neurotrain

Training and Understanding Neurodegenerative Diseases

booklet

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Training and Understanding Neurodegenerative Diseases

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Training and Understanding Neurodegenerative Diseases

NEUROTRAIN

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Cognitive function is the most precious of all human attributes – its loss is considered as one of the most severe and dreadful handicaps for human life quality. The prevalence of neurodegenerative diseases is increasing in the European society and will get even more prevalent in our ageing population.
The focus of this Early Stage Research Training (EST) 'NEUROTRAIN' is to promote training possibilities and to improve our knowledge of neurodegeneration that will lead to prevention and therapy.

Improvement in quality of life is of paramount importance for Health Care providers of all governments in the European Union. Our attention must therefore focus on the ever increasing incidence of neurodegenerative diseases, resulting from an ever increasing life expectancy. In an effort to face this challenge, training and research in neuroscience must be strengthened and intensified. In America and in Europe, the last decade of the past century was defined as the "brain decade" in biomedical research. In order to encourage the synergy between researchers coming from different scientific and humanistic backgrounds, researchers from leading centres in London, Lund, Tuebingen and Munich suggest an exciting training possibility. It is presently possible to reach an understanding of the organisation of higher cognitive functions on the basis of achievements made in different scientific fields. Such knowledge is of utmost importance in order to face the increasing impact of clinical diseases, as it allows the transfer of basic research to therapeutical applications in real time. The specific objective of this Early Stage Research Training (EST) network is the integration and networking of ten outstanding research teams in the context of a well-defined research programme that will formulate and facilitate a structured training programme for researchers in the highly-specialised field of neurodegeneration research. 'NEUROTRAIN' will provide a unique and cohesive but flexible framework for the training and professional development of young researchers. These interacting centres bring together a critical mass of theoretical and applied research using a multidisciplinary approach. Each centre represents an existing working unit, having its own specific expertise and research agenda. Our project objectives have been designed based on the conviction and implicit belief that treatment of neurodegenerative diseases can only be achieved through a comprehensive understanding of genes involved in neuronal development, structure and cellular function in health and disease. This will allow a simultaneous approach for a functional understanding and characterization of disease mechanisms affecting central nervous system (CNS) and retinal neurons and lead to the development of therapeutic approaches, the ultimate end-point of this research programme.

PROJECT 1

Generation of dopaminergic neural precursor cells for Parkinson's disease **(Responsible Supervisor: PD Dr. MINGER, London, UK)**

The Stem Cell Biology Laboratory, led by Dr Stephen Minger, is housed in the new Wolfson Centre for Age-Related Disease at King's College London. The lab is one of the pioneering groups in stem cell biology in the UK. They were one of the first two research teams in the UK to be granted a license to derive human embryonic stem (ES) cells by the Human Fertilisation and Embryology Authority in 2002 and were the first group to successfully generate human ES cells lines and to deposit these cells into the UK Stem Cell Bank. To date, the lab has generated three unique human ES cell lines, one of which harbours the most common mutation that causes Cystic Fibrosis. We will continue to attempt to generate additional human ES cell lines, particularly disease-specific cell lines, that will provide important tools not only for fundamental pathophysiological research, but also as screening tools for new therapeutic compounds and gene targeting strategies.
In addition to our work on human ES cell derivation, much of the effort in the Stem Cell Biology Laboratory is focused on the generation of somatic (e.g. tissue-specific) stem cell populations from human ES cells that may have therapeutic benefit. We are focused on the following clinical targets where cell replacement may have important therapeutic implications: (collaborators to the right)

• Spinal Cord Damage	Prof Stephen McMahon (KCL)
• Multiple Sclerosis	Dr Paul Felts (KCL)
• Parkinson's Disease	Dr Susan Duty (KCL)
• Heart Damage	Dr Anthony Mathur (Bart's Hosp)
• Type I Diabetes	Prof Peter Jones (KCL)
• Retinal Disorders	Prof Robin Ali (Inst Ophthalmology)
• Hepatic Insufficiency	KCL Liver Transplant Unit
• Joint Disorders	Dr Cosimo DeBari (KCL)
• Bone Replacement	Dr Agi Grigoriadis (KCL)
• Tooth Replacement	Prof Paul Sharpe (KCL)
• Haematopoietic Stem Cells	Prof Paul Fairchild (Oxford)

In addition, we have basic science programmes cells in the following areas:

• Lung Development/CF	Novartis Institute
• Huntington's Disease	Prof Gill Bates (KCL)
• Stem Cell Proteomics	Prof Tony Ng (KCL)
• Stem Cell Bioprocessing	Dr Chris Mason (UCL)
• Bioethics of Human ES Cells	Prof Herbert Gottweis (Vienna), Prof John Harris (Manchester), Prof Christine Hauskeller (Exeter) Prof Brian Salter (Norwich) Prof Sarah Franklin (LSE)
• Adult Neurogenesis	Prof Clive Ballard (KCL) Dr Aaron Chuang (GSK)

Relevant Publications

Taylor H and Minger SL. 2005. Regenerative medicine in Parkinson's disease: Generation of mesencephalic dopaminergic cells from embryonic stem cells. Curr Opin Biotech 16, 487-92

Braude P, Minger SL, Warwick RM. 2005. Stem cell therapy: hope or hype? BMJ 330, 1159-60.

Pickering SJ*, Minger SL*, Patel MJ, Taylor H, Black C, Burns CJ, Eknonmou A, and Braude PR. 2005. Generation of a human embryonic stem (hES) cell line encoding the cystic fibrosis mutation using preimplantation genetic diagnosis. Reprod Biomed 10, 390-97.

Milne HM, Burns CJ, Kitsou-Mylona I, Luther MJ, Minger SL, Persaud SJ, Jones PM. 2005. Generation of insulin-expressing cells from mouse embryonic stem cells. Biochem Biophys Res Commun. 328, 399-403

Goncalves MBCV, Boyle J, Webber DJ, Hall S, Minger SL and Corconan JPT. 2005. Timing of retinoid signaling pathway determines the expression of neuronal markers in neural progenitor cells. Dev Biol. 268, 60-70.

Burns CJ, Minger SL, Hall S, Milne H, Ramracheya RD, Evans ND, Persuad SJ, and Jones PM. 2005. The in vitro differentiation of rat neural stem cells into an insulin-expressing phenotype. Biochem. Biophys. Res. Commun. 326, 57-577.

Webber, DJ and Minger, SL. 2004. Therapeutic potential of stem cells in central nervous system regeneration. Curr Opin Invest Drugs 5, 714-9.

Pickering SJ, Braude P, Patel M, Burn CJ, Bolton V**,** Minger SL. 2003. Preimplantation genetic diagnosis as a novel source of embryos for stem cell research. Reprod BioMed, 7, 353-364.

Minger,S.L.; Honer,W.G.; Esiri,M.M.; McDonald,B.; Keene,J.; Nicoll,J.A.; Carter,J.; Hope,T.; Francis,P.T. 2001. Synaptic pathology in prefrontal cortex is present only with severe dementia in Alzheimer disease. *J.Neuropathol.Exp.Neurol.* 60, 929-936.

Minger, S.L., Esiri, M.M., McDonald, B., Keene, J., Carter, J., Hope, T., Francis, P.T. 2000. Cholinergic deficits contribute to behavioral disturbance in patients with dementia. *Neurology* 55, 1460-1467.

PROJECT 2

PINK 1 and molecular basis of cell degeneration in Parkinson's disease **(Responsible Supervisor: Prof. WOOD, London, UK)**

Prof Wood is head of department of molecular neuroscience at the Institute of Neurology, UCL, London. The Institute is closely associated in its work with the National Hospital for Neurology & Neurosurgery, a Department of the University College London Hospitals' NHS Foundation Trust, and in combination they form a national and international centre at Queen Square for teaching, training and research in neurology and allied clinical and basic neurosciences.

The Institute has seven Research Departments encompassing clinical and basic research within in each theme. The dept of Molecular Neuroscience brings a range of molecular and cellular techniques to address basic and applied scientific questions which relate to human neurological disease. The Institute employs a total of around 370 staff, occupies some 11,500sq m of laboratory and office space in five buildings, and has a current annual turnover of £24m. The Institute was awarded a Grade 5*A rating (the highest possible rating) in the 2001 Exercise, a measure of its national and international standing.

Within the institute there are extensive molecular and cellular biological resources and equipment available. These include:

Molecular genetics- High throughout sequencing and genotyping (TaqMan)

Molecular Biology

Quantitative realtime PCR

Cell culture- including confocal suites

Neurochemistry- with expertise in mitochondrial biochemistry

Histology and immunohistochemistry

A new state of the art proteomics facility is currently being established between the Institutes of neurology and Child health which will provide a platform of technologies for use by researchers of UCL.

These techniques and technologies will be made available to the research student to further the project based on recently identified novel genes causing Parkinson's disease- PINK1 and LRRK2 (see publication below- Valente et al 2004, Paisan-Ruiz et al 2004). The challenge is to further understand the molecular and cellular events leading to cell degeneration. The specific aims of this project are to establish a primary human neuronal cell line and a human neuroblastoma cell line which have undergone a variety of manipulations of the PINK1 and/or the LRRK2 gene. These manipulations will include over-expression and knock-down studies using siRNA.

Training:

The Institute has a large postgraduate training programme and is also responsible for teaching inpatient undergraduate neurology to University College medical students Academic and research links with other parts of UCL are being continuously developed.Full details of the Institute's research and teaching activity can be found on the Institute of Neurology website at http://www.ion.ucl.ac.uk

Specifically for this project will offer the research scientist(s) training and experience in:

-Cell culture techniques- particularly handling SHSY-5Y and human neuronal dopaminergic cell lines.

-Western blotting

-Standard and advance molecular genetic/biological techniques (as required)

-Confocal microscopy

-Quantitative RNA analysis

-Antibody characterization

-siRNA techniques

Relevant publications:

Valente EM, Abou-Sleiman PM, Caputo V, Muqit MK, Harvey K, Gispert S, Ali Z, Del Turco D, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, González-Maldonado R, Deller T, Salvi1 S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, Wood NW. Hereditary early onset Parkinson's disease is caused by mutations in PINK1. Science. 2004;304(5674):1158-60.

Healy DG, Abou-Sleiman PM, Ahmadi KR, Muqit MM, Bhatia KP, Quinn NP, Lees AJ, Latchmann DS, Goldstein DB, Wood NW. The gene responsible for PARK6 Parkinson's disease, PINK1, does not influence common forms of parkinsonism. Ann Neurol. 2004;56:329-35.

Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der Brug M, de Munain AL, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de Silva R, Lees A, Marti-Masso JF, Perez-Tur J, Wood NW, Singleton AB. Cloning of the Gene Containing Mutations that Cause PARK8-Linked Parkinson's Disease. Neuron. 2004;44:595-600.

Ahmadi KR, Weale ME, Xue ZY, Soranzo N, Yarnall DP, Briley JD, Maruyama Y, Kobayashi M, Wood NW, Spurr NK, Burns DK, Roses AD, Saunders AM, Goldstein DB. A single-nucleotide polymorphism tagging set for human drug metabolism and transport. Nat Genet. 2005;37:84 -9.

Gandhi S, Wood NW. Molecular pathogenesis of Parkinson's disease. Hum Mol Genet. 2005;14:2749-55.

Gilks WP, Abou-Sleiman PM, Gandhi S, Jain S, Singleton A, Lees AJ, Shaw K, Bhatia KP, Bonifati V, Quinn NP, Lynch J, Healy DG, Holton JL, Revesz T, Wood NW. A common LRRK2 mutation in idiopathic Parkinson's disease. Lancet. 2005;365(9457):415-6.

Sleiman PA, Muqit M, Wood NW. Expanding insights of mitochondrial dysfunction in Parkinson's disease. Nature Reviews Neuroscience. 2006;7:207-19.2006.

Muqit MK, Abou-Sleiman P, Saurin A, Harvey K, Eaton S, Payne Smith MD, Venner K, Gandhi S, Matilla A, Healy DG, Gilks WP, Lees AJ, Holton J, Revesz T, Harvey RJ, Parker PJ, Wood NW, Latchman DS. Altered localization of PARK6 protein, PINK1, to aggresomes following proteasome inhibition in mammalian cells: role of mitochondria in aggregate formation *in vitro* and in Parkinson's disease brain. J Neurochem 2006 (in press)

Gandhi S, Muqit MM, Stanyer L, Healy DG, Abou-Sleiman PM, Hargreaves I, Heales S, Ganguly M, Parsons L, Lees AJ, Latchman DS, Holton JL, Wood NW, Revesz T. PINK1 protein in normal human brain and Parkinson's disease. Brain. 2006 May 15; [Epub ahead of print]

PROJECT 3

Genetics and functional genomics of retinal degenerations
(Responsible Supervisor:
Prof. BHATTACHARYA, London, UK)

Professor Shomi S. Bhattacharya is Head of the Department of Molecular Genetics at the Institute of Ophthalmology, University College London (UCL). The Institute of Ophthalmology is a multi-disciplinary research institue with expertise in electrophysiology, psychophysics, morphology, molecular and cellular biology, biochemistry, histopathology, clinical ophthalmology and epidemiology. It is committed to a research programme that furthers an understanding of the eye and visual system linked with clinical investigations targeted to specific problems in the prevention and treatment of eye diseases. The department of Molecular Genetics within the Institue is fully resourced and equipped with the State-of-the-Art instrumentation for molecular biology oriented research. These include a large capacity for gel electrophoresis, automated DNA sequencing, ultrcentrifugation, over 30 PCR blocks, tissue culture and radioisotope laboratories, biochemistry suite for structural and functional studies and extensive computational facility. The techniques of disease gene mapping, positional cloning, mutation analysis, tissue in-situ hybridisation, in-vitro mutagenesis and biochemical techniques for protein structure and functional analysis are in routine use in the department.

Background:
Molecular Genetics defines a field of study where in the absence of a detectable biochemical defect (true for a majority of inherited diseases), a reverse genetics approach allows the chromosomal assignment and isolation of the disease causing genes. Advent of recombinant DNA technology led to the cloning of chromosome specific DNA markers in the early 1980s. The ability to detect restriction fragment length polymorphisms (RFLPs) in the Human Genome, permitted the use of these markers as genetic tools for linkage studies in families segregating for a particular disease trait.

Inherited retinal dystrophies are a major cause of incurable blindness in the Western World. Amongst these a significant proportion is accounted for by a clinically heterogeneous group of diseases collectively known as Retinitis pigmentosa (RP). Based on pedigree analysis, all three modes of Mendelian inheritance (Autosomal dominant, Autosomal recessive and X-linked) have been observed. RP is initially characterised by night blindness and progressive loss of peripheral vision due to the loss of rod photoreceptor cells. As the disease progresses cone cells in the central retina are also involved, often leading to total blindness in later stages of the disease. The major programme of research in the lab is dedicated to the understanding of the molecular genetic and functional basis of retinal degeneration.

Training:
A comprehensive training will be offered to new candidates joining the lab in the following methods:
1. Linkage mapping using hypervariable marker systems (microsatellites, SNPs)
2. Positional cloning, physical contig mapping
3. Gene prediction software and bioinformatics

4. Mutation screening using high troughput Transgenomics WAVE machine
5. PCR based direct genomic sequencing
6. Expression analysis by RT-PCR, in situ hybridisation, confocal microscopy
7. Western blotting
8. Quantitative RNA analysis
9. Writing of lab reports and manuscripts for publication
10. Poster and seminar presentation skills

List of publications:

Cone-Rod Dystrophy due to mutations in a novel photreceptor-specific homeobox gene (CRX) essential for maintenance of the photoreceptor. Freund, C.L., Gregory-Evans, C.Y., Furukawa, T., Papaioannou, M., Looser, J., Ploder, L., Bellingham, J., Ng, D., Herbrick, J.S., Duncan, A., Scherer, S.W., Tsui, L., Loutradis-Anagnostou, A., Jacobson, S.G., Cepko, C.L., Bhattacharya, S.S. and McInnes, R.R. (1997). Cell, 91: 543-553.

Genetic blindness: current concepts in the pathogenesis of human outer retinal dystrophies. Gregory-Evans, K. and Bhattacharya, S.S. (1998). Trends in Genetics. 14: 103-108.

Mutation in *NRL* is associated with autosomal dominant retinitis pigmentosa. Bessant, D.A.R., Payne, A.M., Mitton, K.P., Wang, Q-L., Swain, P.K., Plant, C., Bird, A.C., Zack, D.J., Swaroop, A. and Bhattacharya, S.S. (1999). Nature Genetics, 21: 355-356.

Restoration of photoreceptor ultrastructure and function in retinal degeneration slow mice by gene therapy. Ali, R.R., Sarra, G.M., Stephens, C., Alwis, M.D., Bainbridge, J.W., Munro, P.M., Fauser, S., Reichel, M.B., Kinnon, C., Hunt, D.M., Bhattacharya, S.S. and Thrasher, A.J. (2000). Nature Genetics, 25: 306-310.

A human homolog of yeast pre-mrna splicing gene, prp31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (rp11). Vithana, E.N., Abu-Safieh, L., Allen, M.J., Carey, A., Papaioannou, M., Chakarova, C., Al-Maghtheh, m., Ebenezer, N.D., Willis, C., Moore, A.T., Bird, A.C., Hunt D.M.and Bhattacharya, S.S. (2001). Mol. Cell, 8: 375-381.

Mutations in HPRP3, a third member of pre-mRNA splicing factor genes, implicated in autosomal dominant retinitis pigmentosa. Chakarova, C.F., Hims, M.M., Bolz, H., Abu-Safieh, L., Patel, R.J., Papaioannou, M.G., Inglehearn, C.F., Keen, T.J., Willis, C., Moore, A.T., Rosenberg, T., Webster, A.R., Bird, A.C., Gal, A., Hunt, D., Vithana, E.N. and Bhattacharya, S.S. (2002). Hum Mol Genet. **11**: 87-92.

Mutant carbonic anhydrase 4 impairs pH regulation and causes retinal photoreceptor degeneration. Yang, Z., Alvarez, B.V., Chakarova, C., Jiang, L., Karan, G., Frederick, J.M., Zhao, Y., Sauve, Y., Li, X., Zrenner, E., Wissinger, B., Den Hollander, A.I., Katz, B., Baehr, W., Cremers, F.P., Casey, J.R., Bhattacharya, S.S., Zhang, K. (2005). Hum Mol Genet. **14**: 255-265.

PROJECT 4

Investigation of disease mechanisms in Huntington's disease **(Responsible Supervisor: Prof. RIESS, Tuebingen, Germany)**

The research work of the Department of Medical Genetics is directed towards deciphering the pathogenesis of neurodegenerative diseases such as Parkinson's disease, spinocerebellar ataxia, dystonia and Huntington's disease. We have a strong background on mutation screening in large patient collections, on the search for disease modifiers, but also on protein and RNA work. To our department belong a service core facility for microarray analysis (Affymetrix based) and a core facility for the generation of transgenic animals. Having this background we generated a large number of transgenic and knock out models of neurodegenerative diseases, in particular also transgenic rat models which are in particular useful for behavioral and treatment studies. Also, we are investigating altered transcriptional pathways in the brain of these models. Finally, the gain insight into the pathogenesis of neurodegeneration we are searching for unknown interacting proteins and if these proteins modify the disease process in vitro.

Histology

- Immunocytochemistry
- Histochemistry

Microarray analysis (Affymetrix)

- Whole genome 500 k SNP analysis
- Transcriptome analysis of human and mouse tissue
- Resequencing analysis

Transgenesis

- Generating transgenic mice and rats
- Knock out technology
- Inducible mouse models
- Behavioral studies

Molecular biology

- PCR
- dHPLC
- Pyrosequencing
- RT-PCR (LC480 Roche)

Microscopy

- Light and fluorescence microscope
- Confocal microscope (shared)

List of publications:

1. Strauss et al.: Loss of function mutations in the gene encoding Omi/HtrA2 in Parkinson's disease. **Hum Mol Genet** 14, (2005), 2099-2111
2. Bauer et al.: Regional and subtype selective changes of neurotransmitter receptor density in a rat transgenic for the Huntington's disease mutation. **J Neurochem** 94, (2005), 639-650
3. Hering et al.: Novel E64D mutation in DJ1 causes early onset Parkinson's disease. **Hum Mut** 24, (2004), 321-329

4. Liani et al.: Ubiquitylation of synphilin-1 and α-synuclein by SIAH proteins and their presence in Lewy bodies imply a role in Parkinson's disease. **Proc Natl Acad Sci USA**, 101, (2004), 5500-5505
5. Bauer et al.: Trinucleotide repeat expansions in TBP is the second most common monogenic cause for a Huntington's disease like phenotype in Caucasians. **J Med Genet** 41, (2004), 230-232
6. Bonin et al.: Microarray expression analysis of gad mice implicates involvement of Parkinson's disease associated UCH-L1 in multiple metabolic pathways. **Brain Res Mol Brain Res** 126, (2004), 88-97
7. Marx et al.: Identification and functional characterization of a novel R621C mutation in the synphilin-1 gene in Parkinson's disease. **Hum Mol Genet** 12, (2003), 1223-1231
8. Rolfs et al.: Clinical features and neuropathology of autosomal dominant spinocerebellar ataxia (SCA17). **Ann Neurol** 54, (2003), 367-375
9. Berg et al.: Specification of 14-3-3 proteins in Lewy bodies. **Ann Neurol** 54, (2003),135
10. Schöls et al.: Do CTG expansions at the SCA8 locus cause ataxia? **Ann Neurol** 54, (2003), 110-5
11. Von Hörsten et al.: Transgenic rat model of Huntington's disease. **Hum Mol Genet** 12, (2003), 617-624.

PROJECT 5

Genetic and molecular basis of Parkinson's disease caused by LRRK2-mutations **(Responsible Supervisor: Prof. GASSER, Tuebingen, Germany)**

The Department of Neurodegeneration at the Hertie-Institute for Clinical Brain Research is devoted to unveil molecular mechanisms of movement disorders based on a combination of human genetics and functional validation in purified protein preparations, cell culture models, yeast, nematode mutants, and transgenic mice. A number of Parkinson's disease genes have been identified by the Gasser lab, most recently PARK8 encoding leucin-rich repeat kinase-2 (LRRK2), a major and dominant cause of Parkinson's disease. The small synaptic phosphoprotein α–synuclein (PARK1 and PARK4) is genetically and pathologically linked to Parkinson's disease. The defining hallmark lesion of Parkinson's disease, the Lewy body, is composed largely of □□ synuclein fibrils. Moreover, Lewy bodies occur in several forms of dementia. In addition to these neuronal inclusions, α–synuclein fibrils are found in glial cytoplasmic inclusions of multiple system atrophy patients. The ubiquitin-ligase parkin (PARK2), mutations of which cause autosomal-recessive juvenile parkinsonism, may be functionally connected to α–synuclein. Two novel recessive genes link Parkinson's disease to mitochondria and oxidative stress, namely the mitochondrial kinase PINK1 (PARK6) and the multifunctional protein DJ-1 (PARK7). The Kahle lab is investigating the structural defects of Parkinson's disease relevant proteins, study the cell biological consequences of these protein aberrations, and develop animal models of Parkinson's and related disorders.

Collectively, we currently focus on the following projects:

PARK1 /α–synuclein

Cell culture models of α–synucleinopathy
- + Study of cellular consequences of post-translational modification (phosphorylation, ubiquitination)

Nemotode models of α–synucleinopathy
- + Genetic networks influencing α–synuclein toxicity

Transgenic mouse models of α–synucleinopathy
- + Synaptic transport
- + Solubility
- + Neuropathology
- + Deterioration of cognitive and locomotor functions

Cross-breeding with additional risk genes

PARK2 / Parkin

Regulation of parkin ubiquitin ligase activity by phosphorylation
Involvement in unfolded protein stress
Yeast two-hybrid screen for interactors

PARK3 / unknown

Fine mapping of the locus

PARK6 / PINK1
Involvement in oxidative stress response

PARK7 / DJ-1
Structure / function analysis
+ Cell culture assays of anti-oxidative activity
+ Biophysical determination of solution structure
Neuropathology

PARK8 / LRRK2
Identification of pathological signal transduction pathways

Selected publications:

1. Kahle et al. (2000) Subcellular localization of wild-type and Parkinson's disease-associated mutant α–synuclein in human and transgenic mouse brain. *J. Neurosci.* **20**:6365

2. Neumann et al. (2002) Misfolded proteinase K-resistant and hyperphosphorylated α–synuclein in aged transgenic mice and in Lewy body disease patients. *J. Clin. Invest.* **110**:1429

3. Kahle et al. (2002) Hyperphosphorylation and insolubility of α–synuclein in transgenic mouse oligodendrocytes. *EMBO Rep.* **3**:583

4. Müller et al. (2005) Multiple regions of α–synuclein are associated with Parkinson's disease. *Ann Neurol.* 57:535

5. Yamamoto et al. (2005) Parkin phosphorylation and modulation of its E3 ubiquitin ligase activity. *J. Biol. Chem.* **280**:3390

6. Springer et al. (2005) A *Caenorhabditis elegans* parkin mutant with altered solubility couples α–synuclein aggregation to proteotoxic stress. *Hum. Mol. Genet.* **14**:3407

7. Gasser et al. (1998) A susceptibility locus for Parkinson's disease maps to chromosome 2p13. *Nat. Genet.* **18**:262

8. Görner et al. (2004) Differential effects of Parkinson's disease-associated mutations on stability and folding of DJ-1. *J. Biol. Chem.* **279**:6943

9. Zimprich et al. (2004) Mutations in LRRK2 cause autosomal-dominant parkinsonism with pleomorphic pathology.*Neuron* **44**:601

PROJECT 6

Genetic susceptibility factors in glaucoma
(Responsible Supervisor: Dr. WISSINGER, Tuebingen, Germany)

The Molecular Genetics Laboratory (MGL) at the University Eye Hospital Tuebingen has been established in 1992. It is one of very few medical genetic research labs embedded in an ophthalmologic clinic in Germany. The lab maintains a patients sample registry which includes more than 15.000 samples of patients with ocular disorders and their family members.

One main focus of our research program is color vision and the hereditary color vision deficiencies. We have performed studies on the structure of the red-/green-opsin gene cluster, with respect to red-/green-colorblindness and blue cone monochromacy. To be mentioned are also the publications of the identification, cloning and characterisation of the causative genes for autosomal recessive achromatopsia (rod monochromacy, total colorblindness).
Another main research project focuses on the causes of hereditary optic neuropathies. In the past we have worked intensively on the maternally/mitochondrially inherited Lebers hereditary Optic Neuropathy /LHON. Recently we are working on the autosomal dominantly inherited Optic Atrophy Type Kjer (ADOA). Our lab was substantially involved in the identification, cloning and characterisation of the first causative gene.
Our most recent project deals with the identification of hereditary factors in glaucoma.

The lab has profound expertise in nucleic acids purification and analysis, including DNA and RNA isolation, DNA and cDNA cloning, pulsed field electrophoresis, Southern and Northern Blot analysis, PCR and RT-PCR, real-time PCR, marker genotyping, mutation detection (SSCP and Heteroduplex analysis) and DNA sequencing. Facilities for mammalian cell culturing are available and more recently, techniques for functional analysis of genes and gene products (e.g. cell transfections, SDS gel electrophoresis, Western blotting, immunocytochemistry, Ca^{2+}-Imaging) have been established.

Key publications:

1. Alexander, C., Votruba, M., Pesch, U., Thiselton, D., Mayer, S., Moore, T., Rodriguez, M., Kellner, U., Leo-Kottler, B., Auburger, G., Bhattacharya, S., Wissinger, B. (2000) OPA1, a gene encoding a dynamin-related GTPase, is mutated in autosomal dominant optic atrophy linked to chromosome 3q28. Nature Genetics 26: 211-215.

2. Kohl S, Baumann B, Rosenberg T, Kellner U, Lorenz B, Vadala M, Jacobson SG, Wissinger B (2002) Mutations in the cone photoreceptor G-protein alpha-subunit gene GNAT2 in patients with achromatopsia. *American Journal of Human Genetics 71:422-425.*

3. Weisschuh, N., Neumann, D., Wolf, C., Wissinger, B., Gramer, E. (2005) Prevalence of myocilin and optineurin
4. sequence variants in german normal tension glaucoma patients. Molecular Vision 11:284-287.

5. Kohl S, Varsanyi B, Antunes GA, Baumann B, Hoyng CB, Jagle H, Rosenberg T, Kellner U, Lorenz B, Salati R, Jurklies B, Farkas A, Andreasson S, Weleber RG, Jacobson SG, Rudolph G, Castellan C, Dollfus H, Legius E, Anastasi M, Bitoun P, Lev D, Sieving PA, Munier FL, Zrenner E, Sharpe LT, Cremers FP, Wissinger B (2005) CNGB3 mutations account for 50%

of all cases with autosomal recessive achromatopsia. European Journal of Human Genetics 13:302-308.

6. Schuster A, Weisschuh N, Jagle H, Besch D, Janecke AR, Zierler H, Tippmann S, Zrenner E, Wissinger B (2005) Novel rhodopsin mutations and genotype-phenotype correlation in patients with autosomal dominant retinitis pigmentosa. British Journal of Ophthalmology 89:1258-1264.

PROJECT 7

Understanding the molecular basis of neuroprotection
(Responsible Supervisor:
Prof. VAN VEEN, Lund, Sweden)

Retinitis Pigmentosa (RP) refers to a group of blinding diseases affecting 1 in 4000 persons in the industrialized world, with symptoms at times noticeable already at an early age. Although progress is being made in elucidating their genetic ethiology (mutations in about 40 genes have so far been identified to be associated with these diseases), very little is known about the exact mechanisms of degeneration and no treatment is yet available. In age-related macular degeneration (AMD), the cone-rich area of the retina is affected and one fears that this type of degeneration will become a more prevalent cause of blindness among the elderly than diabetic retinopathy and glaucoma combined.

As a result of a mutation or following injury, a number of critical alterations are induced in target neuronal cells. As the target cells degenerate, responses are, in addition, elicited in other cells (neuronal & non-neuronal) – responses that are likely to impact on the progression of the disease. Several mechanisms involving, *e.g.,* glial cells under physiological conditions are activated also under pathological conditions. An increased endogenous production of various factors by glial cells is observed in response to neuronal stress and several studies have demonstrated the ability of many of these factors to promote neuronal survival, when applied exogenously. However, it is also well established that activation of non-neuronal cells leads directly and/or indirectly to increased expression of *e.g.,* inflammatory cytokines, matrix metalloproteinases, and other molecules, many of which can contribute to accelerating the progress of the degeneration and/or to limiting the effectiveness of a treatment. In this context, it is particularly interesting to consider what occurs, for instance, in the case of RP, in which mutations are seldom found in cone-specific genes, but mostly in rod-specific genes. Yet, cone cells, which are essential for high acuity and daytime colour vision, are lost in the course of or following rod cell loss, suggesting indirect mechanisms.

The programme will examine the role of non-neuronal cells as modulators/mediators of degeneration of retinal cells. The studies involve examination of various models of retinal cell degeneration in which mutations in genes encoding for rod- and retinal epithelium-specific proteins lead to loss of photoreceptor cells. An understanding of the contribution of non-neuronal cells to secondary pathophysiological responses will allow us to control the activity of these cells, "tipping the scale" in favour of neuroprotection and regeneration – an important aspect when designing therapies.

Selected publications:

Azadi S, Paquet-Durand F, Medstrand P, van Veen T, Ekström PA. (2006) Up-regulation and increased phosphorylation of protein kinase C (PKC) delta, mu and theta in the degenerating rd1 mouse retina. Mol Cell Neurosci. [Epub]

Zhang Y, Kardaszewska AK, van Veen T, Rauch U, Perez MT. (2004) Integration between abutting retinas: role of glial structures and associated molecules at the interface. Invest Ophthalmol Vis Sci. 45:4440-9.

Archer SN, Ahuja P, Caffé R, Mikol C, Foster RG, van Veen T, von Schantz M. (2004) Absence of phosphoglucose isomerase-1 in retinal photoreceptor, pigment epithelium and Müller cells. Eur J Neurosci. 19:2923-30.

Zhang Y, Rauch U, Perez MT. (2003) Accumulation of neurocan, a brain chondroitin sulfate proteoglycan, in association with the retinal vasculature in RCS rats. Invest Ophthalmol Vis Sci. 44:1252-61.

PROJECT 8

Molecular basis of epilepsy and development of gene/cell based therapies **(Responsible Supervisor: Prof. KOKAIA, Lund, Sweden)**

Epilepsy is one of the most devastating neurological diseases affecting about 1 % of the population of the western countries. Despite certain progress in antiepileptic drug development in about 25 % of cases epileptic seizures do not respond to conventional treatment therapies based on enhancement of inhibitory GABAergic synaptic processes in the brain. Recently neuropeptides and neurotrophic factors emerged as strong regulators of excitatory and inhibitory synaptic transmission in the CNS thus offering a potent tool to counteract the seizure activity. However, it is not clear what the role of these agents is in mechanisms of epileptogenesis.

We explore the role of neuropeptides and neurotrophic factors in modulation of excitatory and inhibitory synaptic transmission and epileptogensis in the brain. We use respective knock-out and overexpressing mice in combination with in vivo epilepsy models (kindling) and in vitro approaches (patch-clamp in brain slices). One of the specific aims of this research line is to use both direct and indirect targeted gene transfer of neuropeptides and neurotrophic factors into the different brain regions to investigate mechanistic aspects of their action on synaptic transmission and epileptogensis.

Histology

- Immunocytochemistry
- Histochemistry
- Other common histological stainings

Molecular biology

- *In situ* hybridization
- PCR
- ELISA

Cellular neurophysiology

- Patch-clamp in brain slices using 'blind approach'
- Patch-clamp in brain slices using infrared video visualization of cells
- Patch-clamp in brain slices of GFP-labeled transplanted and endogenous stem cells
- Field recordings in brain slices

Models of epilepsy

- Conventional kindling
- Rapid kindling
- Status epilepticus

Cell culture

- Primary cell culture
- Neurospheres
- Brain slices

Microscopy

- Light and fluorescence microscope
- Stereology equipment

- Confocal microscope (shared)

Selected publications:

1. Kokaia, M., Galanin and kindling, in Kindling 6, M.E. Corcoran and S.L. Moshe, Editors. 2005, Springer Science + Business Media, Inc.: New York. p. 219-227.

2. Kokaia, M. and O. Lindvall, The GDNF family of neurotrophic factors and epilepsy, in Growth factors and epilepsy, D.K. Binder and H.E. Scharfmann, Editors. 2005, Nova Science Publishers, Inc.: New York. p. 191-208.

PROJECT 9

Role of endoplasmic reticulum (ER) and unfolded protein response (EPR) in *in vivo* and *in vitro* models of neurodegeneration **(Resonsible Supervisor: Prof. WIELOCH, Lund, Sweden)**

The brain needs a constant supply of oxygen and glucose in order to generate energy for maintenance of brain function. During cerebral ischemia, when blood flow to the brain is severely hampered by occlusion of brain major arteries, by for example a blood clot (stroke), or due to cardiac arrest, energy production by brain cells rapidly ceases and detrimental processes are activated. Upon reperfusion by for example clot lysis or resumption of cardiac function, energy formation is transiently resumed by neurons. If ischemia is sufficiently dense and prolonged, neurons succumb within hours or days. Several mechanisms have been implicated in this process, including glutamate and calcium toxicity, free radical damage, inflammation, apoptosis and programmed cell death. In brain tissue that survives the ischemic insult, adaptive responses are activated leading to rewiring of neuronal networks and remodelling of synapses.

We have established *in vivo* and *in vitro* rodent models of cerebral ischemia, trauma and oxygen glucose deprivation, respectively. Using these models we focus our research on mechanisms of cell death involving mitochondria and the endoplasmic reticulum, as well as postischemic brain plasticity.

Work and methods description Dr. Toresson

Dr. Toresson recently received funding from the Swedish Research Council to establish his research group within Prof. Wieloch's division. The work is focussed on structural plasticity and its functional implications for physiology and pathology. Within NEUROTRAIN, projects are centred on stress-induced structural dynamics of the endoplasmic reticulum (ER) and how these processes relate to known ER stress pathways. It is known that an ischemic insult leads to cessation of protein synthesis and that at least part of the unfolded protein response is triggered within the ER but how this contributes to protection, cellular damage or death is unknown.

We are using primary and organotypic cultures of the hippocampus to study these dynamic processes by live cell imaging with confocal microscopy. Neurons are transfected with various fluorescent protein constructs and transgenic mice are generated to enable imaging of slices and, in the future, the brain *in vivo*.

Key references:

Rickhag M, Wieloch T, Gidö G, Elmér E, Krogh M, Murray J, Lohr S, Bitter H, Chin D von Schack D, Shamloo M, Nikolich K. (2006) Comprehensive regional and temporal gene expression profiling of the rat brain during the first 24 h after experimental stroke identifies dynamic ischemia-induced gene expression patterns, and reveals a biphasic activation of genes in surviving tissue. *J Neurochem*, 96:14-29.

Toresson, H. and Grant, S. G. (2005). Dynamic distribution of endoplasmic reticulum in hippocampal neuron dendritic spines. *Eur J Neurosci* 22, 1793-8.

Rytter A, Cardoso C, Johansson P, Cronberg T, Hanson MJ, Mattiasson G, Elmer E, Wieloch T (2005) Hyperglycemia during *in vitro* ischemia reveals a cell death mechanism independent of caspase activation and mitochondrial permeability transition. *J Neurochem*, 95:1108-11817

PROJECT 10

Molecular studies in protein folding and pathological mechanisms of mitochondrial dysfunction **(Responsible Supervisor: Dr. UEFFING, Munich, Germany)**

Protein misfolding and aggregation are among the central pathological hallmarks of neurodegenerative disorders, including not only prevalent diseases such as Alzheimer's and Parkinson's disease, but also such rare diseases as Huntington's disease and retinal dystrophies where no therapeutic strategy has been so far developed. We have investigated the role of Cdc48p, the highly conserved yeast orthologue of Valosin-containing protein (VCP/p97). VCP is an effector protein for polyglutamine diseases and when mutated, is an inductor for the progressive dominant human disorder "inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia" (IBMPFD). Expression of VCP mutants with impaired function result in accumulation and aggregation of membrane-associated polyubiquitinated proteins and ER stress. From our recent work on mutant *cdc48*, we propose a direct mechanism of interaction between protein aggregation, ER stress and functional impairment of mitochondria. We aim to investigate the function of normal human VCP versus mutant VCP with respect to pathological mechanism of mitochondrial dysfunction that we have observed with mutant Cdc48p/VCP causing ER stress (Braun et al. *submitted*, Zischka et al. *submitted*). Since identification of molecular mechanisms underlying degeneration and cell death associated with Cdc48p/VCP bears implications for a variety of diseases characterized by abnormal protein deposits, Cdc48p/VCP-mediated cell death may therefore serve as a model for programmed cell death in human degenerative disorders. The analysis and subsequent identification of the systemic consequences of misfolded protein aggregation and their pathophysiological consequences within a cell can contribute to understanding mechanisms of neurodegenerative diseases and potentially lead to novel therapeutic approaches.

The training in this subproject will involve high-end bioanalytical and proteomic methodology including nanoLiquid-Chromatography (nLC) separation of proteins and mass-spectrometry by MALDI-TOF, Q-TOF and MALDI-MS/MS. Functional analysis of normal and mutant protein variants will include survival, stress and apoptosis assays on primary neuronal and glial cell lines, permanent cell lines, analysis of protein-protein interaction, protein modification and aggregation, immunohistochemistry and biochemical assays analysing protein turnover and mitochondrial function, recombinant expression, primary cell culture, cDNA-arrays, bio-computing and animal facilities (German Mouse Clinic) at the GSF.

The GSF Campus with more than 20 different institutes and departments offers a multifaceted research biotope. The Lab of Dr. Ueffing runs the central Proteome Core Facility within the GSF Gene Analysis Center (GAC), an integrated facility for proteomics, gene arrays and DNA analysis. Dr. Ueffing coordinates the German Federal Government funded National Research Cluster 'Analysis of Membrane-coupled Protein Complexes' that focuses on developing proteomic methodology for the analysis of membrane proteins and membranous organelles and partners "INTERACTION PROTEOME" Europe's largest contracted EU research network for proteomics. This offers a variety of training opportunities embedded into an internationally structured

research network. Training extends from human genetics, functional genomics and proteomics to advanced cell biology.

Selected publications:

Braun R.J.*, Zischka H.*, Madeo F., Engelhardt S.M., Wissing S., Büringer D., Ueffing M. Crucial mitochondrial impairment upon *CDC48* mutation in apoptotic yeast. *Revised Manuscript in preparation.*

Zischka H., Braun R.J., Marantidis E.P., Büringer D., Bornhoevd C., Demmer O., Hauck S.M., Reichert A.S., Madeo F., Ueffing M. Differential analysis of *Saccharomyces cerevisiae* mitochondria by free-flow electrophoresis. *Revised manuscript submitted.*