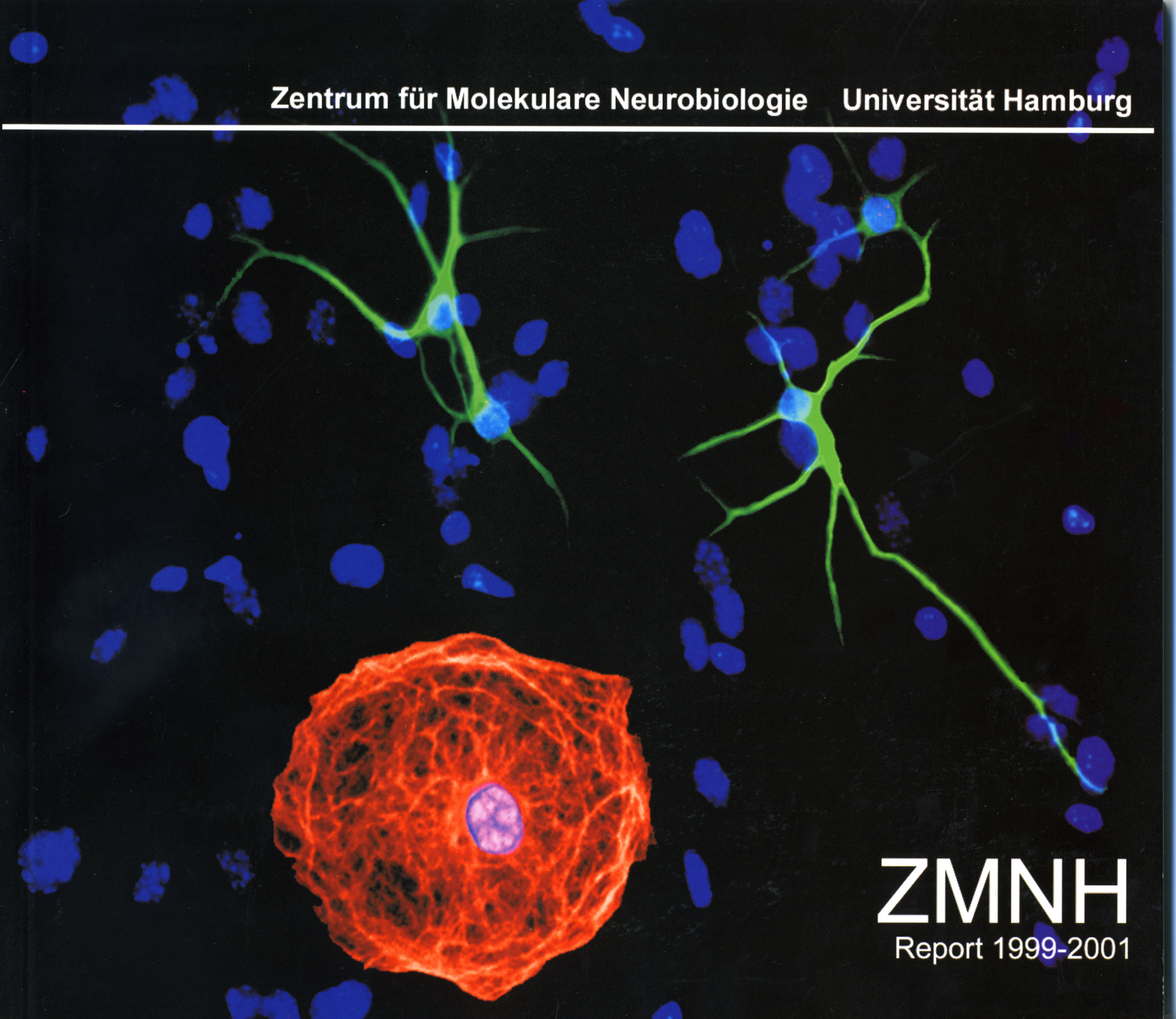
Zentrum für Molekulare Neurobiologie Universität Hamburg



ZMNH

Report 1999-2001

Front Cover: Murine embryonic stem cells were differentiated *invitro* and then stained with antibodies against MAP2 (microtubule associated protein2) green and GFAP (glial fibrillary acidic protein) red to reveal their neuronal and glial identities. The cells were counterstained with DAPI.

Foto: Dieter Riethmacher, Johannes Schmucker

Report 1999-2001

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Layout: E. Bryssinck
Belichtung, Druck
und Buchbinder: Leopold Korst GmbH

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Vorwort

Das Zentrum für Molekulare Neurobiologie ist ein Forschungszentrum der Universität Hamburg am Fachbereich Medizin. Es betreibt Grundlagenforschung auf dem Gebiet der molekularen Neurobiologie und angrenzenden Gebieten. Die gewonnenen Erkenntnisse werden oft schnell auf medizinische und humangenetische Fragestellungen übertragen, wobei zunehmend Mausmodelle eine Rolle spielen. Transgene und '*knock-out* Mäuse', bei denen spezifisch einzelne Gene inaktiviert werden, ermöglichen den Schritt vom Gen zur Physiologie und zur Krankheit. 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 vier von Professoren (Jentsch, Pongs, Schachner, Schaller) geleitete Institute sowie mehrere von Nachwuchswissenschaftlern geleitete, zeitlich befristete Forschergruppen. Diese völlig unabhängigen jungen Gruppen sind zentral für das Konzept des Zentrums. In Verhandlungen mit der Wissenschaftsbehörde konnten wir erreichen, dass die Anzahl der von der Stadt Hamburg finanzierten Forschergruppen ab 2002 von drei (derzeit Riethmacher, Sander, Schimmang) auf vier erhöht wird. Hinzu kommen die von der DFG finanzierten Gruppen: Bach (Heisenberg-Programm) und die neu etablierte Nachwuchsgruppe Kornau des Sonderforschungsbereichs (SFB) 444. Die Gruppe Kornau, über die hier noch nicht berichtet werden kann, wird sich mit Netzwerken synaptischer Proteine befassen. Die Nachwuchsgruppenleiter der vorangegangenen Generation haben inzwischen Rufe auf Professuren angenommen (Michael Wegner auf den Lehrstuhl für Biochemie und Pathobiochemie in Erlangen, und Roger Nitsch für die Abteilung für Psychiatrische Forschung der Universität Zürich), während Dietmar Kuhl sich zwischen mehreren Rufen noch entscheiden muss.

Alle Institute und Gruppen des ZMNH werden durch wissenschaftliche Serviceeinheiten unterstützt. Eine weitgehend eigenständige Verwaltung stellt die notwendige Effizienz und Flexibilität der administrativen Vorgänge sicher. Ein internationaler Beirat evaluiert und berät das ZMNH.

Eng mit dem ZMNH assoziiert ist das Institut für Zellbiochemie und Klinische Neurobiologie (IZKN), das von Professor Richter, dem Gründungsdirektor des ZMNH, geleitet wird. Auch an diesem Institut arbeiten Nachwuchsgruppen sowie eine Serviceeinrichtung.

Vierzehn Jahre nach seiner Gründung (im Jahr 1988) ist das ZMNH fest in die Hamburger Wissenschaftslandschaft integriert. Dies äußert sich in einer großen und weiter zunehmenden Zahl wissenschaftlicher Kollaborationen, insbesondere mit Gruppen der Universitätsklinik Eppendorf (UKE). Hierbei spielen die am Zentrum entwickelten Mausmodelle eine herausragende Rolle. Ihre diversen und manchmal unerwarteten Phänotypen erfordern des öfteren spezifische Expertisen, die am UKE hervorragend vertreten sind.

Die lokale Vernetzung schlägt sich auch in Forschungsverbünden nieder, in denen das ZMNH häufig der Motor ist. Genannt seien der am ZMNH zentrierte Sonderforschungsbereich (SFB) 444 (*Grundlagen neuraler Kommunikation und Signalverarbeitung*; Sprecher Jentsch), das ebenfalls am ZMNH konzentrierte Graduiertenkolleg 255 (*Neurale Signaltransduktion und deren pathologische Störungen*; Sprecherin Schaller), der SFB 545 (*Molekulare Mechanismen genetisch bedingter Erkrankungen*; Sprecher Greten), der SFB 470 (*Glycostrukturen in Biosystemen - Darstellung*

und Wirkung; Sprecher Thiem), sowie die Forschergruppe RNA Transport (Sprecher Richter). Im Rahmen des neu vom BMBF initiierten *'Nationalen Genomforschungsnetzes'* waren wir mit einem Antrag auf dem Gebiet der Nervenkrankheiten erfolgreich. Die Hamburger Gruppierung (Sprecher Jentsch) schließt neben fünf ZMNH Gruppen die Humangenetik des UKE ein.

Forschung am ZMNH

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 nieder. Zu erwähnen sind hier u.a. der Zülch-Preis, der Louis-Jeantet Preis und der Ernst-Jung Preis an Thomas Jentsch, sowie der Preis der Deutschen Stiftung Querschnittslähmung an Melitta Schachner. Auch jüngere Wissenschaftler des Zentrums wurden ausgezeichnet, so C. Kubisch mit dem Heinz Maier-Leibnitz Preis der DFG.

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. auch die Entwicklung des Polypen Hydra, die Entwicklung des Pankreas, Bluthochdruck und Herzrhythmusstörungen, Taubheit, Infertilität und Nieren- und Knochenerkrankungen. Als besonders effizient erweist sich das Zentrum bei der Herstellung und Analyse von *knock-out* Mäusen und herausragende Durchbrüche gelangen in den vergangenen Jahren bei der molekularen Aufklärung monogener menschlicher Erbkrankheiten.

Das Institut für Molekulare Neuropathobiologie (Jentsch) untersucht Fragestellungen des Ionentransportes, wobei die Physiologie und Pathologie eine besondere Aufmerksamkeit finden. Im Brennpunkt stehen Chlorid- und Kaliumkanäle, sowie neuerdings auch Kalium-Chlorid-Cotransporter. Mausmodelle lieferten entscheidende Einsichten in die Funktion intrazellulärer Chloridkanäle und ihre Rolle bei dem Vesikelverkehr und der Endozytose, und in die Rolle von KCl-Cotransport bei synaptischer Inhibition. 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.

Als Schwerpunkt wird im Institut für Entwicklungsbiologie (Schaller) die Wirkungsweise des Neuropeptids Kopfaktivator (KA), seine Interaktion mit Rezeptoren und die Signaltransduktion erforscht. Im Säugetier wirkt KA neuroprotektiv und stimuliert die Zellteilung von neuroendokrinen Zellen und von neuronalen Vorläuferzellen. Auf der Suche nach dem hochaffinen G-Protein gekoppelten KA-Rezeptor wird in heterologen Expressionssystemen eine Serie neuer und Waisenrezeptoren auf Bindung und Wirkung von KA und anderer Liganden untersucht.

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 sind für diese Untersuchungen von besonderem Interesse.

Die Forschergruppe Bach bearbeitet Fragestellungen der molekularen Mechanismen neuronaler Zellspezifizierung während der Embryogenese. Die Forschungen konzentrieren sich auf die Regulation einer Klasse von Transkriptionsfaktoren, die LIM Homeoproteine genannt werden, und die grundlegende Funktionen in der Entstehung und Differenzierung von Neuronen übernehmen. Ingolf Bach und Mitarbeiter konnten zeigen, dass die biologische Aktivität von LIM Homeoproteinen entscheidend durch Protein-Protein Wechselwirkungen mit verschiedenen Kofaktoren beeinflusst wird.

Das Hauptziel des Labors von Dietmar Kuhl ist es, molekularbiologische Ansätze zur Identifizierung und zum Studium von Genen zu entwickeln, die an der synaptischen Plastizität im Säugetiergehirn beteiligt sind. Die Analyse ihrer Expression weist auf eine wichtige Rolle dieser Gene in verschiedenen Formen der neuronalen Plastizität, einschließlich Lernen und Gedächtnis, sowie neurodegenerativen Erkrankungen hin. Seine Forschung bewegt sich von der Identifizierung der Aktivitäts-regulierten Gene zur Analyse der Langzeit-Potenzierung im Gehirn und will bestimmen, welche Konsequenzen diese Gene auf das Verhalten von Tieren und deren Fähigkeit zu lernen und zu erinnern haben.

Die Forschergruppe Riethmacher beschäftigt sich vor allem mit Fragestellungen der Entwicklungsbiologie. Ein besonderer Schwerpunkt ist die Aufklärung der verschiedenen Funktionen des Neuregulin-Signal-Systems sowie des Transkriptionsfaktors Sox10 im peripheren Nervensystems mittels Mausmodellen. Außerdem werden Funktionen des erbB2-Rezeptors sowie der Caspase8 im zentralen Nervensystem mittels des Cre-lox P-Systems analysiert.

Die Forschergruppe Sander beschäftigt sich mit den molekularen Mechanismen, durch die Transkriptionsfaktoren die Entwicklung des zentralen Nervensystems und des Pankreas kontrollieren. Im Speziellen versucht die Gruppe zu verstehen, wie Transkriptionsfaktoren die Entwicklung verschiedener Zelltypen vermitteln und welche Zielgene durch diese Faktoren aktiviert oder reprimiert werden. Um diese Fragestellungen zu beantworten, verwendet die Gruppe die Maus als Modellsystem für genetische, molekulare und genomische Forschungsansätze. Der Gruppe gelang es, wichtige Transkriptionsfaktoren für die Entwicklung von Motorneuronen und von Insulin-produzierenden Zellen im Pankreas zu identifizieren.

Im Mittelpunkt der Forschung der Nachwuchsgruppe Schimmang steht die Entwicklung und Differenzierung des Gehörorgans. Dabei werden besonders die Rollen von Wachstumsfaktoren bei der Induktion des Innenohrs, beim Überleben von Haarsinneszellen und Gehörneuronen und beim Schutz vor Gehörschäden untersucht. Neben der Analyse von transgenen Mausmodellen steht der therapeutische Gentransfer von Wachstumsfaktoren ins Innenohr im Vordergrund.

Stabile Rahmenbedingungen durch eine Ziel- und Leistungsvereinbarung

Im Jahr 2000 konnte das ZMNH eine Ziel- und Leistungsvereinbarung mit der Behörde für Wissenschaft und Forschung, der Universität Hamburg, und dem Fachbereich Medizin abschließen. Diese sehr positive Vereinbarung wurde

jedoch bisher nur zum Teil umgesetzt, da wesentliche Struktur- und Satzungsfragen noch geklärt werden müssen.

Entsprechend dieser Vereinbarung finanziert die Stadt Hamburg in Umsetzung des zwischen Bund und Land vereinbarten Zentrumskonzepts ab 1.1.2002 eine weitere, budget-finanzierte Forschergruppe, deren Leitung wir Ende 2001 international ausgeschrieben haben. Dadurch erhöht sich die Anzahl der Nachwuchsgruppen mit den bisher drei budget-finanzierten Gruppen und den beiden DFG-finanzierten Gruppen auf insgesamt sechs – eine sehr willkommene Entwicklung.

Zudem wird die vorher bestehende Unterfinanzierung des Personaletats des ZMNH weitgehend ausgeglichen. Unter der Voraussetzung positiver externer Evaluation werden Garantien für die Finanzierung des ZMNH-Etats gegeben. Die dem ZMNH zustehende Tierhaltungskapazität für genetisch veränderte Mäuse wird wesentlich ausgedehnt – allerdings bei erheblicher finanzieller Beteiligung der ZMNH-Gruppen. Es ist jedoch absehbar, dass die erhöhte Kapazität bald wieder unzureichend sein wird. Die Ziel- und Leistungsvereinbarung garantiert den Bestand des Instituts für Zellbiochemie und Klinische Neurobiologie auch für die Zeit nach der Emeritierung von Prof. Richter, dem Gründungsdirektor des ZMNH. Obwohl kein integraler Bestandteil des ZMNH, ist dieses Institut sehr wichtig für die kritische Masse auf dem Gebiet der Hamburger Neurobiologie.

Ein zentraler Punkt der Vereinbarung ist die weitere Ausweitung der Selbstständigkeit des ZMNH – eine wichtige Randbedingung für die bei hochkompetitiver Forschung benötigte Effizienz und Flexibilität. Dieser Teil der Vereinbarung, der auch eine neue, noch im Detail auszuarbeitende ZMNH-Satzung vorsieht, sollte nach der Verselbstständigung des Universitätsklinikums Eppendorf nun zügig umgesetzt werden.

Ausblick

Das ZMNH konnte sich innerhalb der letzten 10 Jahre 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 selbstständigen Verwaltung. Die Bundesforschungsministerin Edelgard Bulmahn und der erste Bürgermeister Ortwin Runde haben diesen Erfolg bei einem gemeinsamen Besuch des ZMNH im Mai 2001 positiv gewürdigt.

Mit der Ziel- und Leistungsvereinbarung ist ein wichtiger Schritt in Richtung auf einen Erhalt und Verbesserung dieser Struktur gemacht worden, die Modellcharakter für andere universitäre Forschungseinrichtungen haben könnte. Wir hoffen, dass die vereinbarte größere Selbstständigkeit des ZMNH alsbald umgesetzt wird. Trotz der festgelegten Erweiterung der Tierhaltungskapazitäten ist abzusehen, dass hier bald wieder Engpässe auftreten werden. Diese müssen dringend beseitigt werden. Der durch transgene und *knock-out* Strategien mögliche Schritt vom Gen zum Organismus und zur Krankheit wird für viele Zweige der biomedizinischen Forschung immer wichtiger. Da in naher Zukunft auch in der Universitätsklinik der Bedarf an genetisch veränderten Mäusen stark steigen wird, ist eine strategische Planung der Erweiterung der Tierhaltungskapazität der Universität dringend erforderlich. Diese für die biomedizinische Forschung in Hamburg essentielle Erweiterung wird nicht ohne zusätzliche staatliche Finanzmittel möglich sein.

Durch die anstehende Emeritierung von Chica Schaller wird in der nächsten Berichtsperiode zum ersten Mal eine

Institutsdirektorin ausscheiden. Ich bin zuversichtlich, dass wir bei den Erfolgen und der Ausstrahlung des ZMNH eine sehr gute Wiederbesetzung erreichen können, um die erhebliche Lücke, die das Ausscheiden von Frau Schaller mit sich bringen wird, zu füllen.

Frau Schaller hat das ZMNH in den Jahren 1999 bis 2000 als Direktorin geleitet. Ich möchte ihr für dieses Engagement herzlich danken.

Thomas J. Jentsch

Preface

The Centre for Molecular Neurobiology is a research centre of the University of Hamburg and is part of the faculty of medicine. Its research concerns questions of molecular neurobiology and related areas. In many cases, research results are swiftly applied to problems in medicine and human genetics. This process is greatly facilitated by the generation and analysis of transgenic and *'knock-out'* mice. These mouse models have led to the identification of novel disease genes and have elucidated the pathophysiological basis of human disorders. In addition to research, the ZMNH is involved in teaching. The Centre carries out a two-year graduate course in molecular biology.

The ZMNH comprises four Institutes which are headed by full professors (Jentsch, Pongs, Schachner, Schaller), as well as several Research Groups that are led by young researchers. These independent groups, which generally stay at the ZMNH for a period of roughly five years, are essential to the concept of the Centre. The City-State of Hamburg agreed to finance another research group in 2002. This will increase the number of these junior groups from currently three (Riethmacher, Sander, Schimmang) to four. Two additional research groups are entirely funded by the Deutsche Forschungsgemeinschaft (DFG). Ingolf Bach is a recipient of a Heisenberg Fellowship, and Hans-Christian Kornau has joined us at the end of 2001 as the head of a junior group of the Sonderforschungsbereich 444 (a Collaborative Research Centre of the DFG). His research will be concerned with synaptic protein networks. Of the previous research group leaders, Roger Nitsch is now heading the Department of Psychiatric Research at the University of Zürich, while Michael Wegner is Chair of the Department of Biochemistry and Pathological Biochemistry of the University of Erlangen. Dietmar Kuhl still has to give his preference to one of the several offers made to him by German and foreign institutions.

All groups of the Centre are supported by several scientific service units. The largely independent administration of the ZMNH is crucial for flexible and efficient administrative procedures. A scientific advisory board evaluates the performance of the Centre in regular intervals and provides valuable advice.

The Institute for Cell Biochemistry and Clinical Neurobiology, which is headed by our founding director Dietmar Richter, is closely associated with the ZMNH. This institute also comprises independent junior groups and its service unit provides services both to the ZMNH and to clinical groups.

Fourteen years after its foundation in 1988, the ZMNH is now firmly integrated into the Hamburg research community. There is an ever increasing number of local scientific collaborations, in particular with groups of the University Clinic Eppendorf (UKE). In this respect, the various mouse models generated by researchers of the ZMNH play a prominent role. The broad expertise in various organ systems is often invaluable in analysing their diverse and sometimes unexpected phenotypes.

The ZMNH is firmly integrated into several research networks, in which the Centre is often the driving force. Thus, the SFB 444 (*Basis of neuronal communication and signal transduction,* speaker: Jentsch) is largely centred at the ZMNH, as is the DFG graduate program *'Neural signal transduction and its pathology'* (speaker: Schaller). Groups of the ZMNH participate in the SFB 545 (*'Molecular mechanism of inherited disease*', speaker: Greten), in the SFB 470 (*'Glycostructures in biological systems'*, speaker: Thiem), as well as in the Research Group *'RNA transport'* (speaker: Richter). Recently, the ZMNH was selected as one of the five German centres (speaker: Jentsch) focusing on neurological diseases within the National Genome Network financed by the Federal Ministry for Research and Education. Besides five groups of the ZMNH, it comprises the Institute of Human Genetics of the University Clinic.

Research at the ZMNH

The ZMNH is now widely recognised as one of Germany's premier research institutes in neurobiology. The Centre'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. These include the Prix Louis-Jeantet de médecine and the Ernst-Jung Preis für Medizin to Thomas Jentsch, and the prize of the Deutsche Stiftung Querschnittslähmung to Melitta Schachner. Also younger scientists were honoured by awards. For instance, Christian Kubisch received the Heinz-Maier-Leibnitz Prize of the DFG for his work done at the Centre.

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, and kidney as well as bone diseases. 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 disease.

The institute for molecular neuropathology (headed by Thomas Jentsch) focuses on ion transport processes, in particular on their role in physiology and disease. Its research is primarily concerned with chloride and potassium channels, and, more recently, potassium chloride cotransport. A number of knock-out mouse models has provided crucial 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. The group identified several human disease genes and clarified the pathophysiology of these disorders.

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 institute is involved in human genetic screens for ion channel genes associated with various heart diseases, in the development of animal models to study potassium channel dysfunctions related to vasoregulation, learning and epilepsy, and in structure-based studies of potassium channel function.

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) 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 in the Institute for Biosynthesis of 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 are of particular interest for these investigations.

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 homeodomain proteins, a class of transcription factors that is crucial for the development of neuronal structures and cell types. This group has demonstrated that the biological activity of LIM homeodomain proteins is critically regulated by protein-protein interactions with several associated cofactors.

The main goal of Dietmar Kuhl's laboratory is to develop molecular biological approaches for the identification and study of genes contributing to synaptic plasticity in the mammalian brain. Analysis of their expression indicates a broad role for these genes in different forms of neuronal plasticity, including learning and memory, and neurodegenerative disorders. His research moves from the identification of activity regulated genes to the analysis of long term potentiation in the brain and wants to assess which consequences they convey on the behaviour of animals and their capability to learn and store memories.

The research group of Dieter Riethmacher is engaged in analysing questions of developmental biology. Using mouse models the group focuses on unravelling the functions of the neuregulin-signalling-system and the sox10 transcription factor in PNS development. Additionally the Cre-lox P-system is used to analyse functions of the erbB2-receptor and the caspase 8 in CNS development and pathology.

The Sander laboratory aims to understand the molecular mechanisms by which several classes of transcription factors co-ordinate development of the mammalian central nervous system and pancreas. In their research they combine genetic, molecular, and genomic approaches to gain insight into the molecular mechanisms of synergistic activation and repression of gene expression. In this, the research group has identified key transcriptional regulators of spinal cord motor neuron development, as well as of insulin-producing cells in the pancreas.

The central interest of the research group headed by Thomas Schimmang is the formation and differentiation of the auditory organ. The roles of growth factors during the induction of the inner ear, the survival of sensory hair cells and auditory neurons and their protection from ototoxic damage are studied. Next to the analysis of transgenic mouse models, his group is interested in therapeutic gene transfer of growth factors into the inner ear.

A stable framework for the further development of the Centre

In 2000, the ZMNH negotiated an important agreement with the senator of science of the City-State of Hamburg, the president of the University, and the dean of the faculty of

medicine. Provided the centre is evaluated positively by an external committee, it guarantees stable basic funding by the city-state of Hamburg. A fourth university-funded research group will be established in 2002, and the space for genetically modified mice allocated to the Centre will be significantly expanded. Further, the ZMNH will gain more administrative independence, a point that is crucial for an efficient operation. Several points relating to this autonomy, however, remain to be negotiated in detail.

Outlook

Within the past 10 years, the ZMNH established itself as one of the leading research centres in its field. This remarkable success is not only 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 to the structure of the ZMNH - which judiciously combines rather large institutes with independent young research groups, service groups, and a largely autonomous administration.

The above-mentioned agreement provides an important basis for the future evolution of the Centre. The structure of the Centre and its relative autonomy might have model character for other academic institutions. We hope that the larger autonomy agreed upon in 2000 will be implemented soon. Although the capacity for keeping mice that is available to the ZMNH has been expanded, the associated costs weigh heavily on the groups of the Centre. Further, given the increasing importance of genetic mouse models for the research of the Centre as well as for clinical groups, it is clear that the animal facilities of the University of Hamburg need to be expanded very significantly in the near future. This expansion, which is of strategic importance for biomedical research in Hamburg, will not be possible without additional funding by the state.

The ZMNH is now starting to look for a successor of Chica Schaller, who is going to retire in the next period, as the Director of one of the Institutes. Given the achievements of the Centre and its excellent facilities, I am optimistic that we will be able to attract an excellent scientist to fill the substantial gap that her retirement will create.

Chica Schaller served as Director of the ZMNH from 1999 to 2000. I would like to take this opportunity to thank her very much for her input during that time.

Thomas J. Jentsch

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Professor Gähwiler and Professor Sakmann have stepped down from their positions on the ZMNH advisory board. Their many years of service are greatly appreciated by the ZMNH. Professor von Figura and Professor Hofmann have been elected to replace Professor Gähwiler and Professor Sakmann. The remaining advisory board members have been reelected for an additional term.

Research Projects

Institut für Biosynthese Neuraler Strukturen

Melitta Schachner Camartin

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

1. The L1 family of neural recognition molecules

Udo Bartsch, Christian Bernreuther, Ralf Kleene, Michael Kutsche, Janice Law, Alan Lee, Peggy Putthoff, Melanie Richter, Bettina Rolf, Birte Rossol, Annette Rünker, Sandra Schmidt, Birthe Schnegelsberg, Sinef Yarar, 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 (Fig. 1), 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 determinators 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.

Since L1, a prominent glia-associated neurite outgrowth promoting molecule in the peripheral nervous system, is absent in the central nervous system on glial cells after trauma, we evaluated its neurite outgrowth promoting role in the central nervous system using transgenic mice that overexpress L1 in glial cells during crucial stages of regeneration after a lesion.

In this transgenic mouse, neuronal differentiation and survival and the learning performance in the Morris water maze test are enhanced.

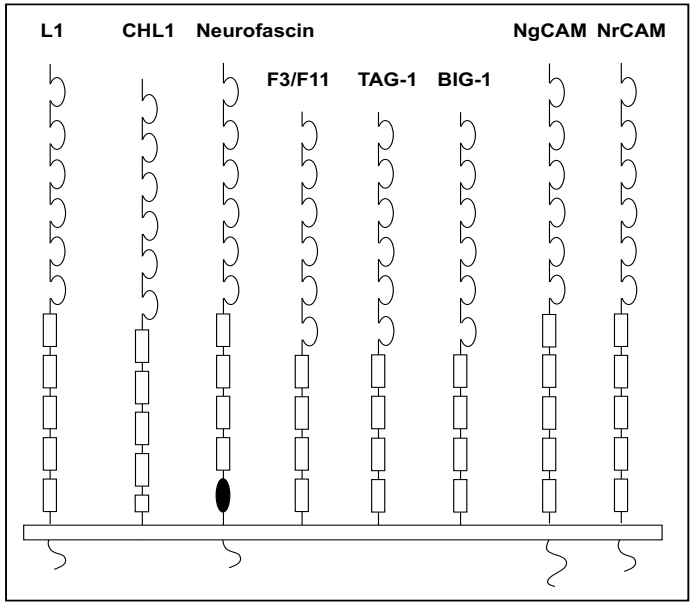


Figure 1. Schematic representation of the structure and the structural motifs of L1-like molecules in vertebrates. The N-terminal ends of the molecules comprise 6 immunoglobulin-like domains followed by fibronectin type III homologous repeats. L1-like molecules can be, like L1, transmembrane glycoproteins,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

Analysis of an L1-deficient mouse mutant generated by homologous recombination has revealed this mutant to be a very good animal model for the inherited human diseases caused by mutations in the L1 gene that are now summarized under the name of CRASH syndrome (comprising mental retardation, aphasia, shuffling gate, adducted thumbs, spastic paraplegia type I, and hydrocephalus) (Fig. 2).

Additionally, analysis of the L1 knock-out mutant in collaborating laboratories has revealed severe impairment of the organogenesis of kidney and spleen in this mutant. A knock-out mouse mutant deficient in the close homologue of L1 (CHL1) shows a much less severe phenotype. Conditional knock-out mutants are being generated and double knock-out mutants carrying defects in genes of the L1 family are being analyzed to further probe the functions of these molecules.

2. Neural recognition molecules and signal transduction

Anja Behrendt, Ulrich Bormann, Suzhen Chen, Achim Dahlmann, Markus Delling, Ling Dong, Torsten Fink, Claudia Friedrich, Silke Gorissen, Ralf Kleene, Iryna Leshchyns'ka, Gabriele Loers, John Neidhardt, Philipp Niethammer, Melanie Richter, Zhi-Cheng Xiao

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. The signalling cascades involving tyrosine and serine/threonine kinases and phosphatases, calcium, G-proteins and the cascades of ras and raf signalling mechanisms are being investigated. Of particular interest is the role of neural recognition molecules in so-called rafts which are characterized as lipid microdomains enriched in cholesterol and sphingolipids. Rafts are proposed to function as platforms for signal transduction events and the attachment of cytoskeletal proteins. One of the advances in our group in

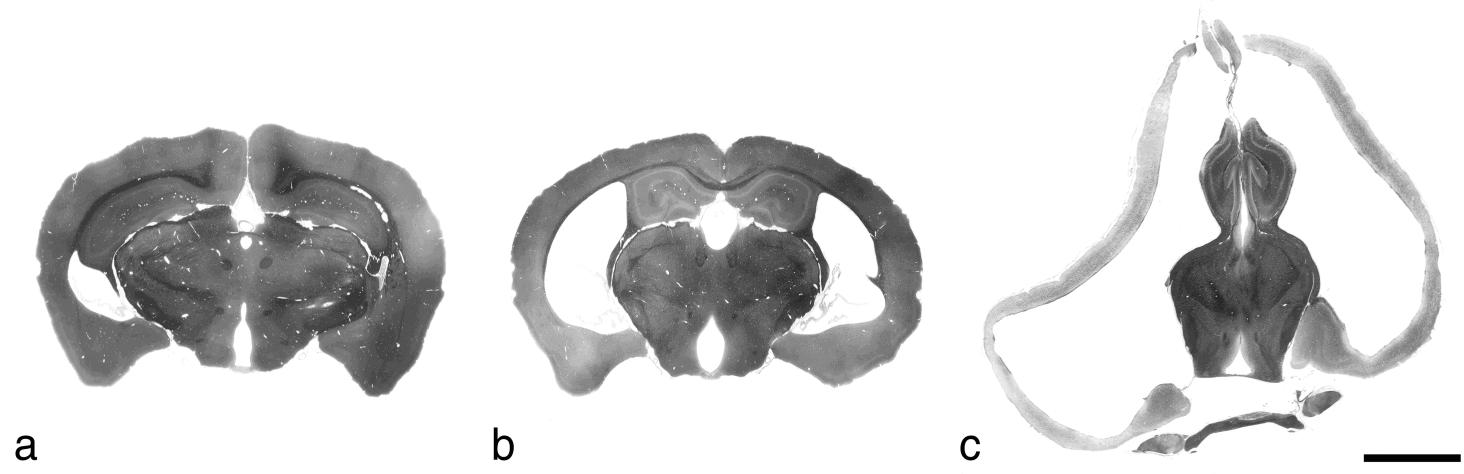


Figure 2. The size of ventricles in wild-type mice and L1 mutants. The lateral ventricles of many L1-deficient mice with a C57BL/6J genetic background (b) are significantly enlarged when compared to wild-type littermates (a). A few mutants display severe hydrocephalus with massively enlarged ventricles (c), thinnened cerebral cortex, and displacement of the hippocampus into a ventro-dorsal orientation. Bar in (c) for a-c: 2 mm.

the recent years was to identify the essential role of lipid rafts for NCAM-mediated signaling processes. The transmembranous isoforms of NCAM can be palmitoylated on intracellular cysteines and are thereby directed into these lipid rafts. The physiological consequences of this lipid raft association turned out to be very important: First, we demonstrated that NCAM-dependent neurite outgrowth is mediated via an "outside-in" co-signaling mechanism involving the fyn-FAK pathway and the FGF receptor. While activation of the fyn-FAK pathway critically depends on the association of NCAM with lipid rafts, probably due to the co-localization of NCAM and the fyn kinase in lipid rafts, the NCAM-dependent activation of the FGF receptor is mediated in the non-raft compartment (Fig. 3). Secondly, 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 defined as an "inside-out" signaling of NCAM. These results could explain how cell adhesion molecules are involved in the regulation of neural activity.

Furthermore, the interactions of adhesion molecules of the L1 family and the isoforms of the neural cell adhesion molecule N-CAM with the cytoskeleton are investigated by using the yeast-two-hybrid system, by direct binding assays with identified cytoskeletal elements, and by co-localization studies using immunocytochemistry. Signal transduction mechanisms evoked by different domains of the extracellular matrix molecules tenascin-C and -R are studied by analyzing the patterns of proteins undergoing changes in expression and

phosphorylation by high resolution 2D gel electrophoresis. In addition, the consequences of triggering of recognition molecules at the cell surface resulting in either repellent or adhesive cell responses is being studied at the transcriptional level.

Conventional and conditional knockout mutants are being generated for recognition molecules involved in cell adhesive and/or cell repellent functions to study their involvement in development, regeneration, and synaptic plasticity.

3. Prion protein and amyloid precursor pproteinprotein as recognition molecules

Suzhen Chen, Muntsin Kolss, Frank Ploeger, Helen Strekalova, Ting Yang

The cellular prion protein (PrP^C) is a membrane glycoprotein anchored by glycosylphosphatidylinositol (GPI). It is mainly expressed in central nervous system, preferentially at the synapse. Extensive studies have focused on the implication of a conformationally altered form of prion protein (PrP^{Sc}) in transmissible spongiform encephalopathies, such as scrapie in sheep and Creutzfeldt-Jacob disease in humans. Although some current studies have shown PrP^C to have antioxidant properties and to trigger signal transduction, the role of PrP^C in normal brain function remains elusive. As a GPI anchored cell surface molecule, PrP^C is HNK-1 carbohydrate-positive and has therefore been postulated to be a recognition molecule, the ligands of which remain to be identified. We are pursuing two approaches toward the understanding of the role of PrP^C in normal brain functions. The first is to investigate the involvement of PrP^C in axon extension, neuronal survival and synaptic plasticity, and to elucidate the underlying signal transduction mechanisms. The second is to identify PrP^C

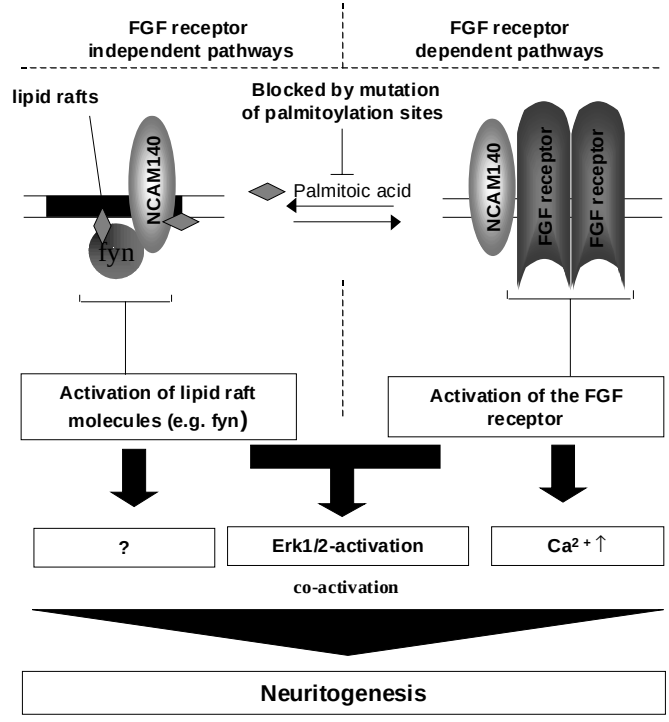


Figure 3. Hypothetical scheme of NCAM140 co-signaling

Within the plasma membrane, NCAM140 is partly present in lipid rafts while the majority is located outside of rafts, thus defining two submembranous compartments in which NCAM140 is present. The presence of NCAM140 in lipid rafts is regulated by palmitoylation of intracellular cysteines by yet unknown mechanisms. Upon homophilic activation, NCAM triggers distinct signal transduction pathways in the two compartments: NCAM140 phosphorylates FAK most likely via raft-associated fyn kinase, while NCAM140 in the non-raft compartment facilitates FGF receptor activated downstream signaling. Both pathways, the fyn-FAK and the FGF receptor pathways merge in the activation of ERK1/2. These pathways can branch upstream of ERK1/2 into other pathways, such as the PLCγ activation or other yet unknown effectors. Activation of both pathways is necessary for NCAM140 to function as a neuritogenic receptor.

binding partners during ontogenetic development and modulation of synaptic activity in the adult. The HNK-1 positive amyloid precursor protein is studied in a similar manner.

4. Carbohydrates and the fine tuning of cell interactions

Martine Albert, Jens Franke, Ralf Kleene, Jens Lütjohann, Maren von der Ohe, Claudia Senn, Miriam Süsskind

We are engaged in studies on different glycans that are expressed by partially overlapping sets of glycoproteins, many of which have been shown to be neural recognition molecules. Some of these neural recognition molecules, e.g. L1, MAG 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. Several glycans have been identified, among them the HNK-1 carbohydrate, oligomannosidic carbohydrates recognized by monoclonal antibodies L3 and L4, the unusual alpha-2,8-linked polysialic acid (PSA), and the Lewis^x antigen recognized by the monoclonal antibody L5. 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 are also identifying carbohydrate peptidomimetics with the view to use 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.

5. Regeneration

Meliha Karsak, Astrid Rollenhagen

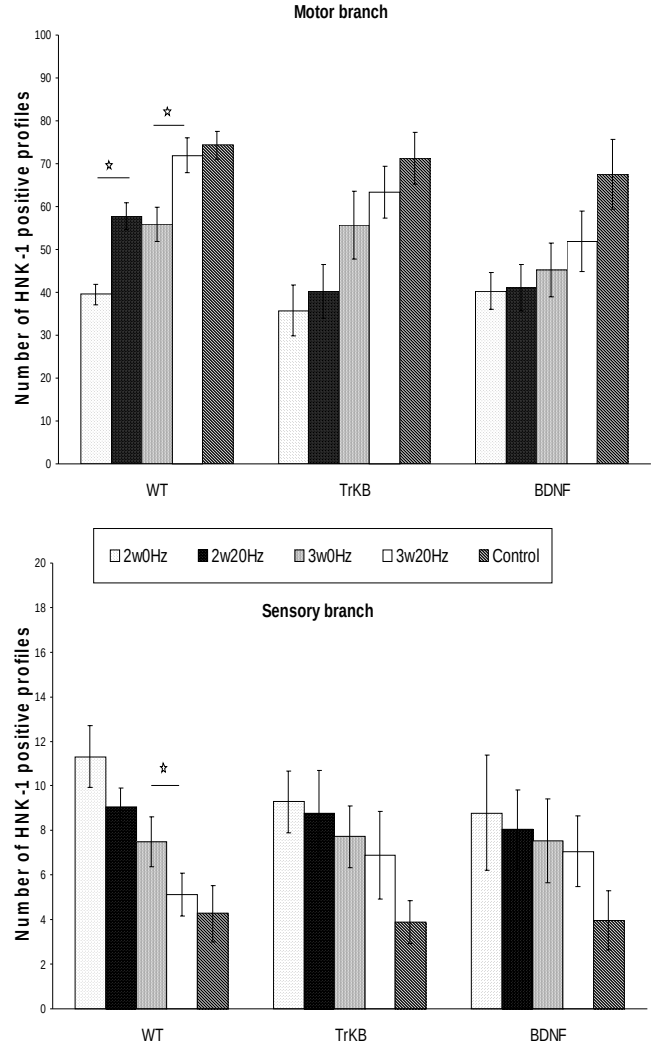
Reinnervation of lesioned mixed motor and sensory peripheral nerves is initially random. Specificity emerges as progressively more motoneurons regenerate axons preferentially into the motor branch, a process termed preferential motor reinnervation. Associated with this process is the selective re-expression of the carbohydrate epitope HNK-1 that is mainly expressed by myelinating Schwann cells in the motor branch.

Our present work is aimed at studying factors contributing to the regulation of HNK-1 expression during peripheral nerve regeneration. Recent experiments show that one factor involved in this process is electrical activity (Fig. 4). Stimulating lesioned mouse femoral nerves with a continuous 20 Hz pulse for 1h leads to an accelerated expression of HNK-1 in reinnervated motor nerve pathways in association with accelerated preferential motor reinnervation. Other factors contributing to the regulation of HNK-1 expression during nerve regeneration are neurotrophins. By using mutants deficient in the brain derived neurotrophic factor (BDNF) and its receptor (TrkB) we showed that HNK-1 expression after lesioning is delayed and that HNK-1 expression is not enhanced by electrical stimulation (Fig. 4). Our observations indicate that electrical activity and neurotrophins are important factors contributing to HNK-1 expression and pathway specific regeneration in the peripheral nervous system. We propose that similar axonal guidance mechanisms may be operant in the central nervous system.

Figure 4. Influence of 1h continuous 20 Hz electrical stimulation on HNK-1 expression of the motor and sensory branches of mouse femoral nerves regenerated after lesion for 2 and 3 weeks.

In lesioned regenerating motor branches HNK-1 expression is lower than in untreated control nerves. Electrical stimulation of the proximal nerve stump for 1h applied immediately after lesion accelerates HNK-1 expression of myelin profiles in wild type (WT) but not in TrkB and BDNF knock-out mutants. In the sensory branch HNK-1 expression is higher in lesioned than in untreated control nerves. After electrical stimulation, HNK-1 expression is downregulated in wild type animals more quickly in stimulated than in non-stimulated nerve branches, but not in TrkB and BDNF knock-out mice.

2w0Hz: Two weeks regeneration without electrical stimulation; 2w20Hz: Two weeks regeneration with 1h 20 Hz electrical stimulation; 3w0Hz: Three weeks regeneration without electrical stimulation; 3w20Hz: Three weeks regeneration with 1h 20 Hz electrical stimulation. * $p<0.028$



6. Biological roles for sulfated carbohydrates – studying the HNK-1 family of sulfotransferases

Matthias R. Evers, Michael Kutsche, Claudia Senn, Guoqing Xia

Carbohydrates attached to proteins and lipids characteristically display complex and heterogeneous structures. 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 now been generated

and characterized. We are interested to investigate whether a family of HNK-1 sulfotransferases may exist. We and others have identified and cloned four more members of this enzyme family. The sulfotransferases GalNAc-4 ST1 and GalNAc-4 ST2 have been shown to synthesize sulfated β1-4-linked GalNAc found on the GGnM epitope characteristic of glycopeptide hormones of the pituitary and to add sulfate to non-terminal β1–4-linked GalNAc found on chondroitin and dermatan. Surprisingly, none of these family members possess the catalytic specificity/activity to sulfate the HNK-1 structure, but it is conceivable that the sulfated glycans share similar functional properties.

7. Detection of differential gene expression in transgenic mice

Kai - Oliver Wesche, Ting Yang

On the way to characterizing gene functions, transgenic mice have been useful in targeting involved molecules. Our observations are now calling for RNA profiling to monitor an altered expression of such candidate genes. The magic word is microarray analysis with its possibility to detect a high number of transcripts. Depending on the chip type, the generated expression profile can contain up to 26.000 genes. We plan to use a partly home-made array that includes previously uncharacterized cDNAs and many of the genes that are of immediate interest to our group. First encouraging results have been obtained by analyzing one of our gene deficient mice.

8. Recognition molecules and synaptogenesis

Alexander Dityatev, Galina Dityateva, Iryna Leshchins'ka, Vladimir Sytnyk, Mirjam Sibbe

Recently, two independent lines of studies have converged in showing that NCAM is a molecule important for synaptogenesis. First, an analysis of synaptic currents and synaptic coverage of neurons maintained in heterogenotypic co-cultures, in which wild type ($NCAM^{+/+}$) and NCAM deficient ($NCAM^{-/-}$) cells are plated in one dish, revealed that NCAM expressing neurons receive more synaptic contacts (Fig. 5) and have larger unitary EPSCs than neighboring NCAM defic ient cells. The preferential synaptic coverage of NCAM expressing cells turned out to depend on activation of the NMDA type of glutamate receptors and the presence of polysialic acid on NCAM. Second, a detailed time-lapse analysis of NCAM distribution along growing neurites revealed moving clusters of NCAM being associated with lipid rafts and intracellular organelles. These clusters could be trapped by approaching growth cones and stabilized (together with associated organelles) in contacts developing into synapses. Absence of NCAM reduced the number of contacts containing these organelles, implicating NCAM in targeting of synaptic components. Our further studies are directed towards the study of molecules interacting with NCAM during synaptogenesis and analysis of other recognition molecules contributing to formation/stabilization of excitatory or inhibitory synaptic contacts.

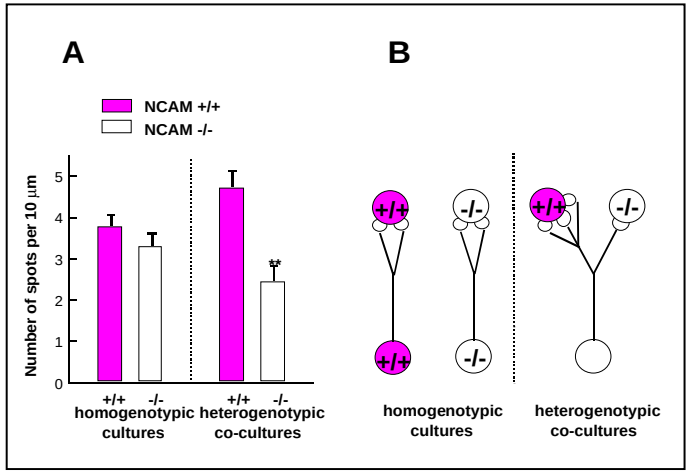


Figure 5. NCAM$^{+/+}$ neurons have more synaptic contacts than NCAM$^{-/-}$ ones in heterogenotypic co-cultures. A: NCAM$^{+/+}$ neurites in co-cultures were more densely covered by synaptophysin-rich spots than NCAM$^{-/-}$ neurons and than neurites in homogenotypic NCAM +/+ and -/- cultures. **Difference between genotypes P<0.01, one-way ANOVA. B: A scheme showing preferential formation and/or stabilization of synapses on NCAM$^{+/+}$ neurites in a choice situation, i.e. in heterogenotypic co-cultures.

9. Recognition molecules and synaptic plasticity

Olena Bukalo, Alexander Dityatev, Sun Mu, Armen Saghatelyan, Benedikt Salmen, Thomas Schuster, Jianrong Tang

We have analyzed several forms of hippocampal synaptic plasticity in mutants deficient in the cell adhesion molecules NCAM and L1, and the extracellular matrix glycoproteins tenascin-C and tenascin-R. Our results show that L1 mutants have normal long-term potentiation (LTP) in the CA1 region of the hippocampus, whereas all other mutants show reduced LTP in CA1. Long-term depression (LTD) in CA1 was reduced in tenascin-C mutants but appeared to be normal in tenascin-R mutants. No abnormalities in LTP recorded in the CA3 region of mutants deficient in tenascin-C and tenascin-R were found. Thus, our data demonstrate that both cell surface and extracellular matrix molecules are specifically involved in several forms of hippocampal synaptic plasticity.

Since many known biological functions of recognition molecules are mediated by carbohydrates associated with these molecules, we analyzed synaptic plasticity in mice deficient in polysialyltransferase ST8SiaIV (producing polysialic acid associated exclusively with NCAM) and HNK-1 sulfotransferase (adding the HNK-1 carbohydrate to several recognition molecules), and after injection of antibody to HNK-1 or enzymatic removal of chondroitin sulfates in slices of the hippocampus of wild type mice. ST8SiaIV$^{-/-}$ deficient mutants showed an age-dependent reduction in LTP and LTD in CA1, which correlated with an age-dependent decline in expression of polysialic acid in ST8SiaIV$^{-/-}$ mutants. HNK-1 sulfotransferase deficient mice showed moderate reduction in LTP and increase in basal excitatory synaptic transmission, a phenotype resembling tenascin-R deficient mice. Enzymatic removal of chondroitin sulfates impaired both LTP and LTD in CA1, for the first time implicating this important carbohydrate structure in the regulation of synaptic plasticity. Injection of HNK-1 antibody, in contrast to all other manipulations, increased LTP in CA1 via reduction of perisomatic inhibition in the CA1 region.

Our current experiments are targeted to verifying the significance of abnormalities found in vitro by in vivo recordings of learning-induced potentiation and analysis of epileptogenesis in mice deficient in recognition molecules. Intense efforts are now directed toward elucidating the

mechanisms by which recognition molecules regulate synaptic plasticity. For example, we have analyzed NMDA receptor-mediated currents, perisomatic inhibition and synaptogenesis in the above mentioned mutants. We try to rescue the abnormalities found in mutants by delivery of recombinant proteins or pharmacological manipulations. Experiments are underway to reconstitute in artificial systems particular components of the synaptic machinery to analyze the molecular determinants involved in interactions between recognition molecules, ion channels, neurotransmitter receptors, synaptic vesicles and the cytoskeleton.

10. Neural recognition molecules and gene expression related to learning

Anja Behrendt, Christoph Sanders, Tanja Schneegans, Thomas Tilling, Stefanie Wagner

Various studies demonstrate the involvement of neural recognition molecules in learning and memory formation, processes that are likely associated with plastic changes at synapses. Recognition molecules may contribute to these alterations in two ways: either by influencing the expression of other proteins or by being up- or down-regulated themselves. We have taken two approaches:

We have analyzed differential gene expression in the hippocampus of GFAP-L1 mice. The transgenic mouse GFAP-L1 expresses L1 ectopically on astrocytes. It had been shown that these animals perform better in a spatial learning task than wild type littermates. We thus assumed that the ectopic expression of L1 may have triggered changes in neural gene expression favourable to learning. Differences in gene expression between GFAP-L1 transgenic and wild type mice were therefore studied by obtaining a comprehensive mRNA expression pattern of both transgenic and wild type hippocampi, using serial analysis of gene expression (SAGE). Of the differentially expressed transcripts, some belonged to known genes while others could be assigned to expressed sequence tags (ESTs). Among the differentially expressed known genes, we selected the most promising candidates for a participation in synaptic plasticity. Experiments to investigate the (direct or indirect) interaction between the corresponding proteins and L1, as well as the functional relevance of these interactions are underway.

We have also analyzed recognition molecule expression in the hippocampus of mice following a passive avoidance task. The step-down-avoidance paradigm is a passive avoidance task, which has been shown to involve the hippocampus. Previous reports have indicated a possible role for NCAM in the acquisition of this task. To investigate whether there are changes in the hippocampal expression of NCAM or other cell recognition molecules following the step-down-avoidance task, we are analyzing mRNA levels of total NCAM, the NCAM180 isoform, L1, CHL1 and tenascin-R by real-time PCR. Hippocampi were prepared from animals at various points of time after training, hopefully enabling us to detect “time windows” of altered recognition molecule expression critical for the formation of memory.

11. Neural recognition molecules and behavior

Joerg Brandewiede, Nikolas Fentrop, Fabio Morellini, Constanze Rehbehn, Sandra Schmidt, Anja Schrewe, Tatyana Strekalova, Stefanie Wagner

Bridging the gap between molecules and behavior is one of the most interesting and challenging tasks in neuroscience. The correlation between molecular and cellular interactions

and functional output at the behavioral level *in vivo* is complicated by the fact that behavior is the output of complex interactions of many cell systems, regulated by the constant cross-talk between genes and environment throughout ontogenic development and also later in the adult. It is therefore crucial that the behavioral tests are designed and conducted appropriately. While in many studies behavioral tests designed for the rat have been uncritically applied to the house mouse, approaches that take species-specific behavioral characteristics into account and use ethological methods could be most useful for interpreting behavioral findings and understanding the biological mechanisms of brain function.

After preliminary observations on general health, home cage behavior, sensory abilities and motor functions, more specific behavioral tasks are conducted to test specific hypotheses on behavioral parameters of transgenic mice: a battery of tests is run to understand whether intra-sexual competition in males, exploratory behavior, reactivity to novelty (novelty seeking behavior) and anxiety may be altered due to genetic manipulation. Alteration of cognitive functions is tested in several learning paradigms such as passive - active avoidance, fear conditioning, water maze, Barnes maze and spatial object recognition tasks.

The role of several neural cell adhesion molecules is studied mainly through two approaches: behavioral analysis of transgenic mice knocked out constitutively and conditionally or overexpressing the gene of a certain neural cell adhesion molecule, and the analysis of recognition molecule expression after stimulation of a particular learning task.

12. Stem cells for the treatment of neurodegenerative disorders

Marius Ader, Udo Bartsch, Stefan Grau, Jinhong Meng, Ester Yu

Stem cells are undifferentiated cells defined by their multipotentiality and capacity for self-renewal. Remarkably, stem cells have recently been identified in the developing and adult nervous system of mammals, including humans. Based on the properties displayed by these cells, there is hope that they can be used for the therapeutic treatment of a variety of neurological diseases. In fact, neural stem cells have already successfully been used in animal experiments to transfer therapeutic gene products to the brain or to replace dysfunctional or degenerated neural cell types. We started our work by studying the ability of transplanted neural stem cells to differentiate into myelin-forming oligodendrocytes. The long-term aim of these studies is to evaluate the use of such cells manipulated to express recognition molecules for the treatment of non-inflammatory dysmyelinating or demyelinating diseases.

To test the capacity of neural stem cells to differentiate into myelinating oligodendrocytes, we have isolated such cells from striata or spinal cords of transgenic mouse embryos ubiquitously expressing enhanced green fluorescent protein (EGFP). Cells were expanded *in vitro* in the presence of epidermal growth factor and basic fibroblast growth factor. The capacity of neural stem cells to differentiate into myelin-forming oligodendrocytes *in vivo* was tested by transplanting them into (i) the retina of wild type mice containing the unmyelinated proximal segments of retinal ganglion cell axons and (ii) the lateral ventricle of severely hypomyelinated mutant

mice deficient in the myelin-associated glycoprotein (MAG) and the non-receptor-type tyrosine kinase Fyn. In both experimental models, transplanted progenitor cells showed widespread integration into the host tissue. Cells transplanted into the retina of wild type mice or the ventricle of MAG/Fyn double mutants showed a preferential integration into the retinal nerve fiber layer or diverse white matter tracts, respectively.

Donor-derived cells differentiated into a variety of morphologically distinct cells types. A significant fraction of these cells was identified as oligodendrocytes, which myelinated unmyelinated host axons in both transplantation paradigms. Differentiated oligodendrocytes and myelin were still detectable in the host tissue six months after transplantation, the latest time point investigated. Remarkably, prolonged survival periods of experimental animals resulted in a significant increase in the number of donor-derived oligodendrocytes and the area being myelinated (Fig. 6).

Experiments are now in progress to study the use of genetically engineered embryonic, neural and stromal bone marrow stem cells for the treatment of a variety of non-inflammatory neurodegenerative diseases and in regeneration. We are particularly interested to improve the integration, survival and differentiation of grafted stem cells by transfecting them with recognition molecules. These morphogenetically important proteins have previously been demonstrated by us to mediate myelination of axons, migration and survival of nerve cells, outgrowth, fasciculation and regrowth of axons, and formation and plasticity of synapses.

13. Cell recognition molecules and axon growth in the nervous system of zebrafish

Catherina G. Becker, Thomas Becker, Robert Bernhardt, Dimitrios Gimnopoulos, Bettina Lieberoth, Niclas Krakat, Jörn Schweitzer

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, oligomannosides, and polysialic acid are also investigated with regard to their possible role as fine tuners of cell interactions. We clone cell recognition molecules and the carbohydrate synthesizing enzymes and analyze their function in developmental axon growth. We localize these molecules by *in situ* hybridization and immunohistochemistry in whole embryos. 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

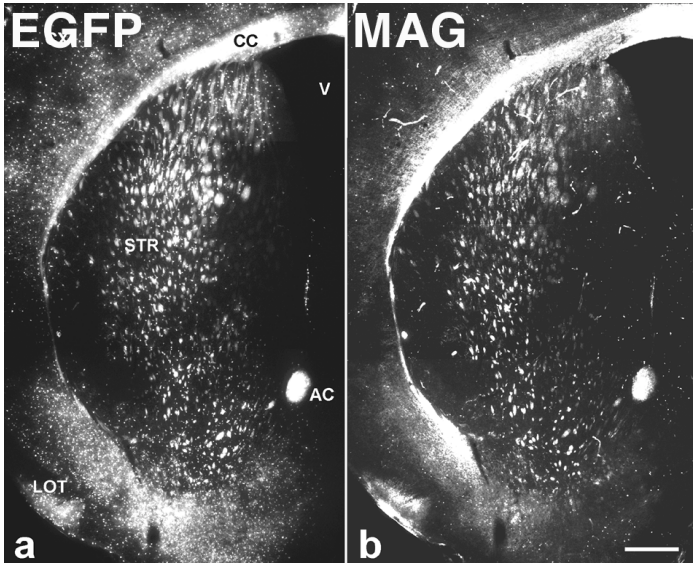


Figure 10. Widespread integration of EGFP-positive cells into a severely hypomyelinated MAG/Fyn-deficient host brain six months after transplantation of spinal cord stem cells. Numerous EGFP-positive cells (a) are detectable in white matter tracts, including the corpus callosum (CC), fascicles in the striatum (STR), anterior commissure (AC) and lateral olfactory tract (LOT). Grafted cells are also present in the cerebral cortex (CX). MAG-immunoreactivity (b) co-localizes with EGFP-fluorescence, and thus demonstrates differentiation of numerous donor cells into myelin-forming oligodendrocytes. STR, striatum.

video-microscopy in transgenic fish and immunohistochemical labeling of specific axons.

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

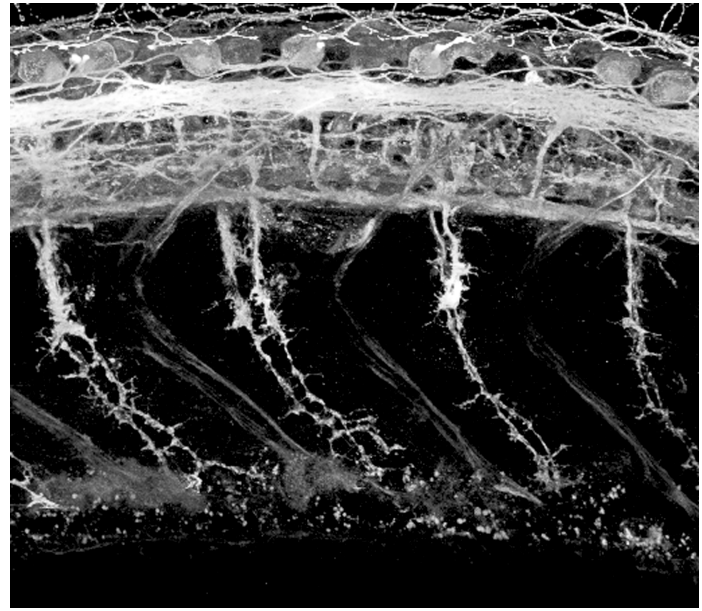


Figure 7. Confocal image stack of the nervous system in the trunk region of a zebrafish larva 24 hours after fertilization labeled with antibody 412 to HNK-1. Two ventrally directed axons of the caudal primary motor neurons per trunk segment are prominently labeled. Rostral is left, dorsal is up.

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 (Fig. 7). 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 factors necessary for repair of the central nervous system after injury.

Support

The work of our laboratory is supported by grants of the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, Canadian Spinal Research Organisation, DAAD, Deutsche Forschungsgemeinschaft, EMBO, European Community, Fonds der Chemischen Industie, Hertie-Stiftung, The Daniel Heumann Fund for Spinal Cord Research, Alexander von Humboldt Stiftung, Christopher Reeve American Paralysis Foundation, Röchling Stiftung, and Volkswagenwerk Stiftung.

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Theses

Diploma

Stephan Grau (1999). Transplantation of neural stem cells into the central nervous system of rodents. ETH Zürich.

Muntsin Kolss (2000). Characterization of putative binding partners of Amyloid Precursor Protein. Universität Hamburg.

Philipp Niethammer (2000). Co-Signaling of N-CAM via the fibroblast growth factor receptor and lipid rafts. Universität Hamburg.

PhD

Marius Ader (2000). The myelinogenic potential of transplanted neural precursor cells. Universität Bielefeld.

Jinhong Meng (2000). Study of in vivo transplantation and in vitro transfection of neural stem cells. University of Xian Medical School, Xian, China.

Maren von der Ohe (2000). Untersuchungen zu Kohlenhydratstrukturen beim neuralen Zellerkennungsmolekül NCAM. Universität Berlin.

Armen Saghatelyan (2000). Untersuchungen zur Funktion des Extrazellulär-Matrix Glycoproteins Tenascin-R und seines assoziierten Kohlenhydrates bei der synaptischen Transmission und Plastizität in der CA1 Region des Hippocampus der Maus. Universität Hamburg.

Claudia Senn (2000). Erzeugung und phänotypische Analyse einer HNK-1-Sulfotransferase knock-out Maus. Universität Hamburg.

Medical dissertations

Christian Bernreuther (2000). Funktionelle Analysen der humanen neuralen Zellerkennungsmoleküle L1 und CHL1. Universität Hamburg.

Torsten Fink (2000). Herstellung von DNA Konstrukten mit humanen Mutationen des neuralen Zellerkennungsmoleküls P0. Universität Hamburg.

Sinef Yarar (2000). Herstellung von DNA Konstrukten der neuralen Zellerkennungsmoleküle L1 und CHL1 zur therapeutischen Anwendung. Universität Hamburg.

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Institut für Entwicklungsneurobiologie

H. Chica Schaller

In early development of the nervous system intercellular communication is important for establishing the wiring pattern. The main aim of our research is to find out how extracellular signals are transmitted over appropriate receptor complexes to the interior of the cell to regulate proliferation, differentiation, pattern formation and behavior.

One of the external signals, which influences early events in neuronal and neuroendocrine development, is the neuropeptide head activator (HA). In the report period we concentrated on characterizing new members of the VPS-10 domain-containing and G-protein coupled receptor (GPR) families as candidates for HA signal transduction. With special emphasis we studied SorLA, the mammalian ortholog to the hydra HA receptor HAB and its function in HA signaling. As second interesting mediator at the outer cell membrane we characterized GRC, a newly discovered Ca^{2+} channel of the TRP family, as responsible for the Ca^{2+} influx triggered by interaction of HA with its receptor.

In two independent research projects S. Hoffmeister-Ullerich and U. Borgmeyer continued their studies on hydra development and transcriptional regulation in early mammalian brain development, respectively. With the guest group of A. Methner we share the interest in GPRs and the technologies to study them.

1. SorLA and its function for HA action

Wolfgang Hampe, Julia Lintzel, Björn Riedel, Inga Franke

The neuropeptide head activator (HA) plays a role in the development of the central nervous system from hydra to man. In a multiheaded mutant of hydra we had identified HAB as HA receptor. SorLA is the only mammalian ortholog to HAB and it binds HA with nanomolar affinity. We analyzed its function in the human neuronal precursor cell line NT2 and the neuroendocrine cell line BON, which both produce HA as autocrine growth factor and respond to HA by entry into mitosis and cell proliferation. Both cell lines express SorLA, as shown by RT-PCR, immunocytochemistry, and western blotting. HA coupled to Sepharose is able to precipitate the endogenous SorLA. Using antisera directed against extra- and intracellular epitopes we found that SorLA exists, like hydra HAB, as membrane receptor and as soluble protein, shed from cells into the culture medium. Cell lines differ strongly in processing of SorLA, with NT2 cells expressing SorLA mainly as membrane receptor, whereas shedding predominates in BON cells. Soluble SorLA lacks the intracellular domain and is released from the transmembrane protein by a metalloprotease. Shedding from cells and brain slices is stimulated by HA and by phorbol ester, and it is blocked by a metalloprotease inhibitor. Inhibition of SorLA shedding and treatment of cells with SorLA antisense oligonucleotides leads to a decrease in the rate of cell proliferation. From this we conclude that SorLA is necessary to mediate the mitogenic effect of endogenous HA. HA enhances the translocation of SorLA from internal membranes to the cell surface and its internalization. In addition, HA stimulates SorLA synthesis hinting at an autocatalytic feedback loop, in which the ligand activates production, processing, and translocation of its

receptor. The VPS10 domain of SorLA heterologously expressed using an adenoviral system, bound HA with nanomolar affinity identifying this part of the SorLA molecule as HA-interaction site. The binding was inhibited by the SorLA propeptide indicating that like in other such receptors proteolytic removal of the propeptide enables SorLA to bind its ligands.

2. Characterization of new members of the VPS10-receptor family

Irm Hermans-Borgmeyer, Meriem Rezgaoui, Guido Hermey, Wolfgang Hampe

In addition to SorLA, which binds HA, another VPS10-domain containing receptor, sortilin, was also shown to bind a neuropeptide, namely neurotensin. Searching for additional VPS10-domain proteins in the database, we found three new putative human and murine neuropeptide receptors (Fig. 1). The new receptors were designated SorCS1, SorCS2 and SorCS3, due to their identical receptors domain composition with imperfect leucine-rich repeats following the VPS10 domain. Their cytoplasmic domains contain signals for internalization and, in SorCS3, for intracellular sorting. In situ hybridization studies for SorCS1-3 revealed highly specific patterns of expression during murine development and in the adult central nervous system. SorCS2 and SorCS3 exhibited predominantly a complementary pattern of expression. In the adult this was most obvious in the hippocampus: While SorCS2 was abundantly expressed in the subiculum and the CA2 region and, at lower levels, in the dentate gyrus, SorCS3 transcripts were primarily found in the CA1 and not in the SorCS2 positive areas.

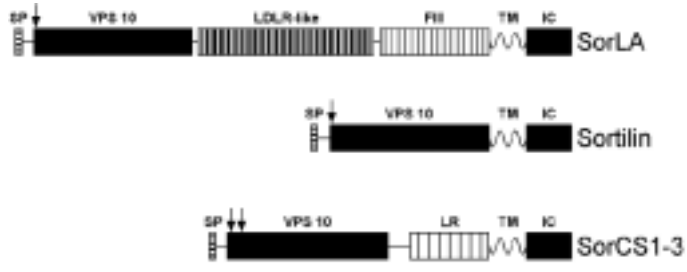

Fig. 1. Domain structure of human VPS10 receptors. The VPS10 domains are drawn as black bars. The furin cleavage motifs for propeptide processing is indicated by arrows. FIII, fibronectin type III module; IC, intracellular domain; LDLR-like domain with homology to the class A and class B repeats and the EGF-like domain of the low density lipoprotein receptor; LR, imperfect leucine-rich repeats; SP signal peptide; TM, transmembrane domain.

Because the temporal and spatial appearance of SorCS-receptor transcripts during brain development suggested a role in establishing synaptic connections in the brain, we checked, in a collaboration with Niels Plath of D. Kuhl's group, whether any of the three SorCS genes was regulated by electrical activity. After stimulation by injection of kainic acid we observed an upregulation of gene expression for SorCS1 and SorCS3 in the hippocampus, the cerebral cortex, and nuclei of the amygdala (Fig. 2).

Using the databases of the human genome project we elucidated the exon-intron structures of the human VPS10-receptor genes. They contain many short exons, separated by introns, several of which extend over more than 50 kb. The three SorCS genes encompass each more than 500 kb of genomic DNA and, therefore, represent some of the largest known human genes. All these genes map to chromosomal localizations of known genetic diseases, many of them

neurological disorders, corresponding to the strong expression of these receptors in the brain.

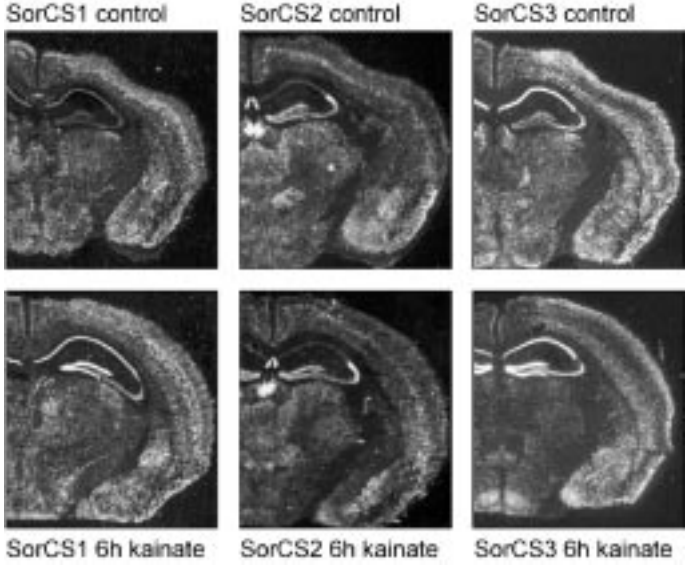

Fig. 2. In situ RNA hybridization analysis of the expression of SorCS1-3 6 h after kainic-acid injection showing an upregulation of both SorCS1 and SorCS3, but no changes in SorCS2 expression.

3. Protein expression of SorLA and SorCS1 in mouse

Irm Hermans-Borgmeyer, Björn Riedel, Meriem Rezgaoui

Antisera generated against SorLA and SorCS1 were used to determine the distribution of the respective proteins in the nervous system. Both were abundant in distinct neuronal populations of the murine brain. In most SorLA- and SorCS1-positive neurons a punctate staining was observed in the somata which extends for SorLA into the proximal dendrites and for SorCS1 into the distal dendrites. SorCS1 immunostaining in some of the positive neurons was associated with the plasma membrane. Colocalization studies performed with SorLA antisera exhibited colocalization with the endosomal marker protein rab5, the Golgi specific protein γ-adaptin, and the transferrin receptor. Electron microscopic analysis of cerebral pyramidal cells confirmed the colocalizations.

Outside the nervous system SorLA was abundant in the Langerhans islets of the endocrine pancreas and in the collecting ducts of the kidney. In the medulla SorLA immunoreactivity was always associated with the luminal plasma membrane of the principle cells, while in the cortex a punctate cytoplasmic staining was detected. These results hint at a highly specialized function of SorLA in the kidney.

4. GRC and HA signal transduction

Wolfgang Hampe, Katrin Boels, Doris Herrmann

Proliferation of neuronal precursor and neuroendocrine cells is stimulated by HA. HA acts as mitogen in the G2/mitosis transition, an effect which is measurable one to three hours after HA application as increase of cells in mitosis. HA signal transduction for mitotic stimulation is mediated by a pertussis-toxin sensitive heterotrimeric G-protein, requires Ca^{2+} influx, down-regulation of adenylyl cyclase, and hyperpolarization of the membrane potential. The latter is achieved by K^+ efflux, for which we had found that the Gardos-type Ca^{2+}-regulated K^+ channel, hIK1, is responsible.

Growth factors stimulate cell proliferation by inducing Ca^{2+} entry into cells. Recently, a new Ca^{2+}-permeable channel was described as growth-factor regulated channel (GRC). Human and rat orthologs were also found and named vanilloid-receptor

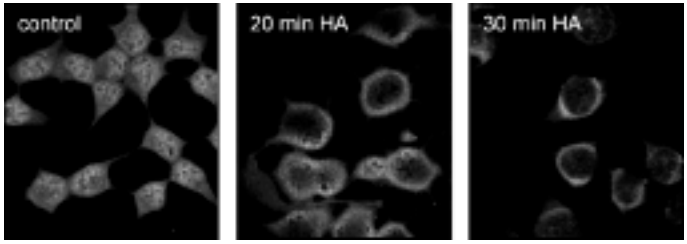

Fig. 3. HA-induced translocation of GRC in NH15-CA2 cells. NH15-CA2 cells were treated for the times indicated with 2 nM monomerized HA, permeabilized by fixation in ice-cold 1% acetic acid in ethanol, and immunostained with the antiserum against GRC.

like (VRL-1), because of their homology to the capsaicin receptor VR1. GRC belongs to the transient receptor potential (TRP) channel family, containing six transmembrane domains, a pore loop, a cytoplasmic amino terminus with three ankyrin repeats, and a cytoplasmic carboxy terminus. We found that GRC is responsible for the Ca^{2+} influx triggered by HA (collaboration with the laboratories of I. Kojima, Japan, and J. Schwarz, Hamburg). In uninduced cells only low amounts of GRC are present at the plasma membrane, but upon stimulation with HA GRC translocates from an intracellular compartment to the cell surface. HA functions as inducer of GRC translocation in neuronal (Fig. 3) and neuroendocrine cells, which express GRC endogenously, and also in COS7 cells after transfection with GRC. HA is no direct ligand for GRC, but its action requires presence of a receptor coupled to a pertussis-toxin sensitive G-protein. CHO cells transfected with GRC and SorLA did not respond to HA, indicating that a cofactor or coreceptor for SorLA is present in COS7, but missing in CHO cells. If heterologously expressed GRC becomes activated by HA, the channel opens leading to Ca^{2+} influx. SK&F 96365, an inhibitor specific for TRP-like channels, blocks Ca^{2+} entry and, as a consequence of this, translocation of GRC is prevented. HA-induced GRC activation and translocation are also inhibited by wortmannin and KN-93, blockers of the PI3- and of the CaM-kinases, respectively, which implies a role for both in HA signaling to GRC (Fig. 4).

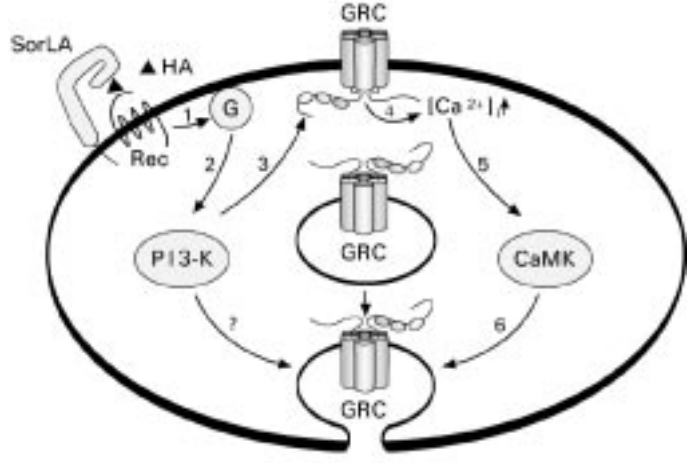

Fig. 4. Model for HA signaling to GRC. After bindung HA the HA receptor SorLA together with a postulated second receptor component (Rec) activates a pertussis-toxin sensitive heterotrimeric G protein (1), which interacts via PI3-K (2) with surface GRC (3) to induce Ca^{2+} influx (4). The increase in Ca^{2+} leads to activation of CaMK (5), which possible together with PI3-K (?), triggers shuttling of more GRC molecules to the plasma membrane (6).

5. New human and murine G-protein coupled receptors

Timo Wittenberger, Suanne Hellebrand, Wolfgang Hampe, Irm Hermans-Borgmeyer, Katrin Boels, Atanas Ignatov,Jens Urny

The GRC experiments indicated that SorLA is only part of the HA-receptor complex. In HA-binding studies using phage display one interesting clone was found coding for a novel type of membrane receptor with high expression in the brain. Its relevance for HA function is under investigation.

Inhibition of HA function with pertussis toxin hinted at G-protein coupled receptors (GPR)s as mediators for HA signaling. To identify GPRs for HA we searched the databases for new GPRs and tested these together with known orphans for binding of HA or other ligands. In one approach we used a comprehensive expressed sequence tag (EST) database search to identify new GPRs. The method proved to be especially useful for the detection of ESTs that do not encode conserved parts of a protein, making it an ideal tool for the identification of members of divergent protein families or of protein parts without conserved domain structures in the EST database. At least 14 of the ESTs, found with this strategy, are promising candidates for new putative G-protein coupled receptors. Six new members of this receptor superfamily, namely GPR84, GPR85, GPR86, GPR87, GPR90 and GPR91 and two receptors identified by searching the human genomic database, GPR99 and GPR100, were further characterized and tested for binding HA or other ligands.

As assay systems we use CHO cells transfected with aequorin as Ca^{2+} sensor and with $G\alpha_{16}$ as promiscous α subunit of trimeric G proteins or, in a collaboration with H. J. Kreienkamp, expression in Xenopus oocytes. We found that GPR 84, 85, 86, 87, 90, 91 and 99 do not respond to HA. We are in the process of analyzing other ligands. The best candidate for a HA receptor is GPR100 which belongs to the neuropeptide receptor family and awaits, like other orphans of this family, ligand testing.

Expression and a chromosomal localization of the new GPRs were studied. GPR85 is predominantly expressed in brain and is identical between man and mouse at the amino acid level. In situ hybridization showed highest transcript levels of GPR85 in the developing cerebral cortex. We localized the human GPR85 gene to chromosome 7q31 and that for GPR84 to chromosome 12q.13.13. A cluster of six closely related GPRs was found on the human chromosome 3q24-3q25. It consists of four orphan receptors (GPR86, GPR87, GPR91, and H963), the purinergic receptor P2Y1, and the uridine 5'-diphosphoglucose receptor KIAA0001. It seems likely that these receptors evolved from a common ancestor and therefore might have related ligands.

6. Foot specific differentiation processes in hydra

Sabine Hoffmeister-Ullerich, Doris Herrmann

Recently we purified two peptides, pedin and pedibin, from *Hydra vulgaris*. The sequences were determined to be EELRPEVLPDVSE for pedin, and AGEDVSHELEEKEKALANHSE for pedibin. Both peptides stimulate foot-specific differentiation during regeneration. Pedin acts as a mitogen for big interstitial cells and it stimulates terminal differentiation of nerve cells. In collaboration with A.

Grens, H. Shimizu, H. R. Bode and T. Fujisawa the role of pedibin was examined in more detail. The results show that treatment of hydra with pedibin lowers the positional value, which means that the tissue becomes more competent for foot formation.

We cloned a full-length cDNA for pedibin. The peptide is synthesized as a precursor of 49 amino acids. A putative cleavage site precedes the peptide as purified from hydra tissue. The precursor, like pedibin, accelerates foot regeneration. Pedibin transcripts are concentrated as expected in the foot region of hydra, but unexpectedly are also present in the head region accumulating in the tentacle bases. The early appearance of pedibin transcripts during phases of cell fate specification like budding and regeneration implies that in hydra pedibin plays an important role in patterning processes of foot and head. This is confirmed by the finding that pedibin also stimulates bud outgrowth.

One target gene for the action of pedibin is *CnNK-2*, a NK-2 homeobox gene, which is expressed in the endodermal epithelial cells in the foot of hydra. *CnNK-2* is upregulated when hydra are treated with pedibin. Another putative target for the action of the peptides is a foot-specific peroxidase, which is present in ectodermal epithelial cells of the basal disk and which was used as a marker for foot-specific differentiation processes. We purified the protein and cloned its complementary DNA. The obtained sequence is unique, with no homology to other proteins. The localization of the mRNA in hydra tissue by in situ hybridization perfectly mirrors the pattern of expression of the enzymatically active endogenous protein.

7. Nuclear receptors in early neuronal development

Uwe Borgmeyer, Eray Gökkurt, Moritz Hentschke, Ute Süsens

An understanding of the mechanisms by which apparently distinct regulatory systems integrate to control neuronal development poses a central problem in neurobiology. Hence one objective of our research is to explore the expression of nuclear receptors in the developing mammalian nervous system. Classical nuclear receptors, such as the sex steroid receptors share several structural features allowing DNA binding and regulation of gene expression. Molecular techniques led to the isolation of gene products (orphan receptors) that appeared to belong to the nuclear receptor superfamily on the basis of their sequence similarity, but lack known ligands. Our aim is to elucidate the function of orphan nuclear receptors and their mechanism of action.

We have focussed on two orphan receptors, the germ cell nuclear factor (GCNF, NR6A1) and the estrogen receptor-related receptor γ (ERRγ, NR3B3) that are both expressed during mouse embryonic development. GCNF is highly expressed in the developing mouse nervous system, the labyrinthine layer of the placenta, and in the developing germ cells. Interestingly, GCNF expression is temporarily regulated when embryonal carcinoma cells are triggered to differentiate by retinoic acid. The genomic structures of the mouse and human GCNF gene are highly conserved. The comparison reveals that skipping of the 45 bp long third exon results in a shorter human protein. Three different human isoforms, GCNF-1, GCNF-2a, and GCNF-2b are generated by alternative splicing. GCNF functions as a constitutive repressor, when it is bound as a homodimer to promoters containing a direct

repeat DNA element 5'-AGGTCAAGGTCA-3' (DR0). Gelshift assays showed that ERRγ binds to the same response element. ERRs are orphan nuclear receptors sharing high sequence homology with estrogen receptors, but have no known endogenous ligands. Northern blot analysis revealed ERRγ transcripts of approximately 5.7 kb at embryonal day 11 (E11), E15, and E17. ERRγ is highly expressed in the nervous system of the developing mouse embryo. The adult pattern of expression is, with few exceptions, established during embryogenesis. Transcripts are preferentially detected in already differentiating areas of the nervous system. *In situ* hybridization on adult mouse brain sections shows strong and differential expression of ERRγ transcripts in the isocortex, the olfactory system, cranial nerve nuclei, major parts of the coordination centers, and in sensory ganglia. Thus, it is tempting to speculate that ERRγ is involved in neuronal differentiation and in the maintenance of mature neurons.

8. Guest group: Protective signaling in the nervous system

Axel Methner, Jan Lewerenz, Patrick Joost, Frank Leypoldt, Naoya Koseki, Julius Steinbeck, Susanne Thomsen

Injury and disease possibly lead to disturbed communication between cells of the nervous system. Cells communicate through different classes of receptors defined by the transduction mechanism used. Ion-channel-linked receptors are involved in rapid synaptic signaling between electrically excitable cells. In contrast, G-protein-coupled receptors (GPRs), the second major class of surface receptors, mediate their signals on a larger time scale, which is more likely to be influenced by gene expression. We try to elucidate and influence disturbed intercellular communication by combining techniques examining differential gene expression, with special emphasis on GPRs and pharmacology.

Neuronal differentiation as a model paradigm. The human teratocarcinoma cell line NT2 can be induced with retinoic acid and cell aggregation to yield post-mitotic neurons. Suppression subtractive hybridization (SSH) was used to detect differentially expressed genes. Microfibril-associated glycoprotein 2 was found to be drastically upregulated and has not been implicated in neuronal differentiation before. SSH also identified DYRK4, a homologue of the Drosophila gene minibrain, which is important in the CNS development in the fly, and led to the characterization of the neurospecific human septin 3, a member of a GTPase family implicated in mitosis and exocytosis. Their upregulation suggests a role for DYRK4 and SEP3 in neuronal differentiation in humans. Additionally, we combined parts of the SSH technique with PCR and probes based on GPR consensus sequences as a screening method for differentially expressed GPRs. Upregulation of one GPR, the β_2-adrenergic receptor was confirmed by quantitative PCR. Currently we try to elucidate the function of this receptor in differentiation by treatment of primary neuronal cultures with specific antagonists.

Resistance to chronic oxidative stress. Oxidative stress plays a role in diverse neurological diseases ranging from Alzheimer´s disease to ischemic stroke. A well characterized model for programmed cell death due to oxidative stress is glutamate-mediated toxicity in the hippocampal cell line HT22. In this paradigm, loss of the intracellular antioxidant glutathione, calcium influx and a burst of reactive oxygen species precede cell death.

We could show that HT22 cells express neuronal marker genes like microtubule-associated protein 2 and middle and high M_r

neurofilament and may thus serve as a model for oxidative stress in the nervous system. Activation of protein kinase C (by the phorbol ester PMA) and inhibition of protein kinase A (by the compound H89) leads to a robust protection of HT22 cells from glutamate toxicity (Fig. 5). Therefore, GPRs may play a role in the regulation of programmed cell death in HT22 cells. To identify genes involved in the sensitivity to apoptotic stressors, we selected HT22 cells resistant to 10-fold higher concentrations of glutamate and subtracted these from their sensitive counterparts. Differentially expressed genes, especially GPRs, are under investigation.

Ischemic preconditioning protects primary neuronal cultures against glucose-oxygen deprivation. Short episodes of ischemia protect mammalian tissues and cells against subsequent ischemia. This phenomenon is called ischemic preconditioning (IP). *In vitro* ischemia is mimicked by glucose–oxygen deprivation (GOD) in an argon atmosphere. In rat primary cortical cultures 60% cell death was observed after two hours of GOD, which was almost totally prevented by a one-hour pretreatment with GOD 24 hours earlier (Fig. 6). cDNA from preconditioned and control cultures was used in SSH to identify differentially expressed genes and GPRs.

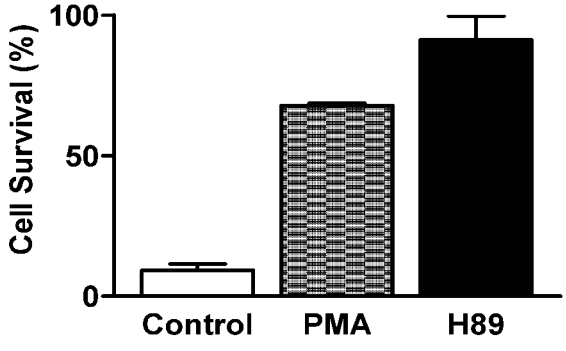

Fig. 5. Modulation of signal transduction pathways downstream of GPR activation leads to enhanced survival after treatment of HT22 cells with glutamate. Cells were incubated with 2.5 mM glutamate in the presence of 10ng/ml PMA, 10 μM H89 or vehicle. Cell survival was measured by a modified MTT assay.

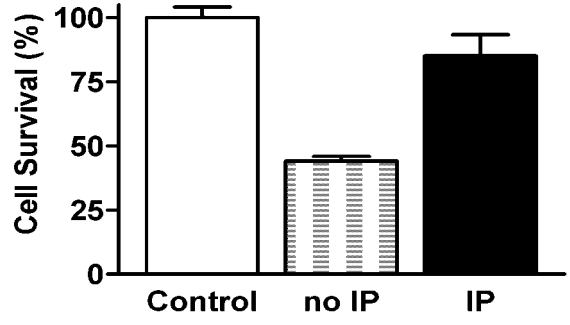

Fig 6. A one-hour preconditioning (IP) with glucose-oxygen-deprivation (GOD) results in a reduction of anoxia-induced cell death due to 2h GOD 24 hours later. Cell survival was measured by a modified MTT assay.

Polymorphisms and mutations leading to altered expression of disease-related genes. In ischemic stroke, a systemic inflammatory reaction is observed which, by favoring coagulation and increasing blood viscosity, may critically influence the impaired blood supply in the peri-infarct brain regions. One of the major determinants of coagulability and blood viscosity is the clotting factor fibrinogen. We were able to show that presence of the less common A allele of the –455G>A promotor polymorphism in the β-fibrinogen gene is associated with increased changes in fibrinogen levels in the first five days after macroangiopathic stroke.

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft (Scha 253, SFB 444, GRK 255, and HO 1296).

Support of guest group Methner

The work in our laboratory is supported by grants from the Deutsche Forschungsgemeinschaft (GRK 255), the Kurt-und-Irmgard-Meister-Stiftung and the Werner-Otto-Stiftung. Susanne Thomsen is funded by the Department of Neurology. We greatly appreciate the continous intellectual and material support, as well as the laboratory space provided by Prof. H. Chica Schaller.

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Institut für Molekulare Neuropathobiologie

Thomas J. Jentsch

The research of our group is concerned with ion transport processes, in particular chloride and potassium channels, as well as in potassium-chloride cotransporters. We investigate the structure-function relationship of the underlying proteins, and study their cell biology, physiology, and role in human disease. These latter aspects are greatly facilitated by a number of knock-out mouse models generated recently in our laboratory.

A main focus of our work is on CLC chloride channels (29), a family of ion channels we discovered several years ago. We have now disrupted the genes of many CLC channels in mice (16,19,22,23). This led to important new insights. For instance, the disruption of the chloride channel ClC-7 unexpectedly led to osteopetrosis (22). This is due to a dysfunction of osteoclasts, the cells that are responsible for bone degradation. ClC-7 currents balance the electrogenic transport of the H^+-ATPase that acidifies the resorption lacuna. Stimulated by these findings, we found mutations in either the ClC-7 chloride channel (22) or the a3 subunit of the H^+-ATPase (15) in human patients with severe juvenile osteopetrosis. ClC-7 is normally present in a lysosomal compartment, but is inserted into the plasma membrane of osteoclasts. Similarly, our results show that ClC-3 through ClC-6 reside normally in membranes of intracellular compartments, where they may contribute to their acidification by electrical shunting of the proton ATPase. ClC-5, a Cl^- channel mutated in Dent's disease, is present in renal endosomes. Our knock-out mouse model (16) revealed that an impaired endosomal acidification led to a broad defect in proximal tubular endocytosis. This entails secondary changes in calciotropic hormones, eventually resulting in the hyperphosphaturia and hypercalciuria (and kidney stones) in Dent's disease. The related ClC-3 channel is also present in endosomes (19), as well as in synaptic vesicles. As the uptake of neurotransmitters into synaptic vesicles is coupled to the proton gradient, this may have interesting consequences for synaptic transmission. Surprisingly, the knock-out of ClC-3 led to nearly complete degeneration of the hippocampus (19). The further elucidation of the roles of the five intracellular CLC chloride channels e.g. in trafficking, sorting, and synaptic transmission is a major goal of our future research. Our knock-out mouse models, which we cross to yield multiple knock-outs, will greatly facilitate this task.

In another area, we focus on the regulation of intracellular Cl^--concentration in neurons. This concentration determines whether the response to the neurotransmitters GABA and glycine is inhibitory (as in most adult neurons) or excitatory (as early in development). ClC-2 is thought to play a role in this regulation, but the disruption of this Cl^- channel led to male infertility and blindness rather than to CNS hyperexcitability that may have been expected (23). By contrast, the disruption of the neuronal K-Cl cotransporter KCC2 led to perinatal death due to the inability to breathe and to a spastic phenotype (21). This was indeed due to an increase in intracellular chloride in motoneurons.

Finally, we pursued our third area of research that focuses on KCNQ potassium channels (32). We had previously shown that mutations in KCNQ2 and KCNQ3, which can form heteromeric channels, may lead to neonatal epilepsy. We

now discovered that a novel member of this family, KCNQ5, can associate with KCNQ3 with which it is co-expressed in broad regions of the brain (10). We showed that KCNQ4 is mutated in a form of dominant deafness (2). It is expressed in hair cells of the inner ear, and surprisingly also in the central auditory pathway (8). Some KCNQ K^+ channels associate with ß-subunits of the KCNE family. We provided evidence that KCNQ1/KCNE3 heteromers are important for Cl^- secretion in the intestine (6).

1. Structure and function of CLC chloride channels

Our previous studies (Nature 383:340 (1996)), together with those of Chris Miller's group, had shown that the *Torpedo* channel ClC-0 is a dimer with two individual pores ('double-barreled channel'). We now extended these studies to other CLC channels by constructing concatemers of ClC-0 with either ClC-1 or ClC-2 (18). In single channel studies, 'double-barreled' channels with one ClC-0 pore, together with one ClC-1 or ClC-2 pore, respectively, were observed. This suggests that CLC channels are 'double barrel' channels in general, and strongly argues that a single CLC protein forms a self-contained pore in the dimeric channel.

Disconcertingly, the human kidney chloride channels ClC-Ka and ClC-Kb do not yield plasma membrane currents upon heterologous expression, even though the genetic evidence (ClC-Kb is mutated in a form of the kidney disease Bartter's syndrome) strongly suggests that they mediate transepithelial transport. We therefore constructed many chimeric channels between the expressible ClC-K1 channel from rat and human ClC-Kb (13). A chimera consisting >80% of ClC-Kb showed currents that differed significantly from ClC-K1. They were abolished by mutations identified in Bartter's syndrome.

ClC-4 and ClC-5, although predominantly present in intracellular compartments, elicit (weak) plasma membrane currents upon heterologous expression. We used site-directed mutagenesis to change their characteristics, proving that these currents are directly mediated by ClC-4 or ClC-5 (1). ClC-5, an endosomal Cl^- channel, carries a so-called PY-motif in its cytoplasmic carboxyterminus. It interacts with a WW-domain protein that contains a ubiquitin protein ligase HECT-domain (20). Mutagenesis showed that the interaction with WW-domain proteins leads to an increased rate of ClC-5 endocytosis presumably due to a ubiquitination of the channel protein (20).

2. The function of ClC-2 revealed by a *knock-out* mouse model

ClC-2 is a ubiquitously expressed plasma membrane Cl^- channel that is activated by hyperpolarization (3), external acidic pH, and cell swelling. It was hypothesised to play an important role in lung development, gastric acid secretion, and in the determination of intracellular chloride in neurons. However, knock-out mice (23) did not show conspicuous abnormalities in these organs. There was neither a reduced threshold to seizure induction that might have been expected when changing neuronal Cl^- concentration. By contrast, we observed a degeneration of male germ cells and photoreceptors (23). Intriguingly, both cell types depend on the transepithelial transport and nutrition by epithelia (formed by Sertoli cells and retinal pigment epithelial cells, which both express ClC-2 normally) that form a blood-organ barrier. We hypothesised that ClC-2 plays an important role in this trans-epithelial transport.

3. Intracellular Cl^- channels ClC-3, ClC-5 and ClC-7: lessons from mouse models and human inherited disease

The role of ClC-3, a broadly expressed Cl^- channel, was highly controversial. Whereas we and others did not detect currents upon heterologous expression, four groups published contradictory results on associated currents. ClC-3 was claimed by Duan and Hume to represent the long-sought swelling activated Cl^- channel. However, we have now shown that swelling-activated currents are not affected in ClC-3 KO mice (19). ClC-3 is present in endosomal compartments as well as in synaptic vesicles. This localisation is mutually consistent, as synaptic vesicles are recycled at the synaptic terminal in part via endosomal intermediates. The rate of synaptic vesicle acidification is reduced in the KO, strongly suggesting that ClC-3 provides a conductive pathway to compensate for the charge transfer by the proton ATPase. As neurotransmitter uptake into synaptic vesicles is driven by the electrochemical potential for protons, ClC-3 may play important roles in synaptic transmission. As synaptic vesicle acidification remained Cl^- dependent in the absence of ClC-3, we suspect that other CLC channels may have similar roles. Surprisingly, the disruption of ClC-3 led to a drastic degeneration of the hippocampus and the retina. Even in the virtual absence of the hippocampus, *Clc3*$^{-/-}$ mice survived for more than a year and were able to learn motor skills.

ClC-5 is predominantly expressed in the kidney. Its mutational inactivation in human patients with Dent's disease leads to low molecular weight proteinuria and the urinary loss of phosphate and calcium, resulting in clinically important kidney stones. The pathophysiological mechanism of that disease, however, remained unclear. We therefore disrupted ClC-5 in mice (16). This led to a severe impairment, but not total

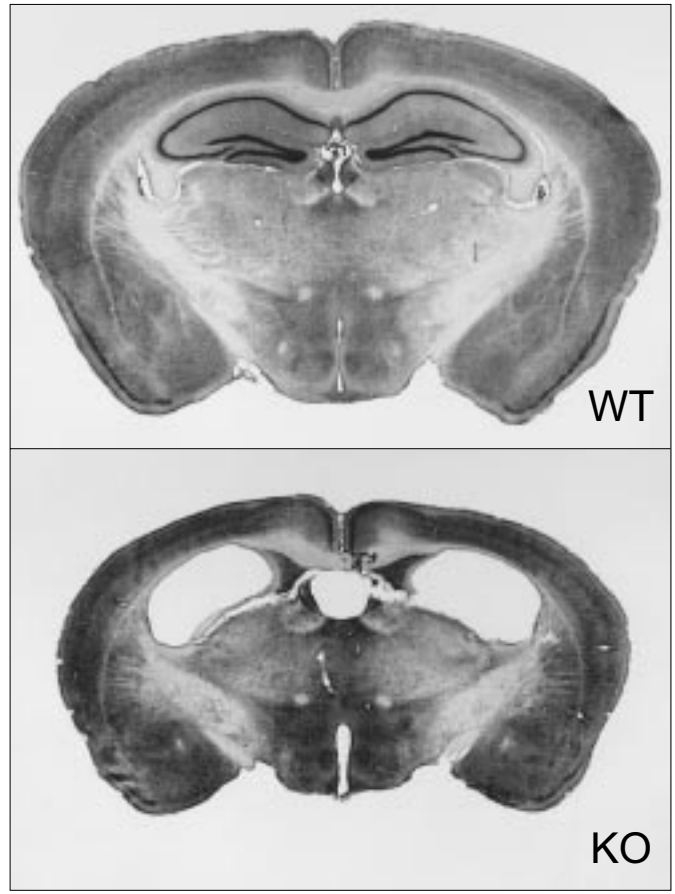

Figure 1. The disruption of the endosomal/synaptic vesicle chloride channel ClC-3 leads to a severe postnatal degeneration of the hippocampus. Cross-sections through the brain of wild-type (WT) and knock-out (KO) 7 months-old mice are shown. For more details see (19)

abolition, of proximal tubular endocytosis. Receptor-mediated and fluid-phase endocytosis, as well as the endocytotic retrieval of certain plasma membrane proteins, are all considerably slowed. This is probably a consequence of a defective acidification of endosomes. Both PTH and 25(OH)VitD_3 are normally endocytosed in the proximal tubule. Our work (16) demonstrates that the impaired endocytosis of both calciotropic hormones can explain the secondary and tertiary symptoms of hyperphosphaturia and hypercalciuria, respectively. Thus, this mouse model enabled us to explain the pathophysiology of Dent's disease.

ClC-7 is a ubiquitously expressed channel that did not yield currents upon heterologous expression and whose function was totally obscure. An exploratory knock-out of ClC-7 (22) resulted in an osteopetrotic phenotype, revealing that ClC-7 is a lysosomal channel that is inserted (together with the proton pump) into the ruffled border of osteoclasts upon their attachment to bone. Here again, the Cl^- channel provides an electrical shunt for the electrogenic pump. A loss of ClC-7 thus leads to a defective acidification of the resorption lacuna, resulting in an inability to dissolve bone. A partial, pharmacological inhibition of ClC-7 may prove beneficial in the treatment of osteoporosis in humans.

The phenotype of the ClC-7 KO mouse prompted us to screen human patients with severe juvenile osteopetrosis for mutations in ClC-7. We found loss of function mutations in the *CLCN7* gene in one out of ten families (22). In several other families, we identified mutations in the a3 subunit of the H^+-ATPase (15), confirming the functional interplay of both proteins.

We now know that five CLC channels (ClC-3 to ClC-7) reside primarily in intracellular organelles. The availability of several knock-out mouse models, which we cross to obtain double and multiple knock-outs, will enable us to address important issues of cell biology such as endocytosis from a new perspective.

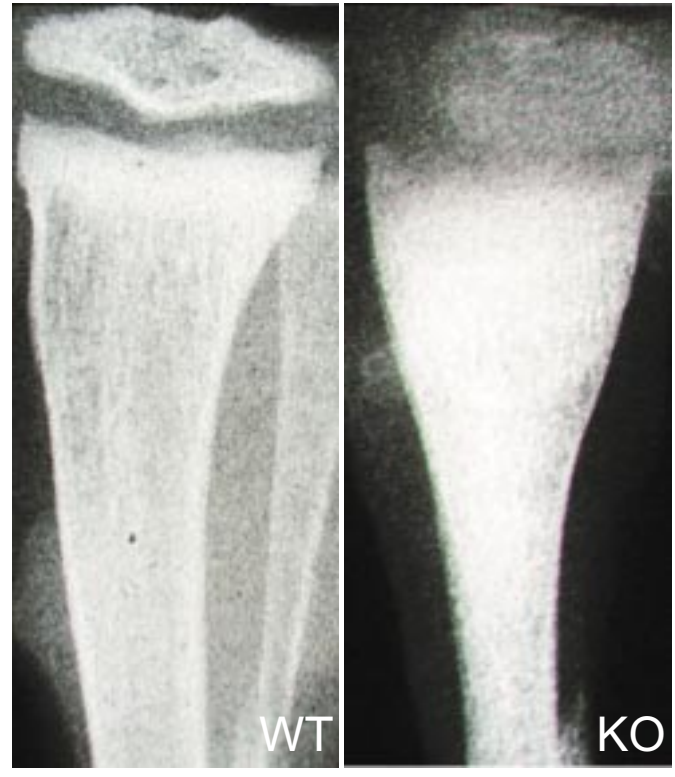

Figure 2. The disruption of the lysosomal channel ClC-7 leads to osteopetrosis. X-ray pictures of WT and KO tibiae are shown. Taken from ref. 22.

4. CLC chloride channels in *C. elegans*

The genome of the nematode *C. elegans* contains six genes encoding CLC Cl^- channels. By using appropriate promoter-GFP constructs in transgenic worms, we demonstrated that some of these channels are highly and specifically expressed in certain cell types (4). Only two of these channels, which both belong to the first branch of the CLC family that contains plasma membrane channels in mammals, could be expressed functionally. Interestingly, we observed at least two different gating processes, one of which was dependent on extracellular chloride (4). This provides a delightful similarity to the Cl^--dependent gating of the double-barreled ClC-0 channel.

5. The K-Cl co-transporter KCC2 is an important regulator of Cl^--dependent synaptic transmission

As ClC-2 was expected to play a role in the regulation of neuronal intracellular chloride concentration, we decided to disrupt KCC2 as well (24). KCC2 is a neuronal isoform of the K-Cl co-transporter gene family. Under most circumstances, K-Cl cotransport will lower intracellular Cl^- below its electrochemical equilibrium. The intracellular Cl^--concentration determines the response to the neurotransmitters GABA or glycine. In early development, this response shifts from excitatory to inhibitory as a consequence of a lowering of the intraneuronal chloride concentration. In the hippocampus of rodents, this shift occurs a couple of weeks after birth.

When we disrupted KCC2 in mice, however, we observed severe deficits already at birth (24). This is due to the fact that KCC2 is upregulated in the brainstem already before birth. Neonatal mice died from the inability to breathe and displayed a spastic phenotype. Patch-clamping of spinal cord motoneurons revealed that the loss of KCC2 entailed an increase in intracellular chloride concentration that led to an excitatory response to GABA and glycine (24).

6. KCNQ2, -3 and –5 potassium channels: a molecular basis for neuronal M-currents

We had previously shown that KCNQ2 and KCNQ3 can form heteromeric channels, and that their loss of function in a form of neonatal epilepsy (BFNC) leads only to a moderate loss of function (Nature 396: 697 (1998)). These heteromers yield currents with properties of the important and highly regulated M-current. The increase in current observed upon KCNQ2/KCNQ3 co-expression is mainly due to an increased surface expression (9). Further, we analysed a novel mutation in the voltage-sensor of KCNQ2 that causes myokymia in addition to BFNC (27). We showed that the novel KCNQ5 protein is also expressed in brain, where it may form heteromers with KCNQ3 (10). Both KCNQ5 and KCNQ3/5 heteromers have properties of M-currents. Our findings suggested the possibility that KCNQ5 might also be mutated in some forms of epilepsy. However, a first screen of appropriate patient's DNA for *KCNQ5* mutations was negative (11).

7. KCNQ4, a potassium channel critical for hearing

The KCNQ4 K^+ channel is mutated in DFNA2, a dominantly inherited form of progressive hearing loss (2). In the ear, KCNQ4 is expressed predominantly in outer hair cells of the organ of Corti (2) and in type I vestibular hair cells (8). KCNQ4 may be required for the basal exit of K^+ from outer hair cells,

which is then recycled to the stria vascularis for a new round of secretion (2,8,30). Surprisingly, KCNQ4 is also expressed rather specifically in the central auditory pathway (8). Whether this implies that there is also a central component in DFNA2 deafness might be clarified in the future by appropriate mouse models.

8. KCNE3: a β-subunit for KCNQ1

KCNE3, a protein with a single transmembrane segment that is homologous to minK (KCNE1), can associate with KCNQ1 (6). Its effect differs dramatically from that of KCNE1: while KCNE1 drastically slows KCNQ1 activation by depolarization and shifts the voltage-dependence to more positive voltages, KCNE3 renders KCNQ1 into a constitutively open channel. Our studies suggest that KCNQ1/KCNE3 underlie the cAMP-stimulated basolateral K^+ conductance in colonic crypt cells (6, 26). This conductance is indirectly necessary for Cl^- secretion as it provides a pathway for the recycling of K^+ that is taken up by the basolateral NaK-2Cl co-transporter.

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft, the European Community, the Federal Ministry for Research and Education (BMBF), the Fonds der Chemischen Industrie, the US Cystic Fibrosis Foundation, the Prix Louis-Jeantet de Médecine, and the Ernst-Jung Preis für Medizin.

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Contribution to a book

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Theses

Diploma

Dissertations

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Awards

K.J. Zülch-Preis der Gertrud-Reemtsma-Stiftung in der Max-Planck-Gesellschaft to Thomas J. Jentsch, 1999.

Wilhelm-Feldberg Prize of the Feldberg Foundation for Anglo-German Scientific Exchange to Thomas J. Jentsch, 2000.

Familie Hansen Preis to Thomas J. Jentsch, 2000.

Heinz Maier-Leibnitz Preis der DFG to Christian Kubisch, 2000.

Prix Louis-Jeantet de Médecine to Thomas J. Jentsch, 2000.

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Institut für Neurale Signalverarbeitung

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. Travelling along the axonal cable, the impulse possesses a stereotypic pattern but when finally invading the presynaptic terminal of a synapse, the site of electro-chemical coupling, this situation changes dramatically. The synapse can modify action potential width as well as respond to changing frequencies of incoming action potentials. The activity of potassium channels makes here an important contribution. The modulatory behaviour of the synapse subserves the translation of electrical signal into a quantized chemical signal, i.e. neurotransmitter release. Consequently, the modulation of incoming action potentials in the synapse is an important molecular basis of synaptic plasticity. In recent years, it has become evident that action potentials also travel back into neuronal dendrites. The back-propagation of action potentials may be limited by the activity of rapidly- inactivating A-type potassium channels which operate in the subthreshold range of membrane potential. The activity of these channels may prevent somato-dendritic action-potential initiation and consequently, backpropagation of action potentials. Back-propagated action potentials can have an important influence on excitatory events at the postsynapse and modulate dendritic spiking correlated with long term potentiation and/or synaptic plasticity. Our interest is to understand the structure, function and physiological role of pre- and post-synaptic voltage-gated potassium channels and their regulatory subunits. The activity of these potassium channels, which is correlated with alterations in neuronal and muscular excitability, is studied by a combination of biochemical, crystallographic, electrophysiological, genetic, molecular biological and physiological approaches.

1. Structure and function of axonal and presynaptic voltage-gated potassium channels and their auxiliary subunits

Robert Bähring, Birgit Engeland, Birgit Hantzsch, Sönke Hornig, Vitya Vardanyan

Potassium channels are both, ubiquitously occurring membrane proteins and highly diverse. The diversity of potassium channels reflects the special needs and fine tuning of a given excitable cell to fulfill its role and function in signal transduction. We are interested in the molecular basis of this diversity and in the characterization of the structural determinants which impose the properties on potassium channels. The cloning and functional expression of many potassium channel cDNAs has shown that most voltage-gated and ligand-gated (e.g. Ca^{2+}) potassium channels are members of a superfamily of ion channels. We have cloned and

extensively characterized the members of three potassium channel subfamilies expressed in the rat nervous system. Altogether this comprises 40 distinct potassium channel cDNAs which encode voltage-gated potassium channels with distinct activation, deactivation and inactivation kinetics, different voltage sensitivities, different pore structures and distinct pharmacologies. Also we have cloned and characterized a family of auxiliary (Kvβ) subunits of *Shaker*-related voltage-gated potassium channels. Kvβ-subunits may function as chaperones for Kvα-subunit assembly to upregulate their cell surface expression. In addition, Kvβ-subunits significantly affect Kv channel gating. In particular Kvβ1 and Kvβ3 subunits may confer rapid inactivation to otherwise non-inactivating Kv channels. Some Kvα-subunits, e.g. Kv1.3 and Kv1.6, are resistant to the inactivating activity of Kvβ1 and/or Kvβ3 subunits. We have extensively characterized the domains involved in the prevention of inactivating activities. Also, the crystal structure of the Kvβ2 subunit shows that Kvβ subunits are oxidoreductase enzymes containing an active site composed of conserved catalytic residues, a nicotinamide (NADPH)-cofactor, and a substrate binding site. We showed by a combination of structural modeling and electrophysiological characterization of structure-based mutations that changes in Kvβ oxidoreductase activity may markedly influence the gating mode of Kv channels. Amino acid substitutions of the putative catalytic residues in the Kvβ1.1 oxidoreductase active site attenuate the inactivating activity of Kvβ1.1 in *Xenopus* oocytes. Conversely, mutating the substrate binding domain and/or the cofactor binding domain rescues the failure of Kvβ3.1 to confer rapid inactivation to Kv1.5 channels in *Xenopus* oocytes. We propose that Kvβ oxidoreductase activity couples Kv channel inactivation to cellular redox regulation.

2. Structure and function of somato-dendritic voltage-gated potassium channels and their modulatory subunits.

Robert Bähring, Britta Callsen, Jens Dannenberg, Manuel Gebauer, Dirk Isbrandt, Christian Peters

Kv4 potassium channels mediate both the transient outward current (I_{To}) in cardiac myocytes and the neuronal subthreshold somatodendritic A-type current. The Kv4 subfamily has four members, Kv4.1, Kv4.2 and two splice variants of Kv4.3, which we have cloned from human cDNA. The gating properties of Kv4 channels reflect their functional roles: not only do these channels inactivate close to the action potential firing threshold, but they also recover from inactivation in the millisecond time range. This property distinguishes Kv4 channels from *Shaker*-related Kv channels. The inactivation of Kv4 channels does not require channel opening. They readily enter inactivated state(s) from pre-open closed state(s). We have performed a detailed kinetic analysis of Kv4.2 channel gating. Our experimental data could be most accurately reproduced by simulations based on an allosteric model of inactivation, where all closed states are connected to inactivated states. Also, the simulations with this model yielded fast recovery kinetics after accumulation in the close-inactivated state(s), just as observed experimentally for Kv4.2 channels.

Recently, small Ca^{2+}-binding and Kv channel-interacting proteins (KChIPs) were discovered. They are encoded in four different genes (KCNIPA-D) and are members of the recoverin/frequenin superfamily of Ca^{2+}-binding proteins. KChIPs are associated with Kv4 channels as shown by immunocytochemical colocalization and co-immunoprecipitation studies. KChIPs are prominently

expressed in brain and in cardiac tissue. Association of KChIPs with Kv4 channels leads to modulation of these A-type potassium channels. We cloned a KChIP2 splice variant (KChIP2.2) from human ventricle. In comparison with KChIP2.1, coexpression of KChIP2.2 with human Kv4 channels in mammalian cells slowed the onset of Kv4 current inactivation (2-3 fold), accelerated the recovery from inactivation (5-7 fold), and shifted Kv4-steady-state inactivation curves by 8 mV to more positive potentials. The features of Kv4.2/KChIP2.2 currents closely resemble those of cardiac transient outward currents (I_{TO}). KChIP2.2 stimulated the Kv4 current density in Chinese hamster ovary cells by ~55 fold. This correlated with a redistribution of immunoreactivity from perinuclear areas to the plasma membrane. Increased Kv4 cell-surface expression and current density were also obtained in the absence of KChIP2.2 when the highly conserved proximal Kv4 N-terminus was deleted. The same domain is required for association of KChIP2.2 with Kv4 α-subunits. We propose that an efficient transport of Kv4 channels to the cell surface depends on KChIP binding to the Kv4 N-terminal domain. Our data suggest that the binding is necessary, but not sufficient, for the functional activity of KChIPs.

3. Large-conductance calcium-activated potassium channels

Ralf Behrens, Saskia Plüger-Stegemann, Oliver Steinmetz*, Ralf Waldschütz**

The large-conductance Ca^{2+}-activated potassium (BK) channels are sensitive to both voltage and free cytosolic Ca^{2+}. BK channels, like other Kv channels, are heterooligomeric complexes of pore-forming BKα-subunits and modulatory BKβ-subunits, membrane-spanning. Association of BKβ with BKα subunits may confer an increased Ca^{2+} sensitivity, different kinetic properties as well as a different pharmacology to BK channels. BKα mRNAs are nearly ubiquitously expressed in line with the detection of BK channels in many different tissues ranging from smooth muscle and kidney tubules to neurons in the central nervous system. In contrast to BKα mRNA, the expression of BKβ mRNAs appears to be restricted to few specific tissues. For istance, BKβ1 subunits are predominantly expressed in smooth muscle cells, whereas BKβ2 subunits were mainly detected in endocrine tissue. We cloned two β subunits of BK channels, hKCNMB3 (BKβ1) and hKCNMB4 (BKβ4). Profiling mRNA expression showed that hKCNMB3 expression is enriched in testis and hKCNMB4 expression is very prominent in brain. We coexpressed BK channel α(BKα) and BKβ4 subunits *in vitro* in CHO cells. We compared BKα/β4-mediated currents with those of smooth muscle BKα/β1 channels. BKβ4 slowed activation kinetics more significantly, led to a steeper apparent calcium sensitivity, and shifted the voltage range of BK current activation to more negative potentials than BKβ1. BKα/β4 channels were not blocked by 100 nM charybdotoxin or iberiotoxin, and were activated by 17β-estradiol.

BK channels are ideally suited to sense both changes in membrane potential and in intracellular Ca^{2+} concentration. BK channel activity in vascular smooth muscle cells (VSMCs) is correlated with the occurrence of spontaneous transient outward K^+ currents (STOCs). The currents produce a K^+ efflux, which causes membrane hyperpolarization and thus diminishes VSMC contraction. BK channel opening depends on local increases in Ca^{2+} in the VSMC cytoplasm, termed Ca^{2+} sparks. Ca^{2+} sparks and STOCs are tightly coupled in that the appearance of a Ca^{2+} spark almost always transiently activates a group of BK channels. Interference with spark

formation or BK channel opening prevents membrane repolarization and causes VSMC contraction. Thus, attenuation of the local release of Ca^{2+} from internal stores by agents such as ryanodine and thapsigargin induces vessel constriction. Similarly, iberiotoxin, which selectively blocks the opening of BK channels, causes membrane depolarization and vasoconstriction in pressurized isolated vessels.

BK channels may serve as negative feedback regulators of vascular tone by linking membrane depolarization and local Ca^{2+} sparks to repolarizing STOCs. To assess this hypothesis *in vivo,* mice with a disrupted BKβ1 gene were generated. Cerebral artery VSMCs from BKβ1 -/- mice generated Ca^{2+} sparks of normal amplitude and frequency, but STOC frequencies were largely reduced at physiological membrane potentials. Our results indicate that BKβ1 -/- mice have an abnormal Ca^{2+} spark/STOC coupling that is shifted to more depolarized potentials. Thoracic aortic rings from BKβ1 -/- mice responded to agonist and elevated KCl with an increased contractility. BKβ1 -/- mice had higher systemic blood pressure than BKβ1 +/+ mice but responded normally to α -adrenergic vasoconstriction and nitric oxide-mediated vasodilation. We propose that the elevated blood pressure in BKβ1 -/- mice serves to normalize Ca^{2+} spark/STOC coupling for regulating myogenic tone. These results are consistent with the idea that Ca^{2+} sparks in arterial smooth muscle cells limit myogenic tone through activation of BK channels. The activation of BK channels by Ca^{2+} sparks reduces the voltage-dependent Ca^{2+} influx and $[Ca^{2+}]_i$ through tonic hyperpolarization. Deletion of BKβ1 disrupts this negative feedback mechanism, leading to increased arterial tone through an increase in global $[Ca^{2+}]_i$.

[1]

4. Correlations of cardiac potassium channel activity and arrhythmias

Patrick Friederich, Susan Hauenschild, Dirk Isbrandt, Axel Neu, Lars Schlichting, Nicole Schmitt, Martin Schwarz*, Anna Solth*

Cardiac arrhythmias are a leading cause of morbidity and mortality in the industrialized world. Mostly, they are due to abnormalities in the cardiac action potential. Shaping of cardiac action potentials depends on a finely tuned orchestra of ion channels. Our research interest focuses on the molecular basis of inherited and acquired cardiac disorders and the study of ion channel function. Mainly, we investigate the molecular basis of long QT syndromes (LQTS) and of sinus node dysfunction (SND).

Long QT syndrome

The long QT syndrome 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 death. The LQT1 locus (*KCNQ1*) has been correlated with the most common form of inherited LQTS. The *KCNQ1* gene encodes KvLQT1 α-subunits, which together with the auxiliary K^+ channel β-subunit IsK (*KCNE1*, minK) form a native cardiac K^+ channels that regulates the slowly delayed rectifier potassium current I_{Ks}. Mutant KvLQT1 subunits may be associated either with an autosomal dominant form of inherited LQTS, Romano-Ward syndrome (RWS), or an autosomal recessive form, Jervell and Lange-Nielsen syndrome (JLNS). In addition to the cardiac phenotype, JLNS patients suffer from severe bilateral congenital deafness. We

have identified a small domain between residues 589 and 620 in the KvLQT1 C-terminus, which may function as an assembly domain for KvLQT1 subunits. KvLQT1 C-termini do not assemble and KvLQT1 subunits do not express functional K^+ channels without this domain. We showed that a JLNS deletion-insertion mutation at KvLQT1 residue 544 eliminates important parts of the C-terminal assembly domain. Therefore, JLNS mutants may be defective in KvLQT1 subunit assembly. The results provide a molecular basis for the clinical observation that heterozygous JLN carriers show slight cardiac dysfunctions and that the severe JLNS phenotype is characterized by the absence of KvLQT1 channel.

In addition to mutations in the *KCNQ1* gene coding for KvLQT1 α-subunits, the disease is caused by mutations in *KCNE1* coding for the small β-subunit IsK. We used single-strand conformation polymorphism and sequencing techniques to identify novel *KCNE1* mutations in patients with a congenital LQT syndrome of unknown genetic origin. In 150 unrelated index patients a missense mutation (V109I) was identified that significantly reduced the wild-type I_{Ks} current amplitude (by 36%) when coexpressed with KvLQT1 in *Xenopus* oocytes. Other biophysical properties of the I_{Ks} channel were not altered. Since we observed incomplete penetrance (only one of two mutation carriers could be diagnosed by clinical criteria), and the family's history was unremarkable for sudden cardiac death, the V109I allele most likely causes a mild phenotype. This finding may have implications for the occurrence of "acquired" conditions for ventricular arrhythmias and thereby the potential cardiac risk for asymptomatic mutation carriers still remains to be determined.

Sinus node dysfunction

Sinus node dysfunction (SND) is the major cause necessitating pacemaker implantation and accounts for approximately half of all cases. The disease commonly occurs in adults with acquired heart disease, as unwanted, but reversible side-effect during antiarrhythmic therapy or in children with congenital heart disease, particularly following corrective cardiac surgery. The term SND (also referred to as „sick sinus syndrome") is applied to a number of sinus nodal and atrial abnormalities. A familial occurrence of SND and lone atrial fibrillation has been reported and suggests the presence of genetic factors in its pathogenesis.

Cardiac pacemaker activity in the sinus node depends on a slow phase of membrane depolarization between action potentials which is mediated by a variety of ion currents. Among these, the hyperpolarization-activated cationic current I_f (synonymously named I_h) is a key component for the control of rhythmic pacemaking activity. Recently, four members of a gene family encoding hyperpolarization-activated cyclic nucleotide-gated cation channels (named *hHCN* genes) have been cloned. The properties of the *in vitro* expressed channels functionally resemble those of human pacemaker channels. Two of the known *hHCN* genes, *hHCN2* and *hHCN*4, are prominently expressed in human heart. Furthermore, it has recently been shown that *hHCN4* expression is most abundant and enriched in the sinus node area of rodent or rabbit heart. This observation makes *hHCN4* a likely candidate gene for encoding subunits of cardiac pacemaker I_f channels in the sinus node. We were able to identify a heterozygous mutation in the *hHCN4* gene in a patient with typical 'idiopathic' SND. Due to a frameshift mutation, the predicted hHCN4 protein sequence is altered such that the mutant protein will contain a truncated C-terminus and therefore lack the cAMP-binding domain (cNBD). Gene expression studies demonstrated the inability of the mutant channel to mediate increased I_f amplitudes in response to higher intracellular cAMP

concentrations. The impairment of I_f channel function is likely to explain the observed cardiac phenotype. We showed for the first time that genetic alterations in a pacemaker channel gene are associated with disturbances in rhythmic impulse activity and that 'idiopathic' SND represents a novel cardiac ion channel disorder.

5. Structural analysis of toxin receptor sites of potassium channels

Florian Albers, Jan Ebbinghaus, Christian Legros, Thomas Licher, Andreas Nolting*

During the last decade, attention has been focused upon the importance of T-cell K^+ channels as potential pharmaceutical targets for modulating immune system function. In particular, the blockade of the voltage-gated K^+ channel encoded by Kv1.3 inhibits T-cell activation, lymphokine secretion, and cell proliferation. Kv1.3 is very scorpion toxin-sensitive. These toxins constitute useful molecular tools to study physiological and structural properties of voltage-gated potassium (Kv) channels. They form a class of basic peptides containing 30 to 40 amino acid residues highly reticulated with three or four disulfide bridges. They are structurally related, sharing a characteristic backbone fold called the cysteine-stabilized α/β motif. The toxins bind with a 1:1 stoichiometry and may inhibit Kv channel activity by plugging the external pore entryway. Amino acid residues between hydrophobic transmembrane segments S5 and S6 (the S5-S6 linker region with the P domain) form the receptor site for scorpion toxin as well as the outer entrance to the Kv channel pore and a substantial part of the ion conduction pathway. Based on the hypothesis that complementary surfaces of scorpion toxin and Kv channel interact, the known three-dimensional structure of scorpion toxins has been exploited to study the topology of the external mouth of Kv channels, e.g. *Shaker* and Kv1.3. The crystal structure of the bacterial K^+ channel, KcsA, and subsequent mutagenesis have revealed a high structural conservation from bacteria to human. We have explored this conservation by swapping subregions of the M1-M2 linker of KcsA with those of the S5-S6 linker of the human Kv-channel Kv1.3. The chimeric K^+ channel constructs were expressed in *Escherichia coli*, and their multimeric state was analyzed after purification. We used two scorpion toxins, kaliotoxin and hongotoxin 1, which bind specifically to Kv1.3, to analyze the pharmacological properties of the KcsA-Kv1.3 chimeras. The results demonstrate that the high affinity scorpion toxin receptor of Kv1.3 could be transferred to KcsA. Our biochemical studies with purified KcsA-Kv1.3 chimeras provide direct chemical evidence that a tetrameric channel structure is necessary for forming a functional scorpion toxin receptor. We have obtained KcsA-Kv1.3 chimeras with kaliotoxin affinities (IC_{50} values of ~4 pM) like native Kv1.3 channels. Furthermore, we showed that a subregion of the S5-S6 linker may be an important determinant of the pharmacological profile of K^+ channels. Using available structural information on KcsA and kaliotoxin, we have developed a structural model of the complex between KcsA-Kv1.3 chimeras and kaliotoxin to aid future pharmacological studies on K^+ channels.

6. Structure and function of Frequenin

Jens Dannenberg, Birgit Grafelmann

Like the KChIPs, Frequenin (Frq) 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. *Drosophila* Frq has been implicated in the facilitation of neurotransmitter release at neuromuscular junctions. Overexpression of Frq in *Drosophila* mutants leads to a pronounced facilitation of neurotransmitter release, that dramatically depends on the frequency of stimulation. Similarly, overexpression of Frq in PC12 cells evokes an increased release of growth hormone in response to agonists like ATP. Comparable observations had also been reported for *Xenopus* Frq suggesting that Frq participates in vesicle secretion. Taken together, these results are consistent with the idea that Frq activity and regulated exocytosis are coupled.

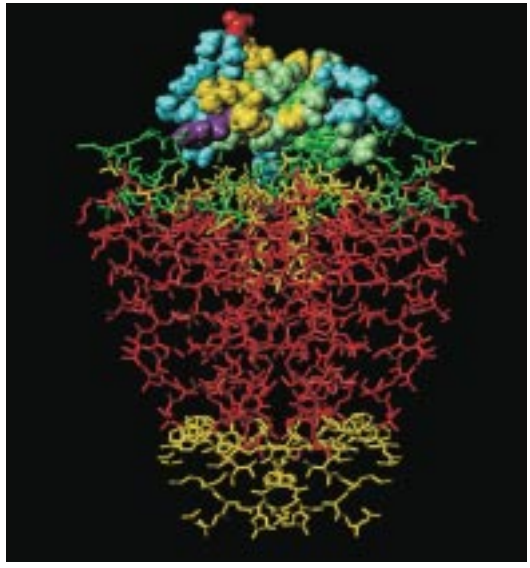

Figure 1. Three-dimensional model of Kaliotoxin- KcsA-Kv1.3 receptor complex. Kaliotoxin is shown as spacefilling model on top. Membrane inserted part of KcsA-Kv1.3 chimera is in red (for details see ref. 16).

Frq is a highly conserved protein, that is expressed in eukaryotic cells from yeast to man. Recently, it has been shown that yeast Frq serves as a subunit of phosphatidylinositol 4-OH kinase PIK1, that regulates secretion at the Golgi. Likewise, ARF1 has been implicated in multiple trafficking events including recruitment of the mammalian PIK1 homolog, PI4Kß, to Golgi membranes. We found that in mammalian cells Frq is localized to the Trans Golgi Network, where it displays a similar distribution like the GTPase ARF1. It is likely that mammalian Frq may fulfill similar functions like the yeast homolog and may be involved in secretion from the Golgi.

Using the two-hybrid system we isolated MLK2 as a Frq-interacting protein. MLK2 belongs to the family of MAPKKKs. By a combination of biochemical assays a minimal domain responsible for calcium-independent binding of Frq has been identified. MLK2 is known to be associated with motor proteins of the kinesin family that are involved in transport of organelles and vesicles. We are investigating the regulation of MLK2-activity by Frq and its consequences for vesicle trafficking.

In order to understand the molecular basis of Frq-function we purified the recombinant protein to homogeneity and crystallized it in the calcium-bound state. Using X-ray diffraction analysis the structure of human Frq was refined to 1.9Å. The overall topology of Frq is similar to related proteins like neurocalcin with three functional calcium binding sites. Furthermore, the structural analysis of Frq revealed the existence of a novel hydrophobic crevice, which is absent in other NCS proteins. To our knowledge, this domain is unique for Frq and may serve as a specific binding region for interacting proteins.

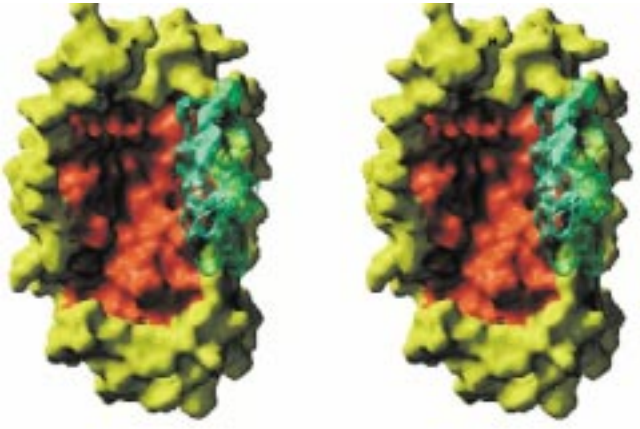

Figure 2. Structure of human Frq.
Molecular surface of human Frequenin, viewed down the large hydrophobic crevice (*orange*).The C-terminal helix J in human Frq (*cyan*) is displayed (for details see ref. 25).

7. Generation of a knockout mouse model for guanidinoacetate N-methyltransferaes (GAMT) deficiency

Dirk Isbrandt, Axel Neu, Andreas Schmidt

Guanidinoacetate methyltransferase (GAMT) deficiency belongs to the family of "creatine disorders" and is an autosomal recessively inherited disease of creatine biosynthesis. It manifests during the first months of life as developmental delay or arrest. Neurological symptoms are heterogeneous, including muscular hypotonia and weakness, poor head control, involuntary extrapyramidal movements and epilepsy, as well as autistic behaviour and absence of active speech development in older patients.

The neurological abnormalities observed in GAMT deficiency might be explained partially by the deficiency of high-energy phosphates in cells with high and fluctuating energy demands, while others appear to be related to the accumulation of the metabolite guanidinoacetate (GAA), which exhibits strong GABAergic properties when applied to neurons in acute murine brain slices.

In order to investigate the pathophysiology of GAMT deficiency using a mouse model, the murine GAMT gene was disrupted in exon 1 by homologous recombination in embryonic stem cells. Northern and Western blot experiments with liver preparations of GAMT-deficient animals proved the absence of GAMT-specific mRNA as well as of GAMT protein. GAMT knockout mice are viable but show an increased neonatal lethality, a marked developmental delay and reduced fertility. Analysis of the guanidino compounds in serum, urine and brain revealed creatine deficiency and accumulation of GAA as observed in the human disease, namely a 14-fold increase in GAA excretion in urine and a 10-fold decrease in creatinine excretion. Proton magnetic resonance spectroscopy (^{1}H-MRS) analysis of GAMT knockout mice revealed a dramatic reduction of total cerebral and muscular creatine levels compared to wild type animals. Phosphorus magnetic resonance spectroscopy (^{31}P-MRS) in addition showed an accumulation of guanidinophosphoacetate in both tissues analysed, which was more pronounced in muscle. In contrast, ATP/ADP and P$_i$ concentrations remained unchanged, which is most likely attributable to a compensatory up-regulation of ATP synthase in brain of GAMT-deficient animals to 175% of the wild type. This corresponds very well with the creatine-dependent regulation of ATP synthase observed in GAMT-deficient skin fibroblasts cultured under creatine-deficient or creatine-supplemented conditions.

In summary, GAMT knockout mice represent a suitable animal model to study the human pathophysiology in GAMT deficiency as well as the physiological role of creatine in different tissues or organs.

Support

The work in our laboratory was supported by grants of the Deutsche Forschungsgemeinschaft, the EU, the Fonds der Chemischen Industrie, GIF, the LeDucq-Foundation, the PROCOP-programme and the BMBF.

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Neuronal cell fate specification

Ingolf Bach

Our laboratory is interested in the molecular mechanisms that underlie neuronal cell fate specification events during embryogenesis. As a model system we are investigating the cofactor-mediated regulation of the biological activity conferred by LIM homeodomain transcription factors during neurogenesis using molecular, biochemical and genetic methods.

The regulation of gene expression is a fundamental biological process controlled by transcription factors. The interactions of distinct cofactor complexes with transcription factors are decisive determinants for the regulation of transcription factor activity and in the past few years the number of identified cofactors has increased drastically. It has been shown that different cofactor complexes can bind to a single class of transcription factors and depending on the bound cofactor complex, a given transcription factor can either activate or repress gene expression.

The LIM domain is an evolutionary conserved protein motif consisting of two zinc fingers that has been demonstrated to promote protein-protein interactions. LIM domains are found in several types of proteins including the LIM-only (LMO) class of nuclear proteins and the LIM homeodomain (LIM-hd) transcription factor family (Fig. 1). LMO proteins consist mainly of a LIM domain, and have been identified as oncogenes. The overexpression of LMO proteins in T-cells that normally express only low LMO levels results in T-cell leukemia (T-ALL). Besides the LIM domain, LIM-hd proteins contain an additional homeo-domain. This homeodomain motif is responsible for the protein's ability to recognize and bind to specific DNA sequences. LIM-hd transcription factors have been demonstrated to confer cell lineage identity and to be responsible for cell fate determination events during the development of organisms as divergent as *Drosophila* and higher vertebrates. The development of motor, inter- and touch receptor neurons has been shown to be dependent on the activity of various LIM-hd proteins. Furthermore, LIM-hd proteins are essential for the formation of many other neuronal and non-neuronal structures such as fore-, mid- and hindbrain, anterior pituitary, eye and limbs. Recent work indicates that the biological activity of LIM-hd transcription factors is regulated by LIM domain-associated cofactors CLIM and RLIM.

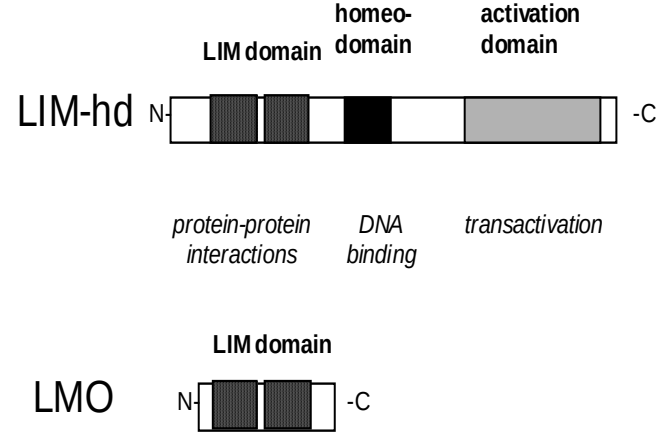

Fig. 1: Structure of nuclear LIM domain-containing proteins. The LIM homeodomain class of transcription factors (LIM-hd) and LIM-only proteins (LMO) are shown.

1. LIM homeodomain transcription factors (LIM-hd)

Because of the crucial and conserved roles mediated by LIM-hd proteins for cell fate specification and organ development during embryogenesis, we searched for new members of this transcription factor family. In a collaborative effort we have identified the new LIM-hd gene *Lhx9* by an RT-PCR based approach using degenerate primers that correspond to conserved homeodomain sequences. Sequence analysis indicated that Lhx9 protein contains a LIM and homeodomain that displays particularly high homologies with Lhx2. We showed by *in situ* hybridizations that mRNA encoding Lhx9 is expressed in pioneer neurons located in several embryonic regions of the developing nervous system, e.g. fore-, mid- and hindbrain and the spinal cord. In the developing nervous system, specific territories called neuromeres were identified on the basis of mitotic zones and fate maps and on the expression patterns of many developmental genes. We found that the expression patterns of Lhx9 mRNA as well as transcripts encoding the LIM-hd proteins Lhx1 to Lhx8 all respected the proposed neuromeric boundaries. Outside the nervous system Lhx9 mRNA is expressed in the forelimb and hindlimb mesenchyme and the urogenital system and can be detected as early as embryonic day (E) 10.5 during mouse development. Thus, the overall spatiotemporal expression pattern of Lhx9 transcripts is similar but distinct from Lhx2. Protein interaction studies revealed that the LIM domain of Lhx9 is able to interact with the CLIM cofactor family. Taken together, these results imply important developmental functions of Lhx9 for several neuronal and non-neuronal organs.

2. CLIM cofactors

The dimer-forming CLIM (cofactor of LIM-hd proteins) cofactor family, consisting of CLIM1 and CLIM2, has been isolated by virtue of its ability to interact with the LIM domains of LIM-hd and LMO proteins. We have previously shown that CLIM and LIM-hd proteins associate *in vivo* with high affinity and form protein complexes on DNA. These protein interactions are required for synergistic gene activation events mediated by LIM-hd proteins *in vitro*. For an analysis of the biological function of CLIM cofactors we have generated a dominant-negative CLIM (DN-CLIM) molecule that can compete with wild-type CLIM for binding to LIM domains. By overexpressing DN-CLIM protein in developing chick wings we have demonstrated that the association of CLIM cofactors with LIM-hd transcription factors is required for the development of distal extremities in vertebrates. Likewise, the *Drosophila* CLIM orthologue Chip has been shown to be crucial for the exertion of the activity of the LIM-hd protein Apterous during wing development, with cellular Chip protein concentrations being decisive determinants. Furthermore, other laboratories have shown that the overexpression of nuclear LMO proteins in the *Drosophila* wing also result in an inhibition of LIM-hd protein activity, presumably by competing away endogenous Chip/CLIM cofactors that are present in limiting amounts (see Figure 1). Thus, both genetic and biochemical results place the CLIM/Chip cofactor family in an essential position for the control of biological LIM-hd protein activity.

The wide mRNA expression pattern of CLIM cofactors in many embryonic regions suggested important functions of CLIM cofactors for the development of other organs and cell types in addition to limbs. Indeed, our recent results obtained by overexpressing the DN-CLIM molecule early during zebrafish development indicated that CLIM cofactors are also important

for proper development of the eyes and the midbrain/hindbrain boundary. The fact that eye development has previously been demonstrated to be dependent on the expression of functional LIM-hd protein Lhx2 suggests a requirement of CLIM cofactors for Lhx2 function. Furthermore, we have identified additional functions of CLIM cofactors in zebrafish for the axonal outgrowth of specific primary sensory and motor neuron subtypes. Interestingly, DN-CLIM overexpression inhibited the development of peripheral but not central axonal projections. Furthermore, the synthesis of LIM-hd protein Isl1 in specific neurons that normally do not express this protein was induced upon DN-CLIM overexpression.

It has been noted that several growth factors are detected in regions that also express LIM homeodomain genes, e.g. members of the fibroblast growth factor (Fgf) family. In a collaborative study we have identified Fgf-8 as an inducer of the LIM homeodomain genes *Lhx6* and *Lhx7* as well as *CLIM2* cofactor expression in developing face and limb buds. Since Fgf-8 is expressed in several other tissues during embryogenesis, these results suggest that Fgf-8 is also responsible for induction/maintenance of CLIM2 expression in other organs, such as the anterior pituitary gland and specific regions of the developing nervous system.

3. RLIM corepressor

We have identified the nuclear RING H2 zinc finger protein RLIM (RING zinc finger LIM domain-binding protein) as a LIM domain-interacting protein that, like CLIM cofactors, can associate with all known LIM-hd and LMO proteins *in vivo*. Besides LIM domains, RLIM is able to interact with CLIM cofactors and with members of the histone deacetylase corepressor (HDAC) complex *in vivo.* In transient cotransfection experiments RLIM inhibits the transcriptional activation capacity of LIM-hd factors, presumably by the recruitment of the HDAC corepressor complex. Furthermore, overexpressing RLIM during chick wing development results in an inhibition of distal wing development. This phenotype and the expression of gene markers affected by this overexpression are remarkably similar to the ones obtained by overexpressing dominant-negative CLIM. These results suggest opposing roles of the cofactors RLIM and CLIM for the regulation of LIM-hd activity.

Our analysis of the RLIM-encoding gene RNF12 revealed that it encompasses 20kb on mouse and human chromosome X, and that it can be activated by various transcription factor families in vitro. This characterization was a prerequisite for the targeted disruption of the Rnf12 gene by homologous recombination in mice, which we are currently pursuing in the laboratory.

Cellular proteins targeted for degradation are ubiquitinated by a cascade of enzymes involving ubiquitin-activating enzymes, ubiquitin-conjugating enzymes, and ubiquitin protein-ligases with the latter enzyme being responsible for the substrate specificity of the ubiquitination reaction. Ubiquitinated proteins are recognized by the 26S proteasome and rapidly degraded. Prompted by the finding that ubiquitin protein-ligases often contain a RING zinc finger motif, we have identified RLIM as a ubiquitin protein-ligase, able to ubiquitinate itself, CLIM and LMO proteins, but not LIM-hd factors *in vitro*. We have demonstrated that RLIM is also able to ubiquitinate CLIM cofactors present in a LIM-hd/CLIM complex. RLIM-mediated LMO ubiquitination can be inhibited by addition of CLIM cofactors, showing a competition of RLIM and CLIM at the level of binding to LIM domains. In cotransfection experiments RLIM was able to induce degradation of CLIM and LMO, but not LIM-hd proteins *in vivo*. This proteolytic activity was

inhibited by mutations in the RING zinc finger of RLIM or by addition of specific inhibitors of the 26S proteasome. Thus, RLIM is a ubiquitin protein-ligase, able to target CLIM and LMO proteins for proteolytic degradation.

By investigating the protein-protein interaction domains we identified a basic domain (BD) in RLIM as responsible for the interactions not only with LIM domains, but also with the dimerization domain (DD) of CLIM cofactors. Although CLIM proteins bound with much higher affinity to LIM domains than RLIM *in vitro*, we demonstrated in ChIP experiments that RLIM was nevertheless able to remove CLIM cofactors from LIM-hd proteins bound to DNA in transfected cells, promoting the formation of an RLIM/LIM-hd complex. This activity was dependent on the presence of a functional RING finger on RLIM. Since the RING finger of RLIM is essential for CLIM ubiquitination and degradation, these experiments directly connect cofactor exchange on LIM-hd proteins with the ubiquitin-protein ligase activity of RLIM. These data place RLIM in a central position for the developmental control of cellular CLIM cofactor levels and lead to the proposed model of LIM-hd regulation. The further ability of RLIM to control protein concentrations of LMO oncogenes argues for an additional role of RLIM in tumor formation. Taken together, these results provide a mechanistic basis for a cofactor exchange on DNA-bound transcription factors.

Support

The work in our laboratory is supported by the Deutsche Forschungsgemeinschaft (grants BA1842/1-3, BA1842/2-1,BA1842/3-1, SFB444 and GRK255).

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Habilitation

Bach, Ingolf (2000). Funktionelle Charakterisierung und entwicklungsbiologische Regulation von LIM Homeoproteinen in Vertebraten. Universität Hamburg.

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Synaptic Plasticity: Learning about Activity-dependent Genes

Dietmar Kuhl

The main goal of my laboratory is to bring to bear molecular biological approaches on the identification and study of genes contributing to synaptic plasticity in the mammalian brain. Analysis of their expression and regulation indicates a broad role for these genes in neuronal plasticity, including learning and memory, epilepsy, and mental diseases. Several of the genes recently identified in our laboratory code for proteins that can directly modify the function of neurons and consequently represent promising targets for therapeutic intervention. Our research moves from the identification of activity regulated genes to the analysis of long term potentiation in the brain and wants to assess which consequences they convey on the behavior of animals and their capability to learn and store memories.

1. Transcriptional profiling of activity-dependent genes

Neurons have the capacity to undergo activity-dependent changes in their molecular composition and structure in order to adjust their synaptic strength. Such synaptic plasticity appears to contribute to a variety of physiological and pathological processes in the adult brain including learning and memory, epileptogenesis, ischemia, drug abuse, and neurological diseases. Since enduring forms of synaptic plasticity require gene induction that is important in defining neuronal connectivity in the brain, it is anticipated that many forms of mental disabilities, including neurodegenerative processes and cognitive disturbances will be understood as cortical or limbic cognates of disturbed activity-dependent gene transcription. We have undertaken a systematic survey to gain a more complete understanding of the genomic response that occurs in hippocampal and cortical neurons as result of synaptic activity (see e.g. Kuhl, D. (2000) In: Advances in Synaptic Plasticity. Cambridge, MA: The MIT Press). We are currently extending these approaches by using our conceptual and experimental tools in conjunction with genome–wide transcriptional profiling. Our aim is the comprehensive identification of genes that experience activity-dependent transcriptional control. We anticipate that this analysis will provide insights into how expression of genes that are activated in coordinated biochemical pathways may contribute to the formation of synaptic plasticity. In as much the identified genes bear the potential to act as direct effectors of neuronal function, they become promising targets for the therapeutic intervention of a variety of diseases that involve disturbances of synaptic plasticity.

2. The role of Pim kinases in synaptic plasticity

Using newly developed subtractive cloning protocols (Konietzko, U. and Kuhl, D. (1998) Nucleic Acids Res. 26, 1359-1361) we identified the proto-oncogene Pim-1 as a gene that is rapidly induced by plasticity-producing stimulation in the brain and is instrumental in the formation of enduring LTP (Konietzko et al. (1999) EMBO J. 18, 3359-3369). Previously, Pim-1 (provirus integration site for Moloney murine leukemia virus) and Pim-2 were identified as a serine/threonine kinase

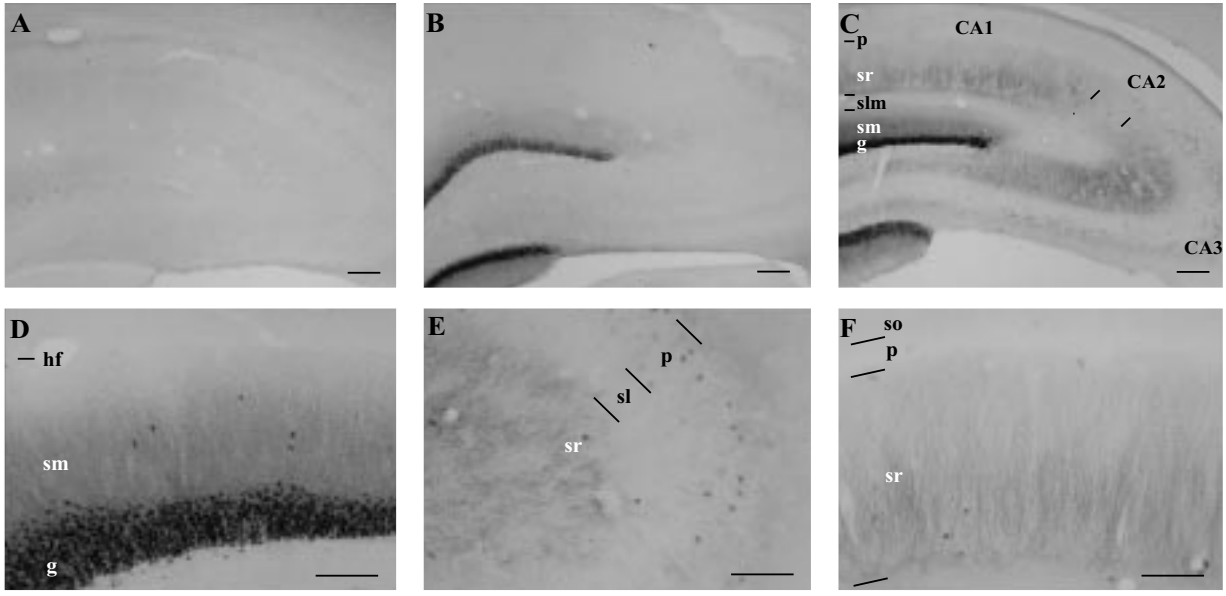

Figure 1. Pim-1 protein is localized to neuronal soma and dendrites of stimulated hippocampal neurons. Hippocampus, cortex, piriform cortex, and striatum of control and PTZ or KA-stimulated rat brain (4 h survival). (A) In control hippocampus a few somata and dendrites show immunoreactivity. (B) After PTZ-induced seizure Pim-1 immunoreactivity is dramtically increased in the granule and molecular layer of the dentate gyrus. (C) After KA-induced seizures immunostaining can in addition be detected in region CA1 and CA3 with prominent staining of dendritic processes. (D-F) High power views of Pim-1 immunoreactivity after KA-induced seizures in (D) granule cells of the dentate gyrus, (E) hippocampal field CA3 and (F) hippocampal field CA1. Sections from the same animal incubated with a serum depleted of Pim-1-specific antibodies had no staining (not shown). Scale bars: (A-C) 200 μm; (D-F) 100 μm. Abbreviations: g, granular layer; hf, hippocampal fissure; p, pyramidal cell layer; sl, stratum lucidum; slm, stratum lacunosum moleculare; sm, stratum moleculare; so, stratum oriens; sr, stratum radiatum.

with oncogenic potential in cells of the different hematopoetic lineages. In addition, by homology screening we identified a third member of this family, pim-3. We find that the pim kinases differ in their regional expression in the brain and their responses to physiological and pathological forms of synaptic activity. Whereas pim-2 is unresponsive to synaptic activity, the expression of both pim-1 and pim-3 is induced by such stimulation. Pim-1 induction is already detectable following weak activity, while that of pim-3 requires higher intensity stimulation. Notably, Pim-1 RNA and protein are virtually undetectable in the unstimulated hippocampus but strongly induced by synaptic activity; the protein being distributed to the nuclei and dendrites of activated neurons (Fig. 1). Pim-1 was consistently induced with LTP producing stimulation,

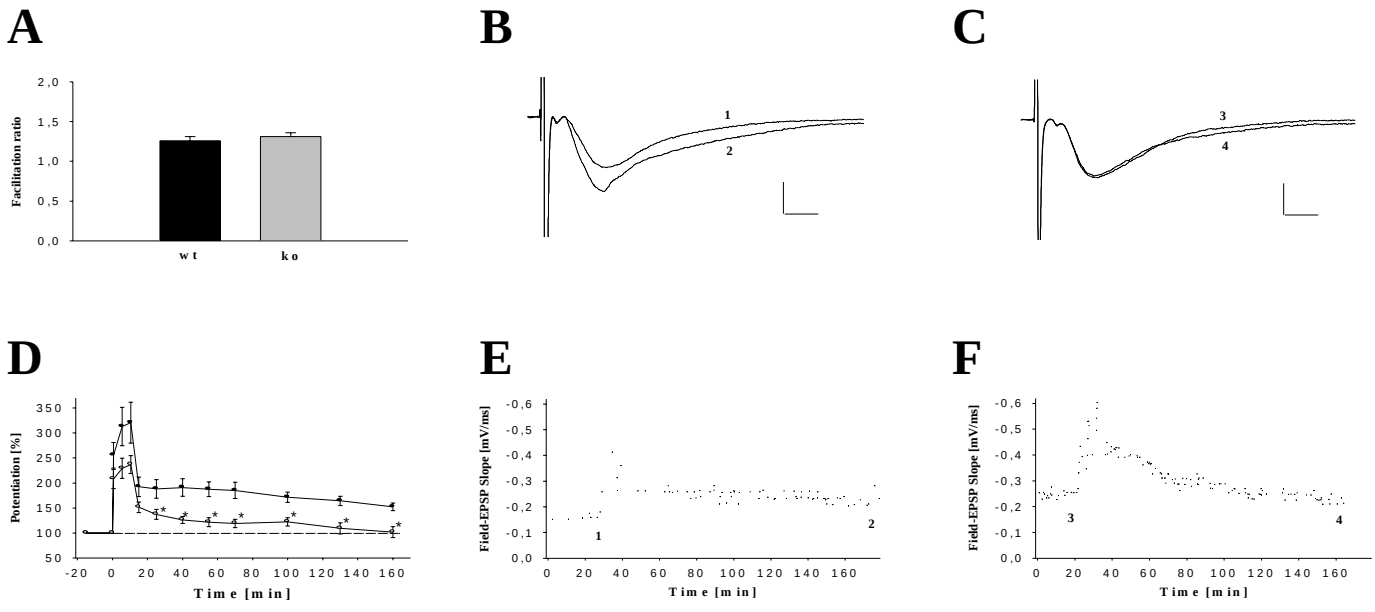

Figure 2. Electrophysiological analysis of hippocampal slices reveals rapidly decaying LTP in Pim-1 knockout mice. Extracellular field potentials were recorded in the stratum radiatum of field CA1. (A) Paired pulse facilitation calculated from the ratio of the second fEPSP slope to the first fEPSP slope at an interpulse interval of 100 ms. Wild-type (wt) mice (n = 10 inputs) and knockout (ko) mice (n = 12 inputs) did not differ significantly in their facilitation ratio. (B) Sample fEPSP traces before and 2.5 hours after the induction of LTP in wild-type mice taken from the experiment shown in (E). (C) Sample fEPSP traces at similar time points in Pim-1 knockout mice taken from the experiment shown in (f). Scale bars in (b) and (c) 0.4 mV and 5 ms. (D) Summary graph of mean fEPSP slope measurements up to 2.5 hours after the induction of LTP with three 100 Hz tetani. Potentiation is expressed as percent of baseline recordings prior to tetanization. The fEPSP slope potentiation of wild-type (n = 7 slices, filled circles) and knockout (n = 6 slices, open circles) mice is plotted against time with the first tetanus assigned to the zero timepoint (Error bars denote S.E.M.). The first three timepoints after tetanization refer to the strongest fEPSP response after each tetanus (posttetanic potentiation). For the following timepoints the average of seven traces from each experiment was used for analysis. Potentiation at the timepoints marked by a star differs significantly between wild-type and knockout mice ($p < 0.05$, Students t-test). In knockout mice LTP is severely impaired. (E) Original recording from a wild-type animal shows stable potentiation of the fEPSP slope after the application of three tetani. Numbers indicate the time points of the traces shown in (B). (F) Similar recording from a knockout animal shows intact PTP and a marked decay in the potentiation of the fEPSP. Numbers indicate the time points of the traces shown in (C).

suggesting that it may be required for this potentiation. To examine the functional role of the pim-1 protein kinase in LTP we analyzed genetically engineered mice deficient for pim-1. These animals develop normally, are fertile, have a normal life span, and our histological analysis does not reveal any gross anatomical abnormalities in hippocampus or other regions of the brain. These mice show normal forms of short-term plasticity, but have lost the ability to establish enduring LTP (Fig. 2). Therefore pim-1 is a nuclear and dendritically localized protein kinase, the expression of which is both induced by and required for LTP. Such properties set pim-1 apart from other kinases previously implicated in LTP. Both serine/threonine and tyrosine specific protein kinases have been implicated in the induction and maintenance of LTP. It has also been proposed that post-translational modifications altering the activity of kinases and kinase regulating proteins may act in the transfer of a short lived LTP to longer lasting potentiations. While Pim-1 can be post-translationally modifed by autophosphorylation, the most remarkable feature of Pim-1 regulation is its dramatic induction at mRNA and protein levels in response to synaptic activity. Thus, the history of a neurons synaptic activity is reflected in the expression level of this kinase. Such mnemonic properties, its localization to the nucleus and dendrites, and the demonstration of its requirement for the consolidation of enduring LTP indicate that Pim-1 may play a pivotal role in regulating the functional changes underlying long-term synaptic plasticity.

3. The role of Polo-like kinases in synaptic plasticity

The polo-like kinases are a family of serine/threonine specific protein kinases, that like Pim-1 are induced as immediate early genes in non-neuronal cells. Guided by this observation and the conceit that certain aspects of cell cycle regulation and differentiation might be co-opted by the brain to serve functional plasticity we examined the influence of neuronal activity on the expression of the polo-like kinases in brain (Kauselmann et al. (1999) EMBO J. 18, 5528-5539). In mammals this family consists of three members, Plk, Fnk, and Snk. Whereas the function of Snk is less clear, Plk and Fnk have been implicated in the control of multiple stages of cell division. Our studies suggest a role for Fnk and Snk outside the cell cycle. Whereas we do not detect expression of Plk in the brain, both Fnk and Snk are constitutively expressed in post-mitotic neurons. Moreover, stimuli that induce seizures or LTP result in a dramatic increase in the synthesis of Fnk and Snk mRNA. This increase is reflected in a concomitant increase of Fnk and Snk protein in somata and dendrites of activated neurons. In yeast Two-hybrid and biochemical studies we find that both kinases interact with Cib, a protein previously shown to bind to Ca^{2+} and the cytoplasmic tail of integrin αIIb. Integrins are membrane spanning, non covalently bound heterodimers that form transmembrane links between the cellular matrix and the actin cytoskeleton. They are frequently clustered into specialized adhesive structures, in which numerous signaling components are concentrated. However, the molecular identity of such signaling centers in the mature nervous system is not known. Further information will be required to determine if Fnk, Snk and Cib are indeed components of such centers and if they mediate signaling in response to Ca^{2+} influx during NMDA receptor-mediated synaptic plasticity. The dramatic increase of Fnk and Snk following specific patterns of synaptic activity, the translocation of the kinase proteins to dendritic arbors and their association with a Ca^{2+} and integrin binding protein suggests that this might be the case.

4. The role of Sgk in synaptic plasticity and neuronal degeneration

Sgk is a kinase that is thought to regulate the mineralocorticoid regulated sodium transport in the kidney. Subtractive cloning protocols allowed us to identify Sgk as a kinase that is dramatically induced with seizures in brain. We find that this induction occurs in two different cell types and is regulated by two independent mechanisms. In oligodendrocytes induction is dependent on glucocorticoid release, whereas in dentate granule neurons transcriptional activation is independent of glucocorticoids but strictly dependent on synaptic activity. Moreover, we find that sgk can increase the conductance of a specific potassium channel by more than fourfold which suggests a functional role of sgk in the repolarization of neurons following synaptic stimulation. These are entirely novel findings with implications for mechanisms of neuropathological processes in brain. Based on these observations we have now generated a complete knockout of sgk as well as animals in which we can delete sgk in a cell-type specific manner. These animals are currently subject of our analysis and will allow us to better understand the specific role of sgk in neurons and oligodendrocytes. Beyond this, we find that animals carrying a complete knock out are impaired in renal sodium retention and, therefore, might prove useful as genetic model of kidney function and disease.

5. The role of arg3.1 in synaptic plasticity and the formation of memories

Although the above experiments establish a link between gene expression and physiological and pathological neuronal plasticity, it remains an open question how transcriptional activation taking place in the nucleus can selectively modify stimulated synaptic sites in the distant dendritic compartment of the neuron. Such selective modifications of synapses that have experienced coincident activity might play an important role in the initiation of Hebbian associative synaptic modifications during long-term potentiation and memory formation. The consolidation of these modifications during the maintenance of memory might then require the local translation of dendritic mRNAs at activated dendritic sites or even single synapses; this could ultimately result in permanent changes in dendritic function, mediated by reorganization of the post-synaptic density or even growth of spines and dendrites. As the requisite coincidence detection occurs only at active synapses, it would ensure the specificity of the resulting synaptic plasticity, an important consideration for the theoretical construct of the Hebbian synapse. Accordingly, the main effort of my lab since the initial identification of arg3.1 (a.k.a. arc) has been to understand the functional role of this gene in NMDA-receptor mediated synaptic plasticity. Among activity-dependent genes arg3.1 is thus far unique, as its mRNA has the potential to be locally translated at stimulated synapses and consequently might play a key role in synapse specific modifications (Kuhl, D. and Skehel, P. (1998) Curr.Opin.Neurobiol. 8, 600-606). Importantly, arg3.1 is the first and only gene whose expression has been directly linked to information processing. Moreover, arg3.1 is reliably induced with LTP-producing stimulation and this inducibility is strictly dependent on the activation of PKA and the MAPK/ERK kinase signaling pathways which have been demonstrated to play specific roles in learning and memory and synaptic plasticity (Waltereit et al. (2001) J.Neurosci. 21, 5484-5493). To further study the functional role of arg3.1 in cognition we generated mutant mice deficient for arg3.1 expression. In these mice the formation of memories in a hippocampal specific learning task are strikingly impaired.

Our biochemical analysis demonstrates that arg3.1 is associated with the NMDA (N-methyl-D-aspartate) receptor. This association is not seen in animals that lack PSD95/SAP90, a protein that can directly bind to the NMDA-receptor. Thus, arg3.1 may act to maintain the structural or functional integrity of the NMDA-receptor complex, and thereby influence signaling pathways that control the magnitude and specificity of synaptic plasticity. We combine in this project a genetic approach with biochemical and physiological analysis to provide first molecular insights into one of the most challenging problems in modern neurobiology: how synapse specificity is achieved. Moreover, we pursue this line of research using our conditional arg3.1 knockout mice which we have recently succeeded in generating and will in the future generate various transgenic lines to rescue the cognitive impairment of arg3.1 mutant animals.

6. Dendritic transport of arg3.1 mRNA

While in the studies described above we show that arg3.1 has a key role in synapse specific modifications, nothing is known about the molecular mechanisms that govern arg3.1 mRNA transport into dendrites. Although dendritic mRNA localization is thought to be achieved by specific RNA-protein interactions, no targeting proteins have yet been identified for any dendritic RNA. To address this issue, we developed the Tri-hybrid Method for the in vivo reconstruction of specific RNA-protein interactions (see e.g. Putz et al. 2000 In Yeast Hybrid Methods. Natick, MA: Eaton Publishing). Using this technique in a genetic screen we identified several clones that specifically interact with arg3.1 mRNA but not with perikaryal-control RNAs. One of these proteins we have named Zinki because it contains a domain of repeated zinc fingers required for the specific binding to arg3.1 mRNA. The expression of Zinki is predominantly dendritic. Moreover, we find that upon dendritic lamina specific stimulation, Zinki vacates those dendritic regions in which translation of the arg3.1 mRNA is enhanced suggesting that Zinki may control translation of the arg3.1 mRNA in this compartment. We are currently creating a conditional zinki knock out mouse and have identified in interaction screens potential partners that interact in a variety of biochemical experiments with zinki in vivo.

7. Future perspectives

The several findings described above in conjunction with our various knock out animals will allow us to go in a variety of different directions. We would like to broaden our research and investigate mechanisms of plasticity that underlie drug addiction or when disturbed might be the cause of mental diseases, psychiatric disorders or play roles in epileptogenesis and ischemia. The main focus of our research, however, will remain on the analysis of learning and memory. Much progress has been made, within discrete levels of analysis, characterizing biophysical, molecular and cellular adaptations associated with plasticity and cognitive functions. However, it has proven difficult to integrate these findings and translate the specific knowledge at each level into an understanding of information processing and storage. A long-term goal of our research is to elucidate how mental functions emerge from specific changes at molecular levels. We see the use of mouse genetics as an important means of building bridges between molecular biology and systems neurobiology and between systems neurobiology and behavior. This provides the rationale for an integrated approach to follow the flow of information from excitatory events in the dendrite through neuronal networks in behaving animals. We hope in this way to extract some of the fundamental rules that govern dendritic information processing in the activity-driven refinement of networks that underlies learning and memory.

Support

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

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Development of the peripheral nervous system

Dieter Riethmacher

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

The tyrosine kinase receptors erbB2, erbB3 and erbB4 recognize the neuregulin family of ligands. By mutating the erbB2 and erbB3 genes in the mouse we have shown that the neuregulin signaling system is an important player in neural crest development. Most of the sympathetic nervous system does not form and Schwann cell precursors aligning axonal projections are virtually absent in mutant embryos. The exact mechanisms underlying these developmental defects are not understood. One major goal of this project is to analyze these mechanisms and identify molecules that become activated by the neuregulin signaling system. The identification of genes that are involved in differentiation, lineage segregation and mobility of neural crest cells will be of high importance for our understanding of peripheral nervous system development.

Another important determinant of neural crest cell development is the transcription factor sox10 and consequently its inactivation in mice leads to several defects in this lineage. Several genes are controlled by sox10 in neural crest cells and interestingly erbB3 is one of them. Phenotypical similarities between erbB3 and sox10 deficient embryos are therefore not surprising.

1. Identification of genes activated by the neuregulin signaling system in embryonic Schwann cells

Phenotypic analysis of erbB3 deficient mice revealed a dramatic reduction of Schwann cell precursors aligning axonal projections already early in development. We do not know the exact mechanism underlying this defect, nor do we know genes that are activated by the neuregulin signaling system in this lineage. We established a cell culture system of embryonic dorsal root ganglia that recapitulates this in-vivo finding and used it to identify genes that are downstream of erbB3 in Schwann cell precursors (Fig.1).

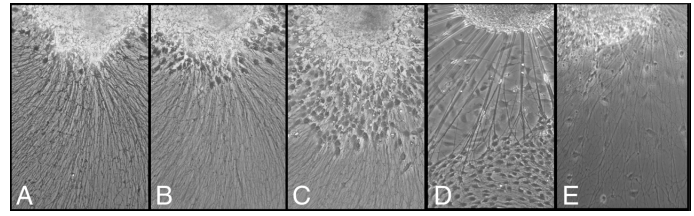

Figure 1. Dynamic of Schwann cell precursor emigration in cultures of embryonic (E12.5) dorsal root ganglia. Wildtype dorsal root ganglia are shown after 24h (A), 32h (B) and 48 h (C) in culture. The absence of Schwann cell precursors in cultures of erbB3 deficient dorsal root ganglia is obvious when wildtype (D) and erbB3 mutant (E) dorsal root ganglia are monitored after 84 h in culture.

The limiting amounts of RNA that can be recovered from our cultures forced us to amplify the RNA before we could carry out the PCR based subtractive hybridization method

(Clontech). The amplification step reduced the reliability of this technique and in our hands many of the identified clones could not be confirmed as differentially expressed. Among the clones that could be confirmed were the erbB3 and the BFABP (Brain fatty acid binding protein) gene.
Another approach we chose was the differential display technique. In order to minimize experimental variations we pooled the RNA´s from several embryos and performed the PCR reactions in doublets. We have isolated 58 differentially expressed fragments most of which are upregulated in the wildtype (45). We have grouped those that have been already identified into several families (structural, cell cycle, signaling, est, unknown) and are currently confirming the differential expression and completing the cDNA´s.

2. Functions of Sox10 in neural crest cells

Sox proteins belong to the HMG (high mobility group)-box-family of transcription factors. Sox10 is expressed in the emerging neural crest cells and is turned off soon in some derivatives but continues to be expressed in all peripheral glia. Moreover sox10 is expressed in oligodendrocytes the myelinating glia of the CNS. This expression starts already during embryogenesis when they are generated and is maintained into adulthood. Analysis of the natural occurring mouse mutant *dom* and the targeted mutant have revealed several functions for sox 10 in distinct neural crest lineages. We have focussed our analysis on the development of the dorsal root ganglia and Schwann cells. In a search of sox10 target genes in the PNS P0 and erbB3 were identified. As the erbB3 gene is silenced in neural crest cells or derivatives in sox10 mutant embryos older than 10.5 we found a substantial overlap of phenotypes in the PNS. The most striking difference however is the complete absence of glial differentiation in the PNS of sox10 mutants as revealed by immunological analysis with the BFABP antibody (Fig.2). Later in development the vast majority of sensory neurons degenerates as was reported already for the erbB3 mutant. The extent of cell death as well as the kinetics is different suggesting that sox10 has multiple functions in this lineage apart from maintaing erbB3 expression.

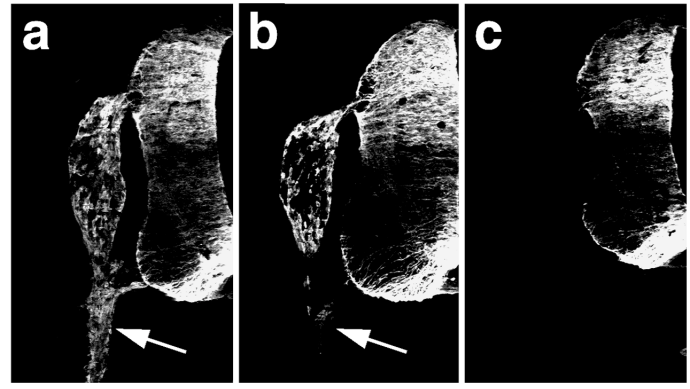

Figure 2. Expression of BFABP in cross-sections of E11.5 embryos. In the wildtype (a) satellite glia in the dorsal root ganglia and Schwann cell precursors aligning axonal projections (arrow) are positive. The erbB3 mutant (b) shows unchanged signals in the dorsal root ganglia and only a few axonal signals (arrow) in close vicinity of the ganglia, whereas the sox10 mutant (c) is completely lacking BFABP staining in the PNS.

3. GCMa, one mammalian homologue of drosophila´s Glial cells missing.

In drosophila GCM was originally identified in a screen for axonal path finding mutants but turned out to be the glial master gene. By intensive screening two homologues in mammals have been identified, GCMa and GCMb. Surprisingly none of the mammalian gcm genes showed strong expression in the nervous system. Targeted

mutagenesis of the gene encoding GCMa in the mouse caused a severe defect in labyrinth formation and led to embryonic lethality between E9.5 and E10 (Fig.3).

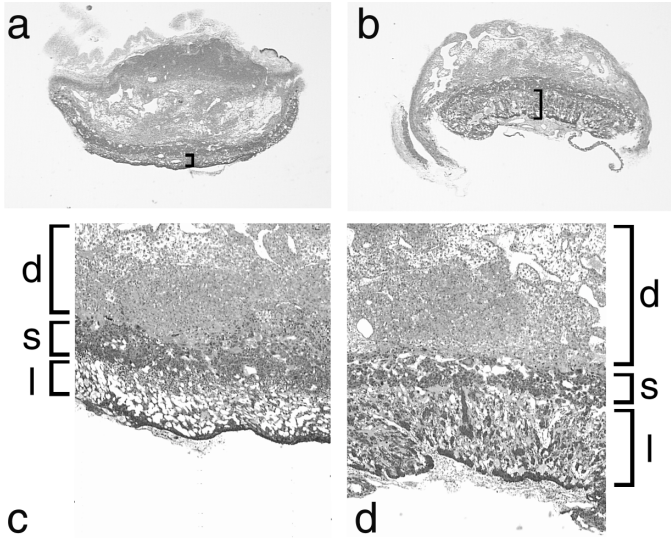

Figure 3. Failure of labyrinth formation in GCMa deficient embryos. Morphology of mutant (a) and control (b) E10 placenta in hematoxilin-eosin-stained semithin cross-sections. Higher magnification shows that decidua (d), spongio-trophoblast layer (s) are unaffected in the mutant (c) whereas the labyrinth layer is thinner and contains cell-free areas but no fetal blood vessels.

Consistent with expression of GCMa, which during embryogenesis is restricted to a defined cell population of the extra-embryonic ectoderm (i.e. trophoblasts), no abnormalities were observed in the embryo proper including the nervous system. Apparently, structure and function sometimes go separate ways.

4. Lineage ablation in mice using Dta

The A subunit of diphtheria toxin has proven to be a powerful cytotoxic agent. We used a construct where a lox-p-flanked Dta gene was placed behind the Lac-Z-reporter gene to generate a mouse strain that expresses this cassette ubiquitously. These mice can be mated with Cre-expressing mice resulting in the activation of the toxin and ultimately in ablation of cells that have undergone cre-mediated recombination. We are currently testing the system by mating our mice with different Cre-expressing strains.

5. Functions of caspase-8 in the PNS and CNS analysed by conditional mutagenesis

The central mediators of apoptosis comprise in mammals a family of at least 14 members, known as caspases. These cysteine proteases are synthesized as catalytically inactive proenzymes that have to be activated. The apoptotic program is instigated by initiator caspases that then trigger an amplifying cascade of effector caspases. Caspase-8 belongs to the initiator caspases and its targeted mutagenesis has revealed an essential function during heart development and caused embryonic lethality at E10.5. It is conceivable that caspase-8 plays central roles in a variety of developmental and pathological processes that cannot be analysed with the conventional knock out strategy. Therefore we decided to generate a conditional knock out for this gene in collaboration with the group of C. Traudtwein. We will focus our analysis on the peripheral and central nervous system

Support

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

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Mechanisms of Pancreas and Central Nervous System Development

Maike Sander

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

Interestingly, despite their different embryonic origin, pancreatic islet cells and neuronal cells in the CNS express remarkably similar sets of transcription factors during development. Previous research has identified that a number of key transcription factors regulate both neuronal differentiation and development of endocrine cells in the pancreas.

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

1. Transcriptional regulation of pancreas development

During pancreas development, a common endocrine precursor cell gives rise to four distinct endocrine cell types, each of which can be readily distinguished by the production of a characteristic hormone. Thus, the endocrine pancreas presents an ideal system to explore the roles of specific families of transcription factors in development.

The Nkx-family of transcription factors comprises a subfamily of the homeodomain transcription factors. Nkx genes are mammalian homologues of the Drosophila NK-transcription factors. All NK- and Nkx-transcription factors share a characteristic decapeptide sequence motif, which has been implicated in co-factor mediated transcriptional repression.

The transcription factor Nkx6.1 was originally cloned from an insulin-producing tumor cell line. Through gene targeting in ES cells, we generated a mouse strain with a null allele for the Nkx6.1 gene. The analysis of pancreatic cell differentiation in the Nkx6.1 mutant mouse revealed a requirement for this transcription factor in the formation of the insulin-producing cells (also called beta-cells) (Fig. 1). In subsequent experiments, designed to further define Nkx6.1 function, we found that Nkx6.1 promotes beta-cell formation from its endocrine progenitor, but serves no functional role in beta-cell survival or proliferation.

At this time, it was already known that another transcription factor, Nkx2.2, also plays a role in beta-cell development. To explore whether Nkx6.1 and Nkx2.2 genetically interact during pancreas development, we generated a double mutant mouse for these two transcription factors. We found that the Nkx2.2 single mutant is identical to the Nkx2.2/Nkx6.1 double mutant

phenotype, demonstrating an epistatic relationship for these two transcription factors in pancreatic endocrine cell differentiation.

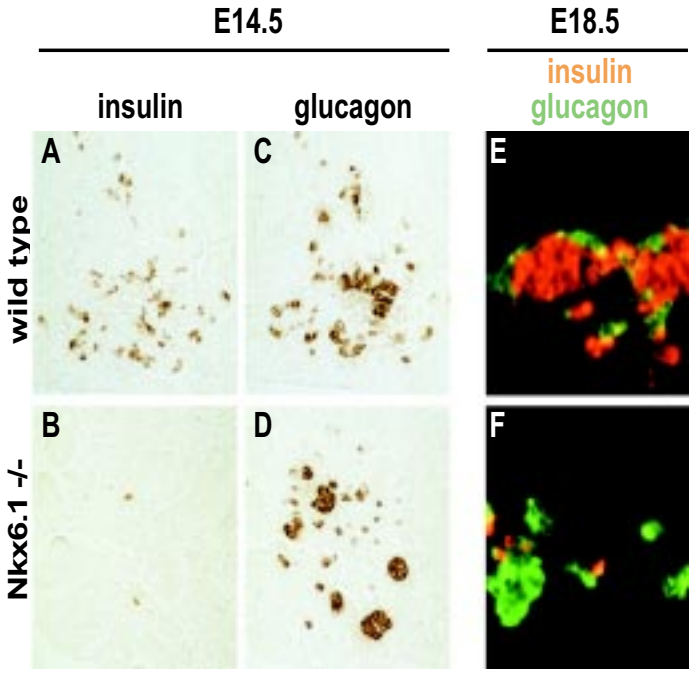

Figure 1. Insulin-expressing cells fail to form in the pancreas of Nkx6.1 homozygous mutant embryos. During embryonic development, the majority of insulin-positive cells develop after embryonic day (E) 13. By E14.5, a large number of insulin-expressing cells have been generated in wild type (A), but not in Nkx6.1 mutant (B) embryos. The number of glucagon-expressing cells is similar in wild type and mutant embryos (C-F). As demonstrated by the reduction in insulin-positive cells in term embryos (F), development of the insulin-producing cells is not delayed in Nkx6.1 mutants.

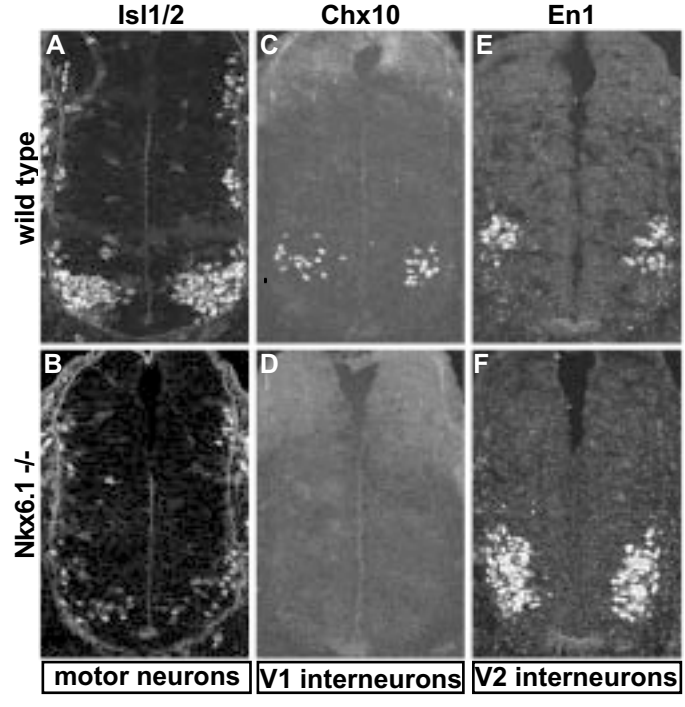

Figure 2. Disruption of motor neuron differentiation, and switch in ventral interneuron fates in Nkx6.1 mutant embryos. Spinal cord sections of E10.5 Nkx6.1 homozygous mutant embryos show almost undetectable expression of the motor neuron marker Isl1/2 (B), and V1 interneuron marker Chx10 (D). The expression domain of the V2 interneuron marker En1 is ventrally expanded in the spinal cord of Nkx6.1 homozygous mutant embryos (F).

2. Nkx6.1 function the development of spinal cord motor neurons

Through an expression analysis of Nkx6.1, we found that Nkx6.1 is not only expressed in the pancreas, but also in the developing central nervous system (CNS). In the mouse embryo, neural expression of Nkx6.1 is first detected in the neural plate, and later in the neuronal progenitors throughout the ventral third of the neural tube. From previous research it was known that spinal cord motor neurons are generated from the ventral progenitor cells that express Nkx6.1. Based upon these results, we studied if Nkx6.1 mutant mice have defects in neuronal patterning and differentiation of spinal cord neurons. Our analysis showed that Nkx6.1 mutants have a block in the generation of motor neurons. This block results from a dorsal-to-ventral switch in the identity of spinal cord neuronal progenitor cells. As a consequence, these progenitors differentiate into a class of more dorsal neurons instead of motor neurons (Fig. 2).

3. Nkx6.1 function in neuronal migration

In the developing CNS, Nkx6.1 is not only expressed at spinal cord levels, but is expressed throughout the entire length of the neural tube, with the exception of the prosencephalon. Therefore, we studied Nkx6.1 function in the development of neurons in the hindbrain. Contingent upon their muscle targets, hindbrain motor neurons can be subdivided into somatic, branchial, and visceral motor neurons. These different classes of motor neurons develop from distinct progenitor domains in the neural tube. In our research, we asked if Nkx6.1 differentially affects the development of these classes of hindbrain motor neurons.

We showed that Nkx6.1 mutant embryos fail to form somatic motor neurons in the hindbrain, but generate normal numbers of hindbrain visceral and branchial motor neurons. After their differentiation, certain branchial motor neurons undergo extensive migration within the hindbrain. Presently, little is known about the regulatory mechanisms that control the migratory paths of these neurons.

To study if Nkx6.1 could play a role in the control of hindbrain motor neuron migration, we tested if mutation of the Nkx6.1 gene in mice affects the migratory behavior of branchial motor neurons. To label the facial branchial motor neurons, we retrogradely injected DiI into the facial nerve of wild type and Nkx6.1 mutant embryos, and found that in Nkx6.1 mutants facial branchial motor neurons fail to migrate into their proper position in the hindbrain. We discovered similar defects in the migratory patterns of other hindbrain branchial motor neurons. Our results demonstrate that a cell-autonomous mechanism involving Nkx6.1 elicits specific gene responses in branchial motor neurons that determine their migratory pathways. In this, it will be interesting to identify cell surface molecules as down-stream effectors of Nkx6.1 in neuronal migration.

Support

Our work is currently supported by the Juvenile Diabetes Foundation International, the Deutsche Forschungsgemeinschaft, the National Institutes of Health, and the Elli Lilly Forschung GmbH. Dr. Shelley Nelson is supported by the Alexander von Humboldt Stiftung, and Dr. Kirsten Kuhlbrodt by the Deutsche Forschungsgemeinschaft.

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Award

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Induction and differentiation of the inner ear

Thomas Schimmang

The inner ear is a complex sensory organ which is induced as a placode next to the developing hindbrain during early embryogenesis. This placode invaginates to form the otic pit and finally closes to develop the otic vesicle. The otic vesicle undergoes a complex series of morphogenetic events which results in the formation of the sensory epithelia and their innervation by the auditory and vestibular ganglia. Our major interest is to define the morphogenetic events leading to the induction of the inner ear in the surface ectoderm of the vertebrate embryo. Additionally, we are analysing the mechanisms which direct the formation and regeneration of auditory neurons and sensory hair cells. At the molecular level we are concentrating on several members of the fibroblast growth factor (FGF) and neurotrophin gene family. As model species we are focussing on the chicken and the mouse and have started collaborations with laboratories using zebrafish and Drosophila. Our experimental approaches involve gain-of-function experiments via viral gene transfer, electroporation and use of transgenic mice. These experiments are complemented by the analysis of knockout mice and primary cell cultures with which we hope to define the molecules necessary for formation of the inner ear and the regeneration of auditory neurons and hair cells.

1. Control of inner ear induction and morphogenesis by FGFs

One of the main focus of our research interest are the molecular events during formation of the inner ear in different species. Based on the knowledge that FGF3 can act as an inducer of the chicken inner ear, we are now performing a systematic analysis of the roles of other FGF members during inner ear development. We are addressing the potential roles of FGF2, FGF8 and FGF10 during induction and early morphogenesis of the chicken inner ear. To do so, we are using viral gene transfer based on Herpes virus and as a complementary approach FGF beads applied to early chicken embryos. Additionally, we have set up the electroporation technique for early chicken embryos in our laboratory. This technique offers an ideal handle to study the functions of genes in chicken embryos via ectopic misexpression (Figure 1). Using this technique, we could demonstrate that electroporation of FGF3 provokes the formation of ectopic inner ear placodes. We have also electroporated genes directly into the otic vesicle, which as a hollow sphere offers an ideal compartment to perform misexpression of genes and study their function. Finally, we have successfully electroporated chicken embryos in the developing neural tube with the aim to find out which genes control inner ear development via the posterior hindbrain. At the moment we are focussing our interest on the mafB gene which has been shown to interact with FGFs in the otic region of the hindbrain.

One of the most relevant points of our research is to analyse the role of FGF3 during mammalian inner ear development. We have decided to perform ectopic overexpression studies in rodents using two different strategies. On the one hand, we are overexpressing FGFs (FGF2, FGF3, FGF10) in rat embryos via intraamniotic injection of Herpesvirus during

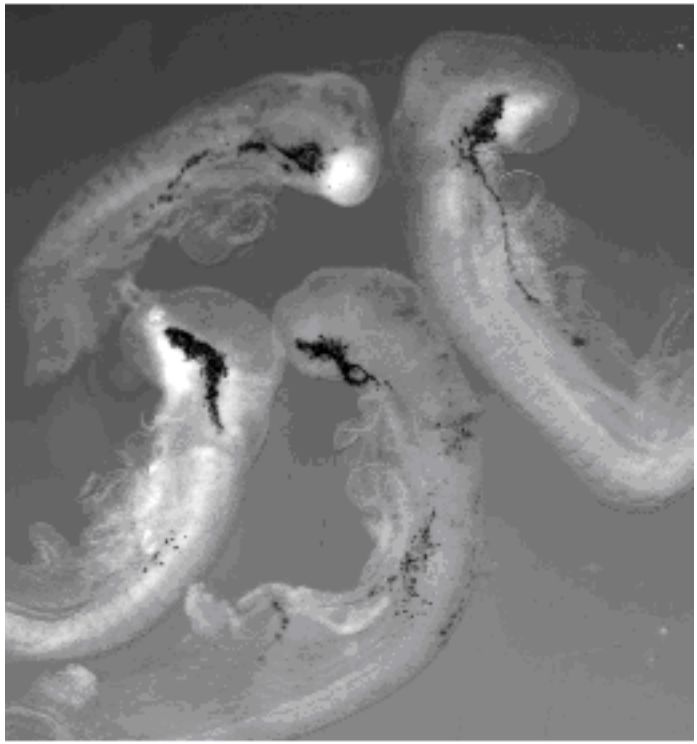

Figure 1. Chicken embryos which have been electroporated with a HSV-1-based expression vector containing the lacZ gene. Note the efficient expression of the reporter gene in the surface ectoderm of the embryos.

development. On the other hand, we are producing transgenic mice expressing these FGFs ectopically under the control of the sek1 enhancer. This enhancer directs ectopic expression in rhombomere 3, anterior to the position of the inner ear localised at the level of rhombomeres 5 and 6. Therefore, this approach will clarify if FGFs expressed by the anterior hindbrain may act as otic inducers. Additionally, misexpression of FGFs will also take place in the dorsal compartment of the otic vesicle. At this location induction of the endolymphatic duct takes place which is defective in FGF3 knockout mice.

2. Analysis and gene transfer of neurotrophins and FGFs in the inner ear

Defective Shc-adaptor binding sites on the neurotrophin receptor TrkB were recently shown to lead to a loss of NT-4-dependent neurons whereas BDNF-dependent neurons appeared unaffected. Interestingly, these studies provided evidence for a loss of vestibular neurons, which so far have not been reported to be under control of NT-4 in vivo. To characterize the impact of the shc-mutation on TrkB signaling in the inner ear, we have analysed the neuronal innervation pattern of vestibular and cochlear epithelia of postnatal and adult mice homozygous for a defective shc-adaptor binding site ($trkB^{shc/shc}$). These results were compared with the innervation pattern observed in mice heterozygous for a defective Shc-adaptor binding site and a defective tyrosine kinase domain $trkB^{shc/-}$, and mice homozygous for the latter allele ($trkB^{-/-}$). To get insight in the role of BDNF for the activation of the involved TrkB pathways we further compared the innvervation pattern with that of BDNF mutants ($BDNF^{-/-}$). We observed that afferent and efferent fibers in $trkB^{shc/shc}$ mutants were severely affected in the vestibular epithelia but not in the cochlear epithelia. A different temporal sequence of fiber loss in the vestibular system of $trkB^{shc/shc}$ mutants versus $trkB^{shc/-}$, $trkB^{-/-}$ and $BDNF^{-/-}$ mutants indicated the parallel existence of BDNF-mediated Shc-dependent and Shc-independent TrkB signalling pathways in the vestibulum. Failure of afferent calyx formation in $trkB^{shc/shc}$ mutants similar to $trkB^{-/-}$ mutants and $BDNF^{-/-}$ mutants, suggested a role of the Shc signaling pathway for the survival or maturation of afferents innervating type I hair cells. In the cochlea, a similar

loss of distinct outer hair cell afferents in trkB$^{shc/-}$, trkB$^{-/-}$ and BDNF$^{-/-}$ mutants was observed, while afferents of trkB$^{shc/shc}$ mutants remained unaffected, indicating that cochlear neurons exclusively use a Shc adaptor protein independent pathway.

Damage to the inner ear may be caused by aging, injury, ototoxic drugs, acoustic trauma or genetic diseases. The majority of hearing loss is caused by damage or loss of sensory hair cells or auditory neurons. Cochlear implants may substitute hair cell function by electrical stimulation of the auditory neurons. However, survival of a critical number of neurons is required for proper function of the prosthesis. To maintain survival of auditory neurons inside the damaged inner ear, BDNF and NT-3 have been introduced via osmotic pumps. Alternatively, viruses may offer a strategy to provide long-term expression of neurotrophins inside auditory neurons and maintain their function. In this context, defective Herpes simplex virus type 1 (HSV-1) vectors offer some characteristics which make them the ideal gene transfer vector for the peripheral nervous system. Most importantly, they infect neurons very efficiently and acquire a state of latency without comprising normal cellular functions. We have produced a HSV-1 based amplicon vector expressing NT-3. This vector efficiently infects isolated auditory neurons and stimulates their survival during distinct developmental stages of the inner ear. Therefore, this vector may present a unique entry point to develop therapies preventing or treating hearing impairment caused by the degeneration of auditory neurons.

The importance of individual members of the fibroblast growth factor gene family during innervation of the vertebrate inner ear is not clearly defined. We have addressed the role of fibroblast growth factor 2 (FGF-2 or basic FGF) during development of the chicken inner ear. We found that FGF-2 stimulated survival of isolated cochlear and vestibular neurons during distinct phases of inner ear innervation. The potential neurotrophic role of FGF-2 was confirmed by its expression in the corresponding sensory epithelia and the detection of one of its high-affinity receptors in inner ear neurons. Finally, we have analysed the potential of the amplicon system based on defective herpes simplex virus type 1 vectors to express FGF-2 in cochlear neurons. Overexpression of FGF-2 in cochlear neurons resulted in neuronal differentiation demonstrating the presence of biologically active growth factor. This study underlined the potential of FGF-2 to control innervation and development of sensory epithelia in the avian inner ear. Furthermore, amplicon vectors may provide a useful tool to analyse gene function in isolated neurons of the vertebrate inner ear.

Support

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

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DNA Sequencing

Willi Kullmann

At the ZMNH a DNA-sequencing facility was established in October 1995. Automated DNA-sequencing started with an ABI Prism 373 DNA sequencer which was replaced by an ABI Prism 377 DNA sequencer in May 1996 to enable faster gel runs with higher throughputs. The latter was then up-graded in 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 flourescence-based detection. Presently an improved set of fluorescence dyes (big dye) is used which greatly reduces the notorious weak G after A pattern characteristics of its predecessor.

The ABI Prism 377 sequenator enables a reading-length of abauot 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 1999 until December 2000 approx . 35000 sequence analyses were performed.

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Morphology

Michaela Schweizer

Synapses in the central nervous system are modifiable, both in the short- and in the long-term, and it has long been recognised that this modifiability, or plasticity, may play an important role in neural function such as learning and memory.

Long-term changes in synaptic function are likely to be associated with structural alterations in the synapse. An increasing number of studies has indicated that synaptic plasticity is intimately associated with the formation of perforated synapses (Buchs & Muller, 1996; Neuhoff *et al.*, 1999). Perforated synapses are characterised by a discontinuous postsynaptic density (PSD) which is always larger compared to those of non-perforated/macular synapses. This suggests that the perforations could function to increase the perimeter length of the PSD, and thereby the size of the active synaptic zone. Thus, the formation of perforations might be a morphological correlate of enhanced synaptic efficacy (Edwards, 1995). The mechanisms that cause perforations are still unknown. An increase in the frequency of perforated synapses was detected after induction of LTP in *in vivo* as well as *in vitro* studies. This suggests that the formation of perforated synapses is an early morphological consequence of synaptic activation.

Thus, it is important to understand the cellular mechanisms leading to synaptic remodelling. Proteases have been connected with several forms of synaptic plasticity. In particular, the tissue type plasminogen activator (tPA) has been suggested to play a role in activity-dependent structural plasticity. tPA is a serine protease that converts plasminogen to plasmin and degrades several other extracellular substrates. This protease has been shown to be involved in tissue remodelling, degradation of extracellular matrix and axon elongation (Seeds *et al.*, 1995). Moreover, tPA is localised in presynaptic boutons (Griesinger & Schweizer, 1997, Soc. Neurosci., abstract) and released in an activity-dependent manner. In addition, it has been shown that tPA mRNA is up regulated in the dentate gyrus after seizure activity and induction of LTP (Qian *et al.*, 1993). tPA knock-out mice show much less neurodegeneration following kainate-induced seizures (Tsirka *et al.*, 1995) and do not exhibit the long lasting form of LTP (Frey *et al.*, 1996). Transgenic mice overexpressing tPA in post-natal neurones have increased and prolonged hippocampal LTP and improved performance in spatial orientation learning tasks. Thus, it is likely that tPA could be implicated in the process of activity-dependent synaptic plasticity by catalysing synaptic remodelling or by binding to a specific cell surface receptor such as LRP (low-density lipoprotein receptor-related protein; Zhuo *et al.*, 2000).

Previous work in our laboratory (Neuhoff et al., 1999) demonstrated that the formation of perforated synapses was strongly dependent on the activity of NMDA-receptors and the serine protease tPA, because their inhibition by APV or tPA-inhibitors, respectively, prevented the activity-dependent induction of perforated synapses. NMDA-receptor activation and tPA activity are also involved in the process of LTP which suggests a mechanistic link between functional and structural plasticity of synapses.

The finding that brief stimulation of NMDA receptors induced breakdown of adhesion molecules (Hoffman *et al.*, 1998a, Endo *et al.*, 1998), and the fact that tPA recognised a sequence of extracellular cell adhesion molecules (Hoffman

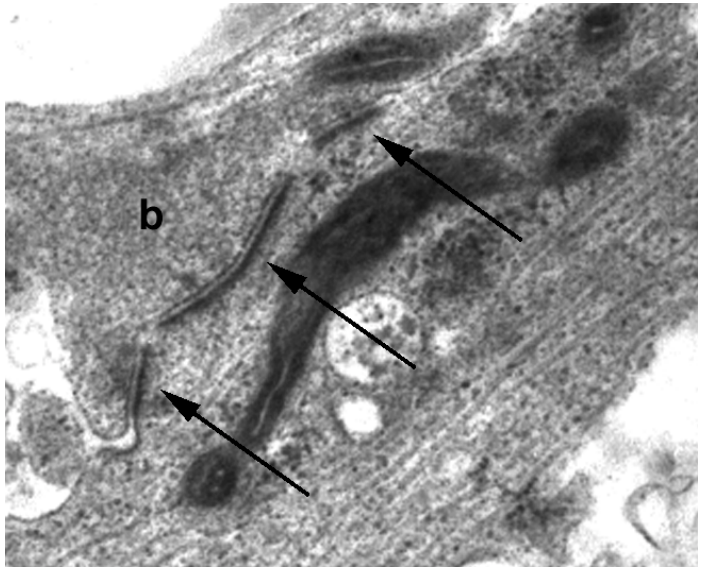

Figure 1. High power electron micrograph of a bouton (b) on a perforated synapse (arrows) from cultured hippocampal neurons

et al., 1998b) suggested that secretion of tPA from presynaptic terminals is induced in response to NMDA receptor stimulation. Adhesion molecules were found at postsynaptic densities (Persohn *et al.*, 1989; Schuster *et al.*, 1998), and cleavage of their extracellular domains might modify the adhesive environment allowing postsynaptic remodelling.

In summary, it is conceivable that cell adhesion molecules must be fragmented in order to allow the formation of perforations. According to our data, it is likely that both pre- and postsynaptic mechanisms - tPA- and NMDA-receptor dependent mechanisms - must act together in order to induce structural remodelling. However, further work needs to be done to define the full sequence of events that are involved in the structural component of synaptic plasticity.

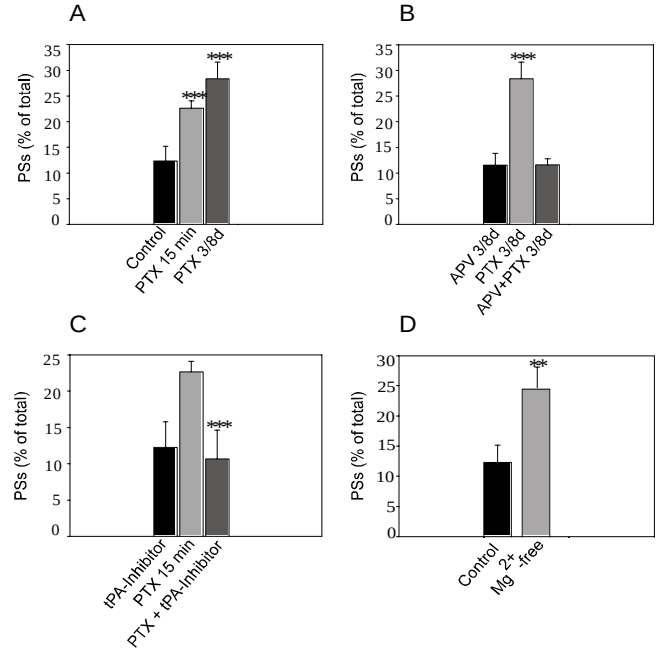

Figure 2. Changes in the number of perforated synapses after stimulation with PTX (A), PTX together with NMDA-receptor antagonist AP-5 (B) PTX and tPA inhibitor (C) and 0 Mg^{2+} and high Ca^{2+}

The facility of morphology is equipped with modern technology for the detection of various antigens in cell culture or tissue sections at the light- and electron microscopical level. Standard fixation protocols are perfusion through the ascending aorta with aldehydes for best structural preservation of brains and other tissues. Detection of specific antibody binding is resolved with signal enhancing techniques like the avidin-biotin complex (ABC) technique. Diaminobenzidine-hydrogen peroxide (DAB) is used as chromogen and allows both light–

and electron microscopical analysis. We further test pre- and postembedding procedures for individual antigen – antibody complexes for more detailed ultrastructural invetigations and the DAB may be replaced by colloidal gold.

Double or triple immunohistochemical staining is routinely carried out with fluorescent labeled secondary antibodies. Confocal-Laser-Scanning microscopy (Leica TCS SPII) offers high resolution detection of antigens. mRNA-expression is monitored on tissue sections by in situ hybridization (ISH). An ultrastructural ISH protocol developed by Susanne Fehr (Prakash et al., 1997) allows the subcellular localization of mRNA from strong nonradioactive ISH.

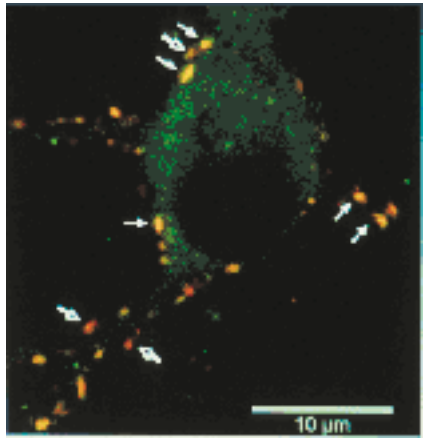

Figure 3. Confocal image of a cultured hippocampal neuron double stained for tissue plasminogen activator (tPA,) and synaptophysin. tPa is present within the soma and partly in dendrites, but is enriched in presynaptic boutons (filled arrows), where it colocalizes with synaptophysin. However some boutons,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

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Thesis

Dissertation

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Mass Spectrometry and Biomolecular Interaction Analysis

Christian Schulze

Quantitative and qualitative analysis of gene products (the 'proteome') is a key to assess their biochemical function. Comparison of discrete expressed proteins withthe corresponding DNA sequence data reveals post-translational processing. Characterization of protein-protein interactions is indispensable for an understanding of the cell as a complex molecular system. At the ZMNH mass spectrometry and a biosensor is available for these studies.

1. Mass spectrometry

Thorough description of the mature form of a protein is a prerequisite for assessing its contribution to cellular function, because protein processing is common. In addition, proteins may be present in different isoforms due to alternative splicing. The occurence of additional forms, however, may also be a result of enzymatic degradation in vitro following cell lysis.

A nice example for this situation is the inositol-5-phosphatase SHIP. SHIP plays a role in the down-regulation of the signalling pathways associated with inositol phosphates and phosphoinositides involved in cytokine receptor signal transduction. The protein is expressed as a 145 kDa full-length protein in human hematopoietic cells, however, a protein of 100 kDa was the major SHIP protein detected in bone marrow and peripheral blood mononuclear cells. Northern blot analysis of total RNA of different SHIP expressing cells showed presence of only one message of 5 kb. Analysisof SHIP on the protein level using Western blot experiments with antibodies against both N- and C-terminal parts of the protein revealed that the 100 kDa form is a C-terminal truncated protein. The 100 kDa form was not detected if cells were lysed in the presence of serine protease inhibitors or in boiling SDS buffer, which suggested involvement of a specific protease. Indeed, only one out of six synthetic peptides covering a domain at which cleavage would give rise to a 100 kDa fragment was degraded by cellular extracts in vitro. Mass spectrometry proved that proteolysis occured at only one position. In conclusion, it is very likely that only the 145 kDa SHIP protein has physiological function.

2. Biomolecular interaction analysis

Protein-protein interaction for functional characterization uses a state-of-the-art surface plasmon resonance (SPR) biosensor (Biacore 3000). A large number of publications appeared which used the SPR technique. The development of new surfaces for immobilization of analytes will widen the application range of the technique.

Basically, SPR is based on the total internal reflection phenomenon. Changes in the mass concentration of macromolecules at a biospecific interface are recorded in real time, so that kinetic information is readily derived. Since the interaction takes place within the evanescent field close to an interface, one of the interactants has to be immobilized on the sensor surface. This procedure, although performed at very mild conditions may lead to inactivation. Maintaining the immobilized protein in an active state is a major issue.

A number of different protein-protein interactions were studied, among which characterization of antibody fragments were the most successful.

Publications

(1) Horn, S., Meyer, J., Heukeshoven, J., Fehse, B., Schulze, C., Li, S., Frey, J., Poll, S., Stocking, C., Jücker, M. (2001). The inositol 5-phosphatase SHIP is expressed as 145 and 135 kDa proteins in blood and bone marrow cells in vivo, whereas carboxyl-truncated forms of SHIP are generated by proteolytic cleavage in vitro. Leukemia 15, 112-20.

Review Article

(2) Schulze, C. (2000). Analysing biomolecular interactions: The surface plasmon resonance technique. Recent Res. Devel. Comparative Biochem. & Physiol. 1, 77-90.

Structure of the Group

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Transgenic Technologies

Michael R. Bösl

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

The transgenic technologies group is routinely performing the injection of recombinant embryonic stem (ES) cells into blastocysts for the generation of chimeric mice and transmission of the modified gene to the germline, and pronuclear injection of DNA constructs into fertilized oocytes for the generation of "classical" transgenic founder animals. The transgenic technologies group is providing ES-cells and feeder cells to the scientists of the center together with protocols and instructions for their proper handling and successful manipulation, and scientific advice for the generation of genetically modified mouse models. The efficient work of our group is documented by the growing number of publications on mouse models by ZMNH researchers. Furthermore, several collaborations exist dealing with the construction and phenotypic characterization of transgenic mouse models.

Publications

(1) Kornak, U., Bösl, M.R., Kubisch, C. (1999). Complete genomic structure of the CLCN6 and CLCN7 putative chloride channel genes. Biochim. Biophys. Acta 1447, 100-106.

(2) Schreiber, J., Riethmacher-Sonnenberg, E., Riethmacher, D., Tuerk, E.E., Enderich, J., Bösl, M.R., Wegner, M. (2000). Placental failure in mice lacking the mammalian homolog of glial cells missing, GCMa. Mol. Cell. Biol. 20, 2466-2474.

(3) Tamai, Y., Ishikawa, T., Bösl, M.R., Mori, M., Nozaki, M., Baribault, H., Oshima, R.G., Taketo, M.M. (2000). Cytokeratins 8 and 19 in the mouse placental development. J. Cell Biol. 151, 563-572.

(4) Piwon, N., Günther, W., Schwake, M., Bösl, M.R., Jentsch, T.J. (2000). ClC-5 Cl^--channel disruption impairs endocytosis in a mouse model for Dent's disease. Nature 408, 369-373.

(5) Stobrawa, S.M., Breiderhoff, T., Takamori, S., Engel, D., Schweizer, M., Zdebik, A.A., Bösl, M.R., Ruether, K., Jahn, H., Draguhn, A., Jahn, R., Jentsch, T.J. (2001). Disruption of ClC-3, a chloride channel expressed on synaptic vesicles, leads to a loss of the hippocampus. Neuron 29, 185-196.

(6) Kornak, U., Kasper, D., Bösl, M.R., Kaiser, E., Schweizer, M., Schulz, A., Friedrich, W., Delling, G., Jentsch, T.J. (2001). Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. Cell 104, 205-215.

(7) Bösl, M.R., Stein, V., Hübner, C., Zdebik, A.A., Jordt, S.E., Mukhopadhyay, A.K., Davidoff, M.S., Holstein, A.F., Jentsch, T.J. (2001). Male germ cells and photoreceptors, both dependent on close cell-cell interactions, degenerate upon ClC-2 Cl^- channel disruption. EMBO J. 20, 1289-1299.

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Teaching, Seminars

Teaching Sommersemester 2000

Seminare für Mediziner und Naturwissenschaftler

ZMNH-Seminar für MedizinerInnen und NaturwissenschaftlerInnen
DozentenInnen und Wissenschaftliche Mitarbeiter des Zentrums und externe Sprecher (2 st.)

Entwicklungsbiologie (nur im SS)
Vorlesung und Seminar für MedizinerInnen und NaturwissenschaftlerInnen
Bach, Schaller (Blockveranst. n. V.)

Praktikum für molekulare Neurobiologie
Bartsch, Becker, Becker, Dityatev, Kleene, Kutsche, Lütjohann, Riethmacher, Sander, Wotjak u.a.
(6 Wo. gztg., n.V.)

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

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

Membrantransport: Zellbiologie und Pathophysiologie (Literaturseminar)
Böttger, Hechenberger, Hübner, Jentsch, Kasper-Biermann, Waldegger, Weinreich, Yamada, Zdebik
(2 st.)

Ionenkanäle (Literaturseminar)
Pongs (2 st.)

Neurale Plastizität (Literaturseminar)
Kuhl (2 st.)

Entwicklungsbiologie und Neurobiologie (Literaturseminar)
Borgmeyer, Hampe, Hermans-Borgmeyer, Hoffmeister, Schaller (1 st.)

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

Neuropeptidwirkung, Signaltransduktion (Forschungsseminar)
Borgmeyer, Hampe, Hermans-Borgmeyer, Hoffmeister, Schaller (2 st.)

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

Ionenkanäle (Forschungsseminar)
Böttger, Hechenberger, Hübner, Jentsch, Kasper-Biermann, Waldegger, Weinreich, Zdebik (2 st.)

Neurale Signalverarbeitung (Forschungsseminar)
Bähring, Isbrandt, Leicher, Legros, Pongs, Schwarz, Waldschütz (2 st.)

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

Molekulare Mechanismen der Neurogenese (Forschungs-
seminar)
Bach, Riethmacher, Sander (2 st.)

Grundlagen der Embryonalentwicklung (Literaturseminar)
Bach, Riethmacher, Sander (2 st.)

Neurale Zellerkennungsmoleküle und Zellinteraktionen bei
der embryonalen Entwicklung und synaptischen Plastizität
(Literaturseminar)
Dityatev, Schachner Camartin, Wotjak (2 st.)

Neurale Zellerkennungsmoleküle (Forschungsseminar)
Schachner Camartin und MitarbeiterInnen (2 st.)

Biochemie (Literaturseminar)
Lütjohann, Schachner Camartin (2 st.)

Zellerkennungsmoleküle und Signaltransduktion (Literatur-
seminar)
Lütjohann, Schachner Camartin (2 st.)

Molekularbiogie (Literaturseminar)
Kutsche, Schachner Camartin (2 st.)

Molekulare Neuropathologie
(Vorlesung und Seminar für MedizinerInnen und
NaturwissenschaftlerInnen)
Jentsch, Riethmacher, Sander, Schaller und
MitarbeiterInnen (2 st.)

Entwicklungsneurobiologie
(Seminar und Vorlesung für MedizinerInnen und
NaturwissenschafterInnen)
Bach, Riethmacher, Sander, Schaller, Wegner und
MitarbeiterInnen (n. V.)

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

Aufbaustudium Molekularbiologie

Vorlesung: Molekularbiologie II (2. Sem.)
Pongs und MitarbeiterInnen (2 st.)

Vorlesung: Molekulare Neuropathologie
Bach, Borgmeyer, Jentsch, Riethmacher, Schaller,
Schimmang und MitarbeiterInnen (2 st.)

Praktikum II
Dozenten und MitarbeiterInnen des ZMNH (10 st. n.V.)

Praktikum IV
Dozenten und MitarbeiterInnen des ZMNH (10 st. n.V.)

Graduiertenkolleg

Signaltransduktion und deren pathologische Störungen
Chica Schaller

Teaching Wintersemester 2000/2001

Seminare für Mediziner und Naturwissenschaftler

ZMNH-Seminar für MedizinerInnen und NaturwissenschaftlerInnen
DozentenInnen und Wissenschaftliche Mitarbeiter des Zentrums und externe Sprecher (2 st.)

Molekulare Biologie I (nur im WS)
Vorlesung und Seminar für MedizinerInnen und NaturwissenschaftlerInnen
Bach, Hermans-Borgmeyer, Jentsch, Riethmacher, Schaller, Schimmang und MitarbeiterInnen (2 st.)

Praktikum für molekulare Neurobiologie
Ader, Bartsch, Becker, Becker, Dityatev, Loers, Kleene, Kutsche, Morellini, Tilling (6 Wo. gztg., n.V.)

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

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

Membrantransport: Zellbiologie und Pathophysiologie (Literaturseminar)
Böttger, Hübner, Jentsch, Kasper-Biermann, Kornak, Piwon, Schroeder, Schwalke, Stein, Strobrawa, Zdebik (2 st.)

Ionenkanäle (Literaturseminar)
Pongs (2 st.)

Neurale Plastizität (Literaturseminar)
Kuhl (2 st.)

Entwicklungsbiologie und Neurobiologie (Literaturseminar)
Borgmeyer, Hermans-Borgmeyer, Hoffmeister, Schaller (1 st.)

Molekulare Aspekte der neuralen Entwicklung
Borgmeyer, Hermans-Borgmeyer (1 st.)

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

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

Ionenkanäle (Forschungsseminar)
Böttger, Hübner, Jentsch, Kasper-Biermann, Kornak, Piwon, Schroeder, Schwake, Stein, Stobrawa, Zdebik (2 st.)

Neurale Signalverarbeitung (Forschungsseminar)
Bähring, Isbrandt, Legros, Pongs (2 st.)

Grundlagen der Embryonalentwicklung (Entwicklungsbiologisches Kolloquium)
Bach, Riethmacher, Sander, Schimmang (2 st.)

Molekulare Mechanismen der Neurogenese (Forschungsseminar)
Bach, Riethmacher, Sander, Schimmang (2 st.)

Aktuelle Aspekte der molekularen Medizin (Literaturseminar)
Bach, Riethmacher, Sander, Schimmang (2 st.)

Neurale Zellerkennungsmoleküle und Zellinteraktionen bei der embryonalen Entwicklung und synaptischen Plastizität (Literaturseminar)
Dityatev, Morellini, Schachner Camartin (2 st.)

Neurale Zellerkennungsmoleküle (Forschungsseminar)
Schachner Camartin und MitarbeiterInnen (2 st.)

Biochemie (Literaturseminar)
Kleene, Schachner Camartin (2 st.)

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Molekularbiologie (Literaturseminar)
Kutsche, Schachner Camartin (2 st.)

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

Methoden der Molekularbiologie (Vorlesung)
Bach, Jentsch, Riethmacher, Schaller, Schimmang (2 st.)

Aufbaustudium Molekularbiologie

Vorlesung: Molekularbiologie I (1. Sem.)
Jentsch, Schaller und MitarbeiterInnen; Bach, Riethmacher, Schimmang (2 st.)

Vorlesung: Einführung in die Neurobiologie (3. Sem.)
Bartsch, Becker, Becker, Schachner, Tilling (2 st.)

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Dozenten und MitarbeiterInnen des ZMNH (10 st. n.V.)

Praktikum III
Dozenten und MitarbeiterInnen des ZMNH (10 st. n.V.)

Graduiertenkolleg

Signaltransduktion und deren pathologische Störungen
Chica Schaller

ZMNH - Seminare 1999

G. P. Hess, Ithaca
Caged ligands, laser-pulse photolysis, and combinatorial synthesis in investigations of signal transmission in the nervous system

F. Lang, Tübingen
Ion channels in the regulation of apoptotic cell death

U. Drescher, Tübingen
The Eph familiy in axon guidance

C. Cuello, Montreal
Cortical synaptic remodelling after aging, lesions and beta-amyloid burden

J. Schouenborg, Lund
New perspectives on the pain pathways - Modular organisaton and experience dependent tuning

T. Herdegen, Kiel
The role of c-Jun N-terminal kinases and c-Jun in neurodegeneration

A. Karschin, Göttingen
Regulation of Kir channels

D. P. Wolfer
Informal - Behavioral analysis of genetically modified mice: memory versus thigmotaxis and passivity

T. K. Kinnunen, Helsinki
Informal - Mechanisms of N-Syndecan-mediated neurite outgrowth

S. Rétaux, Paris
LIM-homeodomain genes and brain regionalisation

J. Ericson, Stockholm
The selection of neural cell fate in response to graded sonic hedgehog signalling

G. Lewin, Berlin
Molcular approaches to mammalian sensory neuron mechanotransduction

C. Schulze, Hamburg
Mass spectrometry methods in molecular biology

A. Konnerth, Homburg
GABA-mediatd synaptic excitation in the immature brain

I. Bach, Hamburg
Developmental roles of LIM homeodomain transcription factors and their associated proteins

J. Spiess, Göttingen
Differential modulation of learning by CRF receptors 1 and 2

D. Riethmacher, Hamburg
The neuregulin signalling system in neural crest development

A. Garrat, Berlin
Conditional ablation in Schwann cells of the receptor tyrosine kinase ErbB2

M. Sander, Hamburg
The homeobox gene Nkx6.1 is required for the development of motor neurons and subsets of interneurons in the mouse spinal cord

C. Munck Petersen, Aarhus
Processing and sorting of sortilin

V. Goncharuk, Moscow
Changes in suprachiasmatic and paraventricular made by hypertension

M. Mattson, Lexington
Novel synaptic signalling cascades involved in plasticity and apoptosis

D. Meijer, Rotterdam
Genetic control of peripheral nerve development

H. Monyer, Heidelberg
Transgenic approaches to modulation of critical molecules involved in oscillatory activity

R. Kleene, Marburg
The role of glycoproteins, lectins, proteoglycans and lipid micro-domains in regulated secretion of pancreatic acinar cells

D. N. Angelov, Köln
Recovery of function after peripheral motor nerve injury: The role of cellular and molecular mechanisms promoting neuronal survival, neurite regrowth, and selective axon guidance.

M. Bastmeyer, Konstanz
The role of cell recognition molecules during axon growth and goldfish and zebrafish

V. Haucke, New Haven
Mechanism of clathrin-coated pit nucleation during synaptic vesicle endocytosis

B. Schwappach, San Francisco
Trafficking and quality control of ion channel proteins: identification of a novel ER retention motif

V. Kovalzon, Moscow
Stress, sleep and hypnogenic peptides

L. Sommer, Zürich
Regulation of cell lineage decisions in neural crest development

J. Ham, London
How to kill a neuron: the molecular mechanisms of programmed cell death in developing sympathetic neurons

H. Beck, Bonn
Synaptic plasticity in human and experimental epilepsy

N. Sewald, Bielefeld
Interactions of chemically modified cyclopeptides with integrins

ZMNH - Seminare 2000

H. Welzl, Zürich
Neurobiologische Grundlagen der konditionierten Geschmacksaversion

B. Bromm, Hamburg
Kortikale Repräsentation von Schmerz

B. Berninger, San Diego
Two factors influencing neurotrophin action on glutamatergic synapses: the postsynaptic target cells type and the functional state of the synapse

W. Stoffel, Köln
Null allelic mutants in the structure-function analysis of ZNS myelin

H. Wässle, Frankfurt
Parallel processing in the mammalian retina

L. M. Boland, Minneapolis
The role of ß subunits in potassium channel gating

P. Nicotera, Konstanz
Role of caspase-dependent and -independent cell death in neurodegeneration

C. Birchmeier, Berlin
Functions of neuregulin and its receptors in development

G. Eichele, Hannover
The genetic basis of the circadian clock in the mammalian brain

J. Bartsch, Bielefeld
Metalloprotease-disintegrins, proteins with dual functions in the CNS and their role in CNS pathology

R. de Kloet, Leiden
Stress in the brain

M. Pratap, Los Angeles
BK channels: molecular studies and subunit composition

J. Fawcett, Cambridge (UK)
Inhibition of axon regeneration in the nervous system. Scars boundaries and inhibitors

D. A. Rusakov, London
Fast inter-synaptic cross-talk: structural and physiological constraints

J. Rubenstein, San Francisco
Forebrain development

B. Attali, Rehovot
Tuning of potassium channels by tyrosine kinases and phosphatases: a molecular switch for myelogenesis

G. Gradwohl, Illkirch
Role of neurogenin transcription factors in cell lineage specification during nervous system and pancreas development

J. B. Schulz, Tübingen
Molecular mechanisms of neuronal apoptosis: New targets for the treatment of neurodegenerative diseases?

A. Pueschel, Frankfurt
Guidance by repulsion: how semaphorins pattern neuronal connections

G. Blobel, New York
Protein targeting

N. M. Soldatov, Baltimore
Ca^{2+} sensors and Ca^{2+}-induced inactivation of Ca^{2+} channels

P. Chavis, Montpellier
Integrin-directed maturation of presynaptic and postsynaptic compartments at a hippocampal synapse

M. Groot, Bad Homburg
Transcriptional regulation during differentiation and apoptosis in PC12 pheochronocytoma cells

ZMNH - Seminare 2001

C. Richter-Landsberg, Oldenburg
Stress responses in oligodendrocytes: implications for neurodegenerative diseases

Murer, Zürich
Structure-function relationship of the type II Na/Pi-cotransporter

O. Paulsen
How information is stored in the brain - neuronal oscillations and synaptic plasticity in the hippocampus

A. Mautes, Homburg
Stress response after experimental spinal cord injury

M. Austen, Cambridge
Identification of new regulators of brain development in zebrafish through subtractive cloning and accelerated forward genetics

W. Wurst, München
Mid-hindbrain development: up- and down-stream of the isthmic organizer

M. Hebrok, San Francisco
Intercellular signalling pathways regulate pancreas formation

C. Rosenmund, Göttingen
Analysis of vesicle priming and release at a central synapse

S. Rose-John, Kiel
Shedding of membrane proteins: molecular mechanisms and physiologic relevance

R. Koester, Pasadena
In vivo imaging of neuronal migration in the developing zebrafish cerebellum

T. Heinzel, Frankfurt
Valproic acid - a novel histone deacetylase inhibitor as a candidate drug for cancer therapy

A. Ullrich, München
Target-specific cancer therapy

D. Frishman, München
Similarity-free prediction of gene function

S. Grissmer, Ulm
Three-dimensional structure of the outer and inner vestibule of a voltage-gated potassium channel

M. Koch, Bremen
Animal models of the neurodevelopmental hypothesis of schizophrenia

J. García Sancho, Valladolid
Subcellular Ca^{2+} domains and oscillations of mitochondrial Ca^{2+} in excitable cells

M. Knipper, Tübingen
Neurotrophins in the inner ear

SFB 444 Seminars

M. Bähr, Tübingen
Secondary neuronal death in trauma and ischemia: Molecular mechanisms and experimental therapy

J. E. Vance, Alberta
The role of lipids in the regulation of axonal growth of rat sympathetic neurons

E. Wanke, Milano
Studies of erg currents in hypothalamic LHRH-secreting neurons

C. McBain, Bethesda
Hippocampal interneurons, precision timing without lasting plasticity

Y. Barde, Martinsried
Signalling death of survival in nerve cells

T. Brady, Dallas
Sculpting the functional architecture of the neuron: myelinating glia and the axonal cytoskeleton

P. H. Seeburg, Heidelberg
Excitatory synaptic functions altered in mice by gene-targeting

M. Kiebler, Tübingen
Dendritic transport of Staufen-GFP in living hippocampal neurons

R. Vallee, Worcester
Cytoplasmic dynein: ist roles in cell division, vesicular transport, and brain development

W. Hevers, Mainz
Developmental heterogeneity of the GABAergic system in cerebellar granule cells

J. Herz, Dallas
Signalling by lipoprotein receptors in the brain

R. Baumeister, München
Of POU proteins and presenilins: studying the differentiation and function of the nervous system in a C. elegans model

L. S. Goldstein, La Jolla
Kinesin molecular motors: transport pathways, receptors, and Alzheimer's disease

K. Scholich, Memphis
Regulation of signalling through adenylyl cyclases

Financing

The ZMNH was financed in 1999 and 2000 by the City-state of Hamburg (FuHH), the Bundesministerium für Bildung und Forschung (BMBF) and by grants from research foundations and industry.
The FuHH provided the basic budget for the four institutes. Since 1999 also the three junior research groups, the central facilities and the building.

In 1999 and 2000 the total budget of the Centre amounted to 10.111 and 10.573 Million EUR, respectively. At present 242 people are employed at the ZMNH (not counting the Institute for Cell Biochemistry and Clinical Neurobiology).

At the time this report went to press, the Center's exact financing data of the year 2001 were not yet available. However, it is estimated that the figures will exceed the amounts of the year 2000.

Financing by FuHH

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

	Personnel costs	supplies incl. equipment
1999:		
FuHH	4.225	2.099
2000:		
FuHH	4.562	2.413

Other financing

In 1999 and 2000 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 provided by Stiftung Volkswagenwerk, the European Community (EC), and others. Outside support amounted to 7.028 Mio. EUR for 1999 and 2000.
The personnel and running costs given by the various funding agencies were (in thousand EUR):

	Personnel costs	supplies incl. equipment
1999:		
BMBF	430	409
DFG	1.221	428
VW Stiftung	134	30
Foundations/Unions	160	41
EC	127	20
Industry and other	48	33
SFB 444, 470, 545	471	235
totalling	2.591	1.196
2000:		
BMBF	332	113
DFG	1.102	538
VW Stiftung	92	17
Foundations/Unions	198	64
EC	198	105
Industry and other	4	26
SFB 444, 470, 545	512	297
totalling	2.438	1.160

Structure of the Centre

Director

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Directorate (Kollegium)

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Dr. Dirk Isbrandt
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Research Groups

Neuronal Cell Fate Specification
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