Newsletter
der
Deutschen Gesellschaft für Neurogenetik

January, 1999
DGNG News No. 9

Society News

Neurogenetics in Freiburg

The fourth annual meeting of the society, the 5th workshop neurogenetics in Germany was held in Freiburg from October 22-24, 1998. It was organized by B. Landwehrmeyer, R. Korinthenberg, C.H. Lücking, D. Morris-Rosenthal and U. Wolf. There were approximately 150 participants, both members and non-members. The workshop focused on the molecular pathogenesis of trinucleotide repeat disorders, mental retardation and abnormal development of the brain, epilepsies, multifactorial diseases, and mouse models for the investigation of neurological disorders. As in previous workshops, the topics were presented by international leaders in their respective fields. J.-L. Mandel (Illkirch) opened the meeting with an excellent overview of trinucleotide repeat disorders. S.W. Davies (London), A. Sittler (Berlin), and L. Lunkes (Illkirch) discussed the most recent findings of intranuclear inclusions and protein interactions in Huntington disease, and H. Orr (Minneapolis) gave an update on his exciting work in spinocerebellar ataxia, type 1. S.E. Antonarakis (Geneva) introduced the audience to the analysis of multifactorial disorders, and P. Heutink (Rotterdam) and K. Jendroska (Berlin) informed on the latest advances of research into temporal lobe dementia and fatal familial insomnia. A. Wynshaw-Boris (Bethesda) presented an animal model of human Miller-Dieker syndrome and isolated lissencephaly, and H. Bujard introduced the audience to his elegant system for generating conditional mouse mutants by cell type-specific control of gene activities via tetracyclines. Additional highlights included a session on epilepsies (P. Szepetowski, Oxford and O. Steinlein, Bonn) and on Parkinson disease (T. Gasser, München, C.B. Lücking, Paris, and R. Krüger, Bochum). Finally, there were interesting presentations on neuronal ceroid lipofuscinosis (A. Jalanko, Helsinki), gene therapy in brain tumors (A. Jacobs), and genes regulated by brain-derived neurotrophic factor, BDNF (A. von Holst, München). This meeting was of the highest scientific standard and - as previous conferences - it certainly fulfilled the major goal of the DGNG to promote interest and active research in Neurogenetics in Germany.

6th Workshop Neurogenetics in Germany, 5th Annual Meeting of the DGNG

The 1999 meeting of the society will be held in Bonn from September 16 to September 18, 1999, and will be organized by Professors T. Klockgether and P. Propping.

Research News

Insertion of a retroposon in Fukuyama congenital muscular dystrophy. Involvement of both muscle (muscular dystrophy) and brain (micropolygyria also known as cobblestone lissencephaly) is the clinical hallmark of Fukuyama congenital muscular dystrophy (FCMD). This autosomal recessive disorder is common in Japan with an incidence of 0.7-1.2 per

10,000 births. The disease locus has been assigned to 9q31 by homozygosity mapping [1]. Haplotype analysis revealed that most (> 80%) Japanese mutations derive from a single ancestor (founder effect) [1]. The investigation of allelic association has narrowed down the FCMD critical region to approximately 100 kb [2]. Now Kobayashi and coworkers [3] have constructed a cosmid contig of this interval. In order to search for chromosomal rearrangements in patients they performed Southern blot analyses. Patient DNA was cleaved with various restriction enzymes, separated on an agarose gel, transferred to a nylon membrane and hybridized to ^{32}P labelled cosmids in the presence of large amounts of sheared human genomic DNA to suppress hybridization to repeated DNA sequences. One cosmid revealed a novel fragment in PvuII, PstI, and BglII digested patient DNA that was not found in control DNA. Detailed analysis demonstrated that a 1.4 kb EcoRI fragment of this cosmid detected the novel restriction fragment that arose from a 3 kb insertion. The authors subsequently used the 1.4 kb EcoRI fragment to screen an adult brain cDNA library. Analysis of several positive clones and application of RACE (rapid amplification of cDNA end pieces) revealed that the cDNA spans 7,349 bp. It has an open reading frame of 1,383bp and is translated into a predicted protein of 461 amino acids. Two transcripts of 6.5 kb and 7.5 kb are detected by Northern analysis. Although the gene is expressed in many tissues, highest expression levels are observed in heart, brain, skeletal muscle, and pancreas. The gene, called fukutin, spans 100 kb of genomic DNA and is composed of 10 exons with the start codon in exon 2. In order to analyze the insertion in patients, Kobayashi et al. constructed a genomic library from a FCMD patient who was homozygous for the insertion. The inserted fragment is in the 3´untranslated region of the gene. It is composed of 41 copies of a hexanucleotide sequence, 27 tandemly repeated copies of a 49 bp sequence, a SINE (short interspersed nuclear element)-type human transposon sequence, a polyadenylation signal and a poly A stretch. These sequences are flanked by a target-site duplication consisting of a 17-base-pair direct repeat, indicative of a retroposon insertion. The insertion results in RNA instability and thus in a loss of gene function. Loss of gene function as the underlying cause of FCMD is supported by the discovery of several patients in whom translation of fukutin is prematurely terminated due to either a nonsense or a frameshift mutation. The authors demonstrated in vitro that fukutin can be secreted. Furthermore it may have a role in the dystrophin-associated complex of proteins that includes sarcoglycan, β-dystroglycan and laminin α2. In agreement with this, the authors found decreased immunostaining of β-dystroglycan and laminin α2 in muscle tissue from FCMD patients. There were also abnormalities of the basal lamina of muscle and brain tissue from patients. Thus fukutin may stabilize the dystrophin-complex and its loss could disrupt the integrity of the basal lamina thus giving rise to the observed symptoms of muscular dystrophy and micropolygyria.

1) Toda T, Ikegawa S, Okui K, Kondo E, Saito K, Fukuyama Y, Yoshioka M, Kumagai T, Suzumori K, Kanazawa I, Nakamura Y (1994) Refined mapping of a gene responsible for Fukuyama-type congenital muscular dystrophy: evidence for strong linkage disequilibrium. *Am J Hum Genet* **55:** 946-950.

2) Toda T, Miyake M, Kobayashi K, Mizuno K, Saito K, Osawa M, Nakamura Y, Kanazawa I, Nakagome Y, Tokunaga K, Nakahori Y (1996) Linkage-disequilibrium mapping narrows the Fukuyama-type congenital muscular dystrophy (FCMD) candidate region to <100 kb. *Am J Hum Genet* **59:** 1313-1320

3) Kobayashi K, Nakahori Y, Miyake M, Matsumura K, Kondo-Iida E, Nomura Y, Segawa M, Yoshioka M, Saito K, Osawa M, Hamano K, Sakakihara Y, Nonaka I, Nakagome Y, Kanazawa I, Nakamura Y, Tokunaga K, Toda T (1998) An ancient retrotransposal insertion causes Fukuyama-type congenital muscular dystrophy. *Nature* **394:** 388-392.

Caspase 9 required for normal brain development. Caspases comprise a family of cysteine-containing, aspartate-specific proteases. They are key effectors of apoptosis (programmed cell death, PCD). To date at least 11 caspases are known in mammals. Caspase 9 (casp9) forms a multiprotein complex of Apaf1 (apoptotic protease activating factor 1) and cytochrome c. There is evidence that cytochrome c released from mitochondria promotes apoptosis by activating the formation of the casp9/Apaf1 complex. Interaction of caspase 9 with Apaf1 is mediated by the CARD (caspase recruitment domain) motifs in the N terminal region of the proteins. Antiapoptotic proteins such as Bcl-X_L can also interact with casp9/Apaf1. The regulation of casp9 by both pro- and antiapoptotic proteins points to an important function of the molecule in apoptosis. In order to learn more about the function of casp9 in vivo, two groups [1,2] have constructed casp9 knock-out mice. The major findings were grossly disturbed differentiation of the brain and prenatal lethality in more than 90% of casp9 -/- homozygous mice. At embryonic day 13 the midbrain region was expanded and at day 16 ectopic growth of brain tissue was pronounced. Perturbations of the brain structure were most severe within cortex and forebrain. The surface of protruding brain tissue was irregular and the structure of the ventricles was highly disturbed. Despite the almost ubiquitous expression of casp9, nonneuronal organs and spinal cord were morphologically normal. Evidently malformations are brain-specific in the absence of casp9. Both groups then investigated normal embryonic brain development. They found significantly more cell death in cerebral cortex and the trigeminal ganglion of wild type mice at embryonic day 12 than in casp9 -/- homozygotes. Thus casp9 plays an important role in PCD during normal differentiation of the brain. It will be interesting to see whether some human malformations of the brain are also caused by mutations of casp9.

1) Kuida K, Haydar TF, Kuan C-Y, Gu Y, Taya C, Karasuyama H, Su MS-S, Rakic P, Flavell RA (1998) Reduced apoptosis and cytochrome c-mediated caspase activation in mice lacking caspase 9. *Cell* **94:** 325-337.

2) Hakem R, Hakem A, Duncan GS, Henderson JT, Woo M, Soengas MS, Elia A, de la Pompa JL, Kagi D, Khoo W, Potter J, Yoshida R, Kaufman SA, Lowe SW, Penninger JM, Mak TW (1998) Differential requirement for caspase 9 in apoptotic pathways in vivo. *Cell* **94:** 339-352.

Intranuclear inclusions in CAG trinucleotide repeat disorders: bystanders or causative agents of neurodegeneration. To date, eight disorders have been identified that are caused by the expansion of CAG repeats within the respective disease genes. These disorders include X-linked recessive spinocerebellar muscular atrophy (SBMA) and autosomal dominant Huntington's disease (HD), dentatorubropallidoluysian atrophy (DRPLA), and at least five subtypes of spinocerebellar ataxias (SCA1, 2, 3, 6,

and 7). All these diseases present with signs of nerve cell loss, a progressive process which usually starts during the third to fourth decade of life. The brain areas predominantly affected are specific for each of the diseases.

The pathological mechanisms underlying nerve cell dysfunction and neurodegeneration are not well known. Similarly, the reason for selectivity of neuronal vulnerability in each disorder is not yet understood. There is now increasing evidence that the protein carrying the expanded polyglutamine tract (encoded by the CAG repeat) has novel biochemical and biological features („gain of function"). Recent studies in HD, DRPLA, SCA1 and SCA3 in both humans and transgenic mice provide the first information about the pathogenic process. Morphologically, the mutated protein accumulates in the nucleus of the most severely affected brain regions as intranuclear inclusion bodies (DGNG News 7, 1997; Davies et al. 1997, Skinner et al. 1997, Schmidt et al. 1998, DiFiglia et al. 1997, Becher et al. 1998). However, until recently it has not been proven that the aggregation of the mutant proteins is by itself toxic. For example, it is also conceivable that neurons promote aggregation of mutant protein as a protective mechanism against more toxic, soluble forms.

These issues were addressed by two recent reports in Cell on ataxin-1 (Klement et al. 1998) and on huntingtin (Saudou et al. 1998). These papers represent major breakthroughs in unravelling the mystery of intranuclear inclusions (NIs). Klement et al. investigated the importance of the nuclear localization and aggregation of mutant ataxin-1 in the pathogenesis of SCA1 in transgenic mice. In earlier experiments this group had shown that an ataxin-1 cDNA containing 82 CAGs under the control of a Purkinje cell specific promoter causes neurodegeneration and neurological symptoms in transgenic mice (Skinner et al. 1997). Extending these studies, they now deleted a consensus nuclear localization signal (NLS, the K772T mutation) in ataxin-1. Ataxin-1 which is usually found in both the cytoplasm and the nucleus is therefore present exclusively in the cytoplasm. Most importantly, transgenic mice containing the 82 CAG trinucleotides as well as the K772T mutation neither developed degeneration of Purkinje cells, nor did they display NIs, nor did they suffer from ataxia (Klement et al. 1998). Thus, intranuclear localization of mutant ataxin-1 is a prerequisite for neurodegeneration to occur. Furthermore, Klement et al. generated transgenic mice with an ataxin-1 cDNA harboring 77 CAG's but lacking a self-association domain. The expressed protein enters the nucleus of transfected cells but does not form aggregates. Surprisingly, transgenic mice generated with this construct developed Purkinje cell pathology, although not in a progressive mode. Thus the generation of nuclear aggregates is not required for the initiation of the neurodegenerative process found in polyglutamine diseases.

This is further supported by findings on huntingtin reported by Saudou et al. 1998. These authors found that blocking of nuclear localization of mutant huntingtin in transfected cultured striatal neurons interfered with its ability to form nuclear inclusions and neurodegeneration. Nuclear localization was blocked by adding a nuclear export signal (NES) to huntingtin. Inhibition of nuclear export with leptomycin B fully restored the ability of mutant huntingtin

to aggregate in the nucleus. Earlier studies had shown that the NIs are ubiquitinated, a process which represents a first step in the ubiquitin/proteasome protein degradation pathway. Although coexpression of mutant huntingtin with a mutated ubiquitin-conjugating enzyme Cdc34p (hCdc34p [CL-S]) decreased the number of nuclear inclusions, the rate of cell death increased dramatically (Saudou et al. 1998). This suggests that ubiquitination of expanded polyglutamine proteins and the formation of NIs represents a cellular mechanism to inactivate toxic soluble nuclear proteins.

Despite this considerable progress, our understanding of the selectivity of neurodegeneration in the respective brain areas characteristic for each of the diseases has not much advanced. For ataxin-1, however, a leucine-rich acidic nuclear protein which is abundant in Purkinje cell nuclei has been shown to interact preferentially with the expanded protein (Matilla et al. 1997). Since abnormal protein-protein interactions could be enhanced by increasing the length of polyglutamine stretches, Sittler et al. searched for proteins interacting specifically with expanded huntingtin using the yeast two-hybrid system. This search has resulted in the identification of SH3GL3, a novel SH3-containing Grb2-like protein. SH3GL3 forms peri- and intranuclear aggregates when co-expressed with truncated expanded huntingtin. It was also found in the neuronal nuclear inclusions of mice transgenic for the HD mutation. Although SH3GL3 is highly expressed in the brain and testis, further expression studies are needed to determine if the expression pattern resembles the region specific cell death found in HD.

1) Becher MW, Kotzuk JA, Sharp AH, Davies SW, Bates GP, Price DL, and Ross CA (1997) Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length. Neurobiol of disease 4: 1-11

2) Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, and Zoghbi HY (1998) Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. Nat Genet 19: 148-154

3) Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, and Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90: 537-548

4) DiFiglia M, Sapp E, Chase K, Davies SW, Bates GP, Vonsattel JP, and Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277, 1990-1993

5) Klement IA, Skinner PJ, Kaytor MD, Hersch SM, Clark HB, Zoghbi HY, and Orr HT (1998) Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. Cell 95: 41-53

6) Matilla A, Koshy BT, Cummings CJ, Isobe T, Orr HT, and Zoghbi HY (1997) The cerebellar leucine-rich acidic nuclear protein interacts with ataxin-1. Nature 389: 974-978

7) Saudou F, Finkbeiner S, Devys D, and Greenberg ME (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 95: 55-66

8) Schmidt T, Landwehrmeyer GB, Schmitt I, Trottier Y, Auburger G, Laccone F, Klockgether T, Völpel M, Epplen JT, Schöls L, and Riess O (1998) An isoform of ataxin-3 accumulates in the nucleus of neuronal cells in affected brain regions of SCA3 patients. Brain Pathol 8: 669-679

9) Sittler A, Wälter S, Wedemeyer N, Hasenbank R, Scherzinger E, Eickhoff H, Bates GP, Lehrach H, and Wanker EE (1998) SH3GL3 associates with the Huntingtin exon 1 protein and promotes the formation of polygln-

containing protein aggregates. Mol Cell 2: 427-436

10) Skinner PJ, Koshy BT, Cummings CJ, Klement IA, Helin K, Servadio A, Zoghbi HY, and Orr HT (1997) Ataxin-1 with an expanded glutamine tract alters nuclear matrix-associated structures. Nature 389: 971-974

With the best wishes for a successful NEW YEAR.

Sincerely yours,

Ulrich Müller
Olaf Riess
Manuel B. Graeber

Protokoll der Mitgliederversammlung 1998

der

Deutschen Gesellschaft für Neurogenetik

Ort:	Neurozentrum - Hörsaalgebäude Killianstraße der Albert-Ludwigs-Universität Freiburg, Breisacherstr. 64 D-79104 Freiburg
Zeit:	Donnerstag, 22.10.1998
Beginn:	18:33 Uhr
Anwesend:	zunächst 26, dann 31 Mitglieder

TOP 1: Protokoll der letzten Mitgliederversammlung

Der Vorsitzende, Prof. Müller (Gießen), eröffnet die Mitgliederversammlung und stellt den Antrag auf Genehmigung des Protokolls der letzten Mitglieder-versammlung. Das Protokoll wird ohne Gegenstimme angenommen.

TOP 2: Bericht des Präsidenten

Prof. Müller berichtet über die erfreuliche Entwicklung der Mitgliederzahl der Gesellschaft, die sich nunmehr auf 161 Mitglieder vergrößert hat.

TOP 3: Bericht des Schriftführers

Dr. Graeber erwähnt das anhaltende Interesse der Zeitschrift NATURE, DGNG-Mitgliedern besonders günstige Konditionen für den Bezug der Zeitschrift einzuräumen. NATURE erbittet hierzu die Adressen-Liste der DGNG. Dr. Graeber fragt nach, ob hiermit Einverständnis bzw. Interesse an dieser Möglichkeit besteht. Dies wird bejaht.

TOP 4: Bericht der Schatz-meisterin, Kassenprüfung

Dr. Egensperger wird zum Vertreter des abwesenden Kassenprüfers bestellt. Dr. Kösel und Dr. Egensperger führen die Kassenprüfung durch. Die Schatzmeisterin, Frau Dr. Koehler, legt die Einnahmen- und Ausgabenrechnung der Gesellschaft dar. Prof. Müller stellt nach erfolgter Kassenprüfung den Antrag auf Entlastung der Schatzmeisterin, der ohne Gegenstimme angenommen wird.

TOP 5: Tagungsorte des DGNG-Meetings 1999 und 2000

Prof. Müller berichtet, daß die Professoren Klockgether und Propping (Bonn) die nächste DGNG-Tagung ausrichten werden (16.-18.09.1999). Dieser Termin überschneidet sich weder mit den großen nationalen noch internationalen Neuroscience- bzw. Genetik-Tagungen. Für die Organisation der DGNG-Tagung im Jahr 2000 konnte Prof. Reichmann (Dresden) gewonnen werden. Prof. Hübner (Berlin) weist darauf hin, daß bei der Planung von DGNG-Tagungen auch Überschneidungen mit Kongreß-Terminen der Neuropädiater beachtet werden sollten.

TOP 6: Vorschläge zur Verbesserung des Newsletters

Verbesserungsvorschläge werden nicht gemacht, jedoch laden Prof. Müller und Dr. Graeber alle DGNG-Mitglieder nochmals herzlich zur Teilnahme an der Gestaltung des Newsletters ein. Prof. Krone (Ulm) fragt an, ob auch Anfragen an die Mitglieder zwecks Forschungsmaterial im Newsletter veröffentlich werden können (z.B. wg. Zell-Linien). Dies wird bejaht.

TOP 7: Verschiedenes

Zu diesem Punkt gibt es keine Wortmeldungen.

Die Mitgliederversammlung endet um 18:47.

Freiburg, den 22.10.1998

Manuel B. Graeber (Schriftführer)
Ulrich Müller (Präsident)

An der Johann Wolfgang Goethe-Universität Frankfurt am Main ist unter den Einstellungsvoraussetzungen des § 39a des Gesetzes über die Universitäten des Landes Hessen (HUG) folgende Professur zu besetzen:

Im Fachbereich Humanmedizin an der Klinik für Neurologie (Direktor: Prof. Dr.H.Steinmetz) des Zentrums der Neurologie und Neurochiriurgie die

Professur (C3) für Experimentelle Neurologie auf Lebenszeit

Die Bewerber/innen sollen auf dem Gebiet der Molekulargenetik und/ oder molekularen Pathogenese neurologischer Erkrankungen besonders ausgewiesen sein. Eine Kooperation mit den am Ort tätigen molekular ausgerichteten Gruppen wird erwartet. Erwünscht ist die Mitarbeit im Sonderforschungsbereich 269 „Molekulare und zelluläre Grundlagen neuronaler Organisations-prozesse“, der Projekte der Universität und des Max-Planck-Instituts für Hirnforschung umfaßt.

Die Bewerberin/ der Bewerber soll habilitiert und wissenschaftlich besonders ausgewiesen sein. Sie/ er soll pädagogische Eignung, Engagement und Erfahrung in der Lehre mitbringen.

Bewerberinen und Bewerber sind gehalten, sich im Falle ihrer Berufung an der Selbstverwaltung der Universität zu beteiligen.

Die Johann Wolfgang Goethe-Universität Frankfurt am Main strebt eine Erhöhung des Anteils von Frauen am wissenschaftlichen Personal an und fordert daher Frauen nachdrücklich auf, sich zu bewerben.

Schwerbehinderte Bewerberinnen und Bewerber werden im Rahmen der geltenden gesetzlichen Bestimmungen bei der Stellenbesetzung bevorzugt behandelt.

Bewerbungen sind innerhalb von 6 Wochen nach Erscheinen dieser Anzeige nach den Richtlinien des Fachbereichs abzufassen und an den **Dekan des Fachbereichs Humanmedizin der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, 60590 Frankfurt am Main,** zu richten.

Vor Abgabe Ihrer Bewerbung bitten wir Sie, sich das Merkblatt über die erforderlichen Bewerbungsunterlagen mit Textauszug des § 39a HUG und anderer Rechtsvorschriften sowie über die Gestaltung des Schriften-verzeichnisses, wie es dem Hessischen Ministerium für Wissenschaft und Kunst vorzulegen ist, unter folgender Internetsadresse

http://www.dekanat.Klinik.uni-frankfurt.de/merkblaetter/

auszudrucken.