggest that the high constitutive expression of arg3.1 in cortex is driven by naturally occurring activation of the NMDA receptor, e. g. by visual experience. Markers for arg3.1 may therefore prove to be useful for monitoring synaptic activity in cortical neurons. Synaptic activity induced by convulsive seizures increased mRNA levels in neurons of the cortex and hippocampus. Induction was independent of new protein synthesis, as is typical of immediate early genes. Unilateral, high frequency stimulation of the perforant path resulted in long-term potentiation and a spatially confined dramatic increase in the level of mRNA in the granule cells of the ipsilateral dentate gyrus. Most strikingly, following LTP and seizure activity the arg3.1 mRNA was localized to the dendrites of the granule cells (Fig. 3). Arg3.1 is distantly related to brain a-spectrin the major constituent of the cytoskeletal network underlying the plasma membrane. The processing of brain spectrin by calcium dependent proteases at the postsynaptic membrane has

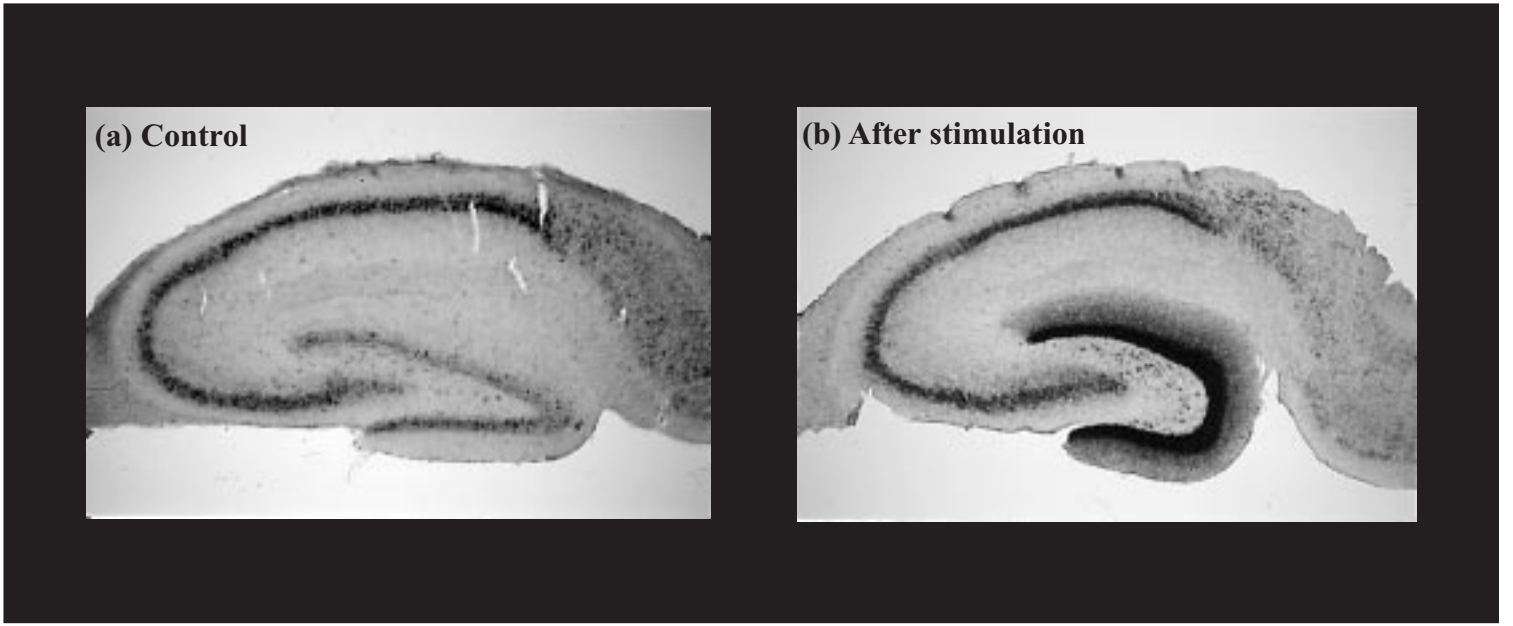


Figure 3. Dendritic arg3.1 mRNA expression is induced by synaptic activity. arg3.1 mRNA was assayed using non-radioactive *in situ* hybridizations. Left panel, hippocampus of a control animal. Right panel, hippocampus of an animal that has experienced plasticity producing stimulation. Note that already 1h following stimulation arg3.1 mRNA is present throughout the dendrites of the dentate molecular layer.

been postulated to be one of the central molecular mechanisms underlying LTP. To our knowledge arg3.1 represents the first example of a gene whose mRNA occurs in the dendrites and which is regulated by synaptic activity. Consequently, arg3.1 mRNA may be locally translated at activated synapses and may have a key role in synapse specific modifications during plastic events in the brain (see Fig 4). More recently we generated mice in which we replaced the endogenous coding region of arg3.1 with a lacz gene. We plan to use these homozygous arg3.1 deficient mice for the analysis of behavior and LTP in the near future. Moreover, we have developed the Tri-Hybrid-System (see Fig. 5), which allowed us to identify several proteins that specifically interact with the arg3.1 mRNA and not with other tested RNAs (Putz, U., Skehel, P., and Kuhl, D. (1996). Nucleic Acids Res. 24, 4838-4840; Putz, U., Kremerskothen, J., Skehel, P., and Kuhl, D**.** (1999). In Yeast Hybrid Methods. L. Zhu, ed. (Natick, MA: Eaton Publishing).

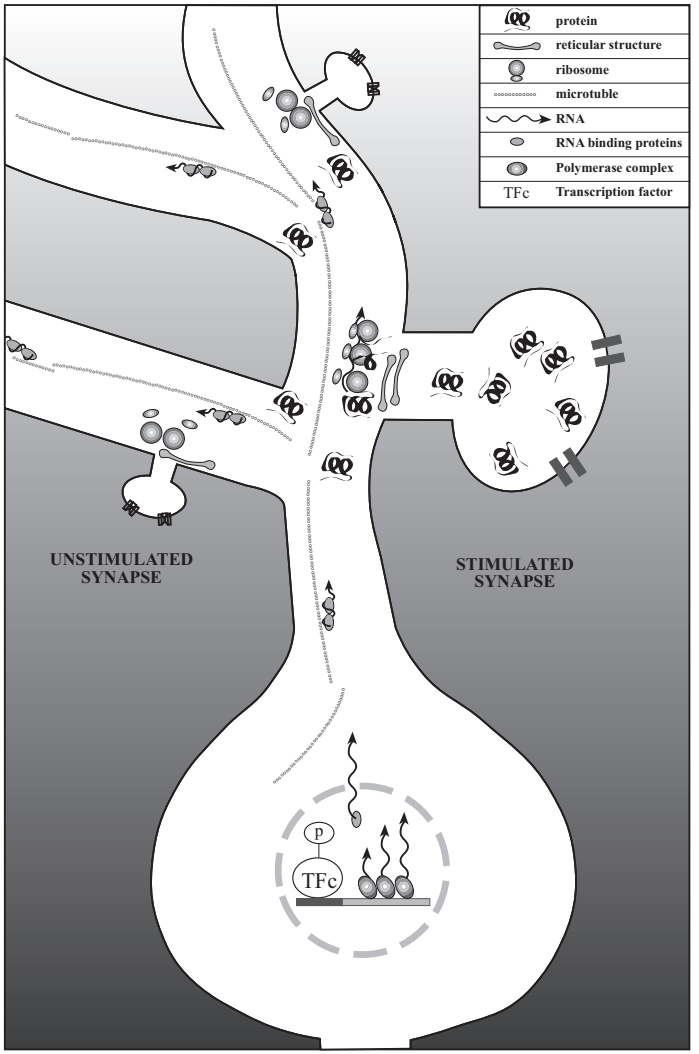


Figure 4. A schematic illustration of synapse-specific modifications by local protein synthesis directed by dendritic mRNA. Trans-synaptic activation of transcription factors leads to induced transcription of specific mRNAs. The transcripts are transported to the dendritic arbor, and enhanced translation of the transcripts takes place at trans-synaptically activated sites.

(in press)). The genes for these arg3.1 mRNA binding proteins are novel but in at least one of them we identified a sequence motif that is known to mediate RNA-protein interactions. In future experiments we want to further characterize these genes and determine what role they play in the dendritic targeting of arg3.1 mRNA and what effect their expression has on the establishment of synapse specific modifications during LTP.

4. Novel effector genes of synaptic plasticity and future perspectives

In addition, the main effort of our laboratory during the last years has been to develop and implement subtractive hybridization techniques. This has enabled us to detect activity-regulated genes expressed at low abundance. Interestingly, several of the newly identified genes encode a class of proteins that share a common function. However, they differ in their induced spatial and temporal expression patterns and their induction follows different thresholds of synaptic activation; whereas some are induced with LTP, others are induced only with pathological stimulation. The latter represent promising candidates for the development of therapeutic agents. These several, recent findings allow us to go in a variety of different directions in the analysis of plasticity and pathological disturbances such as epilepsy

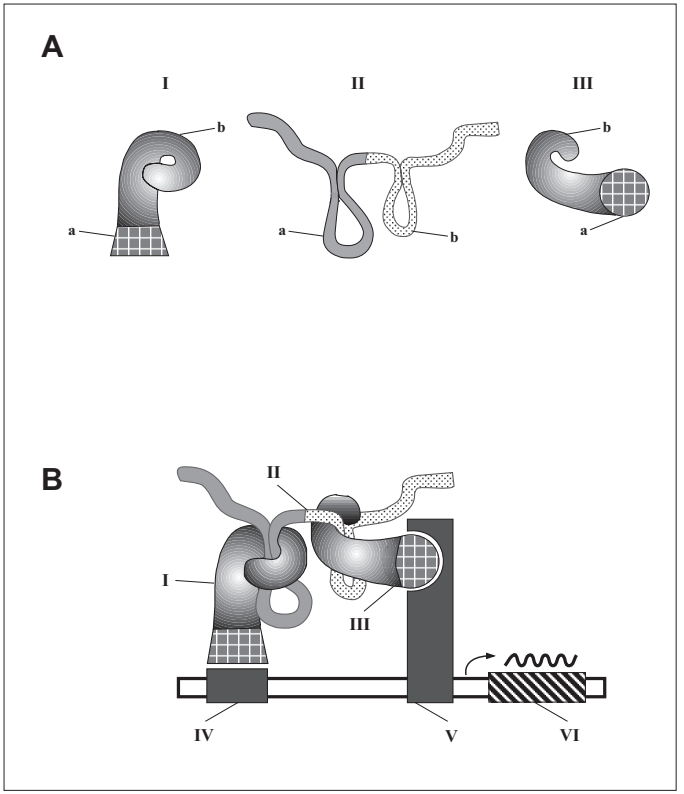


Figure 5. The basic strategy of the Tri-Hybrid method. (**A**) Schematically shows the components. The first hybrid-protein (I) contains the DNA-binding domain of GAL4 (Ia) fused to the RRE-RNA-binding protein RevM10 (Ib). A hybrid-RNA (II) containing the RRE sequence (IIa) and a target RNA sequence X (IIb). The second hybrid-protein (III) contains the activation domain of GAL4 (IIIa) fused to a protein Y (IIIb) capable of recognising the target RNA X on the RNA-hybrid. (**B**) Upon productive interaction of the three hybrids a reconstituted GAL4 transcription factor (I+II+III) bound to a GAL4 responsive promoter (IV) stimulates the basal transcriptional machinery (V) of the lacZ gene and the nutritional reporter gene HIS3 (VI).

and neurodegenerative diseases. The main goal of our laboratory, however, is to bring to bear molecular biological approaches to the study of learning and memory. As exemplified with t-PA and arg3.1 we want to move from the identification of activity regulated genes to the analysis of LTP and assess which consequences they convey on the behavior of animals and their capability to learn and store memories.

Support

The work in our laboratory is supported by by grants from the Bundesministerium für Bildung und Forschung, the Deutsche Forschungsgemeinschaft, and the Fonds der Chemischen Industrie.

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Kauselmann, Gunther (1997). Analyse der Genexpression nach synaptischer Aktivität durch “Differential Display”: Charakterisierung der aktivitätsregulierten Induktion von Glycerol-3-phosphat Dehydrogenase und vier Serin/Threonin Kinasen. Universität Hamburg.

Spiess, Stefan (1999). Untersuchung der Genexpression nach synaptischer Aktivität durch repressiv subtraktive Hybridisierung in Rattus norvegicus: Charakterisierung der aktivitätsregulierten Induktion von Kalirin, Trio und NOR-1. Universität Hamburg.

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Neurodegeneration and Alzheimer's Disease

Roger M. Nitsch

Studies done in our laboratory focuss on genes and proteins involved in the pathophysiology of Alzheimer's disease, the most frequent neurodegenerative illness in humans. In both *in vitro* and *in vivo* approaches, we examine the regulation of APP and presenilin processing by neuronal activity and neurotransmission. Muscarinic receptor-mediated reduction in the formation of amyloidogenic Aβ peptides was developed preclinically, and we currently test this concept in clinical studies. Several additional studies in our laboratory are designed to identify novel genes involved in the pathophysiology of Alzheimer's disease, as well as in neurotransmitter-induced cellular responses.

Identification of differentially expressed genes in selectively vulnerable areas in Alzheimer's disease brain

Isabell Greeve

Differential display was used to identify genes contributing to the pathology of Alzheimer's disease (AD). Tissue levels of mRNA in inferior temporal lobes with histopathologically confirmed neuronal cell loss were compared with those in sensory-motor cortices with minimal loss of neurons in AD brains with post-mortem time intervals of less than 4 hours. Thirty differentially expressed cDNAs generated by forty different primer combinations were cloned and sequenced. One of these cDNAs was identical to KIAA0018 that was previously cloned from a human myeloblast cDNA library, but was not characterized further. We identified several clones with an open reading frame that clearly differed from the previously published sequence. It predicts 516 amino acids of a protein that we termed Seladin-1 (for **sel**ective **A**lzheimer's **d**isease **in**dicator). The cDNA sequence predicts a putative mitochondrial localization signal and an FAD binding domain that is conserved in many oxido-reductases. Expression analysis of the Seladin-1 gene showed high levels of message in adrenal gland, liver, lung and brain, and low expression in skeletal muscle, heart, bladder, uterus, pituitary gland, thyroid gland, salivary gland and mammary gland, but absence of expression in blood and bone marrow cells. Within the central nervous system, high expression was found in substantia nigra, medulla oblongata and spinal cord. *In situ* hybridization studies of *seladin-1* expression in rat brain revealed a clear neuronal expression pattern with strong expression in nerve cell bodies of many brain nuclei. Northern blot analysis confirmed downregulation of *seladin-1* in inferior temporal lobe compared to sensory-motor cortex in *postmortem* brains obtained from Alzheimer's disease patients. In brains from non-demented control subjects, message levels in these brain regions were identical. These results suggest that selectively vulnerable areas in AD brains are associated with decreased expression of the Seladin-1 gene. In human H4 neuroglioma cells stably expressing a Seladin-1-EGFP-fusion protein, Seladin-1 is localized in the endoplasmic reticulum and the Golgi-apparatus. Initial experiments indicate that cells expressing *seladin-1* are more resistant to oxidative stress and to apoptotic stimuli as compared to wildtype control cells. Expression of the protein in *E.coli* for functional in vitro assays and characterization of the protein expression levels in AD

brains and in cells under different conditions with polyclonal antisera raised against Seladin-1 will help to further characterize its function.

Identification of Muscarinic Acetylcholine Receptor-induced Genes

Heinz von der Kammer, Claudia Albrecht, Cüneyt Demiralay, Barbara Hoffmann, Manuel Mayhaus

G-protein-coupled neurotransmitter receptors including muscarinic acetylcholine receptors (mAChRs) are involved in attention, learning, memory and cognition. m1 and m3 AChR subtypes are localized to the somatodendritic cell surfaces of large pyramidal neurons throughout the cortex and the hippocampus, as well as on small cholinergic interneurons in the striatum. In contrast, m2 and m4 AChRs are predominantly present on axons of the large basal forebrain projection neurons that innervate cholinergic target cells throughout the cortex and the hippocampus. Activation of the post-synaptic AChR family by acetylcholine triggers a large variety of distinct signaling cascades including phospholipase D, adenylyl cyclase, phospholipase A2, the generation of diacylglycerol that activates protein kinase C and that couples mAChRs to the ERK-MAP-kinase signaling cascade, activation of endoplasmic reticulum IP3 receptors, stimulation of ligand-operated cell-surface Ca^{2+}-channels, as well as the activity of voltage-gated potassium channels. Cellular responses of mAChRs include the activation of neurite outgrowth, the fine-tuning of membrane potentials, and the regulation of mitogenic growth responses in cells that are not terminally differentiated. mAChRs are also involved in the activity-dependent regulation of post-translational processing of the beta-amyloid precursor protein of Alzheimer's disease by an unidentified protease associated with reduced generation of b-amyloid peptides, the principal component of amyloid plaques in Alzheimer's disease brain. In brain, mAChRs are involved in long-term potentiation and synaptic plasticity. Such plastic alterations in neuronal structure and function have been proposed to be associated with rapid and transient transcription of activity-dependent genes.

In order to identify genes that are regulated by muscarinic acetylcholine receptors, we developed an mRNA differential display approach that yielded highly consistent results. A set of 64 distinct random primers was specifically designed in order to approach a statistically comprehensive analysis of all mRNA species in a defined cell population. This modified DD protocol was applied to total RNA of HEK293 cells stably expressing muscarinic m1 acetylcholine receptors. By analyzing differential bands distinct immediate-early genes were identified that were upregulated by m1AChR activation: *Egr-1*, *Egr-2*, *Egr-3*, NGFi-B, ETR101, *c-jun* , *jun-D,* Gos-3, and *hcyr61*, as well as the unknown gene *Gig-2*.
Our data suggest Egr-1 as a major target among members of the Egr gene family of m1 receptor, because competition experiments with EGR-1 specific antibodies almost completely blocked the binding of nuclear extracts to the EGR recognition sequence that is known to interact with all members of the Egr family. The ability of different muscarinic AChR subtypes in stimulating Egr-1 expression suggests that similar genes are controlled by acetylcholine both in pre- and in post-synaptic neuronal populations. Our data also show that, in addition to Egr-1, the expression of Egr-2, Egr-3, and Egr-4 is

under the control of muscarinic AChRs.
Moreover, our results show binding to, and activation of, EGR-promoter sequences followed by the synthesis of functional protein as a result of mAChR stimulation. EGR-1 increases the promoter activity and gene expression of AChE, a serine hydrolase that catalyzes the hydrolysis of acetylcholine. Our data generated by using the AChE promoter fused to a luciferase reporter show that stimulated m1AChR specifically increased AChE promoter activity. If confirmed for the subcortical cholinergic projection system in brain, EGR-dependent regulation of AChE transcription may be involved in a receptor-coupled feedback control of cholinergic transmission.

Cholinergic signaling in Alzheimer's disease brain is heavily impaired as a result of the early and massive degeneration of the long basal forebrain projection neurons to brain hippocampus and cortex. In as much as EGR-dependent genes in post-synaptic cholinergic target cells are regulated by muscarinic AChR activity, expression of such genes may be decreased in Alzheimer's disease brains. Post-mortem studies are required to test this hypothesis. Drugs designed to activate muscarinic AChRs including AChE inhibitors and m1-agonists currently tested in clinical trials for the treatment of Alzheimer's disease may be expected to stimulate transcription of Egr genes along with EGR-dependent target genes. In vivo studies are required to test whether pharmacological treatments designed to stimulate brain muscarinic AChRs increase AChE gene expression, along with AChE enzyme activity, and accelerated breakdown of acetylcholine.

Identification of disease-causing mutations and genetic risk factors for Alzheimer's disease

In collaborative studies, we identified several novel susceptibility genes for late-onset, sporadic Alzheimer's disease. These include the protease inhibitors cystatin C and alpha2-macroglobulin. Studies are underway to determine whether these proteins are invloved in the regulation of APP and presenilin processing. In collaboration with the Institute of Human Genetics at UKE (U. Finckh, A. Gal), we found 5 novel and 8 previously reported heterozygous mutations in *PSEN1*, *PSEN2*, *APP*, and *PRNP* in dementia patients. Eleven of these mutations were most likely pathogenic and 73% were associated with a positive family history of early-onset dementia. *Post mortem* histopathological analyses confirmed the diagnosis of Alzheimer's disease in 2 patients with mutations in *PSEN1* and *PSEN2*. Moreover, spongiforme encephalopathy was found in one of four patients with *PRNP* mutations, suggesting that hereditary prion disease may occur more frequently than previously expected. No mutations in the above genes were found in control subjects or in patients with late-onset dementia and with positive family history for late-onset dementia.

Alzheimer's Disease Research Group

The DFG-funded Alzheimer's disease research group (Speaker: R.M. Nitsch) is a multidisciplinary consortium of clinical and basic scientists located at both the University Hospital Eppendorf (UKE) and the Max-Planck Society in Hamburg. It studies the molecular pathophysiology of

Alzheimer's disease with a focus on APP, presenilins, tau, ApoE and PrP. A central Memory Disorders Unit located at the Department of Psychiatry (UKE) diagnoses and recruits dementia patients along with matched control subjects for clinical studies of genetic factors, biochemical and neuropsychological markers, as well as for clinical studies of newly-designed therapeuticals.

BMBF-Leitprojekt Molekulare Medizin

This project is designed to identify validated Lead-Target systems for the development of novel treatments of Alzheimer's disease. The BMBF-funded Leitprojekt is coordinated by EVOTEC BioSystems AG, a bio-technology company based in Hamburg.

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft, SFB 444, the Bundesministerium für Bildung und Forschung, the Fonds der Chemischen Industrie, Alzheimer Forschung Initiative and by the European Community.

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Regulation of Neural Gene Expression in Development and Disease

Michael Wegner

Glial cells and neurons arise from the same pool of neuroectodermal stem cells. Whether a cell becomes a glial cell or a nerve cell is determined early in development. After the initial determination event cells continue to proliferate for some time, before they finally undergo terminal differentiation.

The major goal of this project is to study transcriptional regulators that participate in determination and differentiation of neural, in particular glial cells in the developing mammalian nervous system. We currently focus on Glial Cells Missing (GCM) and its mammalian homologs as regulators of early gliogenesis, on POU domain proteins as regulators of terminal differentiation, and on Sox10 as a lineage marker for neural crest and glia. Analysis of these transcription factors will lead to a better understanding of developmental defects, tumor formation and regenerative processes in the nervous system.

Glial transcription factors also play an important role in determining the cell type-specificity of the human papovavirus JC. This opportunistic viral pathogen selectively propagates in CNS glia of immunocompromised patients and by destruction of oligodendrocytes causes a deadly demyelinating disease that is known as Progressive Multifocal Leukoencephalopathy (PML). To better understand the pathogenesis of PML, we analyze the interactions between JC virus and its glial host cell on the level of gene expression.

1. Glial Cells Missing (GCM) and Mammalian Homologs

Glial Cells Missing (GCM) was originally identified in Drosophila as a regulator of early gliogenesis in both loss-of-function and gain-of-function analyses. Whereas absence of GCM led to a loss of glial cells in the embryonic nervous system, overexpression of GCM in neural precursor cells resulted in the generation of surplus glia and a concomitant decrease in neurons. How GCM functioned, however, remained unclear.

Our analyses revealed that GCM fulfilled all criteria of a bona fide transcription factor including nuclear localization, DNA binding and transactivation. Whereas the DNA binding domain was located in the aminoterminal part of the protein, the transactivation domain was present in the 80 most carboxyterminal amino acids of GCM. Intriguingly, the DNA binding domain of GCM exhibited no homology to other known DNA binding domains. It recognized a DNA sequence motif (5'-ATGCGGGT-3') for which no other DNA binding protein has been previously isolated. GCM bound to DNA as a monomer. DNA binding was not dependent on the presence of divalent cations, but could only be observed under reducing conditions. That GCM could indeed function as a transcriptional activator was shown in transiently transfected mammalian cells and Drosophila Schneider cells using a promoter consisting of TATA box and adjacent GCM recognition elements.

Recently, we have isolated a murine homolog of GCM, which we termed mGCMa. Sequence similarity between GCM and mGCMa is highest in the aminoterminal region which contains the DNA binding domain. Comparison between the DNA binding domains of GCM and mGCMa allowed the

identification of conserved amino acids. Substitution of 28 conserved residues by alanines identified seven symmetrically arranged cysteines as the backbone of the DNA binding domain of both GCM and mGCMa. These cysteine residues determine the conformation of the DNA binding domain and are responsible for DNA-binding and its redox-dependency. Although sequence similarity between GCM and mGCMa dropped dramatically outside the DNA binding domain, there is nevertheless topological conservation of functional domains. Like GCM, mGCMa contained a potent transactivation domain in its carboxyterminal 87 amino acids.

This similarity in structure was also reflected by a similarity in function. When an mGCMa transgene is introduced into Drosophila such that mGCMa is expressed throughout the neuroectoderm of the developing embryo, numerous surplus glia are formed at the expense of neurons with total cell numbers in the nervous system remaining relatively constant. When mGCMa expression is directed into the fly's ectoderm rather than neuroectoderm, ectopic glial cells form at the expense of epidermal cells, thus showing that mGCMa can induce glial cell fate both inside and outside the embryonic nervous system of Drosophila in a manner similar to GCM.

2. POU-Domain Proteins

The family of POU-domain proteins comprises a subfamily of homeodomain proteins with predominant expression in the nervous system. Tst-1/Oct6/SCIP, for instance, is expressed both in glial cells and in neurons. Targeted deletion of the Tst-1/Oct6/SCIP gene led to premature arrest of Schwann cell differentiation and a concomitant myelination defect in the PNS. We detected Tst-1/Oct6/SCIP in oligodendrocytes. Contrary to Schwann cells, however, oligodendrocytes also express Brn-1 and Brn-2, two other closely related POU domain proteins. These three POU domain proteins were mostly coexpressed during oligodendrocyte development. Redundancy between them might very well explain the absence of a severe CNS myelination defect following deletion of the Tst-1/Oct6/SCIP gene.

One of the peculiarities of POU domain proteins in general and Tst-1/Oct6/SCIP in particular is their functional dependence on other cellular proteins. Interaction with glial factors, for instance, is believed to account for function of Tst-1/Oct6/SCIP in glial cells. We have previously identified the non-histone chromatin protein HMG-I/Y as an accessory protein for Tst-1/Oct6/SCIP on AT-rich DNA binding elements. Because of its ubiquitous expression, however, HMG-I/Y is unlikely to be responsible for the glia-specificity of Tst-1/Oct6/SCIP.

A better candidate for affecting glia-specificity of Tst-1/Oct6/SCIP function is Sox10. This member of the Sox protein family of transcriptional regulators is selectively expressed in glial cells from mid-embryogenesis onwards. In promoters where binding sites for POU-domain and Sox proteins are in close proximity to each other, Sox10 strongly enhanced Tst-1/Oct6/SCIP function. Other Sox proteins failed to do so, but efficiently interacted with different POU-domain proteins. Brn-1 was the preferred partner for Sox11 in synergistic interactions, and Oct-3/4 has been shown to interact with Sox2. Cooperativity between Tst-1/Oct6/SCIP and Sox10 is, however, always dependent on direct binding of Sox10 to DNA. Thus, Sox10 cannot explain the glia-specificity of Tst-1/Oct6/SCIP on promoters without Sox protein binding sites.

A clue towards understanding glia-specificity of Tst-1/Oct6/SCIP function in the latter cases comes from studying the synergy between Tst-1/Oct6/SCIP and T antigen on the glia-specific JC viral promoter. We had previously shown that synergy in this case is mediated by a direct interaction between Tst-1/Oct6/SCIP and T antigen and did not involve T antigen binding to DNA. Our results now prove that the essential activity of the viral T antigen is the chaperone function of its J domain on the aminoterminal transactivation domain of Tst-1/Oct6/SCIP. We currently favour a model, in which the J domain initially docks onto the POU domain of Tst-1/Oct6/SCIP, and then remodels the transactivation domain of Tst-1/Oct6/SCIP thus making it competent for transactivation. Interestingly, the J domain of T antigen can be replaced by J domains of cellular proteins. Neural J domain proteins have been shown to exist. Thus, it is tempting to speculate that glia-specific J domain proteins are involved in conferring cell-type specificity to Tst-1/Oct6/SCIP.

3. Sox10

Sox proteins carry a high-mobility-group DNA-binding domain similar to the domain of the mammalian sex-determining factor SRY. Similar to other Sox proteins, Sox10 strongly bent DNA, was only a weak transcriptional activator, and efficiently functioned in tissue culture experiments as a modulatory protein for other transcription factors such as Tst-1/Oct6/SCIP, Pax3, and Krox-20.

During embryogenesis, Sox10 is first expressed in the emerging neural crest. Whereas Sox10 is turned off soon in a number of neural crest derivatives, cells that contribute to the forming PNS continue to be Sox10-positive. Later in embryogenesis, Sox10 expression becomes restricted to glial cells of the PNS. Concomitantly, CNS expression starts in a pattern consistent with the presence of Sox10 in oligodendrocyte precursors. In the adult CNS, Sox10 is predominantly found in oligodendrocytes.

The Sox10 gene mapped to a locus on mouse chromosome 15 known to carry the *Dominant megacolon (Dom)* mutation. This mutation affects neural crest development. It is embryonic lethal in the homozygous state and leads to severe PNS defects including a dramatic loss of Schwann cells. In the heterozygous state, mice are viable, but exhibit a combination of pigmentation abnormalities and aganglionosis of the distal colon. We could show that the *Dom* mutation affects the Sox10 gene. The insertion of a single basepair into the open reading frame led to a frame shift which in turn caused the replacement of the carboxyterminal 273 amino acids by an alternate carboxyterminus of 99 amino acids. The shortened Sox10 protein is functionally inactive in tissue culture experiments.

A similar manifestation of pigmentation defects and aganglionosis of the distal colon is also observed in patients suffering from a combination of Hirschsprung disease and Waardenburg syndrome (HSCR/WS). In addition, these patients suffer from sensorineural deafness. We identified the human SOX10 gene on chromosome 22q13 and were able to detect 4 independent heterozygous SOX10 mutations in HSCR/WS patients. The mutations included an amino acid insertion into the DNA-binding domain of SOX10, a frameshift, and two nonsense mutations that resulted in very early chain termination. All led to functional inactivation of the mutant proteins. Our findings on the *Dom* mouse and on HSCR/WS patients clearly prove the relevance of Sox10 for neural crest and glial development.

4. JC virus and PML

JC virus is highly selective for oligodendrocytes because of the glia-specificity of its gene expression. We have shown that Tst-1/Oct6/SCIP might contribute to this glia-specificity by stimulating the expression of viral early and late genes. Among the early genes is the gene for the viral T-antigen. Studies in our lab have shown that T-antigen stimulates the expression of the Tst-1/Oct6/SCIP gene just as Tst-1/Oct6/SCIP stimulated the expression of T-antigen. The existence of such a positive feedback loop probably ensures the coordinate upregulation of both proteins which is important as both proteins form a complex and synergistically activate viral gene expression. This interplay might be a key factor in the efficient infection of oligodendrocytes.

Reports from other labs indicated that JC virus might also lead to formation of glial tumors in man. We investigated glioblastomas, oligodendrogliomas, and astrocytomas of different grades for the presence of JC viral genomes or JC viral proteins. However, we were unable to detect JC virus in any of the tumors tested arguing that JC virus is not a major cause of glial tumors in man.

Support

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Theses

Diploma

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Dissertations

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Neural crest development

Dieter Riethmacher

The embryonic neural crest is a unique group of multipotent cells that is induced at the border between neural plate and epidermis and gives rise to much of the peripheral nervous system, epidermal pigment cells, and a variety of mesectodermal derivatives. In order to become migratory the initially epithelial cells have to undergo an epithelial-mesenchymal transition. Cell-intrinsic as well as extrinsic factors mediate their subsequent differentiation, lineage segregation and mobility.

The tyrosine kinase receptors erbB2, erbB3 and erbB4 recognize the neuregulin family of ligands. By mutating the erbB2 and erbB3 gene in the mouse we have shown that the neuregulin signaling system is an important player in neural crest development. Most of the sympathetic nervous system does not form and Schwann cell precursors aligning axonal projections are virtually absent in mutant embryos. The exact mechanisms underlying these developmental defects are not understood.

The major goal of this project is to analyze these mechanisms and identify molecules that become activated by the neuregulin signaling system. The identification of genes that are involved in differentiation, lineage segregation and mobility of neural crest cells will be of high importance for our understanding of peripheral nervous system development.

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Mechanisms of Pancreas and Central Nervous System Development

Maike Sander

A cascade of molecular events leads to the differentiation of unspecialized progenitor cells into specialized cell types and the activation of cell-type-specific genes. Differentiated cell types are established and maintained by the correct temporal and spatial expression of transcription factors during development. Our research aims to understand how certain transcription factors determine cell lineage decisions, specifically in the pancreas and central nervous system (CNS).

Despite their different embryonic origin, pancreatic islet cells and neuronal cells in the CNS express remarkably similar sets of transcription factors during development. Previous research has shown that a number of key transcription factors regulate both neuronal differentiation, and development of endocrine cells in the pancreas. One such example is the homeodomain transcription factor NKX6.1. By mutating *Nkx6.1* in mice, we have demonstrated that this gene is required for not only the development of motor neurons in the spinal cord, but also of insulin-producing cells in the pancreas.

Given the similarities in the molecules expressed in pancreas and neural tube, we are trying to define conserved developmental pathways utilized by both tissues. Specifically, we aim to identify other genes, and their function within the same developmental pathways as *Nkx6.1*. Our experimental approach employs biochemical methods, as well as animal models, using global and tissue-specific knockouts and over-expression studies.

Publications

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Roles of neurotrophic factors during inner ear development

Thomas Schimmang

The development of the inner ear is an interesting model to study differentiation and induction processes in the peripheral nervous system. Our research objectives address three basic key questions in the areas of developmental biology and neuroscience:

1) Which neurotrophic molecules are involved in the formation and function of the cochleovestibular ganglion?

2) How do we protect the inner ear from neuronal and hair cell damage in vivo?

3) Which signals are required for the induction of the inner ear?

The family of neurotrophins includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4). Most of their functions are mediated by the Trk family of tyrosine kinase receptors. To understand the functional role of neurotrophins in the mammalian inner ear we have analyzed mouse mutants which are defective in Trk receptors. This analysis has revealed a dominant role for TrkB in the vestibular ganglion and TrkC in the cochlear ganglion, whereas signaling via the TrkA receptor is not required. At present we are analyzing a TrkB receptor mouse mutant which is missing the binding site for the shc adaptor protein. In avians neurotrophin signaling has been studied by expressing neurotrophins or their receptors in isolated inner ear sensory neurons. We have used defective Herpes simplex virus type I (HSV-1) vectors which allow the efficient infection and expression of genes inside postmitotic neurons. Using these vectors we have shown that BDNF plays a dominant role during development of the chicken cochlear ganglion, whereas NGF signaling via TrkA is not essential. We have now produced a HSV-1 vector expressing NT-3 to clarify the role of this neurotrophin for neuronal survival in the avian inner ear.

Damage or loss of inner ear sensory neurons and hair cells may be caused by aging, noise, mechanical injury, infections or therapeutic agents. Members of the neurotrophin gene family have been shown to protect neurons from ototoxic damage in vitro. In the case of hair cells promising candidates for hair cell protection include basic fibroblast growth factor (FGF-2) and glial cell line-derived neurotrophic factor (GDNF). To define the potential of these factors to act as therapeutic agents, we are using HSV-1 mediated gene transfer. We have so far demonstrated successful infection of cochlear neurons in rats in vivo and are now testing the protection of neurons against different ototoxic agents. A further study is focused on a mutant strain of mice called hairless, which develop hearing loss with aging. These mice show defects in their auditory sensory epithelia and reduced numbers of cochlear neurons. We will study these mice as a model to test regenerative processes after HSV-1 mediated transfer of neurotrophic factors.

To gain more insight in the molecular basis underlying the induction of the inner ear we have chosen to study the function of fibroblast growth factor 3 (FGF-3) during this process. FGF-3 is expressed in the inner ear or the neighboring hindbrain during induction of the otic vesicle in several species. Based on experiments using antisense techniques FGF-3 has been proposed as the inducer of the chicken inner ear,

whereas knockout mice for FGF-3 only show defects during differentiation of the inner ear. To clarify the role of FGF-3 during avian inner ear development we have chosen a gain-of-function approach using HSV-1 vectors. Ectopic expression of FGF-3 leads to the formation of ectopic otic placodes in chicken embryos. Moreover, FGF-3 also controls size and morphogenesis of the otic vesicle. These results demonstrate that FGF-3 acts during several key steps of inner ear development in avians. To further address the function of FGF-3 in higher vertebrates we are now in the process of expressing FGF-3 ectopically in mammalian embryos.

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Neuronal Cell Fate Specification

Ingolf Bach

Transcription factors are responsible for fundamental biological events that occur during embryogenesis such as tissue-specific gene expression, cell type specification, organ development and terminal cell differentiation. The LIM homeodomain transcription factor family has been demonstrated to confer cell lineage identity and to be responsible for cell fate determination during the development of organisms as divergent as Drosophila and higher vertebrates. Besides their requirement for the development of specific neuronal cell populations such as motor-, inter- and touch receptor neurons, LIM homeodomain proteins are essential for the formation of many neuronal and non-neuronal body structures such as fore-, mid- and hindbrain, anterior pituitary, eye and limbs. Recent work indicates that the biological activitiy of LIM homeodomain factors is regulated by cofactors that are associated with LIM domains of nuclear LIM proteins.

Our research focuses on the specification of neuronal cell types during mouse embryogenesis, conferred by LIM homeodomain transcription factors and their associated proteins. We use molecular, biochemical and genetic approaches to investigate the mechanisms that underly the regulation of the biological activities of LIM homeodomain factors *in vitro* and *in vivo*. The elucidation of these mechanisms is of major importance for a better understanding of nervous system development.

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft.

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Central Service Facilities

DNA Sequencing

Willi Kullmann

Current progress in molecular biology is last but not least based on methods of rapid DNA-sequencing. The originally employed “manual sequencing“ was more and more replaced by “automated sequencing“ because the latter yields faster results and avoids the health and environment risks brought about by radioisotope-labeling routinely used during “manual sequencing“.

At the center of molecular neurobiology (ZMNH) a DNA-sequencing facility was established in October 1995. Automated DNA-sequencing started with an ABI Prism 373 DNA sequencer which was replaced by an ABI Prism 377 DNA sequencer in May 1996 to enable faster gel runs with higher throughputs. The latter was then up-graded in January 1998 from 36 to 64 gel lanes per run.

The biochemical concept underlying the above mentioned DNA-sequencers can be deduced from the chain-termination method developed by Sanger and coworkers in the late seventies. This method uses radioisotope labels in order to detect DNA-fragments, whereas the automated sequencers give preference to flourescence-based detection. Presently an improved set of fluorescence dyes (big dye) is used which greatly reduces the notorious weak G after A pattern characteristics of its predecessor.

The ABI Prism 377 sequenator enables a reading-length of about 450 bases after a gel run time of only 4 hours, whereas the number of bases which can be read after 10 hours amounts to about 750 bases.

Due to the enhanced throughput of the new sequenator, two gels can be run per day. From January 1997 until December 1998 approx 24500 sequence analyses were performed.

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Morphology

Michaela Schweizer

Morphological correlates of activity dependent plasticity in dissociated hippocampal neurons

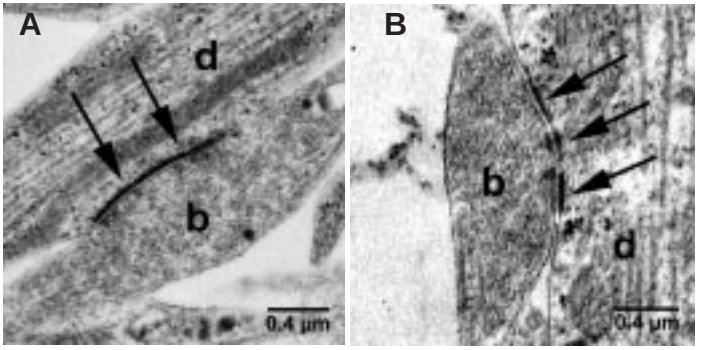
Brain plasticity and mechanisms of learning are based upon modifications of synaptic function. Recent findings favour the idea that changes in synaptic strength are associated with changes in synaptic structure. Thus, detailed knowledge of the ultrastructure of excitatory central synapses is essential for a better understanding of plastic events like LTP and LTD.

Our major goal is to understand the nature of the relationship existing between structural changes and modifications of synaptic efficacy. We use dissociated hippocampal neurons prepared from hippocampi of pre- and postnatal rats. This simplified preparation provides an experimentally accessible *in vitro* model for the study of cellular and molecular properties and of the plastic capabilities of individual identified synapses. High resolution microscopy enables us to describe structural modifications under different conditions of neuronal activity.

One idea is that the formation of perforated synapses is closely related with synaptic plasticity. Perforated synapses are characterized by a discontinuous postsynaptic density (PSD) which is always larger than those of non-perforated/ macular synapses (Fig. 1). This indicates that the perforations increase the perimeter length of the PSD, and thereby the size of the total active synaptic zone. Thus, the formation of perforations might be a morphological correlate of enhanced synaptic efficacy. Defining the cellular mechanisms how changes in neuronal activity are coupled to the morphological event of synapse perforation is likely to contribute to a better understanding of its functional role.

Recent findings have shown that proteases like the tissue type plasminogen activator (tPA) plays a role in activity dependent plasticity and LTP.

Figure 1: High power electron micrographs of a non-perforated **(A)** and perforated synapse **(B)** from 12 day old hippocampal neurons. Boutons



(b) with synaptic vesicles contacting dendritic shafts (d) are shown. Arrows indicate the postsynaptic density

In order to bring some light in steps of structural plasticity we focused on the frequency of perforated synapses in our cell culture system after global stimulation with different drugs.

Our studies demonstrate that the formation of perforated synapses can already be induced by a short-term increase of electrical network activity. A 15 minute stimulation by the $GABA_A$-antagonist picrotoxin induced a two-fold increase in the percentage of perforated synapses (Fig. 2A). This strong increase was blocked when AP-5 was added together with picrotoxin indicating that the formation of perforated

synapses depends on the activation of NMDA-receptors (Fig. 2B). We show that inhibition of the tissue type plasminogen activator significantly interferes with the activity induced increase of perforated synapses (Fig. 2C). This indicates that the proteolytic activities of tPA might be involved in steps which are downstream from the NMDA-receptor mediated synaptic plasticity and lead to morphological changes at synaptic contacts.

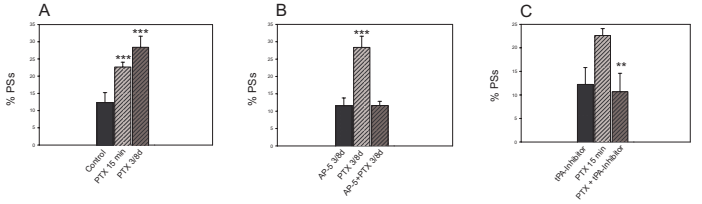


Figure 2: Changes in the number of perforated synapses after stimulation with PTX (A), PTX together with NMDA-receptor antagonist AP-5 (B) and PTX and tPA inhibitor (C).

In a second approach we analyzed the localization of tPA. Immunohistochemical detection of tPA in cultured hippocampal neurons showed that some but not all synaptic boutons expressed tPA. This was shown by double staining with antibodies against synaptophysin and tPA (Fig. 3). The direct relationship between mechanisms of LTP and structural plasticity as well as the formation of perforated synapses needs to be further revealed.

Besides the project described above, the facility of electron-microscopy offers multiple tools for the detection of various antigens in cell cultures or tissue sections at the light and electron microscopical level.

Our standard fixation protocol is perfusion through the ascending aorta with aldehydes for optimal structural preservation of the brain. In order to detect the antibodies bound to individual antigens we use signal enhancing techniques like the avidin-biotin complex (ABC) technique. The development in a substrate such as diaminobenzidine-hydrogen

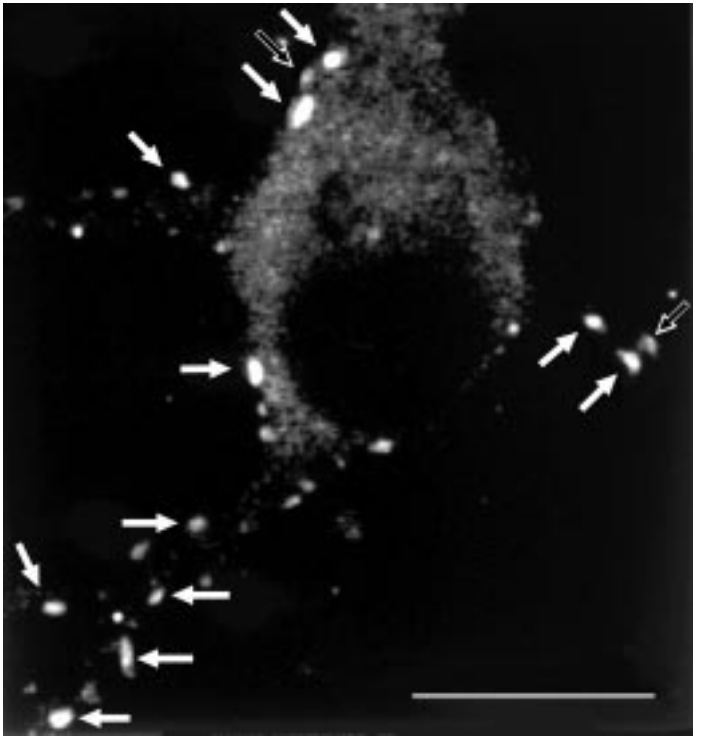


Figure 3. Confocal image of a cultured hippocampal neuron double stained for tissue plasminogen activator (tPA) and synaptophysin. Only tPA signal is shown here. tPA is present within the soma and partly in dendrites, but is enriched in presynaptic boutons (filled arrows), where it colocalizes with synaptophysin. However, some boutons, despite exhibiting strong synaptophysin immunoreactivity show no or only very weak tPA staining (open arrows). Scale bar: 10µm

peroxide allows us both light-and electron microscopical analysis. For a detailed ultrastructural analysis pre- and postembedding procedures have to be tested for each individual antigen - antibody complex. Also the embedding medium and the detection method (DAB - colloidal gold) can be varied.
Double or triple immunohistochemical staining is routinely carried out with fluorescent labeled secondary antibodies. Our Confocal-Laser-Scanning microscope (Leica) enables us to detect up to three fluorophores simultaneously. The much higher resolution of the Confocal-Laser-Scanning microscope (about 0,1µm) compared to conventional fluorescent light microscopes (about 0,3µm) allows us to relate the signal to cellular components with fairly high probability.
Besides the detection of antigens, we can monitor the mRNA-expression on tissue sections by in situ hybridization (ISH). The standard protocol uses paraformaldehyde fixed cryosections, hybridized with ^{35}S- or ^{33}P-labeled cRNA-probes. The bound probe is detected by exposing the sections to x ray-film or by dipping them into photographic emulsion.
If strong hybridization signals are obtained, nonradioactive ISH can improve the spatial cellular resolution of the labeling. Additionally an ultrastructural ISH protocol has been developed by Susanne Fehr (Prakash et al., 1997). If nonradioactive ISH results in a strong staining this method allows the subcellular localization of mRNA in the tissue.

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Dissertation

Griesinger, Claudius (1997). Molekulare Mechanismen struktureller Entwicklungsplastizität. Universität Tübingen.

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Mass Spectrometry and Biomolecular Interaction Analysis

Christian Schulze

Analytical biochemistry involves several techniques to achieve the comprehensive description of biomolecules. Molecular characterization of proteins in terms of amino acid composition and sequence is a field in which mass spectrometry is well established. However, protein function is defined through interaction with other biomolecules and cannot be directly inferred from sequence despite large efforts in computational methods. For functional studies a biosensor (Biacore 2000) is available now (purchased by the Institut für Entwicklungsneurobiologie).

Mass Spectrometry

The key feature of electrospray ionization mass spectrometry (ESI-MS) is the generation of multiply charged molecular ions which fall within a limited range of mass-to-charge (m/z) ratios, typically between m/z 300 and m/z 2,000, irrespective of the analyte molecular weight. The formation of multiply charged molecules and the observed charge-state distributions are influenced by several experimental factors and it has also been found that composition and pH of the analyte solution are important. With respect to biochemistry it is interesting, that the primary and higher order protein structure is reflected in the charge-state distribution. However, systematic studies that correlate analyte structure with charge state have been rare. We were able to shed some light on this relation by analyzing specially designed dendrimer-like multiple antigenic peptides (MAPs) under carefully controlled conditions. MAPs consist of multiple copies of a given immunogenic peptide attached onto a scaffold of lysine residues linked via both *alpha* and *epsilon* linkages, so that a small core matrix of lysine residues bears radially branched synthetic peptides as dendritic arms. The model peptides differed only in the presence of a single arginine residue at the N-terminus on each of the four peptide chains and therefore gave a detailed picture of the contribution of a single basic residue to the charging behavior. N-acetylated MAPs were also examined. The experiments showed that the average charge state exhibits a linear relationship to the number of basic sites. Moreover, the data strongly suggested that the peptide chains of the MAPs are effectively independent, as they are in solution phase. Hence, mass spectrometric data may contribute to a better understanding of protein structure.

Protein identification is most conveniently done by peptide-mass fingerprinting. In peptide-mass fingerprinting, the protein in question is enzymatically degraded and the molecular weight of the fragments is determined by either ESI-MS or matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. These masses are then used to search a modified (actually an *in silico* digested) protein data base. In this lab, a number of proteins from rat brain purified by means of a protein affinity column has been successfully identified, however the approach was somewhat hampered by the low sensitivity of the current instrumentation.

Peptide-mass fingerprinting is extremely useful, but it should be noted, that modifications or disulfide linkages cannot be inferred from the sequence alone, and additional experiments are necessary. In cases, where no data base entry exists, the above outlined approach is not applicable. Edman

degradation is therefore still common to produce sequence information of unknown proteins. This has been used in combination with C-terminal digestion by carboxypeptidases with mass spectrometric readout to obtain the complete amino acid sequence of a carbohydrate-binding protein from the sponge *Axinella polypoides*.

ESI-MS again showed its capability to handle low molecular weight compounds, when the very labile cyclic nucleotide derivative 1-(5-phospho-beta-D-ribosyl)-2'-phospho-adenosine 5'-phosphate cyclic anhydride (c-ADPR-P) was characterized. This compound is formed from a NAD^+ precursor by ADP-ribosyl cyclase from *Aplysia californica*. Application of c-ADPR-P to permeabilized Jurkat cells leads to a release of Ca^{2+} from the intracellular, non-endoplasmatic reticular store. Its role in T cell receptor/CD3-complex mediated Ca^{2+} signaling is still under investigation.

Biomolecular Interaction Analysis

The optical phenomenon surface plasmon resonance (SPR) is used to monitor interactions between biomolecules. The detection principle, termed biomolecular interaction analysis (BIA), depends on changes in the mass concentration of macromolecules at the biospecific interface in real time, so that kinetic information is readily derived. BIA uses a continuous flow technology.

One interactant is immobilized on the sensor surface, and solution containing the other interactant(s) flows continuously over the sensor surface. As molecules from the solution bind to the immobilized interactant, the resonance angle of reflected light changes and a response is registered. Results are presented in a sensorgram. A typical result for the binding of an antibody to its antigen is given in Fig. 1.

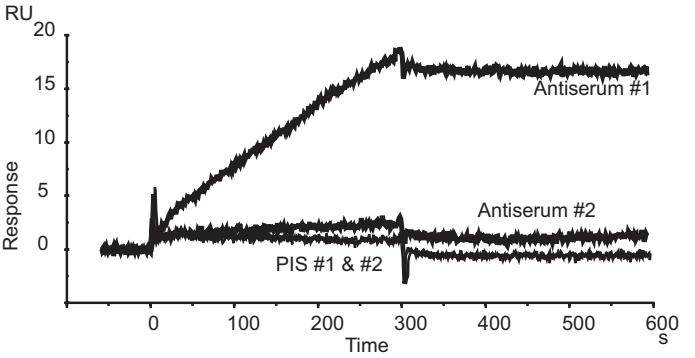

Figure 1: Sensorgram illustrating binding of antibodies against the C-terminal part of SorLA. Results for antisera from two rabbits (#1 and #2) immunized with KLH-linked peptide are shown together with pre-immune sera (PIS).

Under the chosen experimental conditions the signal increase is a measure of the concentration of antibody. The data reveal that both antisera (crude rabbit sera in high dilution) contain binding activity as compared to the pre-immune sera (PIS), but that there is a marked difference in the antibody concentration. Both antisera exhibit quite strong binding.

BIA is suitable for many investigations which address questions of specificity/identity, concentration, affinity, kinetics, and cooperativity. Preliminary data from further experiments show very promising results. It should be noted, that combination of affinity governed purification using BIA and mass spectrometric characterization is a further option. The amount of material obtained from a single injection is well within the range needed for state-of-the-art mass spectrometry.

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Transgenic Technology

Michael R. Bösl

Gene targeting has become a powerful tool to study gene function in the mouse and to develop mouse models for biomedical research. These transgenic animals are essential to understand the role of specific genes at an higher organized level including the cellular and organ level, but especially within the complexity of the whole organism. This includes the role of genes during development, but also age related processes. Thus, transgenic animals are an invaluable tool to bridge the gap from molecular studies to the physiology of higher, integrated functions. Major, well known problems of the classical gene targeting technique are the lack of temporal control and tissue specificity, possible adaptation and compensation mechanisms for the missing gene function during embryogenesis and ontogenesis, and embryonic and neonatal lethality. The combination of gene targeting techniques with site-specific recombination systems such as the Cre/*lox*P system of the bacteriophage P1 or the yeast derived FLP/FRT system allows the development of strategies to circumvent these problems by restricting the mutation to certain cell types and/or a specific time period, but also to introduce large genomic alterations or subtle point mutations. The first successful generation of transgenic mice via additive gene transfer by pronuclear injection was described almost two decades ago, but the use and importance of this technique is still increasing in science and biotechnology and it is indispensable for conditional gene targeting or phenotypic rescue.

The transgenic technology group started its operation in Jan. 1998 with the equipment of a cell culture laboratory and a laboratory for embryo manipulation whose core equipment is an inverse microscope with DIC-optics and micro-manipulators. Mouse colonies for blastocyst donors and foster animals were set up under SPF-conditions. The initial focus has been on the injection of recombinant ES-cells into blastocysts and the generation of chimeric animals. This technique is performed now routinely with high efficiency; within the first year five knockout projects could be processed up to the germline, but already in the first quarter of 1999 the number of projects being processed is higher. The transgenic technology group is providing ES-cells and feeder cells to the scientists of the center together with protocols and advice for their proper handling. Successful pilot experiments have already been performed for pronuclear injection and this technique will be offered as a regular service as soon as the set up of the necessary mouse colonies under SPF-conditions will be finished.

Publication

Bösl, M.R., Takaku, K., Oshima, M., Nishimura, S., Taketo, M.M. (1997). Early embryonic lethality caused by targeted disruption of the mouse selenocysteine tRNA gene (Trsp). Proc. Natl. Acad. Sci. USA 94, 5531-5534.

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Institut für Zellbiochemie und klinische Neurobiologie

Dietmar Richter

The main aim of research in this institute is to understand how nerve cells manage to respond to external and internal signals in order to maintain and regulate their cellular architecture. (i) External signalling is studied using, as tools, G-protein coupled receptors as well as ion channels. The expression patterns of these proteins, the delineation of their ligand binding sites and the structural requirements for endocytosis (somatostatin, corticotropin-releasing factor and thyrotropin-releasing hormone receptors) are examined in vertebrates and invertebrates. GABA- and glutamate-gated channels are studied with the aim of understanding their specific functions in the central nervous system of various species as well as their possible involvement in neurological diseases such as inherited forms of schizophrenia and manic depressive illness. (ii) Internal signalling is studied in those nerve cells known to have developed mechanisms for the subcellular targeting of RNAs to distinct sites such as axons or dendrites. Decentralized local protein synthesis may govern the spatial organization of complex protein repertoires and, thus, may be critical for the generation and maintenance of pattern, polarity and plasticity in nerve cells. *Cis*-elements and *trans*-acting factors that are involved in the subcellular targeting of mRNAs within neurons are currently being investigated.

1. Signal transduction through neuropeptide receptors

Hans-Jürgen Kreienkamp, Ercan Akgün, Dietmar Bächner*, Necla Birgül*, Annette Busch*, Günter Ellinghausen, Michael Glos*, Hans-Hinrich Hönck, Rüdiger Reinking*, Anja Schwärzler*, Claus-Peter Schwartkop*, Heike Zitzer**

Receptors for neuropeptides like somatostatin (SST) are involved in a wide variety of regulatory processes including the control of hormone secretion from neuroendocrine tissues, the modulation of transmitter release from neuronal cells and the regulation of cell proliferation. These phenomena are of considerable clinical importance as SST receptors (SSTRs) are expressed by many neuroendocrine tumours, and treatment of patients with SST analogues may lead to substantial improvement due to the inhibitory effect of SST on hormone release from these tumours and on cellular proliferation.

We have studied the regulatory properties of the various SSTR subtypes (SSTR1–5, all of which may be expressed on different tumour types), i.e. the phenomena of agonist-dependent desensitization and internalization. Expression of recombinant epitope-tagged receptors in a human cell line showed that SSTR1, 2 and 3 undergo agonist-dependent internalization in response to treatment with the native agonists SST14 or SST28; surprisingly, SSTR5 is internalized only in the presence of SST28, whereas SSTR4 is not internalized at all due to an element in its C-terminus. Sequence elements for desensitization and internalization of SSTRs have been identified.

Current work focuses on the identification of proteins that interact with SSTRs, and on the role of these proteins in SSTR signal transduction; using the yeast two-hybrid system we have identified a family of multi-domain proteins which are expressed in brain and other tissues and which interact specifically with the C-terminus of SSTRs.
The physiological function of individual SSTR subtypes is being addressed by using SSTR deficient mice. Mice deficient for SSTR1 have been generated in our laboratory, and are currently being analyzed for their phenotype.

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Jürgen Schwarz, Institut für Physiologie, UKE, Hamburg
Wolfgang Meyerhof, Deutsches Institut für Ernährungs-forschung, Potsdam

2. Characterization of KET, a new protein related to the tumour suppressor p53

Hartwig Schmale, Casimir Bamberger, Julia Bethge, Heidje Christiansen, Gisela Olias**

Taste buds of vertebrates comprise a collection of 40-120 axonless taste receptor cells, together with supporting and precursor cells. The outer margin of taste buds is formed by flattened, concave cells that border the surrounding epithelial cells. Taste cells have a short life-time of about 10 days and are continuously replaced. Several lines of evidence indicate that taste cells and epithelial cells arise from common progenitor cells present in the local epithelium.

From a rat circumvallate (CV) taste papilla cDNA library we have isolated a clone encoding a novel protein, KET, which exhibits significant homology to parts of the tumour suppressor protein p53. The transcription factor p53 is implicated in cell-cycle control mechanisms that monitor the cell's health and thus prevent malignant cell proliferation. Despite the fact that p53 is involved in cell-cycle control and apoptosis, mice deficient for p53 are developmentally normal. This observation suggests that compensatory mechanisms exist during embryogenesis and development.

The KET gene shows remarkable homology to the molluscan p53 and, therefore, may represent a primordial p53 ancestor gene which appeared early in phylogenesis. Comparison of the deduced amino-acid sequence reveals strong similarity to all evolutionary conserved parts of the p53 protein. The identity reaches 75% in the regions which fold to form the sequence-specific DNA binding domain. Therefore, KET and another recently described protein, p73, are members of the new family of p53-related proteins. The human KET protein shares 98% identity with the rat homologue. We have mapped KET to human chromosome 3q27 and the murine homologue to mouse chromosome 16. Both chromosomal regions are deleted during genesis of endocrine pancreas tumours.

The persistence of the KET gene and its conservation point to an important function that cannot be fulfilled by p53. The expression pattern in the adult suggests that KET is involved in tissue-specific differentiation. *In situ* hybridization histochemistry has revealed high levels of KET mRNA in keratinocytes of the tongue epithelium, predominantly in the basal part of the trenches of taste papillae that contain taste buds. In the CV papilla, the onion-shaped taste buds seem to be embedded in keratinocytes expressing the KET gene.

Cells of the taste bud itself, including taste receptor cells which contain gustducin mRNA, do not possess KET transcripts. It is tempting to speculate that KET expression marks a special population of keratinocytes that subsequently enter the taste bud as precursor cells.

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3. Extrasomatic targeting of mRNAs and proteins in neurons

Stefan Kindler, Monika Rehbein, Arne Blichenberg, Michaela Monshausen, Susanne Thiessen*, Birgit Schwanke, Daniel Schober**

Neurons possess distinct cellular compartments that are highly diverse with respect to their protein repertoires. In particular, synapses serving as communication sites between nerve cells are equipped with a highly specialized set of molecules. Synaptic plasticity that underlies learning and memory seems to involve a synapse-specific modification of the protein composition. This adaptation is established by two cellular mechanisms, namely, specific targeting of somatically-synthesized proteins and extrasomatic protein synthesis near synapses. A limited set of mRNAs that seem to be translated in dendrites includes transcripts encoding the somatodendritic microtubule-associated protein 2 (MAP2) and the α subunit of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). To functionally characterize *cis*-acting sequences involved in dendritic mRNA targeting we have expressed tagged MAP2 and CaMKII mRNA fragments in cultured primary neurons. *Cis*-acting dendritic targeting elements (DTEs) are situated in the 3'-untranslated regions (3'UTRs) of both transcripts. Moreover, 640 nucleotides from the 3'UTR of the 10 kb MAP2 mRNA are sufficient to mediate dendritic localization of recombinant transcripts. In UV cross-linking assays, a 90 kDa rat brain protein specifically binds to this DTE. Currently, we are trying to biochemically purify the 90 kDa component and are utilizing the yeast tri-hybrid system to identify additional MAP2 DTE binding proteins.

Unlike MAP2 and CaMKII, members of a recently identified group of proteins, referred to as synapse-associated proteins (SAPs), seem to be exclusively synthesized in the soma and subsequently localized to synapses. SAPs are adaptor proteins that are likely to mediate the assembly of neurotransmitter receptors, ion channels, enzymes and cytoskeletal components into signal transduction complexes. Currently, we are trying to determine domains in SAPs that are involved in their subcellular targeting as well as characterizing their contribution to plastic changes at synapses.

4. Intracellular RNA transport

Evita Mohr, Nilima Prakash, Kerstin Heitmann, Iris Kächele*, Anke Peters*, Susanne Franke**

We are studying cytoplasmic transport of defined mRNA species to distinct subcellular domains in nerve cells. This process is mediated by several determinants including *cis*-acting signals within the mRNA molecule and *trans*-acting RNA binding proteins which ultimately guide mRNAs to their subcellular destinations, most likely along cytoskeletal elements. As a model system, we have chosen the

transcripts encoding the peptide hormone precursors vasopressin (VP) and oxytocin (OT). In the rat, these mRNAs which are abundant in magnocellular neurons of the hypothalamic neurohypophyseal system are not exclusively located in the cell somata. Substantial amounts are also sorted to axons and dendrites.
Microinjection of eukaryotic vector constructs containing the VP or OT cDNA into cell nuclei of *in vitro*-cultured sympathetic neurons (which do not express the peptide hormone genes) indicated targeting of both peptide hormone transcripts to dendrites, while transport to the axonal compartment was not observed. These data suggest that the machinery operating to sort RNA molecules to dendrites might be less cell-specific than that required for axonal transport. *Cis*-acting elements within the VP and OT mRNAs have been delineated.
Current work is aimed at identifying *trans*-acting factors which form part of the dendritic RNA transport machinery, for instance by specific interaction with the dendritic localizer elements. So far, we have characterized a 150 kDa protein which binds specifically to the 3'-untranslated region of the rat OT but not VP mRNA; this is currently being further purified.

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5. Core facility: DNA and protein analysis

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The goal of the core facility of the institute is to provide research groups at the Universitäts-Krankenhaus Eppendorf with state-of-the-art technologies for the analysis of DNA and proteins, namely, automatic non-radioactive DNA sequencing, genome analysis (e.g. microsatellite and AFLP mapping) and protein microcharacterization. At present the facility is equipped with two second-generation DNA sequenators (ABI 377), which meet up-to-date standards in flexibility, capacity and performance. In particular, the direct sequence analysis of PCR products is of growing importance in both pure and applied research and can be performed with high sensitivity and accuracy using the techniques provided by our laboratory.
The protein microsequencing facility has been equipped in the last year by a capillary HPLC blotter that complements the microbore HPLC which is used as a standard tool for the separation of protein digests in the lower picomole range. From such samples, as well as from intact proteins, sequence information is obtained by Edman degradation in order to identify known and to clone unknown proteins. In addition to the activities described above the facility provides research groups with synthetic oligonucleotides and peptides.

6. Molecular biology of amino-acid activated ion-channel receptors and corticotropin-releasing factor (CRF) receptors

Mark G. Darlison, Ute Breitenbach, Stefan E. Grote, Oliver Hannemann*, Olivera B. Nesic*, Sigrun Pohl, Thorsten Stühmer*, Christian Thode**

Molecular biology of vertebrate $GABA_A$ receptors. Complementary DNA (cDNA) cloning studies, in mammals, have revealed that the $GABA_A$ receptor, which mediates rapid inhibitory neurotransmission in brain and which is the site of action of several clinically-important drugs, is

constituted from six different types of subunit (named α, β, γ, δ, ε and π), three of which occur in a variety of isoforms (α1–α6, β1–β3 and γ1–γ3). These polypeptides assemble to yield a number of pentameric receptor subtypes that presumably fulfil subtly different physiological roles in the brain. Two additional polypeptides (β4 and γ4) occur in the chicken, and it appears that these replace the mammalian β1 and γ3 subunits, respectively.

Using *in situ* hybridization, we have shown that the γ4-subunit gene is expressed in the avian brain in structures that are either part of, or receive inputs from, auditory and visual pathways. One of these regions is the intermediate and medial part of the hyperstriatum ventrale, a forebrain area that is known to play a major role in visual imprinting, which is a form of recognition memory. Because of this interesting expression pattern, we have examined the effect of imprinting, on a visual stimulus, on the level of the corresponding transcript in different brain regions. This study has revealed a highly-significant, time-dependent, decrease (25% to 39%, dependent upon the brain region analyzed) in the amount of the γ4-subunit mRNA in trained animals compared to dark-reared controls. These data suggest that a down-regulation of GABAergic neuro-transmission plays a role in learning. Using this chick model, we are also looking at the effect of imprinting training on the expression of immediate-early genes such as arg3.1/*Arc* and ZENK.

The β4 subunit exhibits an interesting feature, and that is it forms robust homo-oligomeric GABA-gated channels in *Xenopus laevis* oocytes. This is in contrast to all other $GABA_A$ receptor polypeptides, which either do not form agonist-gated channels or do so only very poorly. Like native $GABA_A$ receptors, β4-subunit homo-oligomers are sensitive to picrotoxin (a channel blocker), loreclezole (a broad-spectrum anti-convulsant) and barbiturates (allosteric modulators). The cloned cDNA should prove useful in the characterization of ligand-binding sites as well as investigations of receptor assembly.

Molecular biology of Drosophila *glutamate receptors (GluRs).* A glutamate-gated cation channel, that has some interesting properties, is present on insect muscles. This channel is non-selective for cations, exhibits a very high unitary conductance (~120 pS) and can be blocked by certain spider and wasp toxins. To characterize this receptor, we have cloned two novel *Drosophila* full-length cDNAs that encode putative GluR subunits (named DGluR-III and DGluR-IV). Reverse transcription-polymerase chain reaction and *in situ* hybridization studies have indicated that the corresponding genes are expressed at significant levels in adult muscles but not in embryos or larvae.

Molecular biology of teleost fish CRF receptors. The neuropeptide CRF plays an important role in the response of an organism to stress. In mammals, this peptide binds to two receptor types, named CRF-R1 and CRF-R2. To gain insight into the evolution of this receptor family, and to try to understand the physiological roles of CRF and a related peptide (urotensin I) in fish, we have cloned and pharmacologically characterized the homologous receptors from the white sucker *Catostomus commersoni* and the chum salmon *Oncorhynchus keta*.

Collaborations

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Brian J. McCabe and Gabriel Horn, University of Cambridge
Eugene M. Barnes Jr, Baylor College of Medicine, Houston, Texas
Katharina Braun, Leibniz-Institut für Neurobiologie, Magdeburg
Henk Zwiers and Karl Lederis, University of Calgary

7. Molecular evolution and functional analysis of thyrotropin-releasing hormone (TRH) receptors

Thomas Bruhn, Friedrich Buck, Oliver Dammann, Sönke Harder, Ulrike Hubrig, Björn Ehlers**

The tripeptide TRH stimulates the secretion of thyroid-stimulating hormone from the anterior pituitary gland and, therefore, can be considered the driving force of the pituitary-thyroid axis. The TRH receptor (TRH-R) has been cloned from several mammalian species including mouse, rat and man, and exhibits considerable structural similarity. We have cloned TRH receptors from both *Xenopus* and teleost brain and found them to share 77% and 64% sequence identity, respectively, with the human TRH-R. We also identified a novel second TRH-R subtype in both species. This TRH-R2 (subtype 2) exhibits considerable divergence when compared to all known TRH-Rs, which are now referred to as TRH-R1. All TRH-Rs of the subtype 1 and 2 families contain highly conserved amino-acid residues that have been previously implicated in ligand binding. Our data on the molecular evolution of TRH receptors suggest that both TRH and its two receptor subtypes emerged early during evolution indicating the importance of these molecules for lower as well as higher vertebrates.

To identify intracellular determinants essential for signal transduction we have analyzed TRH-R1 mutants bearing deletions and point mutations within the third intracellular loop (IL3). Deletion analysis has revealed that most of IL3, with the exception of short sequences on the N- and C-terminal boundaries of the loop, can be removed without significant loss of receptor activity. Analysis of mutants bearing point mutations within these N- and C-terminal sequences has led to the identification of two residues near the C-terminal boundary of IL3 which are critically important for receptor signal transduction.

Collaborations

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8. Functional characterization of the novel neuropeptide orphanin FQ and other new transmitters

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Physiological functions of orphanin FQ. The neuropeptide orphanin FQ (OFQ) was discovered as the endogenous ligand of an opioid-like receptor. OFQ does not produce analgesic responses as classical opioids do, but instead has been shown to modulate stress-related variables of behaviour and sensory processing when injected into animals: OFQ is able to reverse stress-induced analgesia and produces anxiolytic-like effects. Further research on the physiological effects of OFQ have been hampered by the lack of a selective and high-affinity antagonist. We therefore took a genetic approach and generated OFQ-deficient mice by homologous recombination in embryonic stem cells. Analysis of phenotypic differences revealed that $OFQ^{-/-}$ mice show increased levels of anxiety and elevated nociceptive thresholds, compared to wildtype littermates. Another important function of OFQ for stress adaptation was

discovered via the observation that OFQ-deficient mice fail to develop tolerance following repeated exposure to stressful stimuli. These results suggest that the OFQ system may have important functions in the neural circuitry of stress processing.

Discovery of novel neurotransmitters. By mining the expressed sequence tag (EST) database, we have identified cDNA clones which encode partial sequences with homology to the family of glycoprotein hormone receptors (GPHRs). The three known GPHRs are the targets for lutropin (LH), follicle-stimulating hormone (FSH) and thyrotropin (TSH), which are key regulators of reproduction (LH and FSH) and energy homeostasis and development (TSH). We could identify three novel receptors with significant homology to the known GPHRs. The most striking feature of the new GPHR-like receptors is a long N-terminal extracellular domain which harbours multiple copies of so-called leucine-rich repeats. Since these structures are known to be involved in protein-protein interaction and have been shown to mediate ligand-binding of GPHRs, we assume that the natural ligands for these novel receptors are high molecular weight proteins. The cloned GPHR-like receptors will be used to identify their endogenous ligands in order to study their physiological functions.

Collaborations

Francois Jenck, Hoffmann-La Roche, Basel
Anja Köster, Eli Lilly and Co., Indianapolis
Olivier Civelli, University of California, Irvine
Huda Akil, Mental Health Research Institute, University of Michigan, Ann Arbor

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Diploma

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Dissertations

Olias, Gisela (1997). Heterologe Expression des normalen und eines mutanten Vasopressin-Neurophysin-Vorläufers in einer Hypophysentumorzellinie der Maus als Modellsystem für den familiären hypothalamischen Diabetes insipidus. Universität Hamburg.

Prakash, Nilima (1997). Subzellulärer Transport von Vasopressin mRNA in kultivierten Neuronen der Ratte. Universität Hamburg.

Roth, Adelheid (1997). Internalisierung und Desensibilisierung der fünf Somatostatin-Rezeptor-Subtypen der Ratte. Universität Hamburg.

Breitenbach, Ute (1998). Organisation und funktionelle Charakterisierung der 5'-flankierenden Region des Gens der γ2-$GABA_A$-Rezeptoruntereinheit des Haushuhns (*Gallus gallus domesticus*). Technischen Universität Darmstadt.

Glos, Michael (1998). Genstruktur und Promoteruntersuchungen von Somatostatinrezeptoren der Ratte. Universität Hamburg.

Greten, Florian (1998). Sequenz, Pharmakologie, Gewebeverteilung und Evolution eines μ-Opioidrezeptors aus dem niederen Vertebraten *Catostomus commersoni*. Universität Hamburg.

Habilitation

Bruhn, Thomas (1999). Untersuchungen zur Regulation des Thyrotropin-Releasing Hormone und seines Rezeptors. Universität Hamburg.

Structure of the Institute

Director:	Prof. Dr. Dietmar Richter
Deputy Director:	Prof. Dr. Hartwig Schmale
Research Associates:	PD Dr. Evita Mohr
	Dr. Stefan Kindler
	Dr. Hans-Jürgen Kreienkamp
	Dr. Monika Rehbein
	Dr. Dietmar Bächner*
Graduate Students:	Ercan Akgün*
	Casimir Bamberger
	Julia Bethge*
	Necla Birgül*
	Arne Blichenberg
	Annette Busch*
	Michael Glos*
	Kerstin Heitmann
	Iris Kächele*
	Michaela Monshausen*
	Gisela Olias*
	Sigrun Pohl
	Nilima Prakash*
	Rüdiger Reinking*
	Daniel Schober*
	Claus-Peter Schwartkop*
	Anja Schwärzler*
	Susanne Thiessen*
	Heike Zitzer*
Technicians:	Heidje Christiansen
	Günther Ellinghausen
	Susanne Franke*
	Hans-Hinrich Hönck
	Anke Peters*
	Birgit Schwanke
DNA/Peptide Facility:	Dr. Friedrich Buck
Technicians:	Sönke Harder
	Agata Blaszcyk-Wewer
Laboratory Assistance:	Ruth Heins
Maintenance:	Fahriye Dilli
	Hatice Kayhan
Guests:	Emeritus Professor
	Dr. Gebhard Koch
	Prof. Dr. Wolfgang Meyerhof

Independent Research Groups:

Group Leader:	Dr. Mark G. Darlison
Postdoctoral Fellows:	Dr. Olivera B. Nesic*
	Dr. Thorsten Stühmer*
Graduate Students:	Ute Breitenbach*
	Stefan E. Grote
	Oliver Hannemann*
	Christian Thode*
Group Leader:	Dr. Rainer K. Reinscheid*
Postdoctoral Fellows:	Dr. Xu Li*
	Dr. Alexandra Montkowski*
	Dr. Axel Methner*
Graduate Student:	Marcus Christenn*
Technicians:	Eva-Maria Stübe
	Kristina Knaudt*
Habilitationsstipendiat:	Dr. Thomas Bruhn
Graduate Student:	Oliver Dammann
Technicians:	Ulrike Hubrig*
	Björn Ehlers*

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Internet (Blankenese Conferences): http://www.uke.uni-hamburg.de/blankenese_conferences/

*during part of the reported period

Teaching, Seminars

Wintersemester 1998/99

Aufbaustudiengang Molekularbiologie

Vorlesung und Seminar: Molekularbiologie I
Jentsch, Schaller und Mitarbeiter (2 st.)

Vorlesung und Seminar: Molekulare Neurobiologie
Schachner Camartin und Mitarbeiter (2 st.)

Praktikum I (mit begl. Seminar)
Gentechnologische Methoden
Dozenten und Mitarbeiter des ZMNH (10 SWS n.V.)

Praktikum III (mit begl. Seminar)
Molekular- und Zellbiologische Methoden
Dozenten und Mitarbeiter des ZMNH (10 SWS n.V.)

Wissenschaftliches Forschungsprojekt
Dozenten des Aufbaustudiengangs (gztg. n.V.)

Seminare für Mediziner und Naturwissenschaftler

Vortragsreihe: ZMNH-Seminar für Mediziner und Naturwissenschaftler
Dozenten und wissenschaftliche Mitarbeiter des Zentrums (2 st.)

Praktikum für molekulare Neurobiologie
Jentsch, Kuhl, Nitsch, Pongs, Schachner Camartin, Schaller, Wegner (6 Wo. gztg. n. V.)

Anleitung zum selbständigen wissenschaftlichen Arbeiten
Jentsch, Kuhl, Nitsch, Pongs, Schachner Camartin, Schaller, Wegner (n. V.)

Praktikum: Elektrophysiologische Methoden für Fortgeschrittene
Friedrich, Jentsch (2 Wo. gztg. n. V.)

Literaturseminare

Membrantransport: Zellbiologie und Pathophysiologie
Jentsch und Mitarbeiter (2 st.)

Ionenkanäle
Pongs und Mitarbeiter (2 st.)

Neurale Plastizität
Kuhl (2 st.)

Entwicklungsbiologie und Neurobiologie
Schaller und Mitarbeiter (1 st.)

Zellspezifische Regulation der Genexpression
Sock, Wegner (2 st.)

Neurale Zellerkennungsmoleküle und Zellinteraktionen bei der embryonalen Entwicklung, Regeneration und synaptischen Plastizität
Schachner Camartin und Mitarbeiter (2 st. n.V.)

Alzheimer Krankheit
Nitsch (2 st.)

Forschungsseminare

Neuropeptidwirkung, Signaltransduktion
Schaller und Mitarbeiter (2 st.)

Transsynaptische Regulation der Genexpression
Kuhl (2 st.)

Ionenkanäle
Jentsch und Mitarbeiter (2 st.)

Neurale Signalverarbeitung
Pongs und Mitarbeiter (2 st.)

Molekulare Grundlagen der Gliazell-Differenzierung
Sock, Wegner (2 st.)

Molekulare Grundlagen neuropathologischer Erkrankungen
Nitsch (2 st.)

Neurale Zellerkennungsmoleküle
Schachner Camartin und Mitarbeiter (2 st. n.V.)

Sommersemester 1999

Aufbaustudiengang Molekularbiologie

Vorlesung: Molekularbiologie II
Pongs und Mitarbeiter (2 st.)

Vorlesung und Seminar: Molekulare Neuropathologie
Jentsch, Schaller und Mitarbeiter

Praktikum II (mit begl. Seminar)
Gentechnologische Methoden
Dozenten und Mitarbeiter des ZMNH (10 SWS n.V.)

Wissenschaftliches Forschungsprojekt
Dozenten des Aufbaustudiengangs (gztg. n.V.)

Seminare für Mediziner und Naturwissenschaftler

Vortragsreihe: ZMNH-Seminar für Mediziner und Naturwissenschaftler
Dozenten und wissenschaftliche Mitarbeiter des Zentrums (2 st.)

Vorlesung und Seminar: Molekulare Fragestellungen in Neurologie, Neurochirurgie und Psychiatrie
Methner und Projektleiter des Graduiertenkollegs “Neurale Signaltransduktion und deren Pathologie” (2 st.)

Vorlesung und Seminar: Entwicklungsbiologie
Bach, Wegner, Schaller und Mitarbeiter (2 st.)

Vorlesung und Seminar: Signaltransduktion im Nervensystem
Professoren und Dozenten des Graduiertenkollegs “Neurale Signaltransduktion und deren Pathologie” (2 st.)

Praktikum für molekulare Neurobiologie
Jentsch, Kuhl, Nitsch, Pongs, Schachner Camartin, Schaller, Wegner (6 Wo. gztg. n. V.)

Anleitung zum selbständigen wissenschaftlichen Arbeiten
Jentsch, Kuhl, Nitsch, Pongs, Schachner Camartin, Schaller, Wegner (n. V.)

Praktikum: Elektrophysiologische Methoden für Fortgeschrittene
Jentsch, Waldegger, Weinreich (2 Wo. gztg. n. V.)

Literaturseminare

Membrantransport: Zellbiologie und Pathophysiologie
Jentsch und Mitarbeiter (2 st.)
Ionenkanäle
Pongs und Mitarbeiter (2 st.)

Neurale Plastizität
Kuhl (2 st.)

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Schachner Camartin und Mitarbeiter(2 st. n.V.)

Alzheimer Krankheit
Nitsch (2 st.)

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Neuropeptidwirkung, Signaltransduktion
Schaller und Mitarbeiter (2 st.)

Trans-synaptische Regulation der Genexpression
Kuhl (2 st.)

Ionenkanäle
Jentsch und Mitarbeiter (2 st.)

Neurale Signalverarbeitung
Pongs und Mitarbeiter (2 st. n.V.)

Molekulare Grundlagen der Gliazell-Differenzierung
Sock, Wegner (2 st.)

Molekulare Grundlagen neuropathologischer Erkrankungen
Sock, Wegner (2 st.)

Molekulare Grundlagen neuropathologischer Erkrankungen
Nitsch (2 st.)

Neurale Zellerkennungsmoleküle
Schachner Camartin und Mitarbeiter (2 st. n.V.)

Official Events, Meetings

Official Visitors

29.05.1997

Senator der Umweltbehörde, Hamburg

Dr. Fritz Vahrenholt

03.04.1998

Senatorin für Wissenschaft und Forschung, Hamburg

Frau Krista Sager

19.01.1999

Staatsrätin der Behörde für Wissenschaft und Forschung, Hamburg

Frau Prof. Dr. Marlis Dürkop

Meetings

29.06. - 03.07.1997

17th Blankenese Conference

Neurodegeneration

Organizers
Dietmar Kuhl, Roger Nitsch, Michael Wegner

Session I

Mike Knudson, St. Louis
Bcl-2 gene family and the regulation of programmed cell death

Hermann Steller, Cambridge
Molecular biology of apoptosis

Mark Noble, Salt Lake City
From rodent glial precursor cell to human glial tumor in the oligodendrocytetype-2 astrocyte lineage

Rolf Heumann, Bochum
Does neuronal modulation of P21RAS activity induce neurotrophic effects?

Karl Whitney, Durham
Glutamate receptor autoantibodies and Rasmussen's encephalitits

Session II

Christine M. Gall, Irvine
Seizure regulation of neurotrophic factor expression: implications for protection and plasticity

James I. Morgan, Memphis
The role of transcriptional responses during neurodegeneration

Jeffrey L. Noebels, Houston
Gene dysregulation and cell death in developing epileptic brain

Sidney Strickland, Stony Brook
An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus

Session III

Hans Lassmann, Wien
Patterns of cell death in inflammatory and degenerative diseases of the central nervous system

George C. Ebers, London, Ontario
Genetic susceptibility in multiple sclerosis

Ari Waisman, Köln
The Fragility of the Th1/Th2 Hypothesis in Multiple Sclerosis

Short Communications

Katerina Akassoglou, Athen
Primary and destructive demyelination induced by the central nervous system production of TNF

Paul J. Lucassen, Leiden
Temporal aspects of PrP deposition, microglial activation, cytokine immuneroeactivity and neuronal apoptosis in murine scrapie

Frank Gillardon, Köln
CPP-32, an ICE-related protease, is activated in hippocampal neurons following ischemia and epilepsy

Jochen Röper, Hamburg
Heterogeneous expression of ATP-sensitive potassium channel isoforms in single dopaminergic substantia nigra neurons

Lars Theill, Thousand Oaks
Neuritin: a gene induced by neural activity and neurotrophins that promotes neuritogenesis

Marius Ueffing, München
Identification of PKC-induced neuronal differentiating activity (PNDA) as the rat homologue of human pigment epithelium derived factor

Fred van Leuven, Leuven
Analysis of expression and histopathology in brain of APP/RK transgenic mice

Ulrike Müller, Frankfurt
Transgenic models to understand the physiological role of the amyloid precursor protein gene family

Rick Preddie, Hamburg
Autoimmune response to endogenous pathogenic proteins: a powerful link between inflammation and neurodegeneration

Session IV

Walter Doerfler, Köln
On the molecular biology of the fragile X syndrome

Chica Schaller, Hamburg
Neuroprotective role of the neuropeptide head activator

Session V

Jean C. Manson, Edinburgh
PrP gene dosage and allelic specificity in the transmissible spongiform encephalopathie

Hans A. Kretzschmar, Göttingen
Neuronal cell death in prion diseases

Bruno Oesch, Zürich
Interactions of the prion protein with itself and other proteins

Adriano Aguzzi, Zürich
Modelling the pathogenesis of prion diseases in brain grafts

Eva-Maria Mandelkow, Hamburg
Alzheimer's disease, paired helical filaments and tau protein: structure, aggregation, and phosphorylation

Session VI

Rudolph E. Tanzi, Charlestown
Presenilin and APP processing in Familial Alzheimer's Disease
Sangram S. Sisodia, Baltimore
Molecular biology of presinilin 1

Christian Haass, Mannheim
Presinile because of presenilin: The cell biology of presenilin proteins in mammalian cells and Caenorhabditis elegans

Christopher Eckman, Jacksonville
Analysis of plasma Aβ concentration: The role of Aβ42 in Alzheimer's Disease

Karen K. Hsiao, Minneapolis
The biology of APP transgenic mice

20.08.1997

Symposium
Transgenic mice in biomedical research

Applicants for leader position of ZMNH transgenic facility

Michael Bösl, Hamburg
John McLaughlin, Freiburg
Thomas Theil, London

13./14.10.1997

Site Visit
by the Scientific Advisory Board

Lectures

Dietmar Kuhl
Synaptic plasticity: Learning about activity-dependent genes

R. M. Nitsch
Presenilins in Alzheimer's disease

M. Wegner
Transcription factors and glial development

H. C. Schaller
Head-activator receptor and signal transduction

M. Schachner
Neural recognition molecules in development and regeneration

T. J. Jentsch
New functions for CLC chloride channels

07.05.1998

Berichtskolloquium für das Graduiertenkolleg

Neurale Signaltransduktion und deren pathologische Störungen

Gutachter und Berichterstatter:

Frau Prof. Dr. H. Hörtnagel
Herr Prof. Dr. E. Gundelfinger
Herr Prof. Dr. R. Heumann
Fra u Prof. Dr. D. Schendel
Frau Dr. G. Wandt
Frau S. Mönkemöller

Ralf Bruns (Röper)

Funktionelle und molekulare Charakterisierung eines A-Typ Kaliumkanals in dopaminergen Neuronen der Substantia nigra

Ulrich Putz (Kuhl)
Dendritische Lokalisierung der arg3.1 mRNA

Stefan Grote (Darlison)
Isolierung und Charakterisierung ionotroper Glutamat-rezeptoren

Roland Schäfer(Schwartz)
Charakterisierung eines einwärtsgleichrichtenden Kalium-stroms in laktotrophen Zellen der Ratte

Jörg Schreiber(Wegner)
Transkriptionskontrolle bei der Gliadifferenzierung

Susanne Wegener (Schaller)
Kopfaktivator-Signaltransduktion in neuroektodermalen Zellen

Tanja Kampers (Mandelkow)

Einfluß verschiedener Isoformen des TAU-Proteins auf die artifizielle Bildung paariger helikaler Filamente

04.06.1998

Symposium

New Aspects in Molecular Neurobiology

Applicants for group leader positions

Gerard Drewes, Hamburg
MARK – a novel family of protein kinases as regulators of the microtubule cytoskeleton

Bernd Stahl, Göttingen
Alzheimer's disease-related presenilin interacts directly with a novel armadillo protein

Roger Janz, Dallas
Synaptic vesicle proteins as regulators of neurotransmitter release

Maike Sander, San Francisco
Role of the homeodomain transcription factor Nkx6.1 in pancreatic beta-cell and motor neuron development

Thomas Ciossek, Tübingen
Topographic tetinotectal projection – do Eph tyrosine kinases do it all?

Dieter Riethmacher, Berlin
The roles of erbB2 and erbB3 in the peripheral nervous system

25.06.1998

Site Visit by Projektträger BEO of BMBF, Berlin

Members:
Prof. Dr. Ferdinand Hucho
Frau Dr. Angela Hagen
Frau Bärbel Weiher

Dietmar Kuhl, Hamburg
Learning about Activity-Dependent Genes

Michael Wegner, Hamburg
Transcription Factors in Early Neural Development

Roger Nitsch, Hamburg
Diagnosis and Treatment of Alzheimer's Disease

12./13.10.1998

ZMNH-Retreat

25.11.1998

Workshop

DFG Forschergruppe

Intrazellulärer RNA-Transport

Anne Ephrussi, Heidelberg
Establishment of embryonic polarity in Drosophila by RNA localization and translational control

Hans-Georg Kräusslich, Hamburg
Intracellular transport of retroviral RNA: cis elements and trans-acting factors

Helena Santos-Rosa, Heidelberg
Nuclear mRNA export requires complex formation between Mex67p and Mtr2p at the nuclear pores

Stefan Kindler, Hamburg
Dendritic transport and translation of MAP2 mRNAs

Monika Rehbein, Hamburg
Trans-acting factors of dendritic mRNA targeting

Joel Yisraeli, Jerusalem
RNA and protein localization in vertebrates: A conserved protein family that bridges the gap between microtubule and microfilament-mediated mechanisms

Antoine Triller, Paris
Post synaptic machinery for synthesis of synaptic receptors

Dietmar Kuhl, Hamburg
Learning about activity-dependent genes

Evita Mohr, Hamburg

Peptide hormone encoding mRNAs: analysis of subcellular mRNA transport mechanisms

Jürgen Brosius, Münster

Translational regulation in dendrites mediated by small RNAs?

27.11.-29.11.1998

SFB 444 - Symposium

Grundlagen Neuraler Kommunikation und Signalverarbeitung

R. McKay, Bethesda
From stem cells to the first circuit in the CNS

T. Jentsch, Hamburg
Intracellular CIC-channels

J. R. Schwarz, Hamburg
EAG K currents in rat lactotrophs

J. Roeper, Hamburg
Molecular and functional properties of voltage-gated K channels in dopaminergic midbrain neurons

M. G. Darlison, Hamburg
Expression of ligand-gated ion-channel and immediate early genes in the chick brain and their relationship to imprinting training

B. A. Oostra, Rotterdam
Fragile X-Syndrome is caused by a fragile gene

S. Kindler, Hamburg
Molecular and functional characterization of synapse-associated proteins

D. Kuhl, Hamburg
Learning about activity dependent genes

R. Nitsch, Hamburg
Identification of m1 actylcholine receptor-inducible genes

J. Dannenberg, Hamburg
Frequenin

E.-M. Mandelkow, Hamburg
Regulation of microtubule-associate proteins and micro-tubule dynamics by phosphorylation

P. Seeburg, Heidelberg
Glutamate Receptors

M. Wegner, Hamburg
Structure and Function of the Drosophila protein Glial Cells Missing (MCM) and its mouse homolog

U. Bartsch, Hamburg
Mice deficient in the neural adhesion molecule L1: an animal model for the human hereditary disease CRASH

U. Finckh, Hamburg
In vitro expression of human L1CAM cDNA and its pathogenic variants

I. Hermans-Borgmeyer, Hamburg
A novel type of receptor proteins expressed in the mammalian nervous system

U. Beisiegel, Hamburg
Characterization of lipoproteins and lipoprotein-receptors in neuronal cells of mouse embryos

01.02.1999

Symposium

New Topics in Molecular Neurobiology
Applicants for group leader position

Mathias Treier, San Diego
Signalling control of pituitary gland

Chantal Bazenet, London
Regulators of cell death in the developing sympathetic neuron

Allessandro Cellerino, Pisa
The physiological action of neurotrophic factors on the development of neural connections studied in the retina

Thomas Schimmang, Valladolid
Roles of neurotrophins and FGFs during inner ear development

Jens Coorssen, Bethesda
Studying the late steps of Ca^{2+}-triggered exocytosis

Spiros Efthimiopoulos, New York
Regulation of the secretion of the Alzheimer's amyloid precursor protein. Mechanism for the production of amyloid beta protein

24.03. - 26.03.1999

DFG/BMBF Workshop Alzheimer Forschergruppe

Molekulare Pathomechanismen der Alzheimer-Krankheit

Konrad Maurer, Frankfurt
Aloys Alzheimer - Leben, Werk, Ausblick

Session
Molekulare Genetik der Alzheimer Krankheit

Klaus-Peter Lesch, Würzburg
Serotonin-Transporter: Bedeutung für synaptische Plastiziät und neurodegenerative Prozesse

Ulrich Finckh, Hamburg
Mutationsanalysen und genetische Assoziationsstudien bei der Alzheimer-Krankheit

Bernd Janetzky, Dresden
Biochemische und molekulargenetische Veränderungen des mitochondrialen Energiestoffwechsels bei Patienten mit Morbus Alzheimer

Session
Klinische Marker der Alzheimer Krankheit

Christoph Hock, Basel
Biochemische Marker der Alzheimer-Krankheit

Reinhard Prior, Düsseldorf
Liquor-Diagnostik der ß-Amyloidpathologie mittels Fluoreszenz-Korrelations-Spektroskopie (FCS)

Thomas-Müller Thomsen, Hamburg
Klinische Parameter und biochemische Marker bei Patienten mit Alzheimer Demenz

Session
Zytoskelett-Pathologie

Heiko Braak, Frankfurt
Über die selektive Vulnerabilität bei Morbus Alzheimer und verwandten neurodegenerativen Erkrankungen

Eva-Maria Mandelkow, Hamburg
Tau-Protein, Phosphorylierung und Effekt auf intrazellulären Transport

Jürgen Götz, Zürich
Entwicklung transgener Mausmodelle der Alzheimer-Krankheit

Roland Brandt, Heidelberg
Simulierung einer PHF-ähnlichen Phosphorylierung von Tau

Melitta Schachner, Hamburg
Neurale Zelladhäsionsmoleküle und neurale Degeneration

Session
Apolipoprotein E und Oxidation

Gerd Multhaup, Heidelberg
Oxidativer Stress und APP Metabolismus

Peter Riederer, Würzburg
Oxidativer Stress bei neurodegenerativen Erkrankungen

Ulrike Beisiegel, Hamburg
Lipoproteinoxidation in der Alzheimer Krankheit

Session
Amyloid

Patrick Keller, Heidelberg
Die Rolle von Cholesterol in der APP Prozessierung

Matthias Staufenbiel, Basel
Transgene Tiermodelle der Alzheimer-Krankheit

Ulrike Müller, Frankfurt
Einzelne und kombinierte knockouts der APP Familienmitglieder

Session
Neurodegeneration - Apoptose

Christian Behl, München
Neuroprotektion gegen den Alzheimer-assozierten Nervenzellentod

Christian Kaltschmidt, Freiburg
Eine neuroprotektive Rolle des Transkriptionsfaktors NF-κB in der Alzheimer-Krankheit

Thomas Arendt, Leipzig
Veränderungen der intrazellulären Signaltransduktion und Zellzyklusmechanismen in der Alzheimer-Krankheit

Session
Presenilin

Christian Haass, Mannheim
Presenilin - biologische und pathologische Funktionen

Paul Saftig, Göttingen
Evaluation von Presenilinfunktionen bei presenilindefizienten Mäusen

Ralf Baumeister, München
Analyse von Presenilinfunktionen in *Caenorhabditis elegans*

Helmut Jacobsen, Basel
Endoproteolyse von Presenilin 2: Eine strukturelle und funktionale Charakterisierung

24.06. - 27.06.1999

19th Blankenese Conference

10th Anniversary of the ZMNH
Advances in Molecular Neurobiology

Opening Session

Dietmar Richter, Hamburg
Chica Schaller, Hamburg
Krista Sager, Hamburg
Ulrich Schlüter, Bonn
Jürgen Lüthje, Hamburg
Heinz-Peter Leichtweiß, Hamburg
Reinhard Grunwald, Bonn

Konrad Beyreuther, Heidelberg
Aging without Alzheimer's disease - moving from molecular pathology to prevention

Session I

Betty Eipper, Baltimore
Neuropeptide amidation and the cytoskeleton

Cornelis Grimmelikhuijzen, Copenhagen
Neurohormones and their receptors in invertebrates

Dusan Zitnan, Bratislava
Ecdysteroid-induced expression of ETH gene

Mark Darlison, Hamburg
Co-evolution of neuropeptides and their receptors

Session II

Michael Wegner, Hamburg
Transcription factors in neural development

Christo Goridis, Marseille
Phox2a and Phox2b: master regulators of neuronal types?

Fritz Rathjen, Berlin
Regulation of axonal growth by members of the immunoglobulin superfamily

Jonathan Raper, Philadelphia
A dominant negative receptor for secreted semaphorins

Peter Sonderegger, Zürich
Protease-controlled extracellular signal amplification cascades in the CNS

Evening Session

Klaus-Armin Nave, Heidelberg
Myelin-deficient mice and a family of proteolipid proteins

Hannah Monyer, Heidelberg
Characterization of native glutamate receptors and manipulation thereof in identified cell populations

Session III

Christine Gall, Irvine
Activity and adhesion: Dual synaptic mechanisms regulating brain neurotrophin expression

Dietmar Kuhl, Hamburg
Learning about activity-dependent genes

Craig Garner, Birmingham
Assemblying synaptic junctions of the CNS

Reinhard Jahn, Göttingen
Control of exocytosis in neurons

Eckart Gundelfinger, Magdeburg
Novel protein components of CNS synapses and their role in synaptic assembly and function short communications

Hans-Jürgen Kreienkamp, Hamburg
SSTRIP: a novel human multidomain protein

Session IV

Thomas Jentsch, Hamburg
Disease due to mutations in KCNQ potassium channels

James Morgan, Memphis
Genetic pathways of neuronal death and regeneration

Michael Sendtner, Würzburg
Molecular mechanisms of motoneuron degeneration

Ferdinand Hucho, Berlin
Frozen allosteric states of the nicotinic acetylcholine receptor

Session V

Bart de Strooper, Leuven
Disturbed notch and amyloid precursor protein processing in the brain of presenilin 1 deficient mice

Christian Haass, Mannheim
Proteolytic processing of presenilins - implications for their biological pathological function

Roger Nitsch, Hamburg
Identification of a novel susceptibillity gene for Alzheimer's disease

Heinrich Betz, Frankfurt
Assembly of the glycinergic postsynaptic membrane

Michael Lazdunski, Valbonne
Ion channels with properties of acid- and mechano-sensors

Financing

The ZMNH was financed in 1997 and 1998 by the City-state of Hamburg (FuHH), the Bundesministerium für Bildung und Forschung (BMBF), and by grants from research foundations and industry.
The BMBF funded three research groups, the FuHH provided the budget for the institutes, the central facilities, and the building.

In 1997 and 1998 the total budget of the Center amounted to 15.277 and 16.769 million DM, respectively. At present 220 people are employed at the ZMNH.

Financing by FuHH and BMBF

Personnel and running costs contributed by FuHH and BMBF (in thousand DM):

		Personnel costs	Running costs*
1997	FuHH	6.657	3.098
	BMBF	1.087	358
	totalling	7.744	3.456
1998	FuHH	6.864	2.967
	BMBF	1.164	660
	totalling	8.028	3.627

* excluding investments

Other financing

In 1997 and 1998 members of the Center received support from the Deutsche Forschungsgemeinschaft (DFG) via individual project grants, research groups, SFB's and graduate programs. Further support was provided by the Stiftung Volkswagenwerk, the European Community, and others. Outside support amounted to 9,191 million DM for 1997 and 1998.
The personnel and running costs given by the various funding agencies were (in thousand DM):

		Personnel costs	Running costs*
1997	DFG	1.796	730
	VW Stiftung	114	55
	EEC	145	-
	Foundations, Industry	396	127
	SFB 444, 470, 545	466	248
	totalling	2.917	1.160
1998	DFG	2.074	420
	VW Stiftung	185	21
	EEC	132	5
	Foundations, Industry	537	133
	SFB 444, 470, 545	1.153	454
	totalling	4.081	1.033

* excluding investments

Structure of the Center

Director

Prof. Dr. Dr. Thomas Jentsch
1995 - 1998
tel: 040-42803-4741
fax: 040-42803-4839

Prof. Dr. Chica Schaller
since December 21, 1998
tel: 040-42803-6277
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Institutes

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