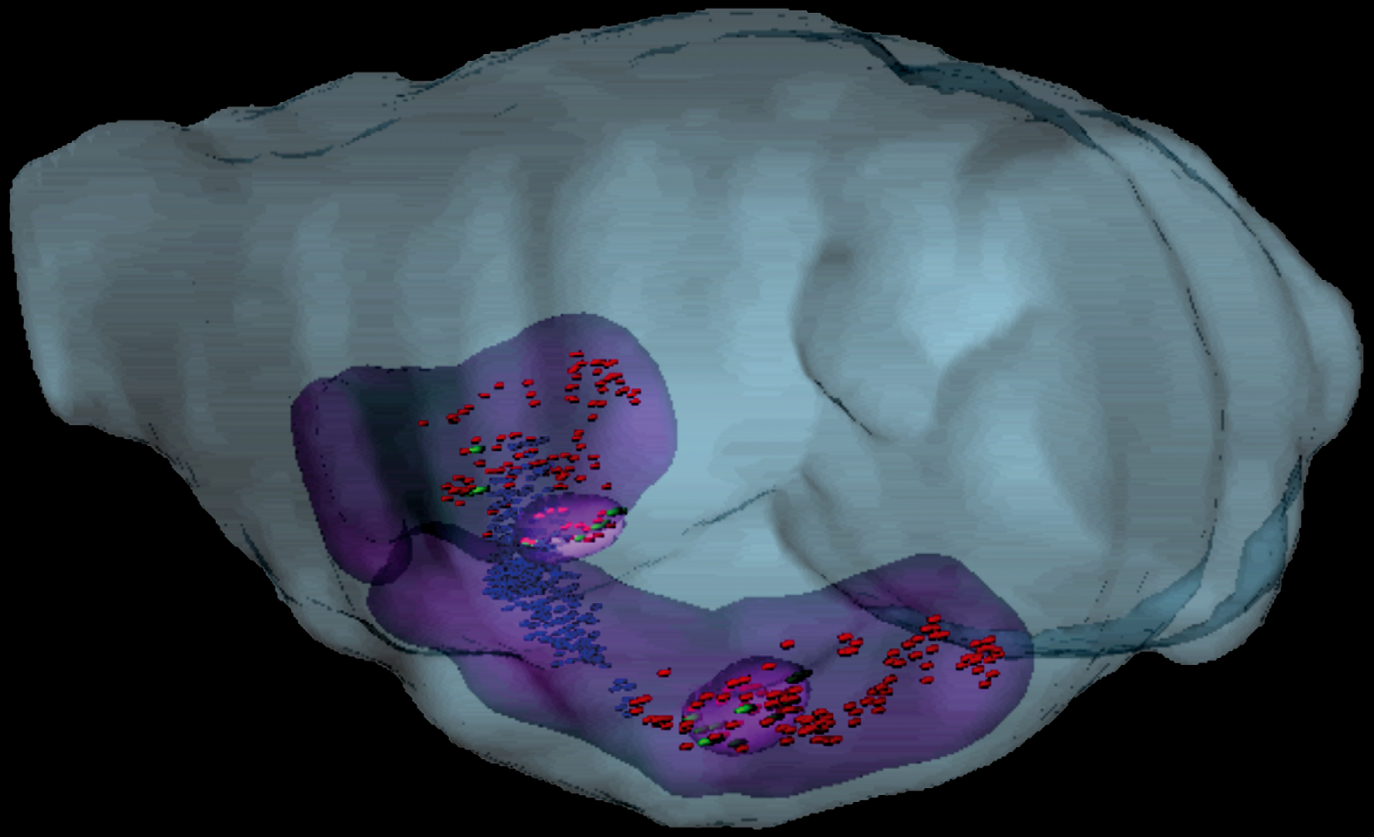
Zentrum für Molekulare Neurobiologie · Universitätsklinikum Hamburg-Eppendorf



ZMNH

Report 2004-2006

Front Cover: Three-dimensional reconstruction of a mouse brain visualizing neural circuits linking odor and pheromone information with the hypothalamic centre of reproduction. Shown are odor and pheromone relaying neurons (red) in the piriform cortex and olfactory amygdala upstream of GnRH neurons (blue).

Foto: Dr. Ulrich Boehm

Report 2004-2006

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Vorwort des Ärztlichen Direktors UKE und des Dekans

Seit seiner Gründung hat sich das Zentrum für Molekulare Neurobiologie (ZMNH) an der Medizinischen Fakultät des Universitätsklinikums Eppendorf (UKE) zu einem hochkarätigen Kristallisationspunkt für die biomedizinische Grundlagenforschung entwickelt, der weit über die Stadt- und Landesgrenzen hinaus einen hohen internationalen Ruf erlangt hat.

Das dem ZMNH zugrunde liegende Konzept eines wissenschaftlichen „Center of Excellence“ hat sich bewährt. Unter den Hamburger Forschungseinrichtungen hat das ZMNH Leuchtturmfunktion. Es war und ist Initiator vieler interdisziplinärer Projekte und hat die neurowissenschaftliche Forschung auf höchstem Niveau vorangetrieben. Die zahlreichen Veröffentlichungen von ZMNH-Arbeitsgruppen in exzellenten internationalen Fachzeitschriften und die vielen renommierten Preise, die an Wissenschaftler des ZMNH verliehen wurden, sprechen in dieser Hinsicht eine eindeutige Sprache. Die beeindruckende Leistungsbilanz des ZMNH ist eine Reflektion mustergültiger Zusammenarbeit zwischen Grundlagen- und klinischer Forschung. Sie hat vielfach zu bedeutenden Weiterentwicklungen in der Diagnostik und Therapie von Krankheiten geführt – zum Wohle der Patienten.

Gegenwärtig befindet sich das ZMNH in einer Phase der Neuorientierung. Dieser Prozess wurde vorangetrieben durch einen bereits stattgehabten bzw. noch bevorstehenden Führungswechsel an der Spitze der vier Institute aus der Gründungsphase. Solche Phasen der Veränderung bergen ein großes Potential für Weiterentwicklungen, stellen aber gleichzeitig auch im Übergang schwierige Herausforderungen für die Beteiligten dar. Fest steht, dass der Modellcharakter des ZMNH für die wissenschaftliche Zukunft des Universitätsklinikums von großer Bedeutung ist. Aus diesem Grund ist auch für die Zukunft die bevorzugte Finanzierung des ZMNH mit über 8 Millionen Euro aus dem UKE Zuführungsbetrag für Forschung fest eingeplant. Damit einher geht die Erwartung, dass die an vielen Stellen schon bestehenden Kooperationen zwischen ZMNH und anderen Instituten bzw. Kliniken des UKE weiter intensiviert werden.

Wir gehen fest davon aus, dass die anstehenden Besetzungen der Führungspositionen innerhalb des ZMNH mit hochkarätigen und international angesehenen Wissenschaftlern bald erfolgreich abgeschlossen sein werden. Mit dem Amtsantritt von Herrn Prof. Roland Martin als Leiter des von der Hertie-Stiftung mitfinanzierten neuen ZMNH Instituts für Neuroimmunologie und Klinische Multiple Skleroseforschung ist ein erster personeller und inhaltlicher Eckpfeiler für eine erfolgreiche ZMNH Zukunft erkennbar geworden. Wir werden alles unternehmen, damit weitere Eckpfeiler rasch folgen werden. Wir freuen uns auf ein noch leistungsstärkeres ZMNH als Motor für die Entwicklung künftiger disziplinen- und einrichtungsübergreifender Verbundforschungsvorhaben innerhalb unseres Universitätsklinikums.

Im April 2007

Prof. Dr. Jörg F. Debatin
Ärztlicher Direktor

Prof. Dr. Dr. Uwe Koch-Gromus
Dekan

Universitätsklinikum Hamburg-Eppendorf

Preface of the Medical Director and the Dean

The Center of Molecular Neurobiology (ZMNH) of the University Medical Center Hamburg Eppendorf (UKE) has rapidly developed into a focal point of excellence in basic biomedical research.

The ZMNH has been the catalyst of a mulitude of interdisciplinary projects and has clearly advanced neuroscience at the highest level. The combination of rich output of seminal scientific work published in most renowned international peer review journals and numerous scientific awards bestowed upon the researchers of the ZMNH attest to the institute's international reputation. The impressive performance of the ZMNH is a reflection of exemplary cooperation between basic and clinical aims. As such it has vastly contributed to improvements in diagnosis and therapy of disease to the benefit of our patients.

Currently, the ZMNH is undergoing a phase of re-orientation. This process was initiated by imminent changes in leadership of the four original institutes. While such phases of change harbour great potential for an even more successful future, they are also a cause for insecurity and doubt. In times like these it seems important to re-affirm the commitment of the University Medical Center Hamburg-Eppendorf to conserve the model character of the ZMNH. Thus, the preferred annual budgeting of the ZMNH exceeding eight million Euro, remains an integral part of all future financial planning. This commitment is bound to the expectation that the already existent level of cooperation between the ZMNH and other institutes and clinics of the UKE will be further intensified.

We are convinced that the eminent replacements in leadership of the ZMNH Institutes with internationally renowned scientists will soon be completed. The taking office of Professor Roland Martin as Head of the new ZMNH Institute of Neuroimmunology and Clinical MS Research, which is co-financed by the Hertie-Stiftung, represents a most important milestone in this process. More steps will quickly follow.

We look forward to an even more successful ZMNH as the catalyst for future interdisciplinary projects, crossing the bridge between basic and clinical science in our University Medical Center.

April 2007

Prof. Dr. Jörg F. Debatin
Medical Director

Prof. Dr. Dr. Uwe Koch-Gromus
Dean

Universitätsklinikum Hamburg-Eppendorf

Vorwort des Direktors ZMNH

Das Zentrum für Molekulare Neurobiologie Hamburg (ZMNH) ist ein Forschungszentrum der Fakultät für Medizin am Universitätsklinikum Eppendorf der Universität Hamburg. Es betreibt Grundlagenforschung auf dem Gebiet der Molekularen Neurobiologie und angrenzender Gebiete, wobei schwerpunktmässig die gewonnenen Erkenntnisse mit medizinischen und humangenetischen Fragestellungen in Zusammenhang gebracht werden. Hierbei spielen die Generierung von transgenen und knock-out/in Mauslinien mit ihrem Potenzial, Genotyp und (patho)physiologischen Phenotyp miteinander kausal verbinden zu können, eine herausragende Rolle. Untersuchungen zur Aktivität einzelner Gene und die Auswirkungen ihrer Mutation für damit assoziierte (patho)physiologische Prozesse bilden einen Mittelpunkt der Forschung am ZMNH, zumal diese Untersuchungen vielfach die molekularbiologischen und zellbiologischen Prozesse erhellen, die mit der Entstehung bestimmter Krankheiten in Zusammenhang stehen. Neben seiner Hauptaufgabe, der Forschung, ist das ZMNH auch in der Lehre aktiv. Dies geschieht vor allem im Aufbaustudiengang Molekularbiologie, der vom ZMNH geleitet wird (Sprecherin Schachner).

Den Kern des ZMNH bilden nunmehr fünf von Professoren geleitete Institute sowie mehrere Forschergruppen, die von Nachwuchswissenschaftlern in zeitlich befristeter Form geleitet werden. Die Institute und Gruppen des ZMNH werden durch wissenschaftliche Serviceeinheiten unterstützt sowie durch eine weitgehend eigenständige Verwaltung, die Effizienz und Flexibilität der administrativen Vorgänge garantiert. Hinzu kommt ein wissenschaftlicher Beirat, der die wichtige Funktion hat, das ZMNH in regelmässigen Abständen zu evaluieren und das ZMNH, den Dekan der Fakultät für Medizin und den Vorstand des UKE in Strukturfragen zu beraten. In der vergangenen Berichtsperiode kam noch eine externe Evaluation hinzu, an der Frau Prof. Dr. Rougon, Prof. Dr. Frotscher und Prof. Dr. Seeburg teilnahmen. Ihnen sei an dieser Stelle herzlichst gedankt für damit verbundene Mühe und Zeitaufwand.

Die Einrichtung und Förderung unabhängiger junger Forschergruppen sind für die Nachwuchsförderung am ZMNH und sein wissenschaftliches Konzept von zentraler Bedeutung. Insgesamt verfügt das ZMNH derzeit über vier etatisierte Forschergruppen (Hoppe, Kneussel, Kornau, (Schimmang), Riethmacher). In der Berichtsperiode arbeiteten zeitweise am ZMNH zwei weitere Forschergruppen, die im Rahmen des Heisenberg-Programms der DFG (Bach) bzw. des Sonderforschungsbereichs (SFB) 444 (Kornau) finanziert wurden. Im Verlaufe der Berichtsperiode haben Ingolf Bach einen Ruf als Associate Professor an der University of Massachusetts Medical School in Worcester, Massachusetts und Thomas Schimmang einen Ruf als Gruppenleiter am Instituto de Biologica y Génetica Molecular (IGBM) in Valladolid angenommen. Die kontinuierliche Wegberufung von Leitern der ZMNH-Nachwuchsgruppen auf Professoren- bzw. Institutsleiterstellen zeigt, dass das Nachwuchsgruppenprogramm des ZMNH ein erfolgreiches Konzept ist, um selbständiges wissenschaftliches Arbeiten junger Nachwuchswissenschaftler mit Erfolg zu fördern. Mittlerweile haben auch einzelne Institute des ZMNH zusätzlich intern Raum und Mittel zur Förderung weiterer Nachwuchsforschergruppen, die den jeweiligen Instituten angegliedert sind, zur Verfügung gestellt.

Achtzehn Jahre nach seiner Gründung (im Jahr 1988) ist das ZMNH inzwischen fest in die Hamburger Wissen-

schaftslandschaft integriert. Dies äußert sich in einer großen und stetig gewachsenen Zahl wissenschaftlicher Kollaborationen, insbesondere mit Gruppen der Universitätsklinik Eppendorf (UKE). Hierbei spielen die am Zentrum entwickelten Mauslinien eine herausragende Rolle, da die diversen und manchmal unerwarteten Phänotypen des öfteren zu neuen Kollaborationen führen bzw. spezifische Expertisen erfordern, die häufig am UKE hervorragend vertreten sind.

Die lokale Vernetzung schlägt sich auch formal in Forschungsverbunden nieder, an denen das ZMNH häufig federführend beteiligt ist. Genannt seien der am ZMNH zentrierte Sonderforschungsbereich (SFB) 444 (*Grundlagen neuraler Kommunikation und Signalverarbeitung*; Sprecher: Jentsch), der zum Ende dieser Berichtsperiode ausgelaufen ist, der SFB 470 (*Glycostrukturen in Biosystemen - Darstellung und Wirkung*; Sprecher: Thiem), die DFG-Forschergruppe 604 (Signaling pathways in the healthy and diseased heart. Sprecher: Eschenhagen), sowie das im Rahmen des vom BMBF initiierten *‚Nationalen Genomforschungsnetzes‘* geförderte Projekt zur Pathologie von Erkrankungen des Nervensystems. Darüberhinaus gibt es viele nationale und internationale Kooperationen, die ihren Niederschlag u.a. in der Ernennung von Frau Schachner als New Jersey Professor for Spinal Cord Research am Keck Center der Rutgers University und damit verbundenen, vom NIH geförderten Projekten (*Genes involved in spinal cord regeneration; therapeutic value of the neural cell adhesion molecule L1 in spinal cord regeneration in mammals*), in der Teilnahme an mehreren EU-Projekten in Forschung (*Molecular Basis of Neurodegeneration in Transmissible Spongiform Encephalopathies; Cell Biology of Rare Monogenic Neurological Disorders Involving KCNQ Channels; European Renal Genome Project; EuroHear*) und Lehre (TEMPUS Master Programme *From Neuron to Cognition*) gefunden haben.

Das ZMNH hat sich als eines der führenden Forschungszentren auf dem Gebiet der Molekularen Neurobiologie etabliert. Neben seinen Publikationen - dem Hauptindikator wissenschaftlicher Leistung - schlägt sich dies auch in dieser Berichtsperiode wieder in einer Anzahl angesehener wissenschaftlicher Preise, insbesondere auch an die jüngeren Wissenschaftler, nieder. Zu erwähnen sind hier u. a. die Verleihung des Martini-Preises an Dirk Isbrandt, des Research Awards of the European Society of Anaesthesiology an Patrick Friederich, der Homer Smith Award und die Hodgkin-Huxley-Katz Prize Lecture an Thomas Jentsch.

Forschung am ZMNH

Die Gruppen des ZMNH beschäftigen sich primär mit Problemen der molekularen Neurobiologie. Schwerpunkte bilden die Funktion und Bedeutung von Ionenkanälen, die neuronale Entwicklung und Zelldifferenzierung sowie Adhäsionsmoleküle und synaptische Plastizität. Neben diesen rein neurobiologischen Fragestellungen erstreckt sich die Forschung auch auf andere Gebiete der Zell- und Entwicklungsbiologie sowie der (Patho-)Physiologie. So erforschen Wissenschaftler des ZMNH sehr erfolgreich u. a. Bluthochdruck und Herzrhythmusstörungen, Taubheit, Infertilität, Nierenerkrankungen, mentale Retardierung, Polyneuropathien und Regeneration des adulten Nervensystems. Als besonders effizient erweist sich das Zentrum bei der Generierung und Analyse von *knock-out* Mäusen, und herausragende Durchbrüche gelangen in den vergangenen Jahren bei der molekularen Aufklärung monogener menschlicher Erbkrankheiten sowie in jüngster Zeit auch bei der Strukturaufklärung von Ionenkanälen in Membranen.

Das Institut für Molekulare Neuropathobiologie (Jentsch bis 2006) untersuchte Fragestellungen des Ionentransportes,

wobei die damit in Zusammenhang stehende Physiologie und Pathologie eine besondere Aufmerksamkeit fanden. Im Brennpunkt standen Chlorid- und Kaliumkanäle, sowie Kalium-Chlorid-Cotransporter. Mausmodelle lieferten entscheidende Einsichten in die Rolle intrazellulärer Chloridkanäle und ihrer Rolle bei der Endozytose, und in die Rolle von KCl-Cotransport bei Osteopetrose, synaptischer Inhibition, Neurodegeneration und Taubheit. Die Gruppe konnte mehrere Erbkrankheiten molekular aufklären und pathophysiologisch erklären.

Die Forschung des Instituts für Neurale Signalverarbeitung (Pongs) beschäftigt sich mit der strukturellen und funktionellen Charakterisierung von Ionenkanälen. Im Mittelpunkt stehen die Charakterisierung neuer Ionenkanalgene, die mit Herzrythmusstörungen assoziert sind, die Entwicklung geeigneter Tiermodelle zur Pathophysiologie von Kaliumkanal-Dysfunktionen und Struktur-Funktions-Untersuchungen zur Pharmakologie und zum Schaltverhalten von spannungsabhängigen Kaliumkanälen. In Zusammenarbeit mit einer Göttinger Arbeitsgruppe um Marc Baldus kam es hier zu einem Durchbruch mit der erstmaligen Strukturaufklärung eines Toxin-Kaliumkanalkomplexes.

Die Forschung am Institut für Zellbiochemie und Klinische Neurobiologie (Richter bis 2005) fokussierte sich auf bestimmte Aspekte neuronaler Antworten auf extra- und intrazelluläre Signale. Besonders interessierte die Analyse von Expressionsprofilen, die Identifizierung von Ligand-Bindungsstellen und entsprechender struktureller Domänen, die in Neuronen die Aufnahme extrazellulärer Signale mit intrazellulären Signalkaskaden verbinden. Als Beispiel einer intrazellulären Signalkaskade wurden molekulare Mechanismen des zytoplasmatischen Transports von mRNA in dendritische und axonale Kompartimente analysiert. Die Ergebnisse zeigten, dass eine dezentralisierte Proteinbiosynthese zu einer distinkten Proteinzusammensetzung in neuronalen Subkompartimenten assoziiert ist mit der Genese und Plastizität neuronaler Morphogenese und Polarität.

Die Arbeiten des Instituts für Biosynthese Neuraler Strukturen (Schachner) beschäftigen sich mit der Aufklärung der Funktionen von Zellerkennungsmolekülen bei der Entwicklung des Nervensystems und bei der Regeneration nach Läsion und Induktion und Aufrechterhaltung von synaptischer Plastizität im erwachsenen Nervensystem. Genetisch veränderte Mäuse als Tiermodelle für menschliche Krankheiten und neurale und embryonale Stammzellen und die Funktionen von Kohlenhydraten bei der Feinregulation der Zellerkennung sind für diese Untersuchungen von besonderem Interesse.

Die Forschung am Institut für Entwicklungsneurobiologie (Schaller bis 2005) fokussierte sich insbesondere auf die Charakterisierung neuer Mitglieder der VPS-10 Domänenenthaltenden Familie G-Protein gekoppelter Membranrezeptoren (GPR) als mögliche Kandidaten für die Ankopplung des Kopfaktivatorpeptids, das in der frühen neuronalen und neuroendokrinen Entwicklung eine maßgebliche Rolle spielt, an intrazelluläre Signalkaskaden. Die Interaktionen des Kopfaktivatorpeptids und anderer Liganden mit GPRs wurde insbesondere in zellulären Modellsystemen erforscht.

Die Arbeiten des Instituts für Neuroimmunologie und Klinische Multiple-Sklerose Forschung (Martin, seit 2006) beschäftigen sich mit grundlegenden Fragen der Immunopathogenese der Multiplen Sklerosen mit einer Fokussierung auf die Rolle Autoantigen-spezifischer T-Zellen, natürlicher T-Killerzellen und ihrer Rezeptoren in Autoimmunerkrankungen, der Antigenpräsentierung im Thymus bzw. in periphären Immunkompartimenten und der Rolle des HLA-Systems für

die Immunpathogenese. Dies wird ergänzt durch neurobiologische Aspekte der Multiplen Sklerose und der Rolle des Immunsystems bei Entstehung und Reparatur von ZNS-Läsionen mit dem Ziel, bessere MS-Tiermodelle und vor allen Dingen bessere und neue Therapien zur Behandlung der Multiplen Sklerose zu entwickeln.

Die Forschergruppe Bach bearbeitet Fragestellungen der molekularen Mechanismen neuronaler Zellspezifizierung während der Embryogenese. Die Forschungen konzentrieren sich auf die Regulation von LIM Proteinen. Diese Klasse von Proteinen bilden Proteinnetzwerke, die grundlegende Funktionen während der Entstehung und Differenzierung von Neuronen übernehmen. Ingolf Bach und Mitarbeiter konnten zeigen, dass die biologische Aktivität von nukleären und cytoplasmatischen LIM Proteinen entscheidend durch Protein-Protein Wechselwirkungen mit Kofaktoren beeinflusst wird.

Die Forschergruppe von Thorsten Hoppe befasst sich mit der Identifizierung und Charakterisierung grundlegend neuer Komponenten des Ubiquitin/Proteasom-Systems, das in allen Eukaryonten den selektiven Proteinabbau vermittelt. Ein besonderer Schwerpunkt liegt dabei auf einer neuen Gruppe von Multiubiquitinierungsfaktoren oder E4 Enzymen. Interessanterweise ist ein Mitglied dieser Proteinfamilie in der Entstehung einer frühen Form der Parkinson'schen Erkrankung (AR-JP) involviert. Darüber hinaus versucht die Arbeitsgruppe weitere Faktoren zu identifizieren, die spezifisch am neuronalen Proteinabbau in *C. elegans* und möglicherweise beim Menschen an der Entwicklung neurodegenerativer Erkrankungen beteiligt sind.

Die Forschergruppe Kneussel bearbeitet Fragestellungen der Sortierung und des Transports von Neurotransmitter-Rezeptoren in neuronalen Dendriten. Die Zielsteuerung dieser Proteine zu und von der Synapse sowie deren Verankerung an der postsynaptischen Spezialisierung sind ein Schwerpunkt der Arbeiten. In diesem Zusammenhang werden Mechanismen der Synaptogenese und neuronaler Plastizität untersucht. Zur Identifizierung von neuronalen Transportkomplexen werden biochemische Screening- Verfahren angewandt. Als Modellsystem dienen die neuronale Zellkultur mit hippokampalen Neuronen und genetisch veränderte Mäuse. Zur funktionellen Charakterisierung von Transportvorgängen wird Time-lapse Video-Mikroskopie an lebenden Neuronen angewandt.

$GABA_B$-Rezeptoren modulieren erregende und hemmende Synapsen im Zentralnervensystem. Schwerpunkt der Arbeiten der Forschergruppe Kornau ist die Analyse von Proteininteraktionen dieser G-Protein-gekoppelten Rezeptoren. Zur Identifizierung von Interaktionspartnern werden biochemische Verfahren mit neuen genetischen Methoden kombiniert. Die funktionellen Auswirkungen der Interaktionen werden in Säugerzelllinien und neuronalen Primärkulturen untersucht.

Die Forschergruppe Riethmacher bearbeitet mithilfe von Mausmodellen Fragestellungen der Entwicklungsbiologie. In den letzten Jahren rückten Moleküle der extrazellulären Matrix in das Zentrum des Interesses. Zwei Moleküle, deren Expression neben anderen Geweben auch im sich entwickelnden und im adulten PNS nachweisbar ist, wurden sowohl mittels KO-Mauslinien als auch biochemisch charakterisiert. Ein weiterer Schwerpunkt lag in der Etablierung und Nutzung eines universellen auf der Cre-LoxP-Technologie basierenden *in-vivo* Zellablationssystems.

Im Mittelpunkt der Forschung der Nachwuchsgruppe Schimmang steht die Entwicklung und Differenzierung des Innenohrs. Dabei wurden insbesondere die Rollen von Fibro-

blasten-Wachstumsfaktoren (FGFs) und Neurotrophinen bei der Bildung der Sinnesepithelien des Innenohrs und deren Innervierung und beim Schutz vor Schäden untersucht. Außerdem wurden durch differentielle Genexpressionsanalyse neue Zielgene des FGF Signalwegs identifiziert.

Ziel- und Leistungsvereinbarung

Im Jahr 2004 konnte nach langwierigen und teilweise nicht einfachen Verhandlungen eine weitere Ziel- und Leistungsvereinbarung ausgehandelt werden, die, bedingt durch die zwischenzeitlich durchgeführte Verselbständigung des UKE, mit dem Dekan des Fachbereichs Medizin und dem Vorstand des UKE für einen Zeitraum von drei Jahren abgeschlossen wurde. Dieser Abschluss hat dem ZMNH, wenn auch für einen kürzeren Zeitraum, weiterhin stabile Rahmenbedingungen und Planungssicherheit gegeben, was eine wesentliche Voraussetzung für eine erfolgreiche wissenschaftliche Arbeit am ZMNH bedeutet. Gegenüber der vorherigen enthält die neue Ziel- und Leistungsvereinbarung eine Vereinbarung über ein Globalbudget, das dem ZMNH eine wünschenswerte Flexibilität in seiner Finanzplanung gibt, aber auch eine einschneidende und schmerzliche Reduzierung der Investitionsmittel sowie bedauerlicherweise eine Überführung der kleinen kreativen und effizienten Werkstatteinheit des ZMNH in die Dienste eines Tochterunternehmens (KME) des UKE.

2002 wurde das Universitätskrankenhaus verselbständigt und erhielt eine neue Struktur. Im Rahmen der mit der Verselbständigung verbundenen Strukturänderungen wurden die im Fachbereich Medizin angesiedelten Institute in Zentren zusammengefasst. Für das ZMNH bedeutete dies, dass das Institut des Gründungsdirektors des ZMNH (Prof. Dr. D. Richter) nicht länger mit dem ZMNH assoziiert war, sondern fünftes Institut des ZMNH wurde. Aufgrund der Emeritierung von Herrn Richter war diese Erweiterung des ZMNH nur von kurzer Dauer. Erfreulicherweise ist es dann durch eine mit der Neurologie gemeinsam durchgeführte erfolgreiche Einwerbung von Mitteln für eine Hertie-Stiftungsprofessur gelungen, als fünftes Institut das Institut für Neuroimmunologie und Klinische Multiple-Sklerose Forschung am ZMNH zu etablieren. Hiermit folgte das ZMNH einer Empfehlung seines wissenschaftlichen Beirats, ausgezeichnete Grundlagenforschung mit kliniknahen, krankheitsrelevanten Aspekten am Universitätskrankenhaus Eppendorf zu verbinden und maßgeblich zu fördern. Mit der Berufung von Herrn Roland Martin hat das ZMNH einen herausragenden klinischen Forscher auf diesem Gebiet gewinnen können. Sehr wesentlich an dieser Besetzung ist, dass Herr Martin neben seinem Institut am ZMNH gleichzeitig an der neurologischen Klinik des UKE als Leiter der dortigen MS-Station tätig ist. Wir erwarten, dass diese Konstruktion für zukünftige klinische Forschung mit einem Schwerpunkt in der Grundlagenforschung Modellcharakter haben wird.

Im Laufe dieser Berichtsperiode wurde die Satzung des ZMNH an die durch die Verselbständigung des UKE entstandenen neuen gesetzlichen Vorgaben angepasst. Damit konnte zwar die Selbständigkeit des ZMNH nicht weiter ausgebaut werden, aber in wesentlichen Teilen nach zähen und zum Teil sehr kontroversen Auseinandersetzungen festgeschrieben und somit eine wichtige Rahmenbedingung für die bei hoch kompetitiver Forschung benötigte Effizienz und Flexibilität realisiert werden.

Ausblick

Das ZMNH konnte sich in der Vergangenheit als eines der auf seinen Arbeitsgebieten besten Forschungszentren

etablieren. Neben der Kreativität und dem Engagement der ZMNH-Mitarbeiter beruht dieser Erfolg auch auf der guten Finanzierung durch die Stadt Hamburg und auf der Struktur des ZMNH – einer gelungenen Kombination größerer Institute, unabhängiger Nachwuchsgruppen, wissenschaftlicher Service-Einrichtungen und einer weitgehend selbständigen Verwaltung. Nach nunmehr 18 Jahren seiner Geschichte ist das ZMNH im Rahmen der Verselbständigung des UKE erwachsen geworden und ist für die Zukunft gut gerüstet. Wir sind guten Mutes, dass das bewährte exzellente Gründungskonzept des ZMNH weiter entwickelt werden kann und die durch den Weggang von Herrn Jentsch entstandene Lücke durch eine hervorragende Neubesetzung bald wieder geschlossen werden kann. Für die vor uns liegende Zeit, die von großen Umbrüchen im akademischen Bereich gekennzeichnet sein wird, sehen wir uns gut aufgestellt und mit innovativen Konzepten und herausragender Wissenschaft exzellent gerüstet.

im April 2007, Olaf Pongs

Preface of the Director ZMNH

The Center for Molecular Neurobiology (ZMNH) is a research center of the Faculty of Medicine at the University Hospital Eppendorf (UKE). Its research concerns questions of molecular neurobiology and related areas. In many cases, research results at the ZMNH are swiftly applied to diagnostic and therapeutic problems in medicine and human genetics. This process is greatly facilitated by the generation and analysis of transgenic and *"knock-out"* mouse lines serving as models to study molecular and cell biological bases of nervous system diseases. The study of corresponding mouse lines has led to identification of novel disease genes and has helped to understand and to elucidate pathophysiologies of human disorders. In addition to its research activities, the ZMNH engages successfully in graduate teaching and training. The Center carries out a two-year graduate course programme in molecular biology and participates in an international TEMPUS study programme.

The ZMNH comprises now five institutes which are headed by full professors as well as several junior research groups directed by young researchers on a time-limited basis. Institutes and junior research groups are supported by central scientific service units. Furthermore, a largely independent administration ensures efficient and flexible administrative support. A scientific advisory board has the important function to evaluate the performance of the ZMNH in regular intervals and to consult the Dean of the Faculty of Medicine as well as the Head of the University Hospital in strategic matters. In the reporting period, an additional external evaluation of the ZMNH took place, in which Drs. Rougon, Frotscher and Seeburg participated. I would like to use this opportunity to thank them again very much for their time and efforts which they employed at a very decisive point for the future of the ZMNH.

The independent junior research groups are essential to the concept of the Center. They ensure a dynamic, innovative and creative range of research activities and the same time enable the ZMNH to provide an excellent support for the scientific and academic careers of junior researchers in molecular neurobiology. In the present budget the ZMNH is able to finance four junior research groups (Hoppe, Kneussel, Kornau, (Schimmang), Riethmacher). Within the reporting period two additional research groups were funded by the Deutsche Forschungsgemeinschaft (DFG). Ingolf Bach was funded by a Heisenberg Fellowship and Hans-Christian Kornau by the Sonderforschungsbereich 444 (a collaborative research center of the DFG). During the reporting period, Ingolf Bach was appointed Associate Professor at the University of Massachusetts and Thomas Schimmang became a scientific member of the Instituto de Biologica Genética Molecular in Valladolid. Thus, the Junior Research Group Programme of the ZMNH continues to be an extraordinary successful concept to support the professional careers of young, independently working scientists. Based on this success, institutes of the ZMNH have extented this concept and support adjunct junior research groups by providing space and resources in their institutes for such groups.

Eighteen years after its foundation in 1988, the ZMNH is now firmly integrated into the Hamburg research community with a substantial and extensive number of local scientific collaborations, in particular with groups of the University Hospital Eppendorf (UKE). In this respect, the various mouse lines generated by researchers of the ZMNH have played a prominent role, since their often surprising and unexpected phenotypes demanded ever so often expertise available at the UKE. This has produced invaluable collaborations in analysing the diverse and at times complex phenotypes of genetically modified mice.

The ZMNH is firmly integrated into several research networks, where it is often the driving force. Thus, the Sonderforschungsbereich 444 ("*Basis of neuronal communication and signal transduction"*, speaker: Jentsch), which ended during the reporting period, was largely centred at the ZMNH. Also, groups of the ZMNH participate in the SFB 470 (*"Glycostructures in biological systems"*, speaker: Thiem) and in the DFG Researchgroup 604 ("Signaling pathways in the healthy and diseased heart", speaker: Eschenhagen). The ZMNH also participates in the National Genome Network focusing on the pathology of neurological diseases financed by the Federal Ministry for Research and Education. Furthermore, researchers of the ZMNH have established many national and international cooperations. Especially noteworthy is the appointment of Melitta Schachner as New Jersey Professor for Spinal Cord Research at the Keck Center of Rutgers University and corresponding NIH supported projects (*Genes involved in spinal cord regeneration; therapeutic value of the neural cell adhesion molecule L1 in spinal cord regeneration in mammals*), several research projects supported within EU-frameworks (*Molecular Basis of Neurodegeneration in Transmissible Spongiform Encephalopathies; Cell Biology of Rare Monogenic Neurological Disoreders Involving KCNQ Channels; European Renal Genome Project; EuroHear*) and, respectively, a EU-TEMPUS master programme *(From Neuron to Cognition*).

The ZMNH is widely recognised as one of Germany's premier research institutes in neurobiology. The Center's scientific success is above all reflected in its publications, which often report breakthroughs in the respective areas. Moreover, in the period covered by the present report, scientists of the

ZMNH have again been awarded several prestigious prizes: the Martini Prize to Dirk Isbrandt, the Research Award of the European Society of Anaesthesiology to Patrick Friederich, the Homer Smith Award and the Hodgkin-Huxley-Katz Prize Lecture to Thomas Jentsch.

Research at the ZMNH

Research at the ZMNH primarily tackles problems of molecular neurobiology. It focuses on the structure, function, and (patho)physiological importance of ion channels, development and differentiation of the nervous system, cell adhesion molecules, and synaptic plasticity. In addition, researchers of the ZMNH investigate other topics of cell and developmental biology, and are concerned with a broad spectrum of pathophysiological conditions and inherited diseases. Thus, important progress has been made in understanding the development of *hydra*, the development of the pancreas, in unravelling mechanisms of cardiac arrhythmia, hypertension, infertility, kidney diseases, mental retardation, polyneuropathies, and regeneration of the nervous system. The ZMNH has proved to be very efficient in the generation and analysis of genetic mouse models, and several breakthroughs were achieved in the molecular genetics and pathophysiology of human inherited diseases.

The Institute for Molecular Neuropathology (headed by Thomas Jentsch until 2006) focused on ion transport processes, in particular on their role in physiology and disease. Its research was primarily concerned with chloride and potassium channels and potassium chloride cotransport. A number of knock-out mouse lines provided important insights into the roles of intracellular chloride channels in vesicle trafficking and endocytosis, as well as into the role of KCl-cotransport in synaptic inhibition, neurodegeneration and deafness. The group identified several genes correlated with channelopathies and characterized the pathophysiology of the associated disorders in corresponding transgenic mouse lines.

The research at the Institute for Neural Signal Transduction (Olaf Pongs) is focused on structural and functional studies of ion channels, in particular potassium channels. The group is involved in human genetic screens for ion channel genes associated with various heart diseases, where they were able to identify new genes and signaling pathways associated with sino-atrial dysfunction. Pathophysiologies related to potassium channel dysfunctions such as high blood pressure, epilepsy, and forgetfulness are investigated and characterized in corresponding transgenic mouse lines. The structural studies of the group provided for the first time an analysis of a potassium channel scorpion toxin complex at atomic resolution.

Research in the Institute of Cell Biochemistry and Clinical Neurobiology (Richter until 2005) was focused on studying responses of neurons to extra and intracellular signalling. In particular, effects of extracellular signals on neuropeptide-hormone and, respectively, taste receptors were investigated. Of special interest were analyses of expression profiles, identification of ligand-binding sites, as well as of structural domains linking the extracellular signal to intracellular signaling cascades in eukaryotic cells. As an example for an intracellular signalling cascade, the molecular mechanisms of selective cytoplasmic mRNA transport to dendrites and axons were analyzed. Apparently, a decentralized protein biosynthesis contributed to the distinct protein composition in subcellular regions associated with genesis and plasticity of morphological patterns and cell polarity.

Research in the Institute for Biosynthesis of the Neural Structures (Schachner) focuses on the function of neural recognition molecules during development of the nervous system, and during regeneration after a lesion and induction and maintenance of synaptic plasticity in the adult. Genetically modified mice as models for human diseases and neural and embryonic stem cells and functions of carbohydrates by fine regulation of cell recognition are of particular interest for these investigations.

One of the external signals that influences early events in neuronal and neuroendocrine development, is the neuropeptide head activator (HA). In the report period the research of the Institute for Developmental Neurobiology (headed by Chica Schaller until 2005) concentrated on characterising new members of the VPS-10 domain-containing and G-protein coupled receptor (GPR) families as candidates for HA signal transduction. Their interaction with HA and other ligands was studied by using various heterologous expression systems.

Research at the Institute for Neuroimmunology and Clinical Multiple Sclerosis Research (Martin) studies molecular mechanisms of immunopathogenesis of multiple sclerosis and tries to implement the results in novel therapies of this devastating disease. Research is focused on the role of auto-antigen-specific T-cells, natural killer T-cells and their receptors in auto-immune diseases, and the presentation of antigens in thymus and peripheral immune compartments. Neurobiological aspects of multiple sclerosis with particular focus on the role of the immune system in damage and repair of CNS lesions are being studied and appropriate animal models which appropriately reflect the complexity of MS disease are being developed. These efforts are linked to the development of better diagnosis and therapies for treatment of MS patients in the adjunct clinic.

The Bach research group investigates questions concerning molecular mechanisms underlying neuronal cell fate specification during embryogenesis. The research interests focus on the regulation of LIM proteins. This class of proteins recruits protein networks that are crucial for the development of neuronal structures and cell types. This group has demonstrated that the biological activity of nuclear and cytoplasmic LIM proteins is critically regulated by protein-protein interactions with cofactors.

The research group of Thorsten Hoppe studies components of the ubiquitin/proteasome system, which is a key player in regulated protein degradation in all eukaryotic cells. The group focuses on new ubiquitination factors (E4 enzymes). Interestingly, one member of this protein family seems to be involved in the development of an early onset form of Parkinson's disease. Furthermore, the research group of Thorsten Hoppe tries to identify additional proteins, which are specifically involved in degradation of neuronal proteins in *C. elegans* and, possibly, may be linked to neurodegenerative diseases in humans.

The Kneussel research group studies dendritic sorting and transport of neurotransmitter receptors. Protein targeting to/from the synapse and postsynaptic receptor anchoring are a particular focus with respect to mechanisms underlying synaptogenesis and synaptic plasticity. Identification of neuronal transport complexes is based on biochemical screening techniques. Primary hippocampal neuron cultures as well as transgenic and gene targeted mice represent model systems for cellular analysis. For functional characterization of protein trafficking time-lapse video microscopy on living neurons is applied.

$GABA_B$ receptors modulate excitatory and inhibitory synapses in the central nervous system. The main focus of the

work of the Kornau research group is the analysis of protein interactions of these G protein-coupled receptors. For the identification of interaction partners biochemical techniques are combined with new genetic approaches. The functional consequences of the interactions are investigated using mammalian cell lines and primary neuronal cultures.

The research group of Dieter Riethmacher utilises mouse models to address questions of developmental biology. In the last years molecules of the extracellular matrix moved into the focus of interest. Two molecules that beside other domains show expression in the developing and adult PNS were characterised, both, with knockout models and biochemically. Another project was to establish and use a universal *in-vivo* cell ablation system based on the Cre-LoxP-technology.

The central interest of the research group headed by Thomas Schimmang is the development and differentiation of the inner ear. The roles of fibroblast growth factors (FGFs) and neurotrophins during the formation of inner ear sensory epithelia and their innervation and protection from damage are studied. Moreover, the analysis of differential gene expression during embryogenesis is used to identify novel targets for FGF signaling.

A stable framework for the further development of the Center

In 2004, the ZMNH negotiated another agreement with the dean of the Faculty of Medicine and the head of the UKE. After long and some times painstaking negotiations the ZMNH obtained a three years duration of this agreement providing, in times of decreasing resources, stable basic funding by the UKE. New in this agreement, apart from its shorter duration, is a global budget, which gives the ZMNH an increased flexibility to administer its financial resources. But the ZMNH suffered significant cuts in the budget for equipment, which may make it very difficult to maintain its technical standard as well as to attract new excellent scientists. Also, regrettably the workshop important to the community of ZMNH researchers for designing and developing new equipment has been outsourced to a hospital owned service company (KME). Nevertheless, the current financial framework should allow the ZMNH to continue to be as successful as it has been in the recent years.

In 2002, the City-State of Hamburg initiated an important structural change by transforming the University Hospital (and with it the Faculty of Medicine) into an independently operating entity. As a consequence, the Faculty of Medicine reorganized itself into Centers, and the institute of our founding director D. Richter was joined to our Center as fifth institute. This welcome expansion of the ZMNH has now been formally completed by appointing Roland Martin to a chair of neuroimmunology and clinical multiple-sclerosis research which is supported by a grant of the Hertie-Foundation. Roland Martin has also been appointed as adjunct professor at the neurological clinics of the UKE. Thus, the ZMNH has been able to materialize a former recommendation of its scientific advisory board to establish a research institute in molecular medicine with strong links to clinical research and the development of experimental therapies. A successful development of this new structure of cooperation between basic and clinical research at the ZMNH may open in the future completely new perspectives for elucidating and tackling bases of human neurological diseases.

At its beginnings, administrative independence of the ZMNH was envisaged as a crucial concept to make the ZMNH as attractive, competitive, and scientific successful as it now

is. Meanwhile, the ZMNH has grown up and has been able to establish itself firmly within the Faculty of Medicine and the UKE as an independent unit of scientific excellence. Fortunately, it was recognized at the end of the day that the administrative framework of the ZMNH will also be crucial for a healthy future.

Outlook

The ZMNH established itself as a leading research center in its field. This remarkable success is due to the creativity and hard work of its scientists, but also to the rather generous basic financing by the City-State of Hamburg and last, but not least to the structure of the ZMNH, which judiciously combines institutes with independent young research groups, service groups, and a largely autonomous administration. The ZMNH has grown up and may be well prepared for challenges in the near future. Thomas Jentsch decided this summer to leave the ZMNH and move to Berlin. Although this leaves a big gap, I am optimistic that we may fill this gap with an excellent successor. Given the achievements of the Centre and its excellent facilities, I have no doubt that the ZMNH will continue to provide outstanding research and teaching facilities in molecular neurobiology. I think that the ZMNH is in a better situation than two years ago to meet forthcoming structural changes in German academia and the tremendous challenges which will come with it.

April 2007, Olaf Pongs

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Research Projects

Institute for Biosynthesis of Neural Structures

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 relay 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.

1. The L1 family of neural cell adhesion molecules

Igor Jakovcevski, Ina Kalus, Nicole Karl, Ralf Kleene, Isabel Köhlitz, Annika Lieberoth, Gabriele Loers, Lars Seiler, Thomas Tilling, Gerrit Wolters, Meifang Xiao, Meike Zerwas**

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.

The L1-like molecules are present in overlapping and distinct subpopulations of neurons at different stages of development and may be important determinants of specific axon 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 NCAM, CD24, and laminin have been investigated.

Like L1 and the close homolog of L1 (CHL1) NCAM is proteolytically processed by a metalloprotease of the ADAM family and this processing is required for neurite outgrowth. It is now well documented that L1 induces neurite outgrowth and neuronal survival in vitro.

In the lesioned adult central nervous system of rodents, application of recombinant L1 (L1-Fc) by osmotic infusion into the contused spinal cord enhances recovery of locomotor functions. L1 can interact with the L1/neuropilin-1 complex in trans-interaction to overcome the inhibitory signal that is

conferred by semaphorin-3A onto neuropilin-1 and relayed via L1 to the cell interior. Conversely, inhibition of an L1 homolog in zebrafish by anti-sense morpholinos reduces the natural regenerative capacities of the central nervous system of this organism by preventing regrowth of descending connections from the brainstem and recovery of naturally occurring locomotor functions. Recombinant monoclonal L1 antibody Fab fragments have been produced and showed in vitro the ability to stimulate L1 and trigger thereby the functions of L1. These antibody fragments will be administered by osmotic infusion in a model of spinal cord injury in adult mice to elucidate the effect of the antibody in vivo on regeneration and recovery of locomotor functions.

Understanding the molecular mechanisms of L1-mediated cell recognition requires a detailed knowledge of the molecule‘s structure. To this end, the extracellular domain of L1 is recombinantly produced in Pichia pastoris and will be used for crystallization and structure analysis.

2. Neural recognition molecules and signal transduction

Anja Behrendt, Claas Cassens, Athena Chalaris*, Carina Figge, Claudia Friedrich*, Ina Kalus*, Gabriele Loers, Tatjana Makhina, Ingo Meier, Mounir Mzoughi, Daniel Novak, Gunnar Poplawski*, Elisa Ramser, Daniela Schneeberger*, Tanja Schneegans*, Thomas Tilling, Ann-Kathrin Tranziska, Gerrit Wolters*

The identification and characterization of intracellular signaling cascades activated by homophilic (self binding) or heterophilic (binding to other molecules) interactions of cell-adhesion molecules such as L1 or the neural cell adhesion molecule NCAM are of central importance for the understanding of adhesion molecule-mediated neuritogenesis and growth cone repulsion.

During the last reporting period, we showed that surface localization of G protein inwardly rectifying K^+ channels (Kir3 channels) is controlled by palmitoylated NCAM isoforms in the trans-Golgi network, a process that can be called an “inside-out” signaling of NCAM. These results could explain how cell adhesion molecules are involved in the regulation of neural activity. In the meantime, we have identified proteins interacting with NCAM and Kir3, and are planning to investigate a possible role of these interacting partners in regulating Kir channel surface localization or NCAM-mediated neurite outgrowth.

Hints on signal transduction mechanisms downstream of L1 have been gained by examining mice ectopically expressing L1 in astrocytes (GFAP-L1 mice). These transgenic animals show increased flexibility and selectivity in spatial learning. Gene expression analyses of GFAP-L1 mice revealed the dysregulation of several molecules involved in transcription, cytoskeletal remodelling, and intracellular signaling cascades. We are investigating several of these “candidate proteins” with regard to their involvement in either regulation of L1 expression or L1-triggered signaling pathways. The transcriptional control of L1 expression is one major mechanism to govern inside-out signaling via L1. Moreover, some of the signaling molecules detected in the initial gene expression screen might also influence L1 surface localization. On the other hand, outside-in signaling via L1 requires transduction not only to the nucleus, but also to the cytoskeleton. Considering the major morphological changes during L1-stimulated neurite formation and growth, cytoskeleton-associated proteins like the ones detected in

our expression screen are therefore attractive candidates for mediating L1 function.

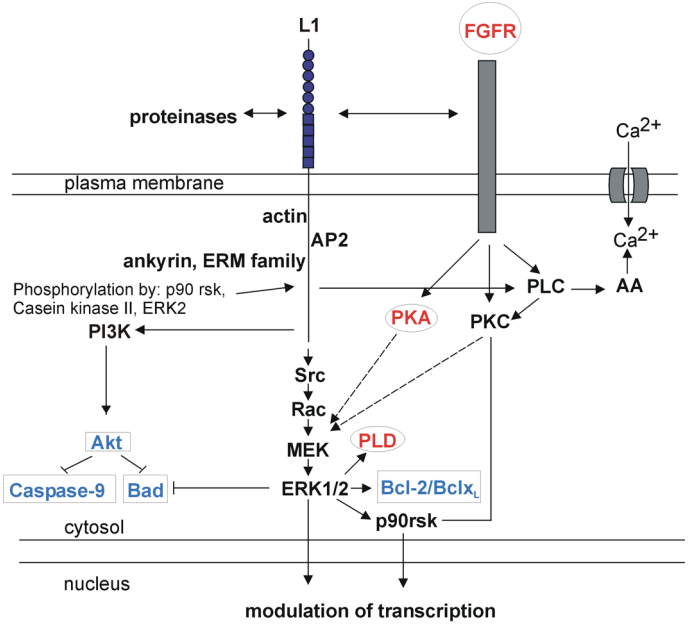

Figure 1: Proposed signal transduction pathways implicated in L1-triggered neuroprotection and neuritogenesis. Proteins involved in L1-mediated neuritogenesis and neuroprotection are shown in black, proteins only involved in neuritogenesis in red (encircled) and proteins only involved in neuroprotection in blue (boxed). Dashed lines represent putative cross-talk between signaling cascades.

Moreover, in GFAP-L1 mice, differentiation and survival of dopaminergic neurons is enhanced. For this reason, these animals were also used in a MPTP model of Parkinson disease to identify target proteins involved in disease amelioration by a proteomic approach.

The finding that L1 acts as a promoter of neuronal survival prompted us to analyze downstream signaling mechanisms. By detailed analyses of several intracellular signaling cascades, we could show that substrate L1-triggered neuritogenesis and neuroprotection depended on distinct but also overlapping signal transduction pathways (Figure 1) and on the expression of L1 at the neuronal cell surface.

3. Prion protein and amyloid precursor protein as recognition molecules

Ute Bork, Vasudharani Devanathan, Ralf Kleene, Iryna Leshchyns'ka, Gabriele Loers, Anna Julia Marquart, Friedhelm Maywald, Antonella Santuccione*, Carsten Schmidt*, Nina Stemmer, Helen Strekalova*, Vladimir Sytnyk*

The cellular form of prion protein (PrPc) is a glycosylphosphatidylinositol (GPI) anchored ubiquitous cell surface glycoprotein. Conversion of PrPc to an abnormal conformer (PrPSc) is a central event in the pathogenesis of prion diseases, such as Creutzfeldt-Jakob disease in humans, bovine spongiform encephalopathy in cattle and scrapie in sheep. Several recently reported observations are consistent with a function of PrPc as a cell adhesion molecule. We confirmed this notion showing that the recognition molecule-related HNK-1 carbohydrate is linked to the protein backbone of PrPc via N-linked glycans. Additionally, we showed that PrPc induces neurite outgrowth and neuronal survival in cell culture of primary neurons via a yet unknown heterophilic receptor involving different signal transduction pathways. We obtained indications that PrP is involved in the regulation of different processes in astrocytes which play important roles

in metabolic support of neurons. We are currently studying the interaction of PrP with glial proteins and characterizing the role of these interactions in astrocytic physiology. Our current studies are focused on studying this aspect that it is essential for PrPc mediated neurite outgrowth, neuronal survival and synaptic plasticity, and the associated triggering of signal transduction mechanisms.

Using different approaches we could identify yet unknown binding partners of the amyloid precursor protein, which is associated to Alzheimer's disease when not correctly cleaved, and the functional roles of these binding partners are currently under investigation.

4. Carbohydrates and the fine tuning of cell interactions

Nuray Akyüz, Frauke Brendel, Shan Bian, Andrey Irintchev, Nainesh Katagihallimath, Ralf Kleene, Michael Knepper, Nina Kurschat*, Annika Lieberoth, Gabriele Loers, Anna-Julia Marquart, Ali Mehanna, Sandra Nickel, Olga Simova*, Shiwei Wang*

We are engaged in studies on different glycans that are carried by partially overlapping sets of glycoproteins, many of which have been shown to be neural recognition molecules. Some of these neural recognition molecules, e.g. L1, MAG, NCAM and basigin, are capable to bind distinct carbohydrate structures, thus functioning as lectins. In vitro assays have shown that glycans themselves are involved in different aspects of cell adhesion, cell migration, outgrowth of neuritic and astrocytic processes as well as in synapse formation and synaptic plasticity. We focus our studies on several functionally important glycans, among them the HNK-1 carbohydrate, oligomannosidic carbohydrates, the unusual alpha-2,8-linked polysialic acid (PSA), the Lewisx epitope and alpha-2,3-linked sialic acid on O-glycans. All of these carbohydrates are involved in the modulation and fine tuning of cell interactions. 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. We have identified carbohydrate peptide-mimetics and are using these as surrogate carbohydrates to trigger or block cell interactions: they are more easily obtained in large amounts than many structurally complex carbohydrates and can be manufactured as better binding ligands with higher metabolic stability. The HNK-1 peptide-mimetics we identified are able to enhance motoneuron growth and survival in vitro and locomotor recovery after peripheral nerve injury in mice. Additionally, we are searching for organic carbohydrate mimetics, which could be even more useful for therapy.

The HNK-1 carbohydrate is a well-characterized example of a protein- and lipid-linked oligosaccharide. This epitope is regulated in its expression independently of the protein backbone, is phylogenetically conserved, and is functional in cell-cell and, particularly, cell-substrate interactions. Interestingly, the sulfate group is essential for most of the functions contributed by this epitope. The enzyme transferring the sulfate group to the oligosaccharide backbone, the HNK-1 sulfotransferase (HNK-1 ST), has previously been cloned and the HNK-1 ST knockout mutant has been generated and characterized. Based on the homology to the HNK-1 sulfotransferase we and others have identified and cloned six more members of this enzyme family. The sulfotransferases GalNAc-4ST1 and GalNAc-4ST2 have been shown to synthesize sulfated beta1-4-linked GalNAc found on the

GGnM epitope characteristic of glycopeptide hormones of the pituitary and to add sulfate to non-terminal beta1-4-linked GalNAc found on chondroitin and dermatan. The sulfotransferases C4ST1, C4ST2, C4ST3 and D4ST1 confer sulfate to beta1-4-linked GalNAc on chondroitin and dermatan.

Except for C4ST3, all HNK-1 ST family members are expressed in mouse brain at certain developmental stages. We generated ST KO mice to investigate whether specific STs are involved in mouse brain development. While homozygous abolishment of the C4ST1 gene causes perinatal lethality, at least some D4ST1 homozygous KO mice survive until adulthood, and show reduced body size compared to the wild-type littermates.

5. Recognition molecules and their roles in the formation and organization of the pre- and post-synaptic machinery

Aksana Andreyeva, Vsevolod Bodrikov, Yana Chernyshova, Babett Baraniec, Doreen Westphal, Aparna Shetty, Vasudharani Devanathan, Dmytro Puchkov, Nan Tian, Shen Li, Ute Eicke-Kohlmorgen, Iryna Leshchyns'ka, Vladimir Sytnyk

It is well established that neural cell adhesion molecules accumulate at synapses and are implicated in certain forms of synaptic plasticity such as long term potentiation. Nevertheless, the exact role that these molecules play at the synapse and mechanisms underlying their functions, are poorly understood. Cell adhesion molecules stabilized at synapses due to their homophilic or heterophilic interactions can provide cues for accumulation of certain synaptic proteins. We confirmed this notion by showing that NCAM associates with the postsynaptic spectrin-based scaffold cross-linking NCAM with the NMDA receptor and CaMKIIα in a manner not firmly or directly linked to PSD95 and α-actinin. Clustering of NCAM promotes formation of detergent-insoluble complexes enriched in postsynaptic proteins and resembling postsynaptic densities. Disruption of the NCAM/spectrin complex decreases the size of postsynaptic densities and reduces synaptic targeting of NCAM/spectrin-associated postsynaptic proteins, including spectrin, NMDA receptors and CaMKIIα, the key enzyme regulating the activity of the NMDA receptors. Degeneration of the spectrin scaffold in NCAM deficient neurons results in inability to recruit CaMKIIα to synapses after NMDA receptor activation, a critical process in NMDA receptor-dependent long-term potentiation. Our combined observations thus indicate that NCAM promotes assembly of the spectrin-based postsynaptic signaling complex which is required for activity-associated long-lasting changes in synaptic strength.

Another function of cell adhesion molecules in synapses is the regulation of distinct signaling cascades resulting in activation of different kinases and leading to changes in gene expression. Similar cascades are often activated by these molecules also at initial stages of neuronal development during neurite outgrowth. In this context, we showed that in response to activation, NCAM utilizes spectrin to translocate protein kinase C to lipid rafts where this kinase activates substrates involved in cytoskeleton remodeling. Spectrin also cross-links NCAM with the receptor type protein phosphatase α (RPTPα), an activator of the tyrosine kinase $p59^{fyn}$ in NCAM mediated signaling cascade. Importantly, to activate $p59^{fyn}$ NCAM co-operates with another recognition molecule, the prion protein. Current work aims at further investigations on the mechanisms by which cell adhesion

molecules NCAM, L1, close homologue of L1 (CHL1) and prion protein promote neurite outgrowth, induce and stabilize axo-dendritic contacts and promote formation, maturation and activity dependent remodeling of the pre- and post-synaptic machinery.

6. Recognition molecules and synaptic plasticity

Stephanie Bonnet, Olena Bukalo, Alexander Dityatev, Galina Dityateva, Martin Hammond*, Gaga Kochlamazashvili, Eka Lepsveridze*, Giorgi Papashvilli, Oleg Senkov, Adriana Stan*, Tiberiu Stan, Luminita Stoenica*

A major interest is to understand how the strength of synaptic connections is modified by neuronal activity. At the molecular level, extracellular matrix molecules (tenascin-R and -C and chondroitin sulfate proteoglycans) and cell adhesion molecules of the immunoglobulin superfamily (NCAM, L1, CHL1) are in the focus of our research. To evaluate the contributions of these molecules to different aspects of synaptic plasticity in juvenile and adult mice, we are using several approaches and experimental models.

We have analyzed several forms of hippocampal synaptic plasticity in mutants deficient in the above mentioned molecules. Our results show that adult L1 and CHL1 constitutive deficient mice have normal long-term potentiation (LTP) at CA3-CA1 synapses, whereas NCAM, tenascin-C and tenascin-R mutants show reduced CA1 LTP. Long-term depression (LTD) in CA3-CA1 synapses is impaired in NCAM and tenascin-C mutants, but appears to be normal in tenascin-R mutants. No abnormalities in mossy fiber LTP in the CA3 region of mice deficient in tenascin-C, tenascin-R, and CHL1 were found. LTP is also normal in perforant path projections to the dentate gyrus of tenascin-C, and CHL1 mutants, but is impaired in NCAM and tenascin-R deficient mice. Thus, our data demonstrate that both cell surface and extracellular matrix molecules are involved in different forms of hippocampal synaptic plasticity. Analysis of the underlying mechanisms revealed that impairments in LTP and LTD in tenascin-C deficient mice are related to a deficit in L-type Ca^{2+} channel-dependent signaling, whereas similar deficits in NCAM deficient mice are due to abnormal function of NMDA receptors.

Since many biological functions of recognition molecules are mediated by carbohydrates associated with these molecules, we analyzed synaptic transmission and plasticity in mice deficient in polysialyltransferases ST8SiaII or ST8SiaIV, which produce polysialic acid associated exclusively with NCAM in immature and mature neurons, respectively. Mice deficient in ST8SiaII have normal LTP in CA3-CA1 synapses, mossy fiber synapses in CA3, and in perforant path projections to the dentate gyrus, but show low levels of synaptic transmission in the latter. Thus, polysialylation of NCAM by ST8SiaII may affect integration of immature granule cells. ST8SiaIV-deficient mutants show an age-dependent synapse specific reduction in LTP and LTD in CA3-CA1 connections, which correlates with an age-dependent decline in expression of polysialic acid in ST8SiaIV-/- mutants. These experiments and data showing that impaired LTP in NCAM deficient mice can be restored by injection of polysialic acid, together with demonstration of modulatory effects of polysialic acid on the NMDA type of glutamate receptors, strongly support the view that polysialic acid synthesized by ST8SiaIV is important for induction of LTP in CA1.

Previously we found an impairment of perisomatic inhibition in the CA1 region of tenascin-R deficient mice and after application of antibody to the tenascin-R carried HNK-1 carbohydrate. These findings stimulated our interest to study regulation of inhibitory synaptic transmission by other recognition molecules. More recently, we found that in juvenile L1-deficient mice perisomatic inhibitory currents and the density of symmetric inhibitory synapses in the CA1 region of the hippocampus are reduced as in tenascin-R deficient mutants. However, in juvenile CHL1 deficient mice there is an increase in perisomatic inhibitory currents, correlating with an increase in density of perisomatic interneurons and their synapses.

Since tenascin-R and -C and chondroitin sulfate proteoglycans accumulate in the extracellular matrix surrounding some inhibitory interneurons in so-called perineuronal nets, we will attempt to unravel how these nets are formed and what are their functions. We showed in vitro that pharmacological blockade of action potentials, transmitter release, Ca^{2+} permeable AMPA subtype of glutamate receptors or L-type Ca^{2+} channels strongly decreased accumulation of net's components; suggesting that two routes of Ca^{2+} signaling are involved in their activity-dependent formation. Enzymatic treatment with chondroitinase ABC, which removes chondroitin sulfates, also resulted in a striking reduction of the major extracellular components and increased the excitability of parvalbumin interneurons.

7. Neural recognition molecules and behavior

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We use an ethological approach with newly established paradigms to evaluate the role of the cell adhesion molecules L1, CHL1 and NCAM, and the extracellular matrix proteins tenascin-C and tenascin-R in a broad spectrum of behavioral responses. Detailed behavioral analysis of constitutively or conditionally deficient mice revealed a variety of alterations caused by the ablation of these molecules, such as: impaired motor function and coordination (tenascin-R), impaired spatial learning and memory (NCAM, L1 and HNK-1), enhanced working and episodic-like memory (tenascin-R), enhanced anxiety (tenascin-R, HNK-1), increased intra-male aggressive behavior (NCAM), impaired social behavior (CHL1), enhanced novelty-induced activity and reduced anxiety (NCAM and L1), enhanced cocaine-induced conditioned place preference (NCAM), altered activity of the stress response and reduced sensitivity of the serotonin 1a receptor (NCAM and L1), reduced attention (CHL1), and impaired synchronization of circadian activity to a Zeitgeber (tenascin-C). Overall, these results indicate that expression of cell adhesion molecules and extracellular matrix proteins during early development and in the adult central nervous system is of paramount importance for the expression of the mouse behavior.

To better interpret the information obtained from the analysis of mutant and transgenic mice, we are investigating the proximate and ultimate causes underlying determined behavioral

responses. Since learning and memory on one hand, and stress and anxiety related behaviors on the other hand are those predominantly affected in our mutants, we focus our attention on these two behavioral systems, which also represent important functional models for synaptic plasticity in the central nervous system. The analysis of genes and proteins expression in the hippocampus of C57BL/6 mice induced by either learning or stressful experiences allowed us to extend and validate the results obtained in the mutants. The correlation of morphological and electrophysiological analyses made on the same mice undergoing behavioral tests led to the formulation of hypotheses on the possible links between structure and function. Finally, the analysis of different behavioral responses of C57BL/6 mice revealed that basal levels of trait anxiety not only predict the large inter-individual variability in the stress response as measured at the behavioral, physiological and molecular levels, but also correlate with hippocampal expression of some cell adhesion molecules and extracellular matrix proteins.

8. Stem cells and neural transplantation

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The long-term aim of these studies is to evaluate the use of embryonic and neural stem cells manipulated to express recognition molecules, extracellular matrix molecules, or carbohydrates for the treatment of neurodegenerative and non-inflammatory dysmyelinating or demyelinating diseases as well as spinal cord injuries. To test the influence of L1 expression on embryonic stem cell-derived neural precursor cells we have generated a murine embryonic stem cell line constitutively expressing L1 at all stages of neural differentiation. L1 transfected cells showed enhanced neuronal differentiation and decreased astrocytic differentiation when compared to non-transfected cells. L1 overexpression also resulted in increased yield of GABAergic neurons and enhanced migration of embryonic stem cell-derived neural precursor cells into the lesioned striatum. Mice grafted with L1-transfected cells showed better recovery of rotation behavior when compared to mice that had received non-transfected cells, thus demonstrating for the first time that a recognition molecule is capable of improving functional recovery in a syngeneic transplantation paradigm. L1 over-expressing stem cells also showed enhanced survival after transplantation in a murine spinal cord injury paradigm. Currently, are investigating the effects of L1 expression in various models of de- and dysmyelinating diseases. We are also currently establishing an embryonic stem cell line inducibly expressing L1 applying the tetracycline system in order to be able to regulate expression of beneficial genes after transplantation *in vivo*.

Furthermore, we introduced a new differentiation and enrichment protocol based on the generation of embryonic stem cell-derived substrate-adherent cellular aggregates that consist predominantly of neurons (>90%) and radial glial cells (>8%). Purified aggregates revealed major advantages over uncommitted and immature nestin$^+$ neural precursors or slightly further pre-differentiated embryonic stem cell-derived neural cells when transplanted into the striatum of quinolinic acid treated adult mice: aggregate-derived cells gave rise to 2-fold more differentiated neurons, migrated over 5-fold longer distances within the host tissue, and did not form tumors up to four months after syngeneic transplantation. We

also investigate the effects of other recognition molecules, such as tenascin-C, tenascin-R and CHL1 on precursor cell behavior. *In vivo* studies have examined embryonic stem cell-derived precursor cells expressing recognition molecules on morphological and functional regeneration in animal models of spinal cord injury, Huntington´s chorea, stroke and Parkinson´s disease.

9. Recognition molecules and axon growth in the nervous system of zebrafish

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Zebrafish offer the unique opportunity to analyze axon growth both during early vertebrate development and during axon regeneration in the adult central nervous system. Embryos are transparent and their relatively simple scaffold of primary axons has been described in detail. The availability of expressed sequence tags and sequencing of the zebrafish genome, which is projected to be complete in the near future, provides easy access to genes of interest, and new methods to perturb gene function are being devised for this important model organism. Our focus is on cell recognition molecules that are important for axonal growth both on the axonal cell surface (L1.1, L1.2, NCAM, and other members of the immunoglobulin superfamily) and in the extracellular matrix (tenascins, proteoglycans). Functionally important unusual glycans attached to these molecules, such as the HNK-1 carbohydrate (Figure 2), oligomannosides, and polysialic acid are also investigated with regard to their possible role as fine tuners of cell interactions. We perturb expression of recognition molecules in vivo by microinjecting RNA (overexpression and knock-down), specific enzymes, peptides, and antibodies into fertilized eggs or embryos. Subsequently, we analyze aberrations of axon growth in these embryos using time-lapse video-microscopy in transgenic fish and immunohistochemical labeling of specific axons.

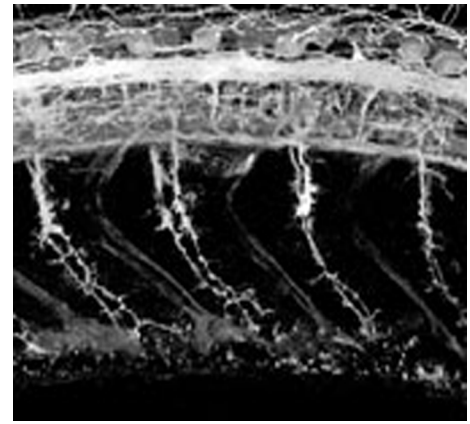

Figure 2: Confocal image stack of the nervous system in the trunk region of a zebrafish larva 24 hours after fertilization labeled with antibody to the HNK-1 carbohydrate. Two ventrally directed axons of the caudal primary motor neurons per trunk segment are prominently labeled. Rostral is left, dorsal is up.

The recognition molecules under study are not only investigated during development, but also in regeneration and synaptic plasticity in the adult, when some of the ontogenetic mechanisms are recapitulated, at least to some extent. In contrast to mammals, which are unable to regenerate injured axons in their central nervous system, adult zebrafish show an impressive regenerative capacity of their central axons. We therefore study recognition molecules, which have functions for developmental axon growth also in axon regeneration. The two systems we are working on are regeneration of optic axons and of supraspinal descending axons in adult

zebrafish. Regeneration of these fibers is analyzed in detail by axonal tracing. The distribution and regulation of recognition molecules is visualized using immunohistochemistry and in situ hybridization. The interactions of regenerating axons with recognition molecules are studied in vivo and in organotypic cell culture. Using these approaches in this model vertebrate we hope to gain insights into important developmental processes and at the same time into the determinants of repair in the central nervous system after injury.

10. Structural substrates of brain dysfunctions caused by mutations of recognition molecules

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Recognition molecules are essential for normal brain development and function. However, the origins of brain dysfunctions caused by constitutive or conditional ablations of genes encoding recognition molecules in mice remain mostly obscure. Using quantitative morphological techniques in combination with electrophysiological and behavioral analyses, we have recently pursued not only to precisely assess structural aberrations, but also to search for structure – function relationships in different mutant mice.

The extracellular matrix glycoprotein tenascin-C (TNC) is widely expressed in the developing neocortex. We addressed the question as to whether constitutive absence of TNC in mice affects cortical physiology and structure. Defined major cell populations (neurons and inhibitory neuronal subpopulations, astrocytes, oligodendrocytes and microglia) were quantified in the somatosensory and motor cortices of adult TNC deficient (TNC-/-) and wild-type (TNC+/+) mice by stereological analysis of immunofluorescence stained sections. In both areas studied we found abnormally high density of excitatory neurons, astrogliosis, low density of parvalbumin-positive interneurons and reduced ratios of oligodendrocytes to neurons and of inhibitory to excitatory neurons in the TNC deficient as opposed to the non-deficient animals. Analysis of Golgi-impregnated layer V pyramidal neurons in TNC-/- animals showed aberrant dendrite tortuosity and redistribution of stubby spines within 1st-3rd order dendritic arbors. Significantly enhanced responses upon whisker stimulation were recorded epicranially over the barrel and the motor cortices of TNC-/- as compared to TNC+/+ animals which might be associated with the diminished inhibitory circuitry. These results demonstrated that TNC is essential for normal cortical development and function.

The cell recognition molecule close homologue of L1 (CHL1) is also broadly expressed in the developing brain and mutations in the encoding gene cause brain dysfunction in humans and mice. To characterize the CHL1 mutant mouse with respect to morphological abnormalities in the brain, we studied the motor and cingulate cortices, cerebral ventricles, hippocampus and substantia nigra of CHL1 deficient (CHL1-/-) and wild-type (CHL+/+) mice at 1, 2, 6 and 12 months of age using morphometry and stereological analysis of major cell types. Abnormal features identified in CHL1-/- animals at all ages studied were enlarged cerebral ventricles and

pyramidal cell layer of the hippocampus. The motor cortex of the mutant animals was deficient in reelin-expressing interneurons at all ages. An age-related decline in the number of parvalbumin-positive interneurons in the hippocampus and of dopaminergic cells in the substantia nigra were observed. Age-related loss of parvalbumin interneurons was paralleled by a decline in long-term potentiation in the CA1 region of the hippocampus. No aberrations were detected in the cingulate cortex indicating that CHL1 deficiency differentially affects different brain regions. Thus, CHL1 appears to affect formation, maturation and survival of inhibitory interneurons and dopaminergic neurons in a region-specific and age-related fashion that may lead to abnormalities observed in some psychiatric diseases.

The extracellular matrix glycoprotein tenascin-R (TNR) has versatile functions in the CNS and its constitutive ablation in mice results in functional aberrations. Recent analyses revealed that spatial learning and memory, as evaluated in the water maze, were normal in adult TNR deficient (TNR-/-) mice. During relearning, however, TNR-/- mice were faster than wild-type (TNR+/+) littermates in finding the relocated platform, suggesting faster extinction or relearning. Stereological analyses of the behaviorally-tested animals revealed abnormally high numbers of principal cells and parvalbumin-positive interneurons in the hippocampus (dentate gyrus and cornu Ammonis, CA) of TNR-/- mice. Although the ratios of inhibitory to excitatory cells in CA1-3 were normal, fewer parvalbumin-positive and -negative GABAergic terminals in CA1 and more parvalbumin-negative terminals in CA3 covered the pyramidal cell bodies in TNR-/- mice. The ratio of inhibitory to excitatory cells in the dentate gyrus was abnormally high and the perisomatic GABAergic input to the granule cells was increased. Some morphological estimates, such as granule cell numbers and their GABAergic perisomatic coverage, correlated strongly with the performance of individual mice during relearning, suggesting causal relationships. The enhanced inhibitory input to granule cells was accompanied by impaired paired-pulse facilitation and long-term potentiation (LTP) in the dentate gyrus of anesthetized TNR-/- mice. Diminished LTP could be restored by abrogation of GABAergic activity. Synaptic plasticity in CA3, studied *in vitro*, was not impaired. Our results indicate that TNR restrains relearning and is essential for acquisition and/or maintenance of normal neuronal numbers, region-specific neuronal connectivity, and synaptic plasticity in the hippocampus.

11. Peripheral nerve regeneration

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Peripheral neurons can regenerate their axons after nerve injury and reinnervate peripheral targets. Despite the robust regenerative potential, the clinical outcome of nerve repair is often disappointing. Regrowth of severed axons to improper targets is considered a major reason for poor functional recovery. A valuable paradigm which allows studies on accuracy of pathway finding and molecular mechanisms determining reinnervation selectivity is the femoral nerve model in rodents. The femoral nerve bifurcates into two major branches, a quadriceps muscle branch containing motor and sensory axons and a purely sensory branch innervating the skin, the saphenous branch. After lesion of the common

nerve trunk, motor axons initially regrow at random into both the proper quadriceps branch and the improper saphenous branch. As reinnervation proceeds, however, the number of correctly projecting motoneurons increases, a phenomenon known as preferential motor reinnervation. The usefulness of the femoral nerve injury paradigm had been restricted by the lack of assays for evaluation of motor functional recovery. To overcome this limitation, we developed and validated a novel video-based motion analysis approach, the single-frame motion analysis, for objective numerical evaluation of quadriceps muscle function which proved to be reliable and sensitive in several studies performed by different investigators.

We had previously identified a molecular cue potentially related to preferential motor reinnervation. An unusual acidic glycan, the human natural killer (HNK) cell glycan (3-sulfoglucuronyl beta1-3 galactoside) known as HNK-1 epitope, was found to be associated with myelin profiles of motor axons, but not of sensory axons, both in intact and regenerating femoral nerves of mice. Now we tested the possibility to promote functional recovery after nerve lesion by application of exogenous HNK-1 carbohydrate. Because of the difficulty to isolate or synthesize this unusual glycan in sufficient amounts, we used a synthetic peptide that functionally mimics the HNK-1 carbohydrate. The glycomimetic was applied into a polyethylene cuff used to reconstitute the continuity of the femoral nerve transected proximal to its bifurcation point. Using the novel video-based approach, we observed that quadriceps muscle function recovered to 93% of normal within 3 months after glycomimetic application. Restoration of function was less complete (71-76%) in control groups. Better functional recovery was associated with larger motoneuron somata, better axonal myelination in the quadriceps nerve and enhanced precision of target reinnervation. Lesion-induced death of motoneurons was reduced by 20-25%. The glycomimetic enhanced survival and neurite outgrowth of both mouse and human motoneurons *in vitro* by 30-75%. Application of a novel cyclic glycomimetic also enhanced functional recovery *in vivo*. We attribute improved outcome of nerve repair after glycomimetic application to neurotrophic effects. Since our results hold promise for therapeutic use in humans, testing of the effects of the glycomimetic treatment on non-human primates is warranted.

Brief electrical stimulation of the acutely lesioned nerve had been identified as another clinically feasible approach increasing precision of axonal regrowth. It had been suggested that the effects of this stimulation were mediated by BDNF and its receptor, TrkB, but the down-stream effectors were unknown. We could show that short-term low-frequency electrical stimulation (1 hour, 20 Hz) of the lesioned and surgically repaired femoral nerve in wild-type mice causes a motor nerve-specific enhancement of HNK-1 carbohydrate expression correlating with previously reported acceleration of muscle reinnervation. Such enhanced HNK-1 expression was not observed after electrical stimulation in heterozygous BDNF or TrkB deficient mice. Accordingly, the degree of proper reinnervation of the quadriceps muscle, as indicated by retrograde labeling of motoneurons, was reduced in TrkB+/- mice compared to wild-type littermates. Also, recovery of quadriceps muscle function, evaluated by the novel single-frame motion analysis approach, and axonal regrowth into the distal nerve stump, assessed morphologically, were considerably delayed in TrkB+/- mice. These findings indicate that BDNF/TrkB signaling is important for functional recovery after nerve repair and suggest that up-regulation of the HNK-1 glycan is linked to this phenomenon.

Previous work had suggested that the extracellular matrix glycoproteins tenascin-C (TNC) and tenascin-R (TNR) might

influence regeneration in the central and peripheral nervous system. We investigated adult mice constitutively deficient in the expression of TNC, TNR, or both, using the facial nerve injury paradigm. Quantitative analysis of vibrissal movements revealed differential effects of the mutations on recovery of whisking: recovery of function was worse in TNC deficient and better in TNR null mice compared to wild-type littermates. In double knockout animals, vibrissal performance was insufficient, but to a lesser extent compared to TNC null mutant mice. Retrograde labeling of motoneurons in the same animals showed that similar numbers of motoneurons had reinnervated the whisker pads in all experimental groups precluding varying extents of motoneuron death and/or axon regeneration failures as causes for the different outcomes of nerve repair. These results provide evidence that TNC promotes and TNR impedes recovery after nerve lesion, findings of particular interest with regard to the scanty knowledge about factors determining success of regeneration in the peripheral nervous system of mammals.

Myelin protein zero (P0), a member of the immunoglobulin superfamily of cell adhesion molecules, is the major protein component of peripheral myelin. Mutations in the human P0 gene cause severe peripheral neuropathies, among them Charcot-Marie-Tooth 1B disease (CMT1B). To investigate the pathological consequences of a specific point mutation in the *P0* gene, we generated two independent transgenic mouse lines expressing the pathogenic CMT1B missense mutation Ile106Leu (P0sub) under the control of the P0 promoter on a wild-type background. Transgenic animals of both lines exhibited ultrastructural abnormalities in peripheral myelin as well as functional deficits strongly resembling the clinical picture of CMT1B caused by the respective mutation in humans. Therefore, our mouse lines can serve as animal models of the severe, tomaculous form of CMT1B.

12. Spinal cord injury and repair

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Recently, we have been interested in the functional roles of several recognition molecules, known to influence neurite outgrowth, cell survival and migration, in recovery from spinal cord injury. Of particular interest was tenascin-R (TNR) because its repellent properties for growing axons in a choice situation with a conducive substrate in vitro have indicated that TNR may impede regeneration in the adult mammalian CNS. We tested whether constitutive lack of TNR has beneficial impacts on recovery from spinal cord injury in adult mice. Using the Basso, Beattie, Bresnahan (BBB) locomotor rating scale, we found that open field locomotion in TNR-deficient (TNR-/-) mice recovered better that in wild-type (TNR+/+) littermates after compression of the thoracic spinal cord. We also designed, validated and applied a motion analysis approach, based on principles used for the single-frame motion analysis after femoral nerve injury, allowing numerical assessment of motor functions. We found, in agreement with the BBB score, that functions requiring low levels of supraspinal control like plantar stepping improved more in TNR-/- mice. This was not the case for motor tasks demanding precision such as ladder climbing. Morphological analyses revealed no evidence that improved recovery of some functions in the mutant mice were due to enhanced tissue sparing or axonal regrowth. Estimates of perisomatic puncta revealed reduced innervation by cholinergic and GABAergic terminals around motoneurons in intact TNR-/- compared to TNR+/+ mice. Relative to non-lesioned animals, spinal cord repair was associated with increase in GABAergic and decrease

of glutamatergic puncta in TNR-/- but not in TNR+/+ mice. Our results suggest that TNR restricts functional recovery by limiting post-traumatic remodeling of synapses around motoneuronal cell bodies where TNR is normally expressed in perineuronal nets.

The close homologue of L1, CHL1, also attracted our attention because of its wide expression in the adult mouse spinal cord and its influences on cell survival and neurite outgrowth shown previously *in vitro*. Assessment of motor functions after spinal cord injury in adult mice revealed better recovery in CHL1 deficient mice compared with wild-type littermates. The better outcome appeared to be associated with lack of CHL1 expression, as opposed to wild-type mice, in astrocytes residing in the lesion scar. *In vitro* analyses using co-cultures of astrocytes and hippocampal neurons showed that neurite outgrowth on CHL1 deficient astrocytes was better than that on wild-type astrocytes suggesting that CHL1 expression in astrocytes is among the factors limiting regeneration in the spinal cord of adult mammals.

Previous work had shown that application of exogenous L1 to the injured spinal cord of rats promotes functional recovery. The positive impact of L1 on spinal cord regeneration could be further demonstrated in recent experiments using transgenic mice. As opposed to wild-type littermates, Thy-1 promoter-driven overexpression of L1 in motoneurons and corticospinal neurons lead to superior functional restoration as estimated by single-frame motion analysis. Moreover, similar augmentation of recovery was achieved when L1 was overexpressed in neuronal and non-neuronal cells in the injured spinal cord of wild-type mice using an adeno-associated vector. These findings not only unequivocally show that L1 is beneficial for recovery from spinal cord injury but also provide basis for design of clinically feasible applications.

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Theses

Bachelor of Science

Petrova, Iveta (2005). Studies on the expression pattern of the cell recognition molecule close homologue of L1 (CHL1) in the postnatal mouse brain: Evaluation of novel probes. University of Applied Science, Bonn-Rhein-Sieg.

Diploma

Cassens, Claas (2004). Charakterisierung der Interaktionspartner der Kir3.2 Untereinheit des Kaliumkanals Kir3 – MUPP und FILIP. Universität Hamburg.

Chalaris, Athena (2004). Die Suche nach Interaktionspartnern des 'multiple PDZ-domain containing Protein' MUPP1 durch 'yeast-two-hybrid' Untersuchungen. Universität Hamburg.

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Ramser, Elisa (2005). Untersuchung der Wechselwirkung von 14-3-3 mit neuralen Proteinen. Medizinische Hochschule Hannover.

Kähler, Birgit (2006). Involvement of the cell adhesion molecules NCAM and L1 in the cocaine-induced activation of the reward system: a behavioral and molecular biology study. Universität Bremen.

Master of Science

Stan, Adriana (2004). Influence of NCAM on serotonergic system: impact for hippocampal synaptic plasticity. University of Bucharest.

Stoenica, Luminita (2004). The role of NCAM and PSA in synaptic plasticity. LTP recordings in vivo. University of Bucharest.

Stan, Tiberiu (2005). Spatial learning and place cell activity in mice deficient in neuronal cell adhesion molecule L1. University of Bucharest.

Kurschat, Nina (2006). Analysis of the epitope of the L1-antibody 324 and search for a polysialic acid peptide mimetic via phage display. Universität Lübeck.

Dissertations (PhD)

Franke, Jens (2004). Isolierung und funktionelle Charakterisierung von Saccharid mimikrierenden Peptiden zur Blockierung der Adhäsion von *Pseudomonas aeruginosa* an Epithelien des Respirationstraktes. Universität Hamburg.

Schrewe, Anja (2004). Learning induced neuronal activation pattern measured by c-fos expression in murine hippocampus and nucleus accumbens. Universität Münster.

Behrendt, Anja (2005). Untersuchungen zur Interaktion zwischen dem Zelladhäsionsmolekül L1 und dem Zytoskelettprotein Cortactin. Universität Hamburg.

Brendel, Frauke (2005). Untersuchungen zu der zuckerabhängigen Interaktion der neuralen Zelladhäsionsmoleküle L1 und CD24 und ihrer funktionellen Bedeutung im Nervensystem der Maus. Universität Hamburg.

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Schneegans, Tanja (2005). Untersuchungen zum Einfluss der Proteine NFI-A und LIS1 auf Expression und Funktion des Zellerkennungsmoleküls L1. Universität Hamburg.

Zerwas, Meike P. (2005). Generation and analysis of a mouse line with neuronal transgenic L1 expression and behavioural analysis of L1 deficient mice. Universität Hamburg.

Lepsveridze, Eka (2006). Synaptic transmission and plasticity in major excitatory hippocampal synapses of L1 conditional and CHL1 constitutive knockout mice *(Mus musculus).* Universität Hamburg.

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Sibbe, Mirjam (2006). In vitro and in vivo analysis of the functional significance of Tenascin-R and -C, CD24 and Semaphorin3A for neural stem cell behaviour and axonal pathfinding in Mus musculus (L.) 1758 and Rattus norvegicus (Berkenhout) 1769. Universität Hamburg.

Medical Dissertations

Angerer, Alexander (2005). Different roles of hippocampal subregions CA1, CA3 and dentate gyrus in spatial learning and memory: a study on behavior and c-fos expression. Universität Hamburg.

Thilo, Barbara Elisabeth (2005). Age dependent and region-specific alterations in the brain of CHL1 deficient mice: An emerging animal-based model of schizophrenia. Universität Hamburg.

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Institute for Developmental Neurobiology

The institute has presently no appointed director succeeding Chica Schaller, who retired in May 2005. Her main research interests were focussed on early development of the nervous system. In her research she has made major contributions to understanding how extracellular signals are transmitted over appropiate receptors to the interior of the cell to regulate proliferation, differentiation, pattern formation, and behavior. In an independent research project U. Borgmeyer continued his studies on nuclear receptors and their effects on transcriptional control in early mammalian development. A. Methner, who shared with the institute a common interest in GPCR cell biology, moved with his guest group to the University of Düsseldorf in 2005.

1. Orphan nuclear receptor signaling

Uwe Borgmeyer, Moritz Hentschke, Ute Süsens, Andrea Zaisser, Irm Hermans-Borgmeyer

Orphan nuclear receptors represent unique molecular tools to elucidate novel hormonal signaling pathways that participate in normal physiological processes and in disease states. These proteins are structurally related to classical nuclear hormone receptors that enhance transcription of distinct target genes in response to endocrine signaling by recruitment of coregulators. The germ cell nuclear factor GCNF which is transiently up-regulated upon differentiation of embryonic stem cells represses several pluripotency genes. Among the direct targets is CRIPTO-1, a co-receptor for the morphogen nodal.

The latest isolated mammalian orphan nuclear receptor is the estrogen-related receptor ERRγ. Just like GCNF, ERRγ binds as a homodimer to derivatives of the direct repeat 5'-AGGTCA-3'. ERRγ response elements in the promoter of genes involved in energy metabolism, as well as activation by PGC-1 coactivators hint toward metabolic regulatory functions in tissues with high energy expenditure.

To investigate the molecular functions of ERRγ, we have established a reporter system which enables us to test the transcriptional activation of mutant proteins and their interaction with coregulator proteins. Using the reporter system, we can demonstrate that different isoforms have unique amino-terminally located activation domains. As a next step we would like to determine the signaling pathways that modify the activity of the protein.

In addition, we have set up biochemical and genetic approaches to identify target genes to get new insights into the biological functions of ERRγ.

2. Control of growth and differentiation processes in Hydra

Sabine Hoffmeister-Ullerich

The aim of this project is a better understanding of the mechanisms controlling growth and differentiation in the basal metazoan Hydra. As a result of these studies, two morphogenetically active peptides, pedin and pedibin, were isolated from hydra tissue and shown to be involved in the regulation of proliferation and differentiation processes. Pedin, a peptide of 13 amino acids, stimulates foot formation in hydra. The corresponding transcripts are 3.8 kb in size

encoding a precursor protein with a size of about 110 kDa, which contains 13 copies of the peptide. Interestingly, the deduced amino acid sequence of the precursor comprises 27 copies of a beta-thymosin-like repeat domain. Hence, we named the precursor protein thypedin. Pedin transcripts are present along the body axis of the animal with slightly higher abundance in the foot to bud region and in the head. Pedin is expressed mainly in epithelial cells of the ectoderm and endoderm. During budding it is present in the evaginating bud. The early appearance of transcripts during phases of cell-fate specification like budding indicates that pedin may be involved in differentiation processes in hydra. This is confirmed by the fact that pedin stimulates bud outgrowth. Thymosin-repeat containing proteins are well known for their regulatory influence on actin polymerisation. Here we show the first indirect evidence that thypedin may be able to interact with actin as well. Since actin polymerisation and depolymerisation processes are known to take place during morphogenetic processes, these findings may hint at new aspects of the function of pedin and its precursor in pattern formation in hydra.

Guest group "Protective Signaling"

Axel Methner

Oxidative glutamate toxicity

Besides the rapid cell death by excitotoxicity, prolonged and excessive amounts of glutamate kills cells by oxidative glutamate toxicity. Here, increased extracellular glutamate depletes cells of cystine by blocking the antiporter X_c^-, which transports cystine into the cells in exchange with extracellular glutamate. X_c^- consists of two subunits, the specific subunit xCT and the 4F2 heavy chain. Cystine is required for the synthesis of glutathione (GSH), the most important antioxidant in the brain and GSH depletion renders the cells incapable of removing reactive oxygen species, which are constantly produced in the mitochondria as well as during certain enzymatic reactions, and ultimately leads to cell death by oxidative stress. We recently selected HT22 cells (HT22R) resistant to glutamate and oxidative stress, which exhibit reduced glutamate-induced glutathione depletion mediated by an increase in the antiporter subunit xCT and X_c^- activity. We could show that glutamate transporters (excitatory amino acid transporters, EAAT) and system X_c^- interact cooperatively to prevent glutathione depletion caused by high extracellular glutamate. These glutamate-resistant cells also exhibit an upregulation of neuroprotective G-protein coupled receptors (GPCR) that protect by increasing Gs and Gq coupled signal transmission, which have been shown previously by us and others to protect from glutamate toxicity.

Mechanisms of ischemic preconditioning

Ischemic preconditioning is a phenomenon that describes the induction of tolerance against ischemia by a preceding smaller ischemia. It takes 24 to 48 hours to develop and depends on new protein synthesis. The proto-oncogene Bcl-2 and its interacting protein BI-1 are both implicated in ischemic preconditioning and more generally in the mechanisms of cell death and survival. BI-1 and Bcl-2 co-localize in the endoplasmic reticulum (ER) and physically interacts with the BH4 domain of Bcl-2. We could show that BI-1 is upregulated in in vitro and in vivo models of ischemia and that it protects against cell death by reducing the ER calcium content.

In 2005, the group relocated to the University of Düsseldorf.

Support of guest group Methner

Deutsche Forschungsgemeinschaft GRK 255 (2002-2005, 35.000 DM und 4 Jahre Doktorandenstipendium)

Stiftung für Alternsforschung (2004, 2 Jahre Doktorandenstipendium)

Serono (2 Jahre Doktorandenstipendium und Sachmittel)

Deutsche Forschungsgemeinschaft (Me1922/9-1, 2 Jahre Doktorandenstipendium, 2 Jahre MTA, Sachmittel)

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Dissertations

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Habilitation

Methner, Axel (2005). Role of G-protein coupled signalling in oxidative glutamate toxicity Fachbereich Medizin. Universität Hamburg.

Dissertations of guest group Methner

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Pantlen, Anna (2006). Klonierung und Charakterisierung der G-Protein gekoppelten Rezeptoren mUPR1, mGpr15 und hVPAC2. Universität Hamburg.

Awards of guest group Methner

Dr. Martini-Preis to Axel Methner, „Schutz durch Schlaganfall? Mechanismus des antiapoptotischen Proteins Bax Inhibitor 1“, 2005

Collaborations

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Structure of the Institute

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	Prof. Dr. O. Pongs (comm.)
Research associates:	PD Dr. Uwe Borgmeyer
	PD Dr. Sabine Hoffmeister-Ullerich*
Graduate students to postdocs:	Dr. Meriem Rezgaoui*
	Dr. Jens Urny*
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Institute for Molecular Neuropathology

Thomas J. Jentsch

Our research is concerned with ion transport processes, in particular with the CLC chloride channel family, as well as with KCNQ K^+ channels and KCC K-Cl cotransporters. We study the structure and function of these ion transport proteins and are very interested in their role for the cell and the organism as a whole. We have elucidated several inherited human diseases that are caused by mutations in Cl^- or K^+ channels and have generated and analysed a large number of knock-out (KO) mouse models. These animal models led our research into different organ systems, including the brain, eye, inner ear, intestine, kidney, and bone.

Much of our research focuses on the family of CLC Cl^- channels, which we discovered at the ZMNH back in 1990. In mammals, there are nine different CLC genes that can be grouped into three branches by homology. The first branch (ClC-1, -2, -Ka and Kb) represents channels which exert their function in the plasma membrane. The members of the other two branches, ClC-3/4/5 and ClC-6/7, respectively, are localized predominantly, but not exclusively, in vesicles of the endocytotic/lysosomal pathway. They are thought to be necessary for an efficient acidification of these organelles. A very surprising recent development was the discovery that the E.coli protein ClC-e1, as well as the mammalian endosomal ClC-4 and ClC-5, do not function as Cl channels, but rather as electrogenic Cl^-/H^+-exchangers (7).

Only one β-subunit, barttin, was known for CLC channels. It associates exclusively with ClC-Ka and ClC-Kb and is crucial for their transport to the plasma membrane. We have recently discovered the second β-subunit for CLC proteins: Ostm1 associates with ClC-7 and is necessary for its stability. Mutations in either Ostm1 or ClC-7 lead to osteopetrosis and lysosomal storage disease.

The crystallization of a bacterial CLC protein by Dutzler, MacKinnon and colleagues greatly facilitates structure-function studies. We are currently exploring the difference between CLC chloride channels and chloride/proton exchangers by a combination of molecular modelling, mutagenesis, and biophysical analysis.

After our identification of barttin and Ostm1 as β-subunits of CLC proteins, we are continuing our search for other interaction partners. A main focus of our work remains the physiology and pathophysiology of CLC channels which we address with mouse models and the elucidation of human diseases. These pathologies (myotonia, renal salt loss, proteinuria, deafness, osteopetrosis, blindness, CNS degeneration…) underscore the importance of Cl^- transport and reveal its diverse functions. As a new addition to CLC pathologies, we have recently reported that the disruption of ClC-6 leads to a lysosomal storage disease that is distinct from the one observed with a lack of ClC-7.

Several years ago, we began studying KCC K-Cl cotransporters because they are likely to influence intracellular Cl^- in neurons, a role also attributed to the ubiquitously expressed Cl^- channel ClC-2. Indeed, our KO mouse models have shown that KCC2 is the main player in determining Cl^- in neurons, with a minor role played by KCC3. The upregulation of KCC2 during development (1) is essential for the 'switch' of the response to GABA from excitatory to inhibitory.

Finally, we continue our studies on KCNQ channels, which we have previously shown to be involved in epilepsy and

deafness. In addition to structure-function investigations, we mainly focus on mouse models and diseases and have recently published our work on KCNQ4 mouse models for human deafness.

After 18 years at the ZMNH, were I established my Junior Research Group in 1988 as one of its first members, we moved to the Research Campus of Berlin-Buch in August 2006. We have established our laboratory in a new building belonging to both the Leibniz-Institut für Molekulare Pharmakologie (FMP) and the Max-Delbrück-Centrum für Molekulare Medizin (MDC), with myself having a joint appointment by both institutions and holding the position of Professor at the Charité, University Medicine Berlin. This brought our time at the ZMNH to an end, a very productive period during which we identified the first CLC protein in 1990, characterized the CLC gene family in considerable detail, and extended our studies to KCNQ K^+ channels and KCC K-Cl cotransporters. We are now continuing our investigations in the very stimulating, excellent research environment of Berlin. I like to express my wish that the ZMNH, which provided very good conditions for our work, continues its success story.

1. Structure and function of CLC Cl^- channels and transporters

A most surprising development in the CLC field was the discovery by Accardi and Miller (Nature 427, 803-807 (2004)) that the *E. coli* ClC-e1 protein, that was used for crystallization by Dutzler and MacKinnon, does not function as a chloride channel, but rather as an electrogenic $2Cl^-/H^+$-exchanger. We therefore investigated whether some mammalian CLCs may similarly mediate a coupled ion exchange. We focussed on ClC-4 and -5, as no single channel data were available for these proteins. Indeed, both ClC-4 and ClC-5 mediate strongly voltage-dependent Cl^-/H^+-exchange, as determined by measurements of internal pH of transfected cells clamped to different voltages using the perforated patch-clamp technique (7). Driving chloride into the cell by positive potential led to an extrusion of H^+ also against electrochemical gradients. By contrast, the *Torpedo* channel ClC-0 did not show exchange properties and rather is a well-behaved ion channel. Mutating a certain glutamate that, in the crystal of ClC-e1, appears to block the access of chloride to the central anion binding site, abolished the coupling of chloride to protons (7). We are currently using site-directed mutagenesis in a combination with biophysical analysis to explore the structural differences between CLC chloride channels and chloride –proton exchangers.

2. CLC mouse models

In order to understand the diverse physiological functions of CLC channels and transporters and to generate animal models for human disease, we have disrupted the mouse genes encoding ClC-2, ClC-3, … to ClC-7. This has not only led to disease models, but has shed considerable light on their physiological function.

ClC-3 to ClC-7 reside in cytoplasmic vesicles of the endocytotic-lysosomal pathway and also on synaptic vesicles (ClC-3). Their subcellular expression overlaps to some extent. They are thought to facilitate the acidification of these vesicles by providing an electric shunt for the proton pump, as we have shown directly for ClC-5 in kidney endosomes and for ClC-3 in synaptic vesicles. The importance of this acidification is best understood in the case of ClC-5, where the knock-out leads to a defect in endocytosis, and in the case of ClC-7, where the defective acidification of the resorption lacuna of osteoclasts results in osteopetrosis in mice and man carrying

non-functional ClC-7. Using the TRAP-promoter to express the ClC-7 cDNA specifically in osteoclasts, we rescued the bone phenotype of ClC-7 KO mice (5). This extends the very restricted lifespan (about 6 weeks) of ClC-7 KO mice only by a couple of weeks, suggesting that these mice rather die from their neurological problems. We have found that the ClC-7 KO has a severe CNS degeneration in addition to osteopetrosis (5). Consistent with the lysosomal localization of ClC-7, the mice present with lysosomal storage disease. It displays the typical features of a neuronal ceroid lipofuscinosis (NCL), including deposits of the subunit c of the mitochondrial ATP synthase. Cytoplasmic deposits were found in neurons of all brain regions, as well as in proximal tubular cells of the kidney. Contrary to our expectation, the lysosomal pH of neurons and fibroblasts in culture were normal (5). We have recently found an important β-subunit for ClC-7 (Ostm1, as discussed in the next paragraph.

With newly generated, KO controlled antibodies we showed that ClC-6 is located on late endosomes, with only trace amounts being on lysosomes (15). ClC-6 KO mice have no immediately visible phenotype, are fertile and have a normal lifespan. However, they display intraneuronal deposits that are also typical for lysosomal storage disease of the NCL type (15). In contrast to ClC-7 KO mice, these intracellular deposits, which stain for all lysosomal markers that were investigated, do not accumulate in the cytosplasm, but rather in initial axon segments. Deposits are particularly severe in dorsal root ganglia, leading to a reduced pain sensitivity of these mice (15). As our studies identified *CLCN6* as a candidate gene for clinically mild forms of human NCL, we screened more than 30 patients with mild NCL for *CLCN6* mutations. We found two missense mutations in two individuals, which, however, do not prove the involvement of ClC-6 in human NCL (15).

To eliminate possible redundancies between vesicular CLC proteins, we are generating and analyzing double-KO animals, which often display more severe phenotypes.

3. Accessory subunits of CLC channels

We had shown in 2001 (Estévez et al. Nature 416, 874) that barttin, a membrane protein with two transmembrane domains that is mutated in Bartter syndrome type IV (presenting with renal salt loss and deafness), acts as an accessory β-subunit of ClC-Ka and ClC-Kb. It ico-localizes with these Cl^- channel α-subunits in renal epithelia and in the stria vascularis of the cochlea. Barttin is necessary for the transport of the channel complex to the plasma membrane. We have generated barttin mouse models which we are currently analyzing.

We have recently identified Ostm1 as a previously unknown β-subunit for ClC-7 (10). Ostm1 is mutated in the *grey lethal* mouse, a spontaneous mutant that has severe osteopetrosis and grey hair (Chalhoub et al., Nature Med. 9, 399-406 (2004)). The small protein encoded by this gene has two hydrophobic areas. Its function was unknown. Also some humans with severe infantile osteopetrosis carry mutations in *OSTM1*. As the phenotype of the *grey lethal* mouse is very similar to that of the ClC-7 KO mouse, we investigated whether there might be some connection. Indeed, ClC-7/Ostm1 form a molecular complex that is targeted to lysosomes and to the ruffled border of osteoclasts (10). In *grey lethal* mice (which lack Ostm1) the ClC-7 protein was barely detectable, and in ClC-7 KO mice the Ostm1 protein was nearly absent. Thus, the stability of either protein depends on the association with its partner (10). The severe downregulation of ClC-7 in *grey lethal* mice explains the pathogenesis of their osteopetrotic phenotype. We also discovered that

grey lethal mice display a lysosomal storage disease just like ClC-7 KO mice (10).

We continue our search for other proteins that may associate with CLCs.

4. Functions of KCC K-Cl cotransporters revealed by KO mouse models

KCC proteins co-transport K^+ and Cl^- ions in an electroneutral manner. There are four different isoforms encoded by different genes (*Kcc1* through *Kcc4*).

KCC proteins play a role in transepithelial transport, cell volume regulation, and in the determination of intracellular Cl^- concentration in neurons. The latter function is crucial for synaptic inhibition, as $[Cl^-]_i$ determines the electrical response of $GABA_A$- and glycine-receptors. The generation and analysis of KCC KO mice allowed us to assess the relevance of K-Cl cotransport for these functions and to discover new, unexpected roles of KCC proteins.

KCC2 is a neuronal isoform that is the main player in determining $[Cl^-]_i$, as shown by the constitutive KO we have previously generated and analysed (Hübner et al., Neuron 30, 515 (2001)). Its transcription is upregulated after birth and parallels the development of an inhibitory response to GABA (1). The initial excitatory effect of GABA, which is due to a high value of $[Cl^-]_i$, is thought to be important for the development of the CNS. We are currently working on several KCC2 mouse models to further understand the role of $[Cl^-]_i$ in brain function.

During the past years, we have knocked out all KCC isoforms. Before the reporting period, we had already published first reports on the KOs of KCC2 -3, and -4 (Hübner et al., Neuron 30, 515 (2001); Boettger et al., Nature 416, 874-878; Boettger et al., EMBO J. 22, 5422-5434). In a new study, we have investigated the mechanism of hypertension in the KCC3 mouse, showing that it is of neurogenic origin (11). We have generated 'floxed' mice for KCC2, KCC3, and KCC4, which we are crossing with various transgenic mouse lines that express the Cre-recombinase in different tissues. We are particularly interested in the impact of a loss of KCC2 in some regions of the CNS, and in the overlapping function of KCCs in epithelial tissues.

The KO of KCC1, which was still unpublished at the time of writing, does not lead to an overt phenotype. In our analysis we focus on the role of KCC1 in the volume regulation of red blood cells (RBCs), a process in which it was thought to play a major role. We showed, however, that only the double KO of KCC1 and KCC3 led to a rather severe inhibition of RBC volume regulation, with KCC3 being more important. K-Cl cotransport is thought to play a major role in the pathogenesis of sickle cell disease. The aggregation of the mutant globin protein depends exquisitely on its concentration. K-Cl cotransport, which decreases cell volume and hence increases the globin concentration, is pathologically activated in RBCs from patients. Therefore, inhibition of K-Cl cotransport has been suggested as a new therapeutic strategy to treat that disease. We are therefore analyzing KCC1/3 double KOs crossed to the SAD sickle cell mouse model of Marie Trudel.

5. KCNQ K^+ channels: structure, function, and role in disease

We have previously cloned KCNQ2, -3, -4, and -5 K^+ channels and have shown that KCNQ2 and KCNQ3 are involved in a rare form of human neonatal epilepsy, while KCNQ4 is mutated in DFNA2, an autosomal dominant, slowly progressive hearing loss in humans.

KCNQ3 can form heteromers with KCNQ2, KCNQ4, and KCNQ5, but not with KCNQ1. In a collaboration with our lab, Michael Schwake and Thomas Friedrich have investigated determinants of KCNQ assembly, using mutagenesis and functional expression as techniques (12). In the same collaboration, we have determined structural requirements for the binding of retigabine (6), a specific activator of M-type KCNQ channels that may be useful for the treatment of epilepsy.

Our recent work on KCNQ channels focused on a mouse model for DFNA2, a human progressive deafness that is caused by mutations in the KCNQ4 K^+ channel. We generated and analyzed KCNQ4 knock-out mice, as well as mice carrying a dominant negative mutation that we had identified in a human patient with DFNA2 (9). When assessed by auditory brain stem responses, the hearing of these mice was nearly normal at the onset of hearing (at two weeks). It rapidly declined over the following two weeks for mice lacking KCNQ4, whereas the hearing loss developed much more slowly with mice heterozygous for the dominant negative mutation. These latter mice are hence an excellent model system for the slowly progressive human hearing loss DFNA2. Morphological analysis revealed a selective degeneration of outer hair cells (OHCs), with inner hair cells remaining intact even in old animals. OHCs are electromotile cells which amplify the mechanical vibrations in the organ of Corti and increase the sensitivity of hearing by about 50-60 dB. The final hearing loss of the animals was indeed compatible with a selective loss of outer hair cells. Patch-clamp recordings of OHCs revealed that the $I_{k,n}$ potassium current was absent from KO mice, proving that it depends on KCNQ4. OHCs were depolarized by about 15 mV. We suggest that this depolarization, which may lead to Ca^{++} influx, is responsible for their degeneration. Otoacoustic emissions, which result from their nonlinear electromotile response, demonstrated a dysfunction of OHCs before they showed signs of degeneration. Inner hair cells were only slightly depolarized and their synaptic function appeared to be normal. Hence, DFNA2 deafness may be totally explained by a loss of sensory outer hair cells (9).

Support

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Publications

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Theses

Dissertations

Rust, Marco; Bielefeld (2004)

Julia Offe; Hamburg (2005)

Blanz, Judith; Hamburg (2005)

Maritzen, Tanja; Hamburg (2005)

Awards and Honours

Homer W. Smith Award (American Society of Nephrology), St. Louis, 2004

Hodgkin-Huxley-Katz Prize Lecture (Physiological Society), London, 2006

Elected Member of the Hamburger Akademie der Wissenschaften, 2006

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Institute for Neural Signal Transduction

Olaf Pongs

A fundamental property of neurons is the generation and propagation of electrical 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. Many genes encode potassium channels yielding a potentially bewildering number of potassium channels with diverse properties. But only a few of the potassium channels have been associated with heritable diseases. Many of the potassium channel-related diseases display an episodic occurrence. Thus, the link between the expression of a dysfunctional potassium channel and the patho-physiological phenotype is often not obvious. In recent years, mutations in a few potassium-channel genes were discovered which were associated with long and short forms of the arrhythmogenic cardiac QT syndrome and, respectively, with a benign form of juvenile epilepsy. We have continued our work on the structure, function and physiology of potassium channels, in particular the cardiac I_{Ks} and I_{Kr} channels and neuronal M-channels. Specifically, we addressed the effects of distinct potassium channel mutations on gating behaviour by combining biochemical, electrophysiological and structural analyses. Also, we generated various mouse models to investiga,te the physiological role of potassium channels involved in central nervous system diseases.

1. Genetics of Human Cardiac Diseases

Jaqueline Alig, Chi-un Choe, Patrick Friederich*, Dirk Isbrandt, Axel Neu*, Gregor Sachse, Steffie Sandke*, Anna Solth*, Kathrin Sauter, Stefan Schillemeit*

Dynamic regulation of contraction rate and force of the heart is an essential property of the cardiovascular system and crucial for the adaptation of blood flow in response to environmental factors. It is, in particular, the stimulation of the sympathetic nervous system (SNS) that in response to exercise or emotional stress can lead to an immediate and dramatic increase in heart rate, which is paralleled by a concomitant decrease in action potential duration of cardiac myocytes and a shortening of the QT interval of the surface electrocardiogram. To a large extent these effects are mediated by activation of β-adrenergic receptors that regulate the activity of specific cardiac ion channels via increases in intracellular cAMP concentrations. Mutations in cardiac ion channel genes that lead to altered channel function or channel regulation may thus lead to syndromes associated with cardiac rhythm disturbances.

Our past and present research focuses on the molecular basis of inherited cardiac disorders such as the long QT syndrome (LQTS) and sinus node dysfunction (SND). By characterizing ion channel dysfunction resulting from gene mutations we aim to get insight into the pathophysiological bases of these diseases.

Long QT syndrome

The long QT syndrome (LQTS) is a cardiac disorder that increases the risk of sudden death. The disease is characterized by a prolongation of the QT interval on the electrocardiogram. Patients suffer from syncopal episodes due to ventricular arrhythmias like *Torsade de pointes* and a high risk of sudden cardiac death. In collaboration with Dr. E. Schulze-Bahr (Department of Cardiology, University Hospital Münster) we identified and functionally characterized novel LQTS-associated mutations in the potassium channel α subunits *HERG* and *KCNQ1* and in their auxiliary subunits *KCNE1* and *KCNE2*. The LQTS-associated mutations caused heterogeneous *in vitro* phenotypes including altered intracellular trafficking, reduced subunit stability, and altered biophysical properties that may explain the clinical phenotype. Interestingly, some C-terminal HERG mutations showed under standard conditions only minute changes in their properties. However, when co-expressed with the protein 14-3-3ε, which normally shifts the conductance-voltage relation for HERG channels towards more negative membrane potentials, the mutant HERG channels were insensitive. The data suggested that β-adrenergic, cAMP-dependent regulation was impaired in the mutant HERG channels. Based on the experimental data we modeled cardiac action potentials under various conditions. The results demonstrated the physiological importance of coupling β-adrenergic stimulation and HERG channel activity, which is key to SNS control of cardiac electric activity.

Sinus node dysfunction

SND is the major cause necessitating pacemaker implantation and accounts for approximately half of all patients requiring a pacemaker. The disease commonly occurs in adults with acquired heart disease, during antiarrhythmic therapy, or after surgically corrected, congenital heart disease. In a significant portion of patients, however, SND appears in the absence of identifiable cardiac abnormalities or other associated conditions ('idiopathic' SND). The cardiac pacemaker current I_f is a major determinant of diastolic depolarization in sinus nodal cells and has a key role in heart beat generation. We previously showed that a form of 'idiopathic' sinus node dysfunction (SND) may be related to a heterozygous mutation in the human *HCN4* gene. The mutation resulted in a mutant HCN4 protein with a truncated C-terminus that lacked the cyclic nucleotide-binding domain. Patch-clamp experiments showed that the mutant HCN4 channels mediated I_f -like currents that were insensitive to increased cellular cAMP levels. Together, the clinical, genetic, and *in vitro* data provided a likely explanation for the patient's sinus bradycardia and the chronotropic incompetence. To futher understand the functional role of HCN channels in cardiac pacemaker activity, we generated transgenic mouse lines expressing specifically in cardiac tissue a dominant-negative HCN4 subunit. This subunit attenuated HCN-channel activity, which apparently leads in the transgenic mice to a bradycardic phenotype comparable to human patients carrying mutations in the HCN4 gene.

2. Regulation of cardiac potassium channel activity in primary cardiomyocytes

Martin Kruse, Li Juan Ma*

We use primary cardiac myocytes in combination with heterologous *in vitro* expression systems to investigate the molecular basis of the regulation of ventricular potassium channel activity by various signaling pathways. For these investigations we have established metods and protocol to record from primary mouse cardiomyocytes potassium

outward currents in the perforated patch-clamp configuration. In contrast to previous electrophysiological work, we could show that mouse cardiomyocytes express voltage-dependent outward currents, of which a significant part is attributable to KCNQ1 mediated currents. The results were based on the distinct pharmacology and tail current kinetics that are typical for the KCNQ1 channel and that are not shared by other voltage-gated potassium channels expressed in cardiomyocytes. In contrast to the L-type Ca-channel, application of isoprenaline to primary mouse cardiomyocytes did not alter cardiac KCNQ1 channel activity. In agreement with this finding, we observed also that an increase in intracellular cAMP concentration did not affect KCNQ1 mediated current amplitudes. Apparently, β-adrenergic stimulation of ventricular mouse cardiomyocytes stimulates Ca-channel activity, without simultaneously altering KCNQ1 channel activity. By contrast, application of phenylephrine significantly reduced KCNQ1 channel activity in the primary cardiomyocyte of the mouse. Consistent with this finding, we observed that a decrease in 4,5-phosphoinositol-phosphate concentration rapidly reduces KCNQ1 channel activity in inside-out patches.

3. Structure-based mutational analysis of the cardiac potassium channel Kv7.1

Lijuan Ma, Iris Meier, Cornelia Siebrands

KCNQ1 is a member of the Kv7 family of K^+ channels, which encodes voltage-gated K^+ (Kv) channels with important physiological functions. Coassembly of KCNQ1 channels with the β subunit KCNE1 generates a delayed-rectifier K^+ current underlying I_{Ks}, a key component in controlling the duration of the action potential in the human heart.

KCNQ1 channels have several remarkable biophysical properties which are altered upon association with KCNE1 subunits. These include a slowing of activation, a suppression of inactivation, an increase in apparent single-channel conductance and a shift of the conductance-voltage relation to more positive potentials. In addition, Rb^+ currents conducted by homomeric KCNQ1 channels are about threefold larger than K^+ currents, whereas heteromeric KCNQ1-KCNE1 have smaller inward Rb^+ currents compared to K^+ currents. The location of MinK relative to KCNQ1 in KCNQ1/KCNE1 channels is a subject of debate. It has been proposed that KCNE1 residues gain exposure to the outer pore vestibule, travel close to the ion conduction pathway near the selectivity filter, and influence the structure of the inner pore vestibule. We used an extensive alanine and tryptophan-scanning mutagenesis comprising amino-acid residues of part of the voltage-sensor (S4), S4-S5 linker region, outer- (S5/TM1), pore- (P) and inner- (S6/TM2) helices as a tool to explore important protein-protein interfaces involved in the gatingon of the human KCNQ1 and KCNQ1/KCNE1 channel, respectively. We mapped the results of our scanning mutagenesis onto the Kv1.2 crystal structure to predict interacting sites between KCNQ1 pore domain, voltage sensor and KCNE1, and between KCNQ1 pore domain and lipid. The emerging pattern is in very good agreement with the proposed structural model for the Kv1.2 channel. Importantly, our results demonstrate that the KCNQ1 channel gates between opened and closed states in a manner which more closely related to the one described for the BK-channel than that for the *Shaker* channel. In particular, the KCNQ1 channel is exquisitely sensitive to changes in intracellular Ca-concentration. This effect is mediated by calmodulin which is tightly bound to C-terminal domain(s) of KCNQ1 channel subunits. Furthermore, mutations in the C-terminus associated with

heritable LQT syndrome may alter the Ca sensitivity of the KCNQ1/KCNE1 channel. The results indicate that Ca is an important modulator of the KCNQ1/KCNE1 channel.

4. Structural analysis of potassium channel complexed with scorpion toxin by NMR spectroscopy

Sönke Hornig, Iris Meyer, Olaf Pongs

Previously, we have constructed a chimeric potassium channel in which a scorpion receptor site was transferred to the bacterial KcsA channel. The resultant KcsA-Kv1.3 channel bound the scorpion toxin kaliotoxin with high affinity in the pMolar range. Thus, we were now able to produce isotope labeled KcsA-Kv1.3 channels in large quantity amenable to high resolution solid-state NMR analysis. The latter was carried out in the laboratory of Marc Baldus at the Max-Planck Institute for Biophysical Chemistry in Göttingen. Using solid-state NMR spectroscopy we showed that high-affinity binding of kaliotoxin to KcsA-Kv1.3 was associated with significant structural rearrangements in both molecules. In particular, we observed unexpected structural changes in the upper third of the selectivity filter facing the extracellular environment of the channel. We proposed that the observed toxin-induced structural changes in the potassium channel represent an important determinant for the high specificity of toxin-potassium channel interaction. The results demonstrated that combining biochemical, electro-physiological and structural analysis is an effective method for analyzing the structure of a potassium channel – inhibitor complex. This is now being extended to other potassium channel-ligand complexes.

5. Functional analysis of neuronal KCNQ/M-channels using transgenic mouse models

Dirk Isbrandt, Quyen Le, Axel Neu, Howard Christian Peters*, Stefan Schillemeit*

Heteromeric voltage-gated potassium channel subunits of the KCNQ (Kv7) family are the molecular correlates of the native M-current that regulates subthreshold electrical excitability of many neurons. The M-current is thought to play a crucial role in the generation of medium afterhypolarisations (mAHPs) and early spike frequency adaptation in hippocampal pyramidal neurons. Mutations in either KCNQ2 or KCNQ3 co-segregate with benign familial neonatal convulsions (BFNC), a neonatal form of epilepsy.

We generated a functional knockout of the native M-current in neurons through inducible expression of a dominant-negative hKCNQ2 subunit under the control of the human prion protein romoter. Electrophysiological studies with acute brain slices revealed that mutant hippocampal CA1 neurons have normal membrane resting potentials, but, when stimulated, exhibited abnormally high action potential frequencies followed by markedly reduced afterhyperpolarizations. Most remarkably, the typical subthreshold resonance of CA1 neurons in the theta frequency range was lost. Furthermore, attenuation of M-channel activity in the brain of transgenic mice was associated with a marked learning and memory deficit. Mutant male mice were conspicuously hyperactive and suffered spontaneous seizures.

6. Kv4.2 A-type channel complexes: Control of surface expression and mechanisms of function

Robert Bähring, Jan Barghaan, Britta Callsen*, Elena Fernandez*, L. Sven Hartmann, Iris Meyer*

Kv4.2 voltage-gated potassium channels represent the molecular substrate of subthreshold-activating somatodendritic A-type currents (I_{SA}) in hippocampal CA1 pyramidal neurons. Due to their subcellular localization and their special biophysical properties Kv4.2 channels act as dendritic "shock absorbers" on one side, but also as key players in dendritic integration and synaptic plasticity.

Neuronal Kv4.2 channels may form ternary complexes with two types of accessory subunits: Cytoplasmic Kv Channel Interacting Proteins (KChIPs) and transmembrane dipeptidyl aminopeptidase-like proteins (DPPs). We coexpressed cloned human Kv4.2 α-subunits with these accessory β-subunits in heterologous cell-lines and studied the effects on channel properties: Both KChIPs and DPPs modulate Kv4.2 gating and increase Kv4.2 surface expression levels. The effects of KChIP and DPP coexpression on Kv4.2 channel inactivation gating are additive and therefore likely to reflect distinct mechanisms of channel modulation mediated by non-overlapping interaction sites.

In a Kv4.2 mutational analysis we identified minimal structural determinants of KChIP binding and KChIP-mediated channel modulation (e.g. Ala14). When mutated (e.g. A14K) these sites exhibit loss-of-function with respect to KChIP interaction. We expressed a KChIP binding-deficient and epitope-tagged α-subunit (Kv4.2A14K-HA-EGFP) in cultured hippocampal neurons and observed no impairment of dendritic targeting and surface expression for this construct. This finding suggests that KChIP binding may not be essential for dendritic transport and recruitment to the plasma membrane of Kv4.2 channels.

The Kv4.2 channel inactivation behaviour differs from the classical *Shaker* N- and C-type mechanisms and is governed by a prominent cumulative closed-state inactivation. We have identified important structural determinants of Kv4.2 closed-state inactivation by combining mutagenesis with the use of pharmacological and electrophysiological tools. Double-mutant cycle analysis of data obtained from alanine-scanning the S4-S5 linker and the distal S6 segment, identified pairs of interaction sites involved in Kv4.2 closed-state inactivation. Based on this information we generated a corresponding double-cysteine mutant and characterized it functionally in the presence of oxidizing and reducing agents. The results were consistent with the idea that temporary uncoupling of voltage-sensor and activation gate underlies Kv4.2 closed-state inactivation.

7. Frequenin

Birgit Grafelmann, Joanna Hermainski, Malte Stockebrand*

Like the KChIPs, Frequenin (Frq), also known as neuronal calcium sensor-1 (NCS-1), is a member of the family of neuronal calcium-sensor (NCS) proteins. KChIPs and Frq are close relatives. Frequenin has attracted much attention, because it may function as a calcium-sensor to modulate synaptic activity and secretion.

Frequenin, is an N-myristoylated Ca^{2+}-binding protein that has been conserved in both sequence and three-dimensional fold during evolution. We demonstrated using both genetic and biochemical approaches that the observed structural

conservation between *Saccharomyces cerevisiae* frequenin (Frq1) and human NCS-1 is also reflected at the functional level. In yeast, the sole essential target of Frq1 is the phosphatidylinositol 4-kinase isoform, Pik1; both *FRQ1* and *PIK1* are indispensable for cell viability. We proposed that the function of NCS-1 in mammals may closely resemble that of Frq1 in *S. cerevisiae*, implicating that NCS-1 may regulate phosphoinisotol-4 phosphate PI-4P) levels in mammalian cells and, thereby, the activity of several membrane proteins sensitive to PI4-P. In order to investigate this further, we have generated mouse lines which overexpress NCS-1 in the forebrain. Alternatively, we have created mouse lines in which NCS-1 has been knocked out. These mice are presently being characterized.

8. Pathophysiology underlying creatine deficiency disorders

Dirk Isbrandt, Ruben Peco

Guanidinoacetate methyltransferase (GAMT) and L-Arginine:Glycine Amidinotranferase (AGAT) deficiencies belong to the family of "creatine deficiency disorders" and are autosomal recessively inherited metabolic diseases of creatine biosynthesis. They manifest during the first months of life with moderate to severe neurological symptoms, often including developmental delay or arrest and epilepsy. Over the past years we have been studying knockout mouse models for both diseases that where generated in our laboratory by 'gene targeting strategies' using homologous recombination in embryonic stem cells. AGAT and GAMT knockout mice show biochemical abnormalities similar to the ones observed in human patients. The mice are valuable tools to study pathophysiological consequences of creatine deficiency in excitable tissues and organs such as brain, cardiac and skeletal muscles, which heavily depend on an intact energy metabolism. Since the mice displayed distinct abnormalities in white adipose tissue composition and body weight, we studied also neuronal, hormonal and metabolic pathways regulating food intake, energy expenditure and body weight.

9. Clinical research group

Patrick Friederich, Michael Chmielinski, Ulrike Eckhoff*, Johnny Kim*, Mark Andre Punke*, Ralf Scholz*, Cornelia Siebrands, Anna Solth**

Hereditary and acquired alteration of potassium channel function is a well recognized cause of severe cardiac arrhythmia and neuronal disease such as epilepsy. Many clinically used therapeutic agents cause severe cardiac and neuronal side effects including cardiac arrhythmia and seizure. Our interdisciplinary research group consisting of basic scientist and clinicians focuses on identifying molecular mechanisms underlying hereditary as well as drug induced states of pathologically altered cardiac and neuronal excitability, in particular to study drug induced cardiac arrhythmia and drug induced seizure by investigating the interaction of local anaesthetics with HERG/MiRP1 and KCNQ2/Q3 channels.

Local anaesthetic induced cardiac arrhythmia

HERG channels underlie the repolarizing cardiac potassium current I_{Kr}. They are critical for the maintenance of normal rhythmicity in human heart. Mutations in the HERG gene may lead to dysfunctional HERG channels and a reduced I_{Kr}. This is correlated with the prolongation of ventricular action potentials as well as an increase in susceptibility to ventricular arrhythmia. Inhibition of HERG channels has also been associated with local anaesthetic induced long

QT syndrome and ventricular fibrillation. HERG channels may associate in human myocardium with minK related peptide 1 (MiRP1) to form I_{Kr}. This auxillary subunit encoded in *KCNE2* has been reported to alter the pharmacological sensitivity of HERG channels. Several mutations in *hKCNE2* as well as the common T8A polymorphisms of MiRP1 have been identified to predispose individuals to drug induced cardiac arrhythmia. Although our data supports the notion that local anaesthetics cause cardiac arrhythmia in part by interacting with HERG channels our results do not support the idea that MiRP1 or mutations in *hKCNE2* are factors involved in proarrhythmic drug action. Besides their cardiotoxic effects accidental intravenous injection, overdosage or rapid systemic uptake of local anesthetics may result in severe neurotoxic side effects such as seizures. By studying the interactions of bupivacaine with retigabine at human KCNQ2/Q3 channels we defined the possibility of a novel therapeutic approach in the treatment of local anesthetic induced seizures. This may be particularly advantageous as retigabine in contrast to commonly used agents for the treatment of local anesthetic induced seizure has not been reported to cause respiratory depression.

10. Olfaction and GnRH neural circuitry

Ulrich Boehm, Ji-Won Kim*, Tanja Klein*, Christian Mayer*, Katja Stein*, Sabine Wehrmann*, Shuping Wen**

The olfactory system detects odorants that elicit odor perceptions as well as pheromones that stimulate instinctive behaviors or hormonal changes. Odor perception and the memories it can evoke involve higher cortical processing areas and neural circuits capable of the plasticity needed for learning. In contrast, the stereotyped behaviors and physiological responses induced by pheromones imply that these effects are mediated by „hard-wired“ neural circuits in the central nervous system. Elucidating the design and neuronal composition of these and other behavioral circuits stands as a challenge to comprehending the mechanisms underlying behavior.

Pheromones released by mammals trigger innate behaviors ranging from intermale aggression to female Lordosis in response to male mounting. Effects of pheromones on reproductive behavior and physiology have been linked to gonadotropin releasing hormone (GnRH), a peptide produced by a subset of neurons in the hypothalamus. In addition to inducing gonadotropin release from the pituitary, GnRH stimulates reproductive behaviors in both male and female mammals via neural circuits in the hypothalamus.

We have recently used genetic transneuronal tracing to visualize the neural circuitry of GnRH neurons in mice. Our experiments show that GnRH neurons receive pheromone signals from both odor and pheromone relays in the brain, and may also receive common odor signals. Moreover, feedback loops are evident whereby GnRH neurons could influence both odor and pheromone processing. Remarkably, ~800 GnRH neurons communicate with ~50,000 neurons in 53 functionally diverse brain areas, with some connections exhibiting sexual dimorphism. Our studies revealed a complex interplay between reproduction and other functions in which GnRH neurons appear to integrate information from multiple sources and modulate a variety of brain functions.

We have started to functionally dissect GnRH neural circuitry by selectively tagging neuronal subpopulations both up- and downstream of GnRH neurons with Cre recombinase in transgenic mouse models. This experimental approach enables us to selectively manipulate different aspects of GnRH neural circuits.

We expect that functional characterization of GnRH neurons and their neural circuitry will contribute to a detailed understanding of the role of GnRH peptide in the brain and unravel mechanisms underlying pheromone effects on reproductive behavior in mice.

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Fernandez, Elena (2004). Characterization of KChIP association with the N-terminus of the α-subunit of Voltage-dependent Kv4.2 potassium channel

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Peco Navio, Ruben (2006). Pathophysiology underlying Creatine deficiency syndromes

Prices

Martini Price to Dr. Dirk Isbrandt (2006)

Research Award of the European Society of Anaesthesiology to Dr. Patrick Friederich (2005)

Karl Horatz-Promotionspreis für Anaesthesiologie und Notfallmedizin to Dr. Anna Solth (2006)

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Dr. Jean-Pierre Montmayeur, CSG-CNRS, Dijon, France.

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Prof. Dr. H. Ehmke, Physiology

Prof. Dr. T. Eschenhagen, Pharmacology

Prof. Dr. Jürgen R. Schwarz, Physiology

Prof. Dr. K. Ullrich, Pediatry

Structure of the Institute

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	Elena Fernandez*
	Birgit Grafelmann*
	Sven Hartmann
	Joanna Hermainski*
	Sönke Hornig
	Simone Hubo
	Tanja Klein*
	Martin Kruse
	Quyen Le
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	Christoph Ogrodowczyk
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	Gregor Sachse
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Institute for Neuroimmunology and Clinical Multiple Sclerosis Research (INiMS)

Roland Martin

The Institute for Neuroimmunology and Clinical Multiple Sclerosis (MS) Research is the result of a joint initiative by the ZMNH and the Department of Neurology, University Clinic Eppendorf (UKE). Its concept won a national competition for funding by the Gemeinnützige Hertie Stiftung, which supports the INiMS during the next 5 years with 1.25 Million Euros. The newly founded Institute opened September 2006 and intends to bridge between the basic science environment at the ZMNH and disease-oriented clinical research. The latter is embedded in the Department of Neurology (Head: Christian Gerloff) and plans to pursue clinical trials, investigate questions related to disease heterogeneity and biomarkers in MS, but will also provide patient care in the outpatient clinic, the MS day hospital and for inpatients.

INiMS is intended to evolve rapidly into a center of excellence in both the areas of basic neuroimmunology and in clinical MS research. Besides interactions with other institutes and junior research groups at the ZMNH, INiMS will collaborate with numerous clinics and institutes at the UKE, among them the Departments of Neurology, Neuroradiology, Neuropathology, Systemic Neurosciences, Neurophysiology, Pharmacology, the Section for Hematopoietic Stem Cell Transplantation, the Institute for Transfusion Medicine, the Bernhard Nocht Institute, and others both nationally and internationally.

1. Priority Programmes in Basic Research

(Group leader: Dr. E. Tolosa)

Etiology of MS including the investigation of genetic susceptibility factors and infectious triggers.

Immunopathogenesis of MS with particular focus on the role of autoantigen-specific T cells in MS, the role of natural killer cells and their receptors in autoimmune diseases, antigen presentation in the thymus and peripheral immune compartments, the role of the HLA system for MS pathogenesis.

Cross-talk, parallels and differences between immune- and central nervous systems with particular focus on shared receptors, activation mechanisms, extracellular and intracellular signaling mechanisms including channels that are shared between immune cells, neural- and glial cells.

Neurobiological aspects of MS with particular focus on the role of the immune system in damage and repair of CNS lesions, negative and positive signals between neurons/glia and immune cells, mechanisms of CNS protection and molecular repair of demyelination, axonal- and neuronal damage.

Development of animal models that reflect better the complexity of the human disease MS and can be employed to study aspects of peripheral immune activation, neuroinflammation, CNS vulnerability as a predisposing factor for MS onset and perpetuation, and of specific therapies.

Systematic development of novel therapies for MS that are based on small molecules, peptides, siRNAs, biologicals such as monoclonal antibodies or gene therapy approaches, vaccination therapies with peptides, cells or plasmids, and finally approaches towards neuroprotection, and molecular repair with cells and/or growth factors. Laboratory-based

research will not only establish the mechanism of action of these therapies, but also be important for monitoring the in vivo mechanisms once these approaches are explored in phase IIa clinical trials.

2. Clinical Priority Programmes

(Head: PD Dr. C. Heesen)

Disease heterogeneity of MS. These efforts will link careful clinical phenotyping, structural and functional neuroimaging to distinguish patients, in whom autoimmune inflammatory pathomechanisms are leading versus those in whom CNS vulnerability and/or compromised repair of lesions appear more relevant, and further systematic biomarker searches by whole genome single nucleotide polymorphism (SNP) typing, whole genome gene expressions studies, proteomics research, and systematic studies of treatment responsiveness to current and future therapies.

The conduct of mechanism of action-oriented phase II clinical trials that are initiated by INiMS. These will target not only the autoimmune inflammatory components of the disease, but also aim at neuroprotection, and eventually also repair. The following, investigator-initiated phase trials shall be started in 2007:

"**E**stablish **T**olerance **I**n **MS** with peptide-pulsed, fixed antigen presenting cells - a MRI-controlled, single center, baseline to treatment cross-over, phase II trial in relapsing-remitting MS patients (ETIMS)"

"Neuroprotection in MS with oral hydroxytyrosol-acetate, an olive oil-derived, orally available and blood-brain-barrier permeating natural phenol – a MRI-controlled, single or two center, baseline to treatment cross-over, phase II trial in relapsing-remitting MS (Protect-MS)"

As part of the systematic escalation of therapy intensity, we will begin to implement a protocol of autologous hematopoietic stem cell transplantation in aggressive relapsing-remitting MS; explore patient selection strategies, prediction of disease activity by MRI outcomes, conditioning regimens and immune repertoire evolution.

Evidence-based medicine in patient education, treatment decision making, rehabilitation and long-term follow-up. Aim is an education programme covering all aspects of MS enhancing self-management strategies (**E**vidence-based **Se**lf Management in **MS**=ESEMUS). These efforts are pursued together with the Fachbereich Gesundheit at the University of Hamburg (Prof. Mühlhauser) in cooperation with inpatient (Rehaklinik Bad Segeberg) and outpatient (Reha Berliner Tor) centers. In 2007 the modules "diagnosis of MS" and "prognosis of MS" will be developed.

Development of outcome measures for phase II trials based on MRI imaging, electrophysiology, clinical- and neuropsychological scores, and later also biomarkers.

Further studies in the field of endocrine-immune interactions in MS, in 2007 focussing on the impact of depression on these system.

For 2007, we plan to initiate the application for a DFG Forschergruppe. Staff of INiMS will participate in teaching activities in the medical and biological faculties.

Support

Our institute is supported by the Gemeinnützige Hertie-Stiftung

Structure of the Institute

Director:	Prof. Dr. Roland Martin
Group leader:	Dr. Eva Tolosa
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Institute for Cell Biochemistry and Clinical Neurobiology

Dietmar Richter

Research in this institute has been focused on how nerve cells manage to respond to external and internal signals in order to maintain and regulate their cellular architecture. (i) External signalling was studied, using as tools, neuropeptide hormone receptors as well as proteins of the taste receptor family. The expression patterns of these proteins, the delineation of their ligand binding sites as well as their structural requirements for intracellular signalling were examined in mammals. (ii) As an example for internal signalling, the molecular mechanisms underlying selective cytoplasmic mRNA trafficking into dendrites and axons were investigated. Due to the retirement of Dietmar Richter in 2005 the institute was closed down and was replaced in 2006 by the Institute for Neuroimmunology and Clininical Multiple Sclerosis Research, which is headed by Roland Martin (see above).

1. Neurotransmitter receptors and their associated signalling proteins

Hans-Jürgen Kreienkamp, Kerstin Berhörster, Agata Blasczcyk-Wewer, Katrin Falley, Hans-Hinrich Hönck, Peter Iglauer, Gwendlyn Kollmorgen, Chong Wee Liew

Signal transduction processes by neurotransmitter receptors are mediated by a series of defined protein-protein interactions. Many proteins that physically interact with transmitter receptors have been, and continue to be identified. The functions of these protein interactions appear to be (i) the targeting of receptors to cellular specializations (e.g. the postsynaptic compartment in a neuron) (ii) anchoring of receptors to the cytoskeleton and (iii) enabling a tight association with elements of a receptor-specific signal transduction machinery, thus providing specificity as well as increased speed and accuracy in signal.

In the past, we have identified specific interacting proteins for each somatostatin receptor subtype (SSTR1-5). Some interacting partners, such as the PDZ domain protein PIST, presumably have a function in the membrane targeting of SSTR3 and SSTR5. Others such as the tight junction protein MUPP1 or the postsynaptic scaffolds PSD-95 (interacting with SSTR4) or SSTRIP/shank (SSTR2) link receptors into very large signalling complexes, such as the postsynaptic density in excitatory synapses of the central nervous system.

2. Sweet taste receptors and their intracellular interacting proteins

Hartwig Schmale, Heidje Christiansen, Jan K. Hennigs, Bettina Walter

The sense of taste contributes to food palatability, which promotes food intake and to sensory-specific satiety, which promotes termination of intake. Reduced sensitivity to these sensory-based signals may contribute to the over-consumption of energy that leads to obesity. In order to prove the hypothesis that taste has an impact on food preference and eating behaviour and therefore may be one factor associated with the development and progression of human eating disorders it is essential to understand basic mechanisms of sweet taste transduction.

Taste perception is mediated by defined transduction pathways involving ion channels and membrane receptors.Taste

stimuli such as sugars, artificial sweeteners, amino acids including glutamate and several bitter compounds bind to G-protein-coupled taste receptors thereby activating different intracellular second messenger systems. Two families of receptors, T1R -sweet and -umami receptors and T2R -bitter receptors, have been identified. To identify proteins involved in the signalling pathways of T1Rs we have used the yeast two-hybrid system to screen a human keratinocyte cDNA library with the C-terminal domains of rat T1R1 and T1R2 as "baits". The Calcium Integrin-binding protein CIB/calmyrin showed strong and specific binding activity towards the C-terminal 29 amino acids of rT1R2. Binding of rT1R2ct to calmyrin was verified by GST-pulldown assays, co-immunoprecipitation and co-localization studies of rT1R2ct and calmyrin .

3. Dendritic mRNA targeting and the genesis and plasticity of synaptic signalling complexes

Stefan Kindler, Monika Rehbein, John Jia En Chua, Krishna H. Zivraj, Christiane Schröder-Birkner, Birgit Schwanke

Neurons possess distinct cellular compartments that are highly diverse with respect to their protein repertoires. In particular, synapses serving as communication sites between neurons are equipped with a highly specialized set of molecules. Synaptic plasticity underlying learning and memory involves a synapse-specific modification of the protein composition. This adaptation is established by two cellular mechanisms, namely, synaptic targeting of somatically-synthesized proteins and extrasomatic protein synthesis near synapses. The small group of dendritic mRNAs includes transcripts encoding the microtubule-associated protein 2 (MAP2) and the α subunit of the Ca^{2+}/calmodulin-dependent protein kinase II (αCaMKII). The *cis*-acting dendritic targeting elements (DTEs) in both messages have been mapped to the 3'-untranslated regions of both transcripts. Proteins that bind to the MAP2-DTE have been identified and biochemically as well as immunochemically characterized.

The four members of the family of synapse-associated protein 90/postsynaptic density-95-associated proteins, SAPAP1-4, are adapter proteins of the postsynaptic density (PSD). They interact with different synaptic scaffolding proteins, cytoskeletal and signalling components, and are therefore considered to assemble functional multi-protein units at synapses. In the hippocampus, SAPAP 1, 2 and 4 transcripts are restricted to cell body zones, whereas SAPAP3 mRNAs are also detected in molecular layers. Thus, SAPAP3 is one of the few PSD components whose activity-dependent local synthesis in dendrites may directly contribute to an input-specific adaptation of dendritic spine function.

4. Intracellular vasopressin mRNA transport

Evita Mohr, Hatmone Miroci, Stefanie Reinhardt

Specific sorting of a subset of cellular mRNAs to defined cytoplasmic regions takes place in different organisms and cell types including neurons. Nerve cells harbor a still growing number of different mRNA species in dendrites and, infrequently, in the axon. mRNA transport is specified by molecular entities encompassing distinct sequence elements within the RNA molecule (*cis*-acting elements) and proteins (*trans*-acting factors) which associate with those elements to build up a complex transport machinery. The current line

of thinking is that proteins with key functions in synaptic plasticity are, at least in part, produced by local dendritic translation of their mRNAs.

We have shown earlier that the mRNA encoding the rat vasopressin (VP) precursor is sorted to axons and dendrites of hypothalamic magnocellular neurons. Magnocellular neurons, like many other nerve cell types, are capable of synthesizing cytosolic as well as membrane-bound and secretory proteins within their dendrites while the axonal compartment appears to lack protein synthesizing capacity. VP is secreted from the nerve terminals into the systemic circulation. However, substantial amounts are also released from the dendrites of magnocellular neurons into the brain. This raises the question as to whether the dendritic portion of the precursor originates, at least in part, from local translation. When expressed in cultured nerve cells from superior cervical ganglia (SCG) VP transcripts are delivered to dendrites while axonal targeting of the mRNA does not appear to take place (or is beyond the level of detection). In order to determine whether the VP precursor is produced in the dendrites of SCG neurons we have made use of a naturally occurring mutant version of this secretory protein. In the Brattleboro (BB) rat a frameshift mutation in the VP precursor-encoding gene leads to synthesis of a protein with an altered amino acid sequence of its C-terminal part which renders it incapable of leaving the site of its synthesis, the rough endoplasmic reticulum (RER). In SCG neurons microinjected with an appropriate expression vector the RER-bound BB rat VP precursor was restricted to those parts of the dendrites that also contained the corresponding mRNA indicating on-site synthesis of the mutant protein.

In vitro protein/RNA-interaction studies with rat brain cytosolic extracts demonstrated specific binding of the multifunctional poly(A)-binding protein (PABP) to a segment within VP mRNA that is required for dendritic sorting. The physiological consequences of these PABP/RNA interactions include functions such as translational control, mRNA stabilization/destabilization and presumably mRNA localization. The translational state of mRNAs subject to dendritic sorting appears to be influenced by external stimuli. PABP could, thus, be a component required to regulate on-site synthesis within dendrites of the VP precursor and possibly of other proteins.

5. Neuropeptide receptors and regulation of mammalian food intake behavior

Dietmar Bächner, Felix Francke

Food intake is a complex behavior regulated by a large number of peripheral and central signals. Orexigenic hormons induce food intake behavior in response to energy deficiency and anorexigenic hormones, produced in response to food intake, act as satiety signals. Understanding the regulation of appetite is becoming increasingly important as overweight and its morbid form obesity is increasing worldwide, with adverse consequences for human health.

Our work focuses on the signal transduction of the Melanin-concentrating hormone, MCH, a key neuropeptide hormone controlling food intake and energy homeostasis. Using a reverse-pharmacological approach, we identified the endogenous MCH-receptor, MCH-R1, and showed coupling to Gi/o and Gq-type G-proteins. The MCH-R1 interacting zinc-finger protein (MIZIP), and Neurochondrin (NCDN), a neurite outgrowth promoting factor, were identified as C-terminal interacting proteins.

6. Biochemical Analytics Group

Friedrich Buck, Sönke Harder

The core competence of the lab is the analysis of DNA and proteins, using state-of-the-art technologies, in particular mass spectrometry techniques.

DNA sequencing has been performed with a capillary electrophoresis based DNA sequenator (ABI 3100) as well as with the traditional gel electrophoresis based technique (ABI 377).

Protein/proteom analysis was carried out using mass spectrometric techniques using either an electrospray tandem mass spectrometer (QTOF II, Micromass) or a laser desorption (MALDI) mass spectrometer (Reflex IV, Bruker). Both techniques are complementary to each other: MALDI measurements allow the rapid screening of large numbers of samples, while the more elaborate and time consuming ESI tandem mass spectrometry allows the thorough characterization of complex samples.

Support

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Theses

Diploma

Joanna Hermainski (2005). Identifizierung von neuen Interaktionspartnern des postsynaptischen Proteins Densin-180. FU Berlin.

Inga Schapitz (2006). Zelluläre Funktion von Ataxin-10. Universität Hamburg.

Dissertations

Kerstin Berhörster (2006). Die Funktion des Insulinrezeptorsubstratproteins von 53 kDa für Dendriten und Synapsen in der Maus. Universität Hamburg.

Cornelia Brendel (2004). Charakterisierung von Staufen1-enthaltenden Ribonukleoprotein Partikeln. Universität Hamburg.

Marcus Christenn (2005). Charakterisierung von Somatostatinrezeptor-Subtyp 4 interagierenden Proteinen in der Ratte (*Rattus norvegicus*). Universität Würzburg .

John Jia En Chua (2006). Translational regulation of mRNAs encoding SAPAP3 in *Rattus norvegicus*. Universität Hamburg.

Felix Francke (2005). Funktionelle Untersuchungen an den MCH-Rezeptor 1 interagierenden Proteinen MIZIP und Neurochondrin. Universität Hannover.

Peter Iglauer (2005).Transport und Translation der dendritisch lokalisierten shank1 mRNA in *Rattus norvegicus*. Universität Hamburg.

Chong Wee Liew (2004). Regulation der epithelialen *tight junctions* durch den humanen Somatostatinrezeptor-Subtyp 3. Universität Hamburg.

Carola Mullin (2004). Identifizierung und Charakterisierung des Poly(A)-Bindungsproteins aus *Rattus norvegicus* (Berkenhout, 1769) als trans-agierender Faktor der dendritisch lokalisierten Vasopressin mRNA. Universität Hamburg.

Arne Quitsch (2004). Die Funktion postsynaptischer Proteinkomplexe in der neuronalen Morphogenese von Säuger. Universität Hamburg.

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Krishna H. Zivraj (2005) Regulation of dendritic MAP2 mRNA targeting by MARTA2 in *Rattus norvegicus*. Universität Hamburg.

Awards

Gebhard Koch-Promotionspreis für Zellbiochemie und Neurobiologie to Chong Wee Liew, December 2005.

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Neuronal cell fate specification

Ingolf Bach

The main goal of the research carried out in our laboratory is to understand the molecular mechanisms that underlie cell fate specification events during neuronal development in vertebrates. As a model system we investigate the regulation protein networks recruited by LIM domain proteins, applying molecular, biochemical and genetic methods.

The LIM domain is a conserved cysteine-rich zinc-coordinating motif that mediates protein-protein interactions. LIM domains are found in a variety of proteins including LIM homeodomain transcription factors (LIM-HD), nuclear LIM-only (LMO) proteins, as well as cytoplasmic LIM kinases (LIMK). LIM domains are crucial motifs for the assembly of cellular protein networks necessary for basic cellular functions such transcriptional regulation, embryogenesis as well as for cytoskeletal regulation.

LIM-HD factors are crucial regulators of neuronal development and cell type specification. LMO proteins also regulate developmental processes and have additionally been implicated in oncogenesis. Both classes of proteins participate in a nuclear protein network consisting of LIM-HDs, LMOs and LIM domain-binding cofactors CLIM (cofactor of LIM-HD proteins, also known as NLI or Ldb) and RLIM (RING finger LIM domain-binding protein). We have previously shown that LIM-HDs need the association of CLIM cofactors to exert much of their biological activity. Recently, the involvement of post-translational modifications as mechanism of LIM-HD network regulation has become clear in particular modifications by the ubiquitin/proteasome system. Cellular proteins targeted for degradation are ubiquitinated by a cascade of enzymes involving ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). By bridging substrate proteins and E2 enzymes, the E3 enzymes are responsible for substrate specificity of the ubiquitination reaction. In general, poly-ubiquitinated proteins are recognized by the 26S proteasome and rapidly degraded. We have identified RLIM as a ubiquitin ligase able to bind to and target CLIM cofactors for proteasomal degradation thereby inhibiting LIM-HD activity in vivo.

The LIM domain-containing LIMK1 localizes to the cytoplasm and represents a key molecule in the regulation of the cellular actin cytoskeleton. Activation of the Rho/Rock signaling pathways results in the phosphorylation and activation of LIMK1, enabling it to phosphorylate the actin depolymerizing factors ADF/cofilin. Phosphorylation of cofilin leads to an increase in actin polymer formation. It is thought that this activity is directly responsible for the various cellular functions identified for LIMK1 such as morphogenesis, cell motility, tumor cell metastasis as well as neuronal axon growth.

1. Expression of nuclear LIM-HD network proteins during murine neural tube development

The LIM homeodomain (LIM-HD) class of transcription factors have been shown in numerous examples to regulate neuronal differentiation during the development of many species from *C. elegans* to humans. In particular, the development of motor- and interneurons has been shown to be dependent on the activity of various LIM-HD proteins during neural tube development. As recent work indicates that the biological activity of LIM-HD transcription factors is regulated by LIM domain-associated cofactors CLIM and

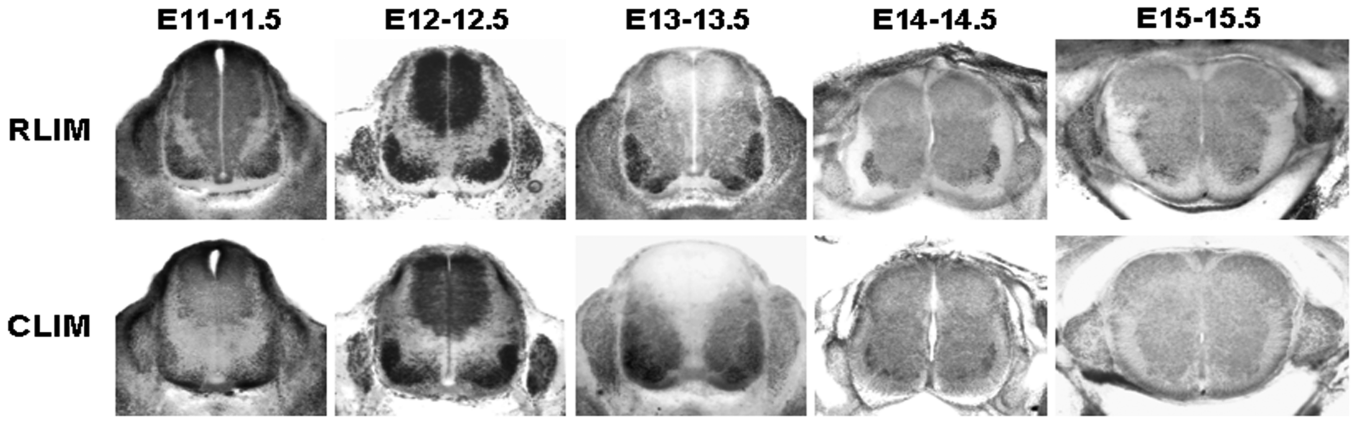

Figure 1. Dynamic expression of RLIM and CLIM during neural tube development. Images of immunohistochemical experiments on transversal sections showing the neural tube of mouse embryos at E11.5 to 15.5 at the thoracic level using antiserum directed against RLIM (top) and CLIM (bottom). Note the similar dynamics of RLIM and CLIM expression.

RLIM, we investigated the comparative expression pattern of LIM network proteins in the developing murine neural tube using specific antisera that we have generated in our laboratory. Immunohisto-chemical stainings at E12.5 showed that both CLIM and RLIM co-localize with Isl and Lhx3 proteins in the ventral neural tube, where specific motor and interneurons reside.

Furthermore, we were able to show a dynamic expression pattern of both cofactors in the neural tube in E8.5 to E15.5 embryos with widespread early expression of both cofactors. At E11.5 we observed higher expression of both proteins in the dorsal and ventral expression domains. Expression of CLIM and RLIM condensed at E12.5 until at E13.5, when dorsal expression faded and both cofactors are detected mainly in ventral neural tube regions. At E14.5 signal strength of ventral expression fades until at E15.5 only relatively little expression of CLIM and RLIM is detected at the protein level (Fig. 2). This down-regulation occurs only at the protein level as in situ hybridization experiments showed that mRNAs of CLIM and RLIM are robustly expressed throughout embryonic neural tube development. Thus, these results indicate that post-translational regulation of CLIM and RLIM occurs. Next, we examined co-localizations of LIM network proteins Isl1,2, Lhx3, CLIM and RLIM using various combinations of specific antisera in co-immunohistochemistry. Consistent with connected biological functions, these experiments showed that both CLIM and RLIM are co-localizing with LIM-HDs Isl1,2 and Lhx3 in ventral neural tube regions. Surprisingly, we found that RLIM co-localized with CLIM in ventral neural tube neurons at E12.5. As we and others identified RLIM as a ubiquitin ligase able to target CLIM for proteasomal degradation these results point towards the

intriguing possibility that the RLIM-mediated degradation of CLIM is regulated during embryogenesis (Ostendorff et al., 2006).

2. The RLIM-like ubiquitin ligase Rnf6 regulates axon outgrowth

For the establishment of neuronal circuits, axons of developing neurons are directed by guidance cues to their correct destinations. The growth cone is located at the tip of the outgrowing axon and senses guidance cues in the environment and translates this information into changes in the cytoskeleton thereby determining the direction of growth. Ubiquitination events are crucial for the development of neuronal projections. However, little is known as to the identities of the molecules involved in this processes.

A search of the GenBank database identified Rnf6 as the protein with the highest sequence homology to RLIM. In particular, functional domains such as the RING finger and the basic domain displayed high sequence homologies. We raised specific Rnf6 antibodies and investigated the distribution of Rnf6 protein during mouse neural tube development. We detected high Rnf6 expression in axonal projections of DRG and ventral neural tube neurons.

Examining mouse primary hippocampal neurons we found that Rnf6 is localized in the cytoplasm of neurons, with particularly high expression in growth cones during axon outgrowth (Fig. 2). Using RNAi technology we were able to show that a partial knock-down of Rnf6 led to increased axon outgrowth indicating inhibitory functions of Rnf6 for axon development. Because of the known connections of RLIM-like ubiquitin ligases with LIM domains and the fact that LIMK1 had previously been implicated for axon growth we tested LIMK1 might serve as substrate for ubiquitination events mediated by Rnf6. Examining their potential to interact, we demonstrated in co-immunoprecipitations that both proteins are associated with each other in cultured hippocampal neurons. Next we showed in vitro that Rnf6 is a ubiquitin ligase able to mediate the polyubiquitination of LIMK1 leading to proteasomal degradation. In growth cones of developing axons we detected only little overlap in the expression of Rnf6 and LIMK1 (Fig. 2) that was proteasome-dependent. In a series of experiments using specific knock-down and overexpressions of various GFP-tagged Rnf6 full length and mutant molecules, we demonstrated that Rnf6 targets LIMK1 for proteasomal degradation in hippocampal growth cones. Furthermore, we were able to functionally link both molecules during axonal outgrowth: Whereas the

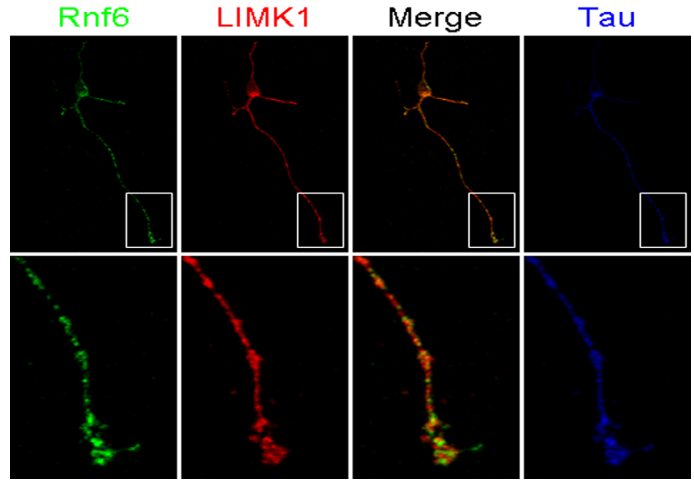

Fig. 2: Rnf6 expression partially co-localizes with LIMK1 in neuronal projections. Upper panel: Primary hippocampal neuron (36h) stained with antisera against Rnf6 (green) LIMK1 (red) and Tau (blue). Boxed regions are enlarged in lower panel. Note co-localization of Rnf6 and LIMK1 in cytoplasm of the cell body whereas expression in the axonal growth cone appears mutually exclusive.

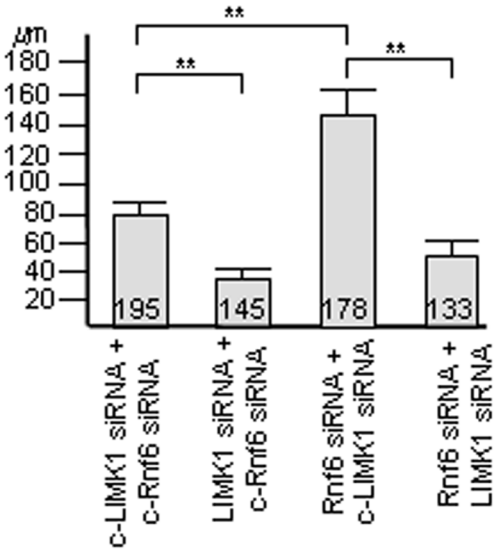

Fig. 3: Rnf6 is functionally linked with LIMK1 during axon outgrowth. Primary hippocampal neurons were co-transfected 24h after culturing with indicated siRNAs and pEGFP-C3 vector. Statistical analysis of axon length of Tau-positive projections using analySIS software. Total number of measured axons is indicated. (**) P<0.0001. Error bars indicate SEM of three independent experiments

specific knockdown of LIMK1 resulted in shorter axons when compared to controls, the knockdown of Rnf6 increased axon length. This latter effect was directly dependent on the presence of LIMK1 because knocking down both Rnf6 and LIMK1 still resulted in shortened axons (Fig. 3). Thus, we conclude that the ubiquitin ligase Rnf6 mediates proteasomal regulation of local LIMK1 levels, thereby playing a central role in controlling actin dynamics in subcellular structures such as axonal growth cones. (Tursun et al., 2005).

Support

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Theses

Diploma

Kerstin Cornils (2005). Thesis title: “Funktion und Stabilität von Proteinen im LIM Proteinnetzwerk während der Zebrafischentwicklung“. Universität Hamburg.

Dissertations

Michael Bossenz (2004). Thesis title: “*In vivo* Analyse des LIM Domäne-bindenden Kofaktors RLIM (RING finger LIM domain-binding protein)“. Universität Hamburg.

Anne Schlüter (2004). Thesis title: “Funktionelle Analyse von Rnf6, einer RLIM-ähnlichen Ubiquitin-Ligase“. Universität Hamburg.

Marvin Peters (2004). Thesis title: “Funktionen der Ubiquitinligasen RLIM und Rnf6 in der Regulation von LIM-Domänen Proteinnetzwerken“. Universität Hamburg.

Baris Tursun (2005). Thesis title: “Expression und Funktion der RING-Finger Protein-Familie Rnf6/RLIM während der Neuronalentwicklung in Mus Musculus“. Universität Hamburg.

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Guest scientists:	Naoko Ishigaki*
Technicians:	Birte Viehweger*
	Michaela Braun*
	Andrea Zaisser*
Secretary:	Renate Erb*

*during part of the reported period

Neuronal Protein Degradation

Thorsten Hoppe

Protein degradation by the ubiquitin/proteasome system is prevocal for cellular regulation pathways, cell cycle progression, signal transduction and development. Substrates modified by multiubiquitin chains are usually marked for proteolysis by the 26S proteasome, a multicatalytic protease complex. Ubiquitylation of proteins requires a cascade of enzymes. Ubiquitin-activating enzyme, E1, hydrolyzes ATP and forms a high-energy thioester bond between an internal cysteine residue and the C-terminus of ubiquitin. Activated ubiquitin is then passed on to ubiquitin-conjugating enzymes, E2s, which form similar thioester-linked complexes with ubiquitin. Finally, ubiquitin is covalently attached to the substrate protein by ubiquitin-protein ligases, E3s, which often interact with the substrate directly. Recently, the yeast protein UFD2 has been described as an additional conjugation factor, E4, which binds to the ubiquitin moieties of preformed conjugates and catalyzes multiubiquitin chain assembly in conjunction with E1, E2, and E3. E4-mediated multiubiquitylation is needed for proteasomal targeting and subsequent proteolysis of specific model substrates.

The human CHIP protein (Carboxyl-terminus of Hsc70 interacting protein) also displays E4 function, since it positively regulates the ubiquitylation activity of the E3 enzyme Parkin. Therefore it might play a role as an E4 enzyme in the development of autosomal-recessive juvenile Parkinsonism (AR-JP), one of the most common forms of Parkinson's disease. Additionally, CHIP shows an important role in the regulation of the microtubule associated protein tau and seems to be involved in the pathogenesis of tauopathies. Mammalian UFD2a, an ortholog of the yeast E4 enzyme UFD2, has been identified as a rate-limiting factor in the degradation of pathological forms of Ataxin-3, which are responsible for spinocerebellar ataxia type 3 (SCA3, also known as Machado-Joseph disease). Furthermore, the aberrant expression of a mutant Ufd2/D4Cole1e fusion protein has been implicated in slow wallerian degeneration mice.

Our research focuses primarily on functional aspects of E4 enzymes. The identification and analysis of novel genetic and physical interaction partners and substrates will help decipher mechanistic and developmental aspects of ubiquitin chain elongation and the specific role in neurodegeneration. Another major aspect is the identification of new components of the ubiquitin/proteasome system involved in neuron-specific protein degradation. Therefore we currently establish a tissue specific *in vivo* degradation assay in a living organism. The isolation of new genes implicated in neuron-specific protein degradation and their genetic and biochemical characterization will give further insights into the connection between the ubiquitin/proteasome system and neurodegenerative diseases like Parkinson's and Alzheimer's disease.

1. Regulation of myosin assembly

The assembly of myosin into thick filaments during muscle development is still a largely unexplored phenomenon. Recent data suggest that the organization of myosin into sarcomeric structures is the result of a regulated multi-step assembly pathway that requires additional factors. Candidates for this process are members of a protein family containing a UCS (UNC-45/CRO1/She4p) domain, which have been indicated to be necessary for proper myosin func-

tion. One founding member of this family is UNC-45, for which homologs have been identified in a variety of organisms, from yeast to humans. UNC-45 functions both as a molecular chaperone and as an Hsp90 co-chaperone for myosin during muscle thick filament assembly. Consequently, mutations in *C. elegans unc-45* result in paralyzed animals with severe myofibril disorganization in striated body wall muscles.

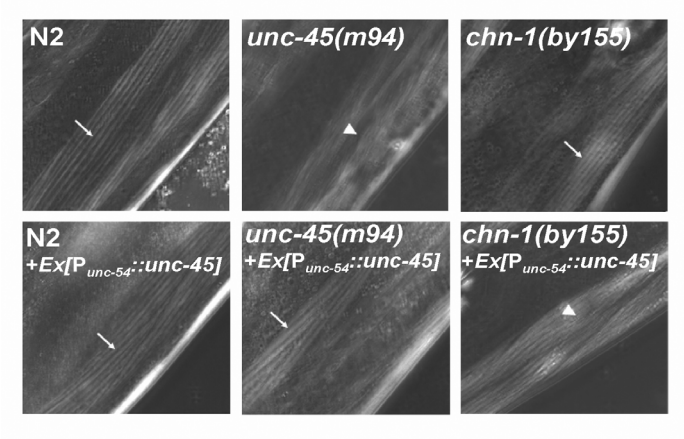

Figure 1.: Polarized light microscopy of body wall muscles of N2, *unc-45(m94)* and *chn-1(by155)* strains expressing or not *unc-45* under the muscle specific *unc-54* promoter (*Ex[*P_{unc-54}*::unc-45]*). Note that the muscle cells of N2, *chn-1(by155)*, N2+*Ex[*P_{unc-54}*::unc-45]*, and *unc-45(m94)*+ *Ex[*P_{unc-54}*::unc-45]* animals have well organized sarcomeres in which long A bands (bright bands, marked by arrows) alternate with long I bands (dark bands), whereas those of *unc-45(m94)* and *chn-1(by155)*+*Ex[*P_{unc-54}*::unc-45]* animals have disorganized sarcomeres in which alternating A and I bands are difficult to identify in equivalent regions (arrowheads).

Our recent work revealed that protein levels of the myosin chaperone UNC-45 are subject to stringent regulation, which appears to be dependent on the *C. elegans* orthologs of UFD2 and CHIP, UFD-2 and CHN-1. We were able to show that either UFD-2 or CHN-1 alone, in collaboration with E1 and E2, conjugates UNC-45 with one to three ubiquitin moieties. Therefore, both CHN-1 and UFD-2 work independently as E3 enzymes in this pathway. However, in combination, CHN-1 and UFD-2 dramatically increase the ubiquitylation of UNC-45. Movement defects of *unc-45* thermosensitive (*ts*) mutants are suppressed in animals lacking CHN-1 or UFD-2 most likely due to stabilization of the corresponding UNC-45 (*ts*) proteins. Interestingly, analysis of body wall muscle cells by polarized light microscopy showed that the muscle structure of *chn-1* and *ufd-2* knockout worms is comparable to that of wild-type; however overexpression of transgenic *unc-45* leads to strong sarcomeric assembly defects. Therefore, the amount of UNC-45 protein present in the muscle cells is critical for proper thick filament function and muscle formation.

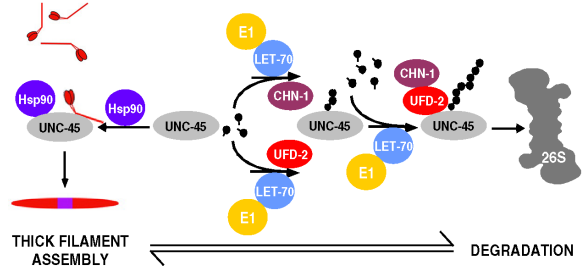

Figure 2.: Hypothetical model for CHN-1/UFD-2 dependent regulation of the myosin chaperone UNC-45. UNC-45 is able to bind myosin and Hsp90 simultaneously and thereby functions both as a molecular chaperone and as an Hsp90 co-chaperone for myosin in muscle thick filament assembly. Additionally, UNC-45 interacts with CHN-1 or UFD-2 and each of them alone in collaboration with the ubiquitin-activating enzyme (E1) and the ubiquitin-conjugating enzyme (E2) LET-70 mediates only triple ubiquitylation of UNC-45. However, the E3/E4-complex formed by CHN-1 and UFD-2 multiubiquitylates UNC-45 which probably leads to degradation of UNC-45 by the 26S proteasome. Thus, the protein level of the myosin chaperone UNC-45 seems to be tightly regulated and this appears to be necessary for the correct assembly of myosin in body wall muscle cells.

Conceptually, beside the regulation of sarcomere assembly, these findings support a novel mechanism in which two E3 enzymes, UFD-2 and CHN-1, team up to achieve E4 function. The assembly of such an E3/E4 complex in multicellular organisms could be controlled by tissue specific co-expression of both E3 enzymes in a developmentally-regulated manner. Furthermore, different combinations of E3 enzymes might result in alternative substrate specific complexes with E4 activity.

2. *C. elegans* model for Parkin/PDR-1

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting about 1-2 % of the population over the age of 65. PD is mainly characterized by motor dysfunctions, resulting from selective loss of dopaminergic neurons in the substantia nigra. A neuropathological hallmark of idiopathic PD is the formation of cytoplasmic protein inclusions called Lewy bodies. A major component of these aggregates is the presynaptic protein α-synuclein, implicated in many biological processes. Moreover, rare dominant mutations of α-synuclein as well as genomic multiplications have been identified to be causative for the disease.

In contrast to the rare α-synuclein mutations, most cases of familial PD are linked to mutations in the *parkin* gene, causing autosomal recessive juvenile Parkinsonism (AR-JP). Human Parkin consists of four known domains: an N-terminal ubiquitin-like domain (UBL) and two RING finger domains flanking a cysteine-rich in between RING finger (IBR) domain. The UBL domain has been implicated in proteasome binding, substrate recognition, and regulation of Parkin stability. Like many proteins with RING finger domains, Parkin acts as an E3 ubiquitin ligase *in vitro*.

Intriguingly, we identified that beside its role in the E3/E4 complex regulating myosin assembly, CHN-1 alternatively cooperates with the *C. elegans* Parkin homolog PDR-1 throughout substrate ubiquitylation. Strikingly, in contrast to *pdr-1* loss-of-function mutants, the in-frame deletion mutant *pdr-1(lg103)* is hypersensitive towards proteotoxic stress conditions. Induction of protein folding stress in the lumen of the endoplasmic reticulum (ER) results in severe developmental defects lethal to *pdr-1(lg103)* animals. Moreover, the expression of *pdr-1* is regulated by IRE-1, PEK-1, and ATF-6, which are activators of the unfolded protein response (UPR), an intracellular signalling pathway that counteracts stress caused by accumulation of misfolded proteins in the lumen of the ER. Our genetic findings indicate that PDR-1- and IRE-1-dependent pathways are partially redundant throughout ER-stress. In summary, our biochemical and genetic data suggest that PDR-1 plays an essential role in the regulation of unfolded protein stress pathways *in vivo*.

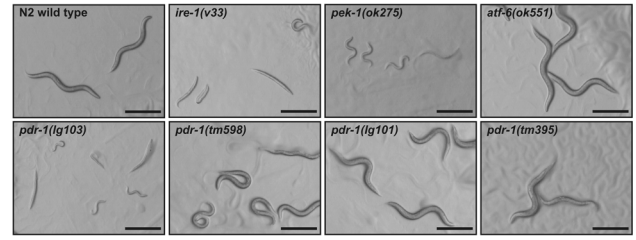

Figure 3.: The in-frame deletion *pdr-1(lg103)* causes hypersensitivity to ER stress. Most of the N2 wild-type animals reach adulthood, whereas most of the *ire-1(v33)* and *pek-1(ok275)* mutant animals either arrest or die. *pdr-1(lg103)* animals, in contrast to other *pdr-1* deletion alleles, show the same ER stress hypersensitivity as mutants of the UPR.

These findings are in line with the recent observation showing PD mimetics like 6-OHDA, MPP^+, and rotenone to specifically induce ER stress and to activate the UPR in cultured

neuronal cells. In addition cell culture experiments have identified that the expression of human *parkin* is up-regulated in response to unfolded protein stress. It is thus conceivable to propose a widespread involvement of ER stress and the UPR in the pathophysiology of PD which indicates a more general function of Parkin rather than degrading a specific substrate.

3. ER-associated protein degradation

ER-associated protein degradation (ERAD) is a quality control process that selectively eliminates misfolded and unassembled proteins in the secretory pathway. Nascent polypeptides are transported into the ER in an unprocessed state through the heterotrimeric SEC61 channel. Subsequent folding and posttranslational modifications are assisted by ER resident chaperones and involves N-linked glycosylation and disulphide-bond formation. However, imperfect maturation and exogenously induced ER stress leads to an accumulation of misfolded proteins in the ER lumen. These proteins are eventually degraded by the cytosolic ubiquitin/proteasome system and must therefore be retro-translocated. Blocking the ERAD pathway aggravates ER stress, leading to the activation of the UPR. Both ERAD and UPR are connected to coordinate the disposal of misfolded proteins even in the absence of acute stress.

Although many molecular details of ERAD remain unknown, recent work has identified the cytosolic AAA (ATPase associated with various cellular activities) ATPase p97 to be a major component of the retro-translocation machinery. p97 is conserved from archaebacteria to human and is also known as VAT in archaea, CDC48 in yeast, TER94 in insects and VCP in mammals. p97 forms a homohexamer with subunits arranged in a barrel-like structure with a pore in the centre. Each subunit is composed of an N-terminal domain and two copies of a conserved ATP-binding domain (D1 and D2) that are responsible for nucleotide binding and hydrolysis. The N-domain is positioned on the outer face of the barrel, which allows further interaction with cofactors and substrates. Beside its function in the ERAD pathway p97 is involved in a variety of diverse cellular processes, including homotypic membrane fusion and cell-cycle regulation and this specificity is determined by distinct N-domain binding cofactors.

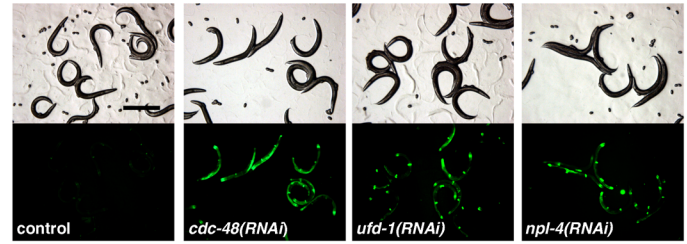

Figure 4.: Inactivation of cdc-48.1/cdc-48.2, *ufd-1* or *npl-4* causes ER stress. The indicated genes were inactivated by RNAi in wild-type worms expressing GFP under an UPR-inducible *hsp-4* promoter (*hsp-4::gfp*). *C. elegans hsp-4* is a homolog of the mammalian ER-resident chaperone BiP.

Two of the most extensively studied p97 binding partners are p47 and the Ufd1/Npl4 heterodimer, which form alternative complexes with p97 required for different types of reactions. The p47 cofactor enables p97 to perform homotypic membrane fusion of post-mitotic Golgi vesicles. In contrast, the Ufd1/Npl4 adapter is needed for the function of p97 in ubiquitin/proteasome dependent protein degradation, including the ERAD pathway and the mobilization of the ER-membrane bound transcription factor SPT23. During ERAD, p97 and Ufd1/Npl4 are involved in the retro-translocation of misfolded proteins into the cytosol before ubiquitylation and

proteasome mediated degradation. Thereby, the p97$^{Ufd1/Npl4}$ complex seems to be required for substrate recognition.

Recently, we identified a CDC-48$^{UFD-1/NPL-4}$ complex in *C. elegans* functionally similar to the mammalian p97$^{Ufd1/Npl4}$ complex. Unlike all other organisms, *C. elegans* bears two p97 homologs. CDC-48.1 and CDC-48.2 appear to be essential for the elimination of misfolded proteins from the ER lumen. Depletion of either p97 homolog causes ER stress and results in hypersensitivity to stress conditions that induce high levels of unfolded proteins within the ER lumen. Moreover, the interaction studies suggest that CDC-48.1 and CDC-48.2 interact with UFD-1 and NPL-4, forming a complex related to yeast CDC48$^{UFD1/NPL4}$ or mammalian p97$^{UFD1/NPL4}$. Recent results identified the novel p97-interacting protein Derlin-1 and its *C. elegans* homolog as a central membrane component of the retro-translocation machinery. In line with these data, our findings propose that the function of the p97 ATPase complex appears to be evolutionarily conserved throughout all ERAD pathways from yeast to man.

Support

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Thesis

Diploma

Barikbin, Roja (2006). Identifizierung von Interaktionspartnern und Funktionen des Proteins Ataxin-3/ATX-3 innerhalb des Ubiquitin/Proteasom-Systems in *C. elegans*.

Awards

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Protein Trafficking and Synapse Formation

Matthias Kneussel

Neurotransmitter receptors are subject of microtubule-based transport between intracellular organelles and the neuronal plasma membrane. Receptors that arrive at plasma membrane compartments diffuse laterally within the plane of the cellular surface. To achieve immobilization at their sites of action, cytoplasmic receptor residues bind to submembrane proteins, which are coupled to the underlying cytoskeleton by multiprotein scaffolds.

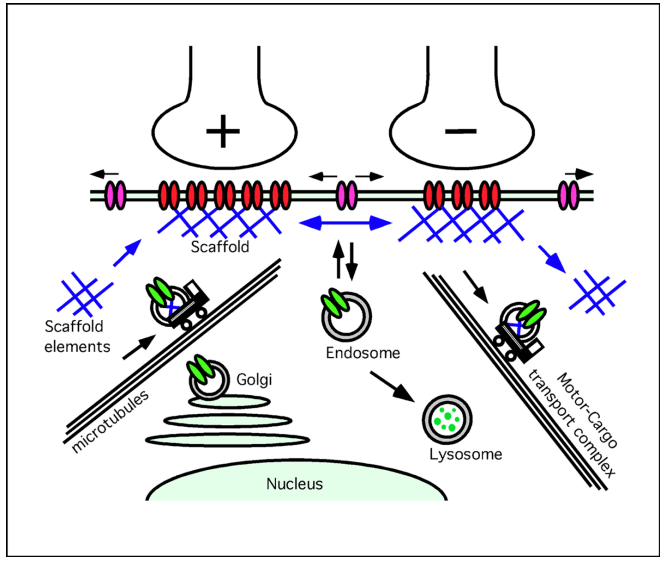

Figure 1. Schematic representation of neurotransmitter receptor and scaffold dynamics underlying the regulation of synaptic plasticity. Receptors and scaffold elements are subject of recruitment between intracellular organelles and the plasma membrane. Surface receptors are either clustered through submembrane interactions at axo-dendritic contacts or display membrane diffusion across the plane of the cellular surface.

Besides their role in diffusion-trapping of surface receptors, scaffold components also undergo rapid exchange to/from and between postsynaptic specializations, leading to a dynamic equilibrium of receptor-scaffold complexes. Moreover, scaffold components serve as adaptor proteins that mediate specificity in intracellular transport complexes.

Our research group is concerned with sorting, transport and clustering mechanisms of synaptic proteins to, from and at central nervous system synapses underlying the regulation of synaptic plasticity. We apply the yeast two-hybrid screening technique as well as mass spectrometry approaches to identify protein-protein interactions between motor protein complexes and synaptic cargo elements. Biochemical *in-vitro* binding assays and immunocytochemistry are used to characterize new protein complexes in cultured hippocampal neurons. Furthermore, time-lapse video microscopy in living neurons allows dynamic studies of intraneuronal co-transport to and from synaptic sites. Mutagenesis, RNAi and dominant-negative approaches are used to interfere with functional transport reactions. The generation of transgenic mice that express fluorescent fusion proteins allows the dynamic visualization of synaptic components under defined physiological parameters in tissue slices.

1. Microtubule-dependent transport of synaptic receptor-scaffold complexes

To shed light into the dynamics of submembrane scaffold elements, we used the multimeric scaffold element gephyrin, which locates at inhibitory postsynaptic membrane specializations, as a model protein to ask whether scaffold elements

rapidly cycle to/from and between synapses. According to the trapping of diffusible surface membrane receptors through synaptic receptor-scaffold interactions, the number of scaffold molecules might be proportional to the number of synaptic receptors that locate at synapses at a given time. It is therefore plausible that scaffold dynamics could contribute to the regulation of synaptic strength.

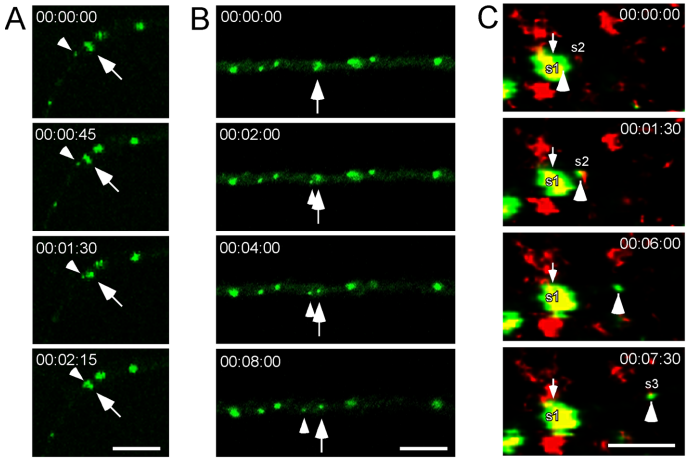

Figure 2. GFP-gephyrin transport packets (green) are rapidly added to (A) or removed from (B) postsynaptic scaffold formations. Note that mobile particles are on average smaller in size, as compared to preexisting submembrane scaffold formations. (C) Application of FM-dyes (red), that label active presynaptic terminal boutons, revealed that GFP-gephyrin units (green) leave synaptic contacts (yellow) in the minute timescale and rapidly cycle between individual synapses over time. (modified after: Maas et al., 2006 - Journal of Cell Biology).

Analysis of GFP-gephyrin dynamics in living neurons revealed that gephyrin transport units enter and leave postsynaptic scaffold formations of gephyrin within several minutes, suggesting that postsynaptic scaffolds undergo rapid remodeling in the minute range (Maas et al., 2006). By application of FM- dyes that allow for the identification of active terminal boutons in living neurons, we revealed evidence that gephyrin furthermore cycles between individual synapses in the minute range, suggesting that postsynaptic scaffolds are rapidly remodeled via activity-dependent signals.

Since gephyrin represents a glycine receptor (GlyR) binding protein at postsynaptic sites, we asked whether intracellular recruitment of gephyrin would represent co-transport with trans-Golgi network-derived intracellular vesicles that carry the inhibitory GlyR. Indeed, applying double time-lapse video microscopy using GFP-variant fusion proteins, we detected co-transport of GlyR-gephyrin complexes within neuronal dendrites (Maas et al., 2006). Functional analysis revealed that these transport reactions are microtubule-dependent processes. Notably depolarization or GlyR blockade with the antagonist strychnine caused a significant increase in the transport velocities of gephyrin particles, suggesting for crosstalk between activity-dependent mechanisms and the intracellular transport machinery (Maas et al., 2006).

Experiments to identify the driving force of a GlyR-gephyrin transport complex in neurons identified the cytoplasmic dynein motor complex as a retrograde recruitment system that removes GlyR-gephyrin packets from synapses to intracellular compartments (Maas et al., 2006). Consistently, functional blockade of dynein-mediated transport, as well as specific interference with gephyrin-dynein interactions blocked GlyR-gephyrin recruitment over time.

Our laboratory is involved in the functional characterization of a number of synaptic transport complexes from both excitatory and inhibitory synapses that have been identified in our systematic screening approaches using neuronal motor proteins as baits. Future experiments are expected to address the functional relationships between synaptic

transmission and the intracellular machinery that recruits protein cargo along cytoskeletal tracks toward and/or from sites of activity.

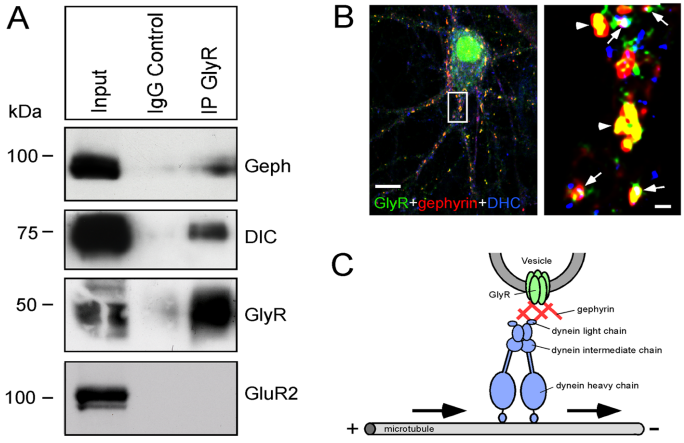

Figure 3. Identification of a glycine receptor (GlyR)-gephyrin transport complex that couples to the dynein motor complex for retrograde recruitment within dendrites. (A) Immunoprecipitaion from intracellular fractions of rat brain lysate. (B) Triple colocalization of endogenous GlyR (green), gephyrin (red) and dynein heavy chain (blue) in cultured hippocampal neurons. (C) Schematic representation of the motor-cargo complex (modified after Maas et al., 2006 – Journal of Cell Biology).

2. Receptor clustering at synaptic and extrasynaptic sites

Receptor clustering at synaptic contacts represents an important mechanism to achieve efficient transmission in apposition to axon terminal boutons that release the neurotransmitter. Despite the role of gephyrin in the postsynaptic clustering of GlyRs, data from our and other laboratories have shown that gephyrin also represents a critical factor for the synaptic clustering of most $GABA_A$ receptors ($GABA_A$Rs). Notably, data from gephyrin knockout mice have revealed that certain subtypes of $GABA_A$Rs, including the $\alpha5$ subunit, cluster independent of gephyrin. In fact, $\alpha5$ subunit-containing $GABA_A$Rs mainly locate at extrasynaptic plasma membrane positions, however are occasionally detected at inhibitory synapses. Moreover, mouse mutants affecting $GABA_A$R $\alpha5$ function, display an increase in spatial learning and a phenotype in trace fear conditioning.

In an attempt to identify the protein that mediates $GABA_A$R $\alpha5$ clustering, we isolated the ERM-family protein radixin, which is known to bind to both membrane proteins and the submembrane actin cytoskeleton (Loebrich et al., 2006). Our data revealed that a phosphorylation-dependent intramolecular activation process is required to mediate radixin binding to $\alpha5$ subunit-containing $GABA_A$Rs.

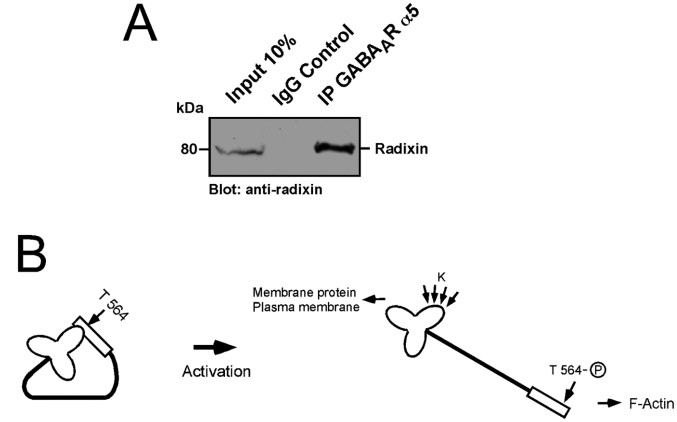

Figure 4. The ERM-family protein radixin binds to $\alpha5$ subunit-containing $GABA_A$Rs. (A) Immunoprecipitation experiment form rat brain lysate. (B) Schematic representation of the intramolecular activation of radixin, which is a prerequisite for both $GABA_A$R and F-actin binding (modified after Loebrich et al., 2006 – EMBO Journal).

Knock-down of radixin in neuronal cultures, as well as knock-out of radixin in mice revealed that radixin is essential for $GABA_AR$ α5 clustering. However, under these circumstances, unclustered receptors remain in the neuronal plasma membrane, as detected by electrophysiological analysis (Loebrich et al., 2006). Together, our data suggest for a functional role for $GABA_AR$ α5 cluster formation at extrasynaptic membrane positions. Our current analysis addresses the question, whether physiological triggers allow extrasynaptic $GABA_AR$ α5 clusters to participate in neuronal transmission at synapses, a question that addresses possible reserve pool functions of extrasynaptic receptors. In addition, we analyse whether neuron-glia contacts could be a trigger to extrasynaptically cluster $GABA_AR$ α5 receptors, a subtype that is otherwise involved in tonic inhibition.

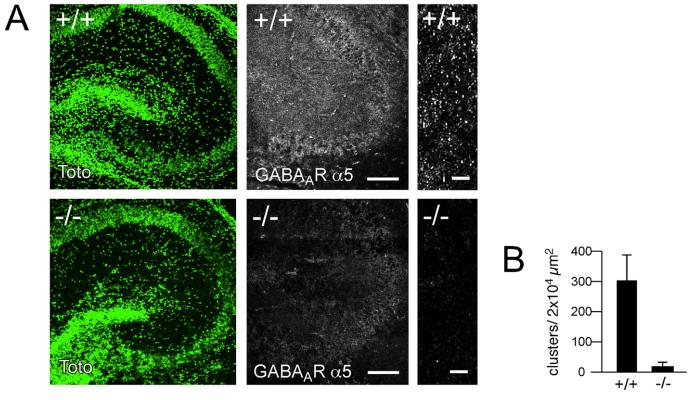

Figure 5. Hippocampal tissue sections derived from radixin knockout mice display loss of $GABA_AR$ α5 subunit clusters. (A) Toto staining reveals the normal anatomy of the hippocampal regions from both genotypes. White puncta in the magnified image (right) represent $GABA_AR$ α5 clusters. (B) Quantification of cluster number per area (modified after Loebrich et al., 2006 – EMBO Journal).

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft (grants KN556/1-2, 1-3, 3-1; SFB 444, B7).

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Molecular analysis of $GABA_B$ receptor complexes

Hans-Christian Kornau

$GABA_B$ receptors mediate pre- and postsynaptic modulation of central nervous system synapses. These abundant $G_{i/o}$ protein-coupled receptors, although activated exclusively by the major inhibitory neurotransmitter GABA, are found at both glutamatergic and GABAergic synapses. $GABA_B$ receptors localized on axon terminals confer inhibitory effects on transmitter release by inhibiting voltage-dependent calcium channels as well as adenylate cyclase activity; activation of postsynaptic receptors opens G-protein-regulated inwardly rectifying potassium (GIRK) channels eliciting slow hyperpolarization. Cloning of cDNAs and expression in heterologous systems demonstrated that functional $GABA_B$ receptors assemble from two related subunits, $GABA_{B(1)}$ and $GABA_{B(2)}$, each encompassing seven transmembrane domains.

Further studies revealed that $GABA_{B(1)}$ is the ligand-binding subunit, whereas $GABA_{B(2)}$ is essential for the coupling to G-proteins. A coiled-coil interaction between the intracellular C-termini of the subunits mediates receptor assembly in the endoplasmic reticulum (ER) and, importantly, masks an ER retention signal in $GABA_{B(1)}$. This ensures that only heterodimeric, functional receptors reach the cell surface. The coiled-coil domains constitute hotspots for interactions with the $GABA_B$ receptor: several proteins were shown to interact with the coiled-coil domains of either $GABA_{B(1)}$ or $GABA_{B(2)}$, often mutually exclusive with receptor heterodimerization.

Gene targeting of $GABA_B$ receptors in mice revealed that both subunits are essential for all classical pre- and postsynaptic biochemical and electrophysiological $GABA_B$ responses. Knockout mice exhibited spontaneous, primarily clonic epileptic seizures, hyperalgesia, hyperlocomotor activity, hypothermia and impairment in passive avoidance learning. In $GABA_{B(2)}$ knockout mice, however, the specific $GABA_B$ receptor agonist baclofen elicited an atypical electrophysiological response. It is not known whether this current is a consequence of the genetic manipulation or if it is present, but masked by the large outward current mediated by GIRK channels in wild-type mice. It may be an indication for assembly of $GABA_{B(1)}$ with an unknown subunit independent of $GABA_{B(2)}$. Roles of $GABA_{B(1)}$ and $GABA_{B(2)}$ in addition to their functions as subunits in the $GABA_{B(1)}$/$GABA_{B(2)}$ heterodimer have also been suggested by their independent maturation during postnatal development.

Two functional subtypes of the $GABA_B$ receptor have recently been identified. Alternative transcriptional start site selection results in the $GABA_{B(1)}$ subunit variants a and b, which differ by the presence of a pair of sushi-repeats in $GABA_{B(1a)}$ (Fig. 1). These isoforms show differences in their spatial and temporal expression patterns with $GABA_{B(1a)}$ being more abundant during development and $GABA_{B(1b)}$ showing increased expression in the adult brain. Using gene-targeting differential functions of the $GABA_{B(1)}$ isoforms in hippocampus and cortex were unravelled (Vigot et al. (2006) Neuron 50, 589-601; Perez-Garci et al. (2006) Neuron 50, 603-616). $GABA_{B(1a)}$ is the variant responsible for the inhibition of presynaptic transmitter release and localizes to axons in transfected hippocampal neurons, whereas the $GABA_{B(1b)}$ isoform is restricted to somatic and dendritic compartments and mediates the slow postsynaptic inhibition. Interestingly, the postsynaptic $GABA_B$ receptors

at excitatory synapses in dendritic spines are colocalized with the GIRK effector channels, while in dendrites they appear to be distributed independently (Kulik et al. (2006) J. Neurosci. 26, 4289-4297). Moreover, the $GABA_B$-GIRK pathway in dendritic spines is subject to a new form of long-term potentiation (LTP) in hippocampal CA1 pyramidal neurons (Huang et al. (2005) Cell 123, 105-118). High-frequency stimulation leading to NMDA receptor activation, calcium influx and CaMKII activation induces not only excitatory LTP, but also an inhibitory, $GABA_B$-GIRK-mediated LTP, i.e. both the glutamate-evoked excitatory postsynaptic currents and the $GABA_B$-mediated inhibitory postsynaptic currents are persistently increased. It is unknown yet if this potentiation of the late inhibitory currents is the result of an increased amount of $GABA_B$-GIRK clusters or of facilitated signalling between the GPCR and the channel. The inhibitory LTP may sharpen the coincidence detection of excitatory events by weakening late-arriving inputs. The tight colocalization of $GABA_B$ receptors and GIRK channels and the functional importance of this signalling pathway for the excitability of dendritic spines suggest a physical coupling of the two membrane protein complexes.

To improve our understanding of the synaptic modulation by $GABA_B$ receptors we are studying their physical interactions using biochemical and genetic tools.

1. Biochemical purification of $GABA_B$ receptors from mouse brain

Genetic screens, primarily with the yeast two-hybrid system, have revealed a number of interaction partners of the cytoplasmic C-terminal domains of the $GABA_B$ receptor (see also 2. and 3.). However, a biochemical analysis of native $GABA_B$ receptor complexes might in addition reveal indirect interaction partners as well as integral membrane proteins associated with the receptor. Given that the amount of $GABA_B$ receptors within a membrane protein extract from rodent brain is very limited, excellent high-affinity antibodies would be necessary to immunopurify $GABA_B$ receptors from such preparations. To circumvent this restriction, we decided to express $GABA_B$ receptor subunits encompassing tags suitable for their purification in the mouse brain.

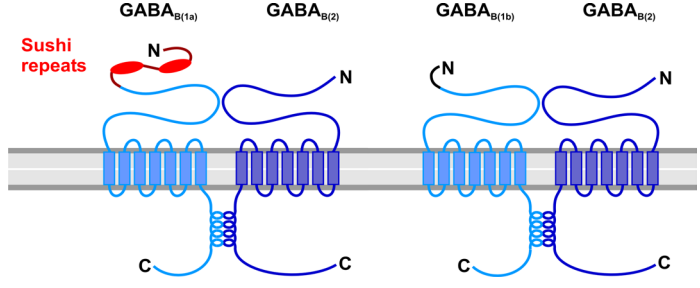

Figure 1. Two molecular subtypes of the $GABA_B$ receptor. Alternative transcriptional start sites result in two isoforms of $GABA_{B(1)}$, which differ by a pair of N-terminal sushi repeats. Both isoforms assemble with $GABA_{B(2)}$, but the resulting heterodimeric complexes are differentially localized to subcellular sites and mediate distinct physiological functions. To unravel subtype-selective complexes, we specifically tagged the $GABA_{B(1a)}$ isoform.

As outlined above, $GABA_{B(1a)}$ in contrast to $GABA_{B(1b)}$ contains two sushi repeats at the N-terminus. Sushi repeats have been found in proteins of the complement system and are proposed to mediate interactions with proteins of the extracellular matrix. Gene targeting revealed that the sushi repeats in $GABA_{B(1a)}$ are essential to target the $GABA_B$ receptor to presynaptic axonal elements. Thus, specifically tagging $GABA_{B(1a)}$ may allow to identify its select set of interaction partners, possibly including factors responsible for the sushi-mediated localization and effectors of $GABA_B$ receptors in nerve terminals.

To isolate $GABA_B$ receptor complexes from mouse brain membranes we are using a tandem affinity purification strategy based on a very successful approach to analyze protein complexes in yeast (Gavin et al. (2002) Nature 415, 141-147). We fused combinations of tags to the N- or the C-terminus of $GABA_{B(1a)}$ and confirmed that they did not change the signalling properties of the receptor in transfected cells. To generate mice expressing tagged $GABA_{B(1)}$ subunits we introduced DNA sequences encoding the specific affinity tags into a bacterial artificial chromosome (BAC) carrying all elements of the $GABA_{B(1)}$ gene by ET recombination. These site-directed manipulations allowed a C-terminal modification affecting both subunit variants $GABA_{B(1a)}$ and $GABA_{B(1b)}$ as well as an N-terminal, $GABA_{B(1a)}$-specific modification. The manipulated BACs were purified and then injected into 1-cell stage mouse embryos and implanted into pseudo-pregnant females by the transgenic mouse facility of the ZMNH. In both cases several transgenic founders could be identified by polymerase chain reaction and southern blot analysis. Offspring of the founders was subsequently shown by western blot analysis to express tagged $GABA_{B(1)}$ subunits of the expected respective molecular weights in contrast to wild-type mice. These transgenic subunits are expressed in addition to the endogenous subunits and are expected to be targeted to the same intracellular locations and incorporated into the same protein complexes as the endogenous subunits. Consistent with this hypothesis, purifications of the tagged receptors from mouse brain membranes contained $GABA_{B(2)}$ as detected by western blot analysis.

Currently, we purify $GABA_B$ receptor complexes from brain preparations of the transgenic mice and analyze them for the presence of additional components using mass spectrometry.

2. Identification and analysis of protein interactions using β-lactamase complementation

Recently, several techniques for studying protein interactions in mammalian cells have been developed utilizing reconstitution of an enzymatic activity by protein fragment complementation. One of these approaches, the β-lactamase complementation assay (Galarneau et al. (2002) Nat. Biotechnol. 20, 619-622; Wehrmann et al. (2002) Proc. Natl. Acad. Sci. U.S.A. 99, 3469-3474), is based on the prokaryotic enzyme β-lactamase encoded by the ampicillin resistance gene widely used as a plasmid selection marker. This small (29 kDa) monomeric enzyme can be split into two fragments designated α and ω, respectively (Fig. 2). Neither of these fragments displays β-lactamase enzyme activity on its own, nor does mere coexpression of the two fragments result in β-lactamase activity. In contrast, if the α and the ω fragment are fused to an interacting pair of proteins, the fragments are capable of assembling a functional β-lactamase enzyme. Thus, β-lactamase activity can be used as a read-out for the occurence of protein interactions.

An important advantage of the β-lactamase complementation system - besides the small size and monomeric nature of the β-lactamase enzyme, the non-toxicity and the absence of endogenous β-lactamase activity in mammalian cells - is the existence of a membrane-permeant, fluorescent β-lactamase substrate compatible with fluorescence activated cell sorting (FACS) procedures. This substrate, designated CCF2/AM, consists of a cephalosporin core linking a 7-hydroxycoumarin to a fluorescein (Zlokarnik et al. (1998), Science 279, 84-88). Upon excitation of the coumarin moiety at a wavelength of 409 nm, energy is transferred via an intramolecular FRET

effect to the fluorescein, resulting in fluorescence emission at a peak wavelength of 520 nm (green fluorescence). β-lactamase-catalyzed metabolization of CCF2 results in elimination of the fluorescein moiety and in a shift of the peak wavelength of the fluorescence emission to the emission maximum of coumarin (460 nm, blue fluorescence). Thus, β-lactamase activity can be quantified in living mammalian cells by measuring the emitted fluorescence intensities at the fluorescein and the coumarin emission peak frequencies, respectively. Ratiometric analysis, i.e. calculating the quotient between blue and green fluorescence intensities, allows evaluation independent of cell number, cell size and loading efficiency. Additionally, β-lactamase activity in cell lysates can be quantified by means of the colorimetric substrate nitrocefin, whose β-lactamase-catalyzed change of color (yellow to red) can be monitored by absorption measurements at 490 nm.

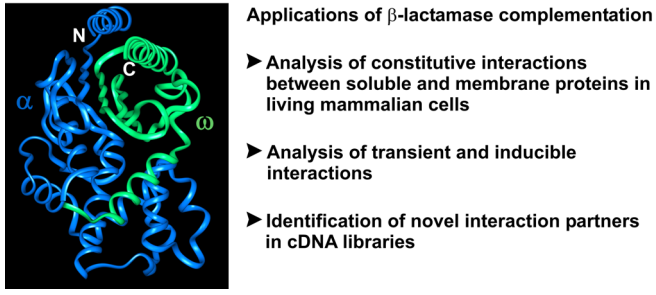

Figure 2. β-lactamase complementation. The monomeric enzyme β-lactamase can be split into two fragments designated α and ω, which reconstitute β-lactamase activity if they are fused to two interacting proteins. β-lactamase activity can be detected either in living cells using the membrane permeant fluorescent dye CCF2/AM or in cell lysates using the colorimetric substrate nitrocefin, thus allowing a variety of applications of β-lactamase complementation.

We have set up the β-lactamase system in our laboratory by applying the technique to well-known protein interactions of the $GABA_B$ receptor complex. Sensitivity as well as specificity of the assay system were characterized by FACS analyses of Flp-In-3T3 cells coexpressing various soluble and transmembrane α and ω fusion proteins. Using this approach we monitored intra- and extracellular interactions mediating assembly of $GABA_{B(1)}$ and $GABA_{B(2)}$ (Fig. 3). Starting from these results, we extended the β-lactamase complementation system into a mammalian cDNA library screening system applicable not only for soluble, but also for transmembrane bait proteins. Screening a rat forebrain cDNA library using this system identified known binding partners of $GABA_B$ receptors in addition to new candidate interactors (see 3.). Finally, the β-lactamase system allows the analysis of transient, inducible interactions. For example, the $GABA_B$ receptor is a target for several protein kinases, among them protein kinase A, and a phosphorylation-dependent binding of a candidate interactor could be demonstrated using β-lactamase complementation.

3. Analysis of $GABA_B$ receptor coiled-coil interactions

The intracellular coiled-coil interaction between the C-terminal domains of $GABA_{B(1)}$ and $GABA_{B(2)}$ plays a pivotal role for assembly and trafficking of the heterodimeric $GABA_B$ receptor. The tight binding between the amphipathic alpha-helices is one of the main sites stabilizing the receptor complex. An RSRR (R for arginine, S for serine) ER retention signal in $GABA_{B(1)}$ secures that the ligand-binding subunit is not reaching the cell surface as a monomer. The coiled-coil binding between $GABA_{B(1)}$ and $GABA_{B(2)}$ masks the ER retention signal in $GABA_{B(1)}$ allowing plasma membrane trafficking of

the heterodimeric functional receptor. Thus, the ER retention signal and the coiled-coil interaction are part of a quality control mechanism for the surface expression of $GABA_B$ receptors. In addition, the coiled-coil domains of $GABA_{B(1)}$ and $GABA_{B(2)}$ constitute binding sites for two transcription factors, ATF4 and CHOP, and a part of the coiled-coil domain of $GABA_{B(1)}$ is necessary for binding of 14-3-3 family members. These interactions and $GABA_B$ receptor heterodimerization are mutually exclusive. An RNA-binding coiled-coil domain protein, Marlin-1, was shown to interact with the $GABA_{B(1)}$ C-terminus as well.

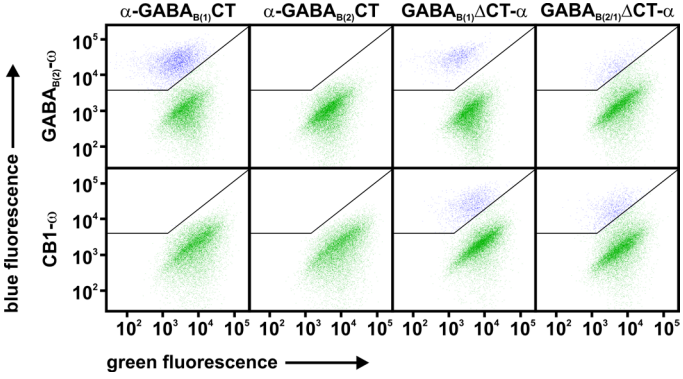

Figure 3. Monitoring $GABA_B$ receptor interactions using β-lactamase complementation. Flp-In-3T3 cells stably expressing either the $GABA_B$ receptor subunit $GABA_{B(2)}$ or the cannabinoid receptor CB1 C-terminally fused to the β-lactamase ω fragment ($GABA_{B(2)}$-ω, CB1-ω) were infected with retroviruses encoding soluble or transmembrane α fusion proteins. Cells were loaded with CCF2/AM and subjected to FACS analysis. An increase in blue and a decrease in green fluorescence is indicative of β-lactamase activity. The intracellular C-terminal domain of $GABA_{B(1)}$ ($GABA_{B(1)}$CT), in contrast to the intracellular C-terminus of $GABA_{B(2)}$ ($GABA_{B(2)}$CT), interacted with $GABA_{B(2)}$, but not with CB1. In addition, an extracellular interaction between $GABA_{B(1)}$ and $GABA_{B(2)}$ was detected: in cells expressing $GABA_{B(2)}$-ω, a $GABA_{B(1a)}$ α fusion protein lacking the intracellular C-terminus ($GABA_{B(1)}$ΔCT-α) resulted in an increased β-lactamase activity (see the separated population of blue cells) as compared with a chimeric construct in which the extracellular domain (ECD) of $GABA_{B1}$ was replaced by the ECD of $GABA_{B2}$ ($GABA_{B(2/1)}$ΔCT-α). This dependence on the ECD of $GABA_{B(1)}$ was not observed upon coexpression with CB1-ω. The considerable β-lactamase background activity observed upon coexpression of two transmembrane proteins fused to α and ω, respectively, presumably results from their limited diffusion and/or increased local concentration.

In cDNA library screens with the $GABA_{B(1)}$ C-terminus as a bait using the ras recruitment system (Broder et al. (1998) Curr. Biol. 8, 1121-1124) as well as the β-lactamase complementation system (see 2.) we identified several of the known interaction partners and in addition a new, functionally uncharacterized, coiled-coiled domain interaction partner. The new interaction was confirmed biochemically. A competition assay in Flp-In-3T3 cells using β-lactamase complementation revealed that $GABA_B$ receptor heterodimerization and the novel interaction of $GABA_{B(1)}$ are mutually exclusive. Studies in transfected neurons support the hypothesis that the novel coiled-coil interaction partner transiently localizes $GABA_{B(1)}$ to specific sites in the ER, before it is displaced by $GABA_{B(2)}$ and the dimeric receptor is trafficked to its site of action at the plasma membrane.

Support

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Thesis

Dagmar Zunner (2006). Identifizierung und Analyse von direkten Proteininteraktionen des $GABA_B$-Rezeptors in Säugern. Universität Hamburg.

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Development of the peripheral nervous system

Dieter Riethmacher

Our main interest in the past years was the analysis of differentiation processes during the development of the peripheral nervous system. The erbB3 mutation results in a complete loss of Schwann cells along axonal projections, impaired fasciculation and neuronal cell death (sensory and motoneurons) and we thus set out to identify genes that are downstream of erbB3. Using cDNA of dorsal root ganglia (drg) of wildtype and erbB3 deficient embryos we identified a number of genes with potential implications for the development of the PNS.

Another topic of our research was the development of a universal *in-vivo* cell ablation tool. As our first mouse model had some limitations we generated an improved model for universal cell ablation and demonstrated its functionality. The ablation of distinct cell populations inside an intact organism now enables us to answer specific questions of developmental and pathological relevance.

1. Matrilin-2 in the PNS

We identified matrilin-2 (matn2) in our gene expression analysis using cDNA of dorsal root ganglia. In addition to the published expression domains we also detected expression in embryonic and adult Schwann cells. In order to characterize potential functions of matn2 in the PNS we used recombinant protein in several *in-vitro* assays. We could show that Matn2 strongly promotes Schwann cell attachment and migration. Apart form the impact on Schwann cell behavior, matn2 also promoted neurite outgrowth. As a mutant mouse had been generated and is lacking any obvious phenotypes (Mates et al. 2004), we specifically investigated PNS development in these mice. We were, however, unable to detect any abnormalities by histological means. Drg cultures from matn2 deficient embryos showed statistically significant reductions of axonal length and the number of migrating Schwann cells. This clear defect identified in our *in-vitro* drg culture system must be compensated *in-vivo*.

As our expression analysis revealed a strong upregulation of matn2 after nerve lesions we analyzed matn2 deficient animals in a functional assay after femoral nerve injury. Interestingly matn2 deficient animals showed a severe delay of recovery compared to control animals pointing towards a functional role of matn2 in the PNS during nerve regeneration.

2. Periostin and Schwann cell development

Recently, we identified periostin as strongly down-regulated in our gene expression analysis using cDNA of dorsal root ganglia (drg) of wildtype and erbB3 deficient embryos. Periostin is a secreted extracellular matrix protein with a molecular mass of app. 93 kDa. that belongs to the family of fasciclin-like cell adhesion proteins. Fasciclin I, the periostin homologue in insects is reported to be involved in fasciculation and adhesion in neuronal development. Periostin as a ligand for $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins is supposed to promote integrin-dependent cell adhesion and cell migration as well as metastasis.

Expression analysis revealed periostin transcript and protein in the developing heart, bone, periodontal ligament and in

Schwann cell precursors. We found that after stimulating the erbB3 receptor with its ligand neuregulin-1, periostin transcripts are upregulated in Schwann cell cultures. A similar upregulation of periostin expression is seen when an oncogenic variant of the erbB2 receptor is overexpressed in various cell types.

Drg explants from wildtype and periostin deficient embryos were employed to assess the relevance of periostin in Schwann cell differentiation and migration. Periostin had no effect on axonal outgrowth. However, the overall number of migrating Schwann cells was significantly reduced (Fig 1A,B).

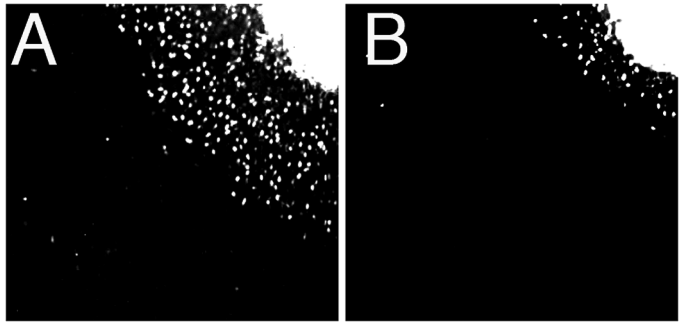

Figure 1. Immunofluorescence analysis of drg explant cultures from wildtype (A) and periostin deficient mice (B) with the Schwann cell specific Sox 10 antibody. The average number of migrating Sox 10 positive cells revealed a reduction to 57% in cultures of periostin deficient animals compared to control cultures (drg explants from 38 embryos at 12,5 dpc of 5 different litters).

Our findings identify periostin as a target of the neuregulin signaling system and furthermore suggest its implication in the migration of Schwann cell precursors, possibly via integrin signaling.

3. Maid (GCIP) is involved in cell cycle control

Maid, another gene found in our gene expression analysis, was originally isolated as a maternally described helix-loop-helix (HLH) protein. Later it was shown to be able to interact with cyclin D1. During embryonic development Maid is expressed in high levels in the liver, dorsal root ganglia (drg) and the central nervous system (CNS).

In order to analyse the function of Maid in vivo, we created a mouse carrying a null mutation of the Maid gene by homologous recombination. Mice homozygous for the mutated allel were viable and did not show any obvious developmental phenotypes. Interestingly, aged maid deficient animals developed liver tumours. Males develop liver tumours earlier in life compared to females, as was already known from the literature (Fig.2A). Further histological analysis classified these tumours as hepatocellular adenomas (HCA) and hepatocellular carcinomas (HCC). So lack of Maid triggers tumour development in the liver. This effect is liver specific as no other tissue showed an increase in tumour rate.

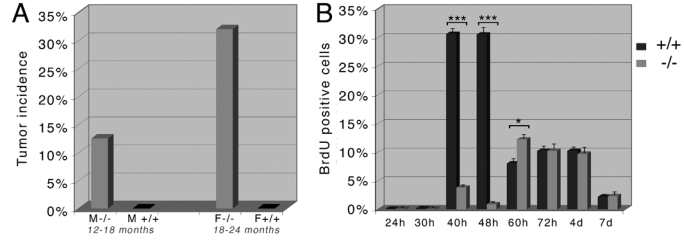

Figure 2. Tumour incidence and proliferation of hepatocytes. (A) Liver tumour incidence in aged maid deficient animals. In accordance with the literature males develop liver tumours earlier in life compared to females. (B) Delay of proliferation in hepatocytes after P.H. in young maid deficient animals.

As earlier experiments had revealed an upregulation of Maid after P.H. (partial hepatectomy), we performed P.H. experiments, using wildtype and maid deficient animals. In accordance with results from the literature in wildtype mice a maximum of DNA synthesis was found 40 to 48 hours after P.H. (Fig.2B). In contrast, homozygous mutant mice did not show this peak. Instead a delayed and less prominent peak of DNA synthesis could be observed 60 hours after P.H. in those animals (Fig.2B). In wildtype mice a strong cyclin A expression was first detected 48 hours after P.H., while in livers of Maid -/- mice an increase was observed only 60 hours after P.H.. The delay in cyclin A expression is in good agreement with the proliferation assay. Our results indicate that Maid is required in mediating the G1/S-phase transition of hepatocytes during cell cycle progression after P.H. (Sonnenberg-Riethmacher et al. accepted for publication).

4. Cell ablation in mice

We have generated and characterized the R26:LacZ/DT-A line in which a *loxP*-conditional DTA allel was introduced into the ubiquitously expressed ROSA26 locus (Brockschnieder et al. 2004). We showed that a wide spectrum of different cell types could be efficiently ablated by mating it to different Cre-expressing mouse strains. Unexpectedly, however, homo- but not heterozygous animals of the R26:LacZ/DT-A line developed some degenerative abnormalities in a variety of tissues. We reasoned that these abnormalities were most likely caused by a leaky expression of small amounts of toxin from the unrecombined LacZ^{flox}DT-A cassette. Therefore we monitored the expression of DTA in these animals and were able to detect DTA specific transcripts in homo- and heterozygous animals. This result indicates that cells can tolerate very low levels of endogenously expressed DTA molecules, while above a certain threshold, cell death inevitably occurs. To avoid this unwanted toxin expression we inserted an additional transcriptional regulatory sequence (polyA-signal) following the LacZ-ORF and showed that this modification prevented the formation of any defects in homozygous animals without diminishing the ablation efficacy upon Cre-mediated recombination. (Brockschnieder et al. 2006)

In an attempt to identify novel genes in oligodendrocytes we used our R26:LacZ/DT-A line to deplete animals of all oligodendrocytes. These animals were then used in microarray expression profiling experiments. This effort resulted in the identification of several promising candidates, one of which is Ermin, a novel cytoskeletal molecule exclusively expressed by oligodendrocytes. The expression pattern and subcellular localisation (outer cytoplasmic lip of the myelin sheath) suggests a role during the late wrapping and/or compaction phases of myelinogenesis (Brockschnieder et al. 2006).

Our mouse line enables us to unravel the function of distinct cell types by specifically ablating cell populations inside an organism. We were interested in the role of V1 spinal interneurons, that are part of neuronal networks (the central pattern generators (CPGs)), for locomotor outputs. We could show that V1 interneurons shape motor outputs during locomotion and are required for controlling the speed. These data showed that surprisingly inhibition is important for speed (Gosgnach et al. 2006).

In another set of experiments ablating the PKD2L1 expressing taste receptor cells resulted in a devastating loss of sour taste without affecting the salt responses in these animals (Huang et al., Nature 2006), further substantiating the usefulness of our ablation model.

Support

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Riethmacher, Dieter (2004). Funktionelle Analyse von Signal-transduktionsprozessen während der Entwicklung von Vertebraten.

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Prof. Dr. Michail S. Davidoff, Anatomisches Institut

Prof. Dr. Thomas Eschenhagen, Institut für Experimentelle und Klinische Pharmakologie und Toxikologie

Structure of the Group

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Development and differentiation of the inner ear

Thomas Schimmang

The inner ear is induced as an auditory placode that closes to form the otic vesicle and then undergoes a complex morphogenetic process to form the mature sensory organ. We are interested in several aspects of the auditory organ, including its development, formation and regeneration of auditory neurons and hair cells and gene transfer into the inner ear. At the molecular level we are focussing on two large gene families, the neurotrophins and its receptors and the Fibroblast growth factors (FGFs). To analyse the functions of these gene families *in vivo* we are using avian (chicken) and mammalian (mice) model systems. These experiments are complemented with *in vitro* studies using cultures of hair cells. We perform gain-of-function (viral expression) and loss-of-function (knock-out mice) experiments to define the roles of neurotrophin and FGF signaling in the inner ear. Next to these studies we are performing a genome-wide screen to identify genes involved in otic vesicle development.

1. The role of BDNF during age-related hearing loss

A decline in neuronal plasticity during the adult life span has been proposed to be associated with a reduced level of the effectors of plasticity responses (e.g., BDNF). Alteration of plasticity is also correlated with age-related hearing loss (presbycusis), but to date no detailed studies of BDNF expression have been performed in the young or aging mature cochlea. We have used rat and gerbil animal models for presbycusis which displayed hearing loss in the final third of the animals' natural life span. We have demonstrated for the first time a co-localization of BDNF protein, transcripts III and IV, and TrkB protein in cochlear neurons with a declining distribution towards low-frequency processing cochlear turns. BDNF protein was also found within the neuronal projections of the cochlea. A significant reduction of BDNF transcripts in high-frequency processing cochlear neurons was observed during aging, though this did not coincide with a major reduction of BDNF protein. In contrast, BDNF protein in peripheral and central projections was drastically reduced. Our results suggest that reduced BDNF protein levels in auditory nerves over age may be a crucial factor in the altered brainstem plasticity observed during presbycusis.

2. Functions of FGF-2 during development and maintenance of cochlear sensory epithelia

The development of the auditory sensory epithelium in vertebrates is a complex process controlled by cell proliferation, cell fate decisions and cell differentiation. Moreover, regenerative processes recapitulating these developmental steps have been described after damage to the mature inner ear. FGFs and their receptors have been suggested to play decisive roles for the development and maintenance of the auditory sensory epithelium. Amongst FGFs, FGF2 has been postulated as a key regulator involved in the proliferation, differentiation and regeneration of sensory hair cells. We have addressed the potential functions of FGF2 during the formation and regeneration of the auditory epithelium in chicken and mice. Using viral gene transfer, based on Her-

pes simplex type 1 virus (HSV-1; Figure 1), we showed that ectopically applied FGF2 drastically increases the number of cells expressing early hair cell markers during embryonic development in avians. Intriguingly, FGF2 did not stimulate cell division during this process. These data suggest that FGF2 plays a role during differentiation of sensory hair cells in avians. To address the potential functions of FGF2 during murine inner ear development we analysed FGF2 mouse mutants. Mice lacking FGF2 showed normal formation of the inner ear and no abnormalities were observed at the adult stage. Moreover, FGF2 mouse mutants showed similar hearing thresholds compared to those observed in control mice before and after noise damage. Therefore, endogenous FGF2 appears not to be essential for the development or functional maintenance of the auditory organ in mammals.

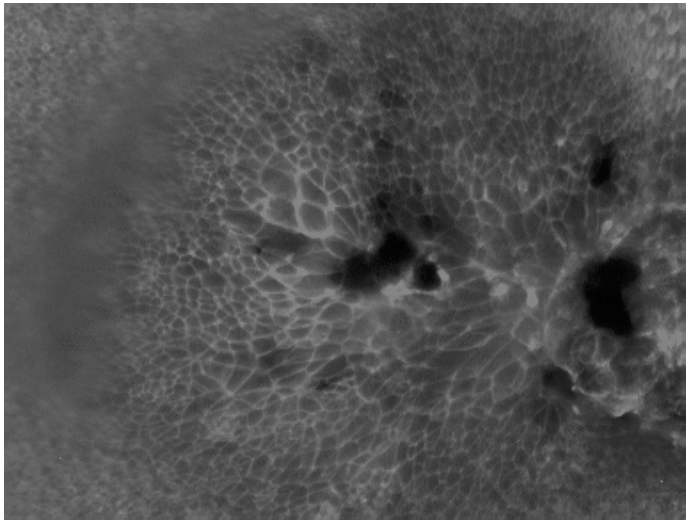

Figure 1. Avian cochlear sensory epithelium stained with phalloidin. Dark patches correspond to cells infected with HSV-1 vectors.

3. Screening for FGF-regulated genes

FGFs have been shown to control formation and differentiation of multiple organ systems in the developing vertebrate embryo. The analysis of differential gene expression during embryogenesis is therefore a potent tool to identify novel target genes regulated by FGF signalling. We have applied microarray analysis to identify differentially regulated genes in FGF mutant mouse embryos. Surprisingly, transcripts corresponding to vomeronasal receptors (VRs), which so far have been only detected in the vomeronasal organ (VNO), were found to be downregulated in FGF mutant embryos. VR expression was detected in the developing olfactory pit (Figure 2) and the anlage of the VNO.

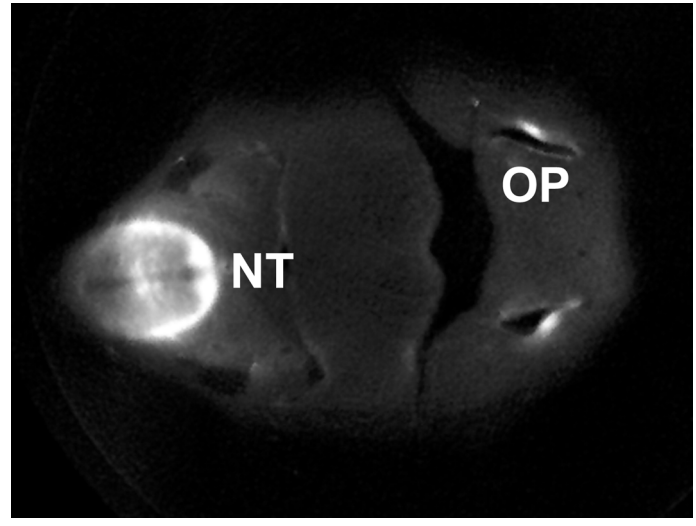

Figure 2. Section through a mouse embryo at embryonic day 12 showing VR expression in the neural tube (NT) and olfactory pit (OP).

Interestingly, several FGFs can be detected in the developing olfactory pit during mouse embryogenesis. FGF signalling may thus control expression of VRs in the olfactory pit and formation of the VNO. Moreover, VR expression was detected in unexpected locations within the developing embryo including retina, dorsal root ganglia and neural tube.

Support
The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft and the Spanish Ministry of Education.

Publications

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Thesis

Dissertation

Zelarayan, Laura (2005). Roles of fibroblast growth factors during induction and morphogenesis of the inner ear of the mouse and chicken. University of Hamburg.

Collaborations

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Dr. Thomas Theil, Anatomical Institute, University of Tübingen

Dr. Marlies Knipper, Tübingen Hearing Research Center (THRC), University of Tübingen

Dr. Marian Ros, Departamento de Anatomía y Biología Celular, Universidad de Cantabria, Santander, Spain

Dr. Dieter Riethmacher, Center for Molecular Neurobiology Hamburg

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DNA Sequencing

Willi Kullmann / S. Hoffmeister-Ullerich

The DNA-sequencing facility of the ZMNH 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 June 1999 from 64 to 96 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 fluorescence-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 2004 until September 2006 approximately 40,000 sequence analyses were performed.

Structure of the Group

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Morphology

Michaela Schweizer

As a part of the general services of the ZMNH the Morphology Unit performs investigations of neurobiological questions and supports scientists in many areas of microscopy. The histological characterisation of genetically engineered animals is becoming a major approach in our facility. We investigate the histopathology of selected organs or tissues of interest to study the effect of genetic modifications. We give advice on morphological questions, teach and train researchers in the application of microscopical techniques. We introduce and establish new techniques and guarantee efficient use of the respective equipment.

1. Offered services

- Performance of light- and electron microscopical investigations
- Advice and practical instruction in the application of histochemical techniques
- Instruction of researchers in operation of microscopes and accessories
- Introduction of useful new (immuno-) histochemical techniques and/or equipment

2. Techniques

- Morphological studies of many kinds of tissues with light-, confocal laser scanning-, or transmission electron microscopy
- Patho-histological analysis of the whole body of transgenic mice
- Histo (cyto) chemical staining procedures
- Immunohisto (cyto) chemistry
- In situ hybridisation
- Pre- and postembeeding immunogold labelling techniques

We prepare cell and tissue samples for scientific histological and (immuno-)histochemical light and fluorescence microscopy. All preparation steps, (including fixation, sectioning with vibratome, cryotome or microtome, staining, mounting etc.) are performed by the group. The Morphology Unit has at its disposal both conventional and fluorescence microscopes (Zeiss Axiophot), as well as one confocal scanning laser microscope (Leica SP2) in inverted configuration.

We process cells and tissues for conventional transmission electron microscopy (Zeiss 902) and offer immunolocalisation of gene products applying pre- and postembedding protocols. We take care to preserve both, antigenicity and structural integrity.

We localise m-RNA expression routinely by hybridisation of radioactively labelled cRNA-probes to cryo-sections of fresh-frozen tissues or to cultured cells. The hybridisation signals are shown autoradiographically using high resolution X-ray-films or application of photographic emulsion followed by light microscopy.

All results are documented in high resolution digital images.

Publications

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Mass Spectrometry, Biomolecular Interaction Analysis, and Bioinformatics

Christian Schulze

Transcription factors are key players in the control of gene expression. They recognize short nucleotide motifs in the upstream region of a gene and participate in activation or repression of transcription. The availability of high quality DNA sequences, and documented binding preferences for transcription factors allows predicting potential sites. The service unit also offers other bioinformatics services.

1. Biomolecular interaction analysis

Protein-protein interaction for functional characterization is performed using a surface plasmon resonance (SPR) biosensor (Biacore 3000). The technique is based on the total internal reflection phenomenon. Changes in the mass concentration of macromolecules at a biospecific interface are recorded and displayed in real time. Numerical evaluation of the data is used to derive reaction rates and binding constants.

The application area of a surface plasmon resonance (SPR) biosensor is not limited to the investigation of protein-protein interaction; also protein-RNA interaction can easily be monitored. A detailed description of a RNA recognition system is given in (1).

2. Bioinformatics

A computational method was developed that can identify target genes for transcription factors in sets of orthologous non-coding DNA sequences. The method can handle sets of specified genes as extracted from gene expression analysis or complete genomes. Given a base species, gene oriented genomic sequences upstream the transcription start sites are loaded automatically from EnsEMBL. Transcription factor recognition motifs represented by position weight matrices (PWM) taken from Transfac are used to search for putative motifs. The program is written in Perl, results may be visualized using DAS resources at EnsEMBL.

Publication

(1) Horke, S., Reumann, K., Schulze, C., Grosse, F., and Heise, T. (2004). The La motif and the RNA recognition motifs of human La autoantigen contribute individually to RNA recognition and subcellular localization. J. Biol. Chem. 279, 50302-50309.

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Transgenic Mouse Facility

Irm Hermans-Borgmeyer

The transgenic mouse facility supports scientists of the ZMNH and of the UKE in all aspects of transgenic mice production.
The injection laboratory is equipped with two injection set ups, one for pronucleus and one for ES cell injection.
The cell culture laboratory serves to provide ES cells and mouse embryonic fibroblast and can be used by scientists to carry out their gene targeting experiments.
The molecular biology laboratory is used for genotyping and preparation of DNA fragments.
Since spring 2006 we are equipped with a SPOF mouse room. All mice are now generated on a SPOF level.
On average we perform 50 injection projects per year, half of which are pronucleus injections.

We offer as service:

- The generation of transgenic mice by pronucleus injection of DNA constructs into one cell stage mouse embryos
- The injection of recombinant mouse ES cells into mouse blastocysts
- ES cells and mouse embryonal fibroblasts for ES cell culture
- Supervision of gene targeting experiments carried out in the cell culture laboratory of the facility
- Cre- and Flip-Deleter mice
- Reporter mouse lines
- Help with the design of experiments
- Help with the preparation of DNA for pronucleus injection and electroporation into ES cells
- Help with the analysis of recombinant ES cell clones as well as of the generated mouse lines

Publications

(1) Hermey, G., Plath, N., Hübner, C.A., Kuhl, D., Schaller, H.C., and Hermans-Borgmeyer, I. (2004).The three sorCS genes are differentially expressed and regulated by synaptic activity. J. Neurochem. 88, 1470 -1476.

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(6) Susens, U., Hermans-Borgmeyer, I., Urny, J., and Schaller, H.C. (2006). Characterization and differential expression of two closely related G-protein-coupled receptors, GPR139 and GPR142, in mouse tissue and during mouse development. Neuropharmacology 50, 512-520.

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Seminare und Vorlesungen für Mediziner und Naturwissenschaftler

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Bähring, Boehm, Isbrandt, Pongs

Molekulare Aspekte der neuronalen Entwicklungsbiologie
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Aufbaustudiengang Molekularbiologie

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Praktikum II
DozentInnen und Wiss. MitarbeiterInnen des ZMNH

Praktikum IV
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DFG Seminare

GRK 255 *(bis 31.12.2005)*
Neurale Signaltransduktion und deren pathologische Störungen
Sprecherin: Melitta Schachner Camartin

DFG Forschergruppe 604 *(seit 2006)*
Signaling pathways in the healthy and diseased heart
Sprecher:
Heimo Ehmke, Thomas Eschenhagen, Olaf Pongs

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Seminare und Vorlesungen für Mediziner und Naturwissenschaftler

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Schachner Camartin (2 st.)

Molekularbiologie
Schachner Camartin (2 st.)

Aktuelle Themen aus der Neurobiologie
Hermans-Borgmeyer (2 st.)

Zebrafische: Entwicklung und Regeneration
Becker, Becker, Schachner Camartin (2 st.)

Forschungsseminare

Struktur und Funktion von Ionenkanälen
Bähring, Boehm, Isbrandt, Pongs (2 st.)

Neuropeptidwirkung, Signaltransduktion
Borgmeyer, Hoffmeister-Ullerich, Schaller (2 st.)

Ionenkanäle
Fuhrmann, Jentsch, Kurth, Scheel, Zdebik (2 st.)

Neurale Signalverarbeitung
Bähring, Boehm, Isbrandt, Pongs (2 st.)

Molekulare Mechanismen der Neurogenese
Bach, Hoppe, Kornau, Riethmacher, Schimmang (2 st.)

Neurale Zellerkennungsmoleküle
Schachner Camartin und MitarbeiterInnen (2 st.)

Praktika

Molekularbiologische Methoden in der Neurobiologie
Bähring, Boehm, Isbrandt, Pongs

Praktikum für molekulare Neurobiologie
Becker, Becker, Bernreuther, Dityatev, Irintchev, Leshchyns'ka, Loers, Kleene, Morellini, Schmidt, Sydnyk, Tilling u.a.

Elektrophysiologische Methoden für Fortgeschrittene
Jentsch, Neagoe, Scheel, Zdebik

Neurobiologische Methoden für Fortgeschrittene
Bähring, Boehm, Isbrandt, Pongs

Molekulare Aspekte der neuronalen Entwicklungsbiologie
Borgmeyer, Hermans-Borgmeyer, Hoffmeister-Ullerich

Aufbaustudiengang Molekularbiologie

Vorlesung: Molekularbiologie I (1. Sem.)
Bach, Hermans-Borgmeyer, Hoppe, Jentsch, Kornau, Riethmacher, Schaller, Schimmang und itarbeiterInnen (2 st.)

Einführung in die Neurobiologie/Immunologie (3. Sem.)
Vorlesung u. Seminar f. MedizinerInnen und NaturwissenschaftlerInnen
Bartsch, Becker, Becker, Irintchev, Schachner Camartin, Tilling (2 st.)

Praktikum I
DozentenInnen und Wiss. MitarbeiterInnen

Praktikum III
DozentInnen und Wiss. MitarbeiterInnen des ZMNH

DFG Seminare

GRK 255 *(bis 31.12.2005)*
Neurale Signaltransduktion und deren pathologische Störungen
Sprecherin: Melitta Schachner Camartin

DFG Forschergruppe 604 *(seit 2006*)
Signaling pathways in the healthy and diseased heart
Sprecher:
Heimo Ehmke, Thomas Eschenhagen, Olaf Pongs

ZMNH - Seminars 2004

J.-L. Bessereau, Paris
Genetic analysis of receptor clustering at C. elegans neuromuscular synapses

V. Stein, San Francisco
Stargazin and psd-95 regulate synaptic plasticity

R. Mains, Farmington
Kalirin: multiple domains, multiple functions

M. Covarrubias, Philadelphia
Molecular physiology of somatodentritic A-type potassium channels

I. Soltesz, Irvine
Plasticity of hippocampal circuits in epilepsy

Z.C. Xiao, Singapore
Neurite outgrowth inhibitors at nodes of Ranvier

J.L. de la Pompa, Madrid
Notch regulates cardiac valve formation and ventricular trabeculation in vertebrates: implications for tumor progression

H. Ulrich, Sao Paulo
RNA aptamers as modulators of neurotransmitter receptor activity implicated in disease and neuronal differentiation

ZMNH - Seminars 2005

E. Ponimaskin, Göttingen
Signal transduction mediated by serotonin receptors: new pathways and dynamic distribution

W. Witke, Moterotondo
Cytoskeletal dynamics in neurons: F-Actin depolymerizing factors and the role in neuronal migration and brain development

Z. J. Huang, Cold Spring Harbor
Constructing GABAergic circuits in neocortex and cerebellum: from subcellular organization to activity dependent plasticity

W. Loescher, Hannover
Current status and future directions in the pharmacotherapy of epilepsy

J. Rettig, Homburg/Saar
Molecular mechanisms of LDCV exocytosis from adrenal chromaffin cells

J. Kirsch, Heidelberg
Determination of inhibitory synaptogenesis in time and space

M. R. Kreutz, Magdeburg
Synapto-dendritic Ca^{2+}-signaling via Caldendrin and its binding partners

A. Aszodi, Martinsried
Role of cell matrix interactions in sceletal development

U. Cavallaro, Milano
CAMs and receptor tyrosine kinases: a cross-talk that prevents tumor metastasis?

C. Bargmann, New York
Guideposts and cell communication in synaptic specification

H. F. Epstein, Galveston
Regulation of myosin assembly and function

G. Sauter, Hamburg
Tissue microarrays for high-throughput tissue analysis

A. Vellodi, London
Lysosomal disorders and the brain

T. Roeder, Kiel
Control of behavior and metabolism by an ancient hormonal system

R. S. Nowakowski, Piscataway
System Biology: The New Neuroscience?

A. El-Husseini, Vancouver
Protein traffic at nascent synapses

G. Winterer, Düsseldorf
Molecular & physiological effects of nicotine: attention, stress & reward

J.-M. Fritschy, Zürich
Role of GABA-A receptors for synapse formation and plasticity

C. Eckmann, Dresden
Regulating cell fate switches at the translational level – a theme of GoLD

P. Doherty, London
Role of novel DAG lipases in neurogenesis and axonal growth

M. Heine, Bordeaux
Participation of mobile glutamate receptors in synaptic signalling

P. Jonas, Freiburg
Properties of GABAergic synaptic transmission in the hippocampal principal neuron-interneuron network

ZMNH - Seminars 2006

V. Bolshakov, Belmont
Mechanisms of synaptic plasticity in fear learning

B Schwappach, Heidelberg
Isoform-specific role of 14-3-3 proteins in the trafficking of multimeric membrane proteins

M. Häusser, London
Sensory coding by single neurons at the input layer of the cerebellar cortex

W. Reutter, Berlin
Biochemical engineering of surface glycan structures: biological implications

T. Sommer, Berlin
The function of Ubiquitin ligases of the ER-membrane in protein dislocation

O. Brüstle, Bonn
Engineering embryonic stem cells for neural repair

T. Smart, London
Molecualr regulation of the GABA-A receptor

A. Gottschalk, Frankfurt
Characterization of nAChR-associated proteins in Caenorhabditits elegans, and the remote control of a worm by blue light

O. Yifrach, Beer-Sheva
Intrinsically-disordered protein segments of voltage-activated potassium channels: implication for synapse assembly and maintenance

P. Washbourne, Eugene
Intracellular dynamics of NMDA receptors

A. Bragin, Los Angeles
Pathological high frequency oscillations in the epileptic brain: mechanisms of generation and functional role

H. Kettenmann, Berlin
Properties of microglia

E. Wanker, Berlin-Buch
Neuroproteomics: linking human proteins to cellular pathways and disease processes

M. Missler, Göttingen
Synaptic cell-adhesion molecules neurexins at the cross-road between synapse function and structure

O. Paulsen, Oxford
Synaptic control of spike timing in hippocampal neurons

D. J. Glass, Massachusetts
Signaling pathways that mediate skeletal muscle atrophy and hypertrophy

Z. Xiao, Singapore
Nodes of Ranvier: an important axonal domain for CNS degeneration and regeneration

V. Haucke, Berlin
Regulatory mechanism in clathrin- and adaptor-mediated membrane traffic

C. Bernard, Marseille
Temporal lobe epilepsy as a plasticity machine

U. Fischer, Würzburg
The molecular basis of spinal muscular atrophy

P. Mombaerts, New York
Olfaction targeted

J. L. Weiss, Sheffield
Ca^{2+} channel regulation by the Ca^{2+} sensor NCS-1/frequenin: function in neuronal outgrowth

S. Kins, Heidelberg
Alzheimer's Disease: function and trafficking of the APP family members

M. Koegl, Heidelberg
Nuclear receptors and automated Yeast Two-Hybrid screening: from basic research to drug discovery

ZMNH PhD-Seminars 2004-2006

N. Brose, Göttingen
The role of neuroligins in synaptogenesis and synaptic function

C. Petit, Paris
Human hereditary deafness: From genes to the molecular mechanisms underlying the development and the physiology of the auditory hair cells

H. Bujard, Heidelberg
Controlling gene activities in vivo via tetracyclins

J. Penninger, Wien
Learning from SARS infections?

O. Hobert, New York
MicroRNAs and neuronal fate determination in C. elegans

SFB 444 Seminars 2004-2005

F. Barros, Oviedo
Molecular determinants for regulation of ERG potassium channels by TRH

Y. Yaari, Jerusalem
KCNQ/M channels in the brain: what do they do?

C. Dotti, Turin
Early events of neuronal differentiation

J. M. Barral, Martinsried
Multi-domain proteins follow different folding pathways in prokaryotes vs. eucariotes

M. Manrino, Pisa
Role of megalin in the regulation of thyroid homeostasis

M. Sassoe-Pognetto, Turin
Organization and plasticity of a dendrodendritic synaptic circuit

D. Hoffman, Bethesda
Activity dependent redistribution of the A-type K^+ channel subunit Kv4.2 in hippocampal CA1 neurons

DFG Research Group 604 Seminars 2005

K. Stangl, Berlin
The ubiquitin-proteasom system: A general cellular regulation system with specific cardiovascular relevance

M. Boiani, Münster
Factors affecting nuclear reprogramming after somatic cell nuclear transfer into mouse oocytes

DFG Research Group 604 Seminars 2006

A.A. Genazzini, Novara
NAADP: the ins and outs of an unusual Ca2+-releasing second messenger

P. Lipp, Homburg
Protein kinase C signalling in living cells: the PKC-membrane 'pas de deux'

B. Attali, Tel Aviv
Calmodulin is essential for cardiac IKs channel gating and assembly: impaired function in long QT mutations

M. Glatzel, Hamburg
Modeling conformational dementias in genetically modified mice

K.R. Chien, La Jolla
Heart like Blood: Cardiopoiesis and the Islet-1 Cardiovascular progenitor story

R. Fischmeister, Paris
Intracellular cyclic nucleotide compartmentation and cardiac function: act locally, think globally

V. Flockerzi, Homburg
Coupling of TRCP-channels to voltage dependent Ca channels

B. Kaupp, Jülich
Regulation of pacemaker activity in the heart - a matter of life and death

J. Nargeot, Montpellier
New insights in T-type calcium channel physiological functions

A. J. Kaumann, Cambridge
Myocardial 5-HT4 receptors

L. Pott, Bochum
GIRK channels in atrial and ventricular myocytes

Financing

The ZMNH was financed in 2004, 2005 and 2006 by the City-state of Hamburg (FuHH), the Bundesministerium für Bildung und Forschung (BMBF), the Deutsche Forschungsgemeinschaft (DFG) and by grants from research foundations and industry.

The FuHH funded the basic budget for the four institutes, the central facilities, the building and the junior research groups. Initially financed by the Hertie-Foundation, a fifth institute (Neuroimmunology and Clinical Multiple Sclerosis Research) was established in September 2006.

In 2004, 2005 and 2006 the total budget of the Centre amounted to 12.375, 12.373 and 11.857 million EUR. Presently 222 people are employed at the ZMNH (inclusive fellowships).

Financing by FuHH

Personnel, supplies and equipment contributed by FuHH (in thousand EUR):

	personnel costs	supplies/ running costs	investment costs
2004:	5.366	2.794	154
2005:	5.652	2.794	71
2006:	5.652*	2.794*	85

Other financing

In 2004, 2005 and 2006 members of the Centre received support from the Bundesministerium für Bildung und Forschung (BMBF) via individual project grants and research groups and from the Deutsche Forschungsgemeinschaft (DFG) via individual project grants, research groups, SFB's and graduate programs.

Further support was given by the European Community, Foundation Leducq, Philip Morris USA Inc., Hertie Foundation, Roechling Foundation, Werner Otto Foundation and others. Outside support amounted to 11.030 million EUR for 2004-2006.

The personnel and supply costs provided by the various funding agencies were (in thousand EUR):

	personnel costs	supplies incl.equipment
2004:		
BMBF	331	240
DFG	1.291	489
EC	129	65
Foundations	270	16
Industry and other	120	65
SFB 444, 470, 545	724	321
total	2.865	1.196
2005:		
BMBF	332	169
DFG	1.125	399
EC	103	374
Foundations	279	150
Industry and other	65	79
SFB 444, 470	531	250
total	2.435	1.421

* plus 540 thousand EUR personnel and 100 thousand EUR supplies p.a. for the new Institute for Neuroimmunology and Clinical MS-Research

	personnel costs	supplies incl. equipment
2006*:		
BMBF	354	161
DFG	945	345
EC	95	124
Foundations	590	67
Industry and other	22	37
SFB 444, 470	259	114
total	2.265	848

*as at Sept. 30, 2006

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Prof. Dr. Chica Schaller*
Dr. Dirk Isbrandt*
Dr. Dieter Riethmacher*
Dr. Anselm Zdebik*
Jürgen Dralle

* during part of the reported period