

## **Publications de l'UMR 8602 (CNRS) (1999-2002)**

**2002**

**CHADEFAUX-VEKEMANS B., COUDE M., MULLER F., OURY J.F., CHABLI A., JAIS J.P., KAMOUN P.**

Methylenetetrahydrofolate Reductase Polymorphism in the Etiology of Down Syndrome.

*Pediat. Res.*, 51 (6), 766-767, 2002

(Services cités : Biostatistique, UMR 8602)

A methylenetetrahydrofolate reductase polymorphism (677 C/T mutation) was recently implicated in the etiology of Down syndrome. We studied a cohort of 85 women carrying fetuses with Down syndrome and found no difference in the frequencies of the three groups of subjects (C/C, C/T, T/T) between Down syndrome mothers and controls.

**TAKAMATSU Y., NAKAGOSHI H., RACHIDI M., LOPES C., NISHIDA Y., OHSAKO S.**

Characterization of the dCaMKII-GAL4 driver line whose expression is controlled by the *Drosophila* Ca(2+)/calmodulin-dependent protein kinase II promoter.

*Cell Tissue Res.*, 310 (2), 237-252, 2002

(Services cités : UMR 8602)

Transgenic flies that can drive GAL4 expression under the control of the 7 kb 5'-region of the *Drosophila* Ca(2+)/calmodulin-dependent protein kinase II (dCaMKII) gene (dCaMKII-GAL4) were established. Characteristic features of this dCaMKII-GAL4 driven reporter expression were compatible with the endogenous dCaMKII expression pattern: The dCaMKII-GAL4 driven reporter gene was expressed preferentially in the central nervous system of the embryo and larvae. Reporter expression was also observed in the brain, thoracic ganglion, and gut of the adult. The whole-brain distribution and projections of dCaMKII-GAL4-expressing cells in the adults were visualized three-dimensionally by using UAS-linked reporter genes. Prominent signals of nuclear-localized beta-Gal reporter gene expression were found in extensive brain regions, especially in the Kenyon cells of the mushroom body (MB), cells in the pars intercerebralis, and subesophageal ganglion (SOG). tau reporter gene expression highlighting neurite projections was detected in the MB lobes, median bundle, antennal lobe glomeruli, and fibers of clusters in the SOG, ventrolateral protocerebrum and superior lateral protocerebrum. These observations agree with those of a previous study mapping the dCaMKII-dependent memory circuits in courtship conditioning. Interestingly, green fluorescent protein reporter gene expression in adult MB lobes was predominantly observed in the alpha and beta lobes with a core-deficient pattern, but not in the alpha' and beta' lobes, similar to Fasciclin II immunoreactivity.

**2001**

**BELARDINELLI M.C., CHABLI A., CHADEFAUX-VEKEMANS B., KAMOUN P.**

Urinary sulfur compounds in down syndrome.

*Clin. Chem.*, 47 (8), 1500-1501, 2001

(Services cités : Biochimie Médicale, UMR 8602)

**CHABERT C., CHERFOUH A., DELABAR J.M., DUQUENNE V.**

Assessing implications between genotypic and phenotypic variables through lattice analysis.  
*Behav. Genet.*, 31 (1), 125-139, 2001

(Services cités : UMR 8602)

A previous paper assessed a "Molecular Mapping of Twenty-Four Features of Down Syndrome on Chromosome 21" (Delabar et al., 1993), by analyzing the genotype s/phenotypes of patients suffering from partial trisomy. The mapping was defined through implications-each feature was mapped to the conjunction of cytogenetic bands that were shared by all patients having that feature. In the present paper, we extend that approach to determine how far those implications depart from defining equivalences. Finding equivalences is important. Local equivalences permit a genetic characterization of a feature. And if global equivalences held for all features, that set of bands would be sufficient to characterize the various phenotypes observed in individuals with partial trisomy 21. To extend the earlier approach, we examine the structure of equivalences as well as the structure of implications. We examine both conjunctions of bands and conjunctions of features. The use of Galois lattices permits simultaneous evaluation of both kinds of structures. Each Galois lattice is labeled with a basis (minimal generating set) of implications going from conjunctions of features into bands and those going from conjunctions of bands into features. Analysis reveals that about half of the conjunctions of bands that characterize the genetic structure embody equivalences. This allows us to improve the genetic description of features and to specify minimal sets of questions that need to be investigated to make the global genetic description more precise. [References: 11]

#### **KAMOUN P.**

Mental retardation in down syndrome: a hydrogen sulfide hypothesis.

*Med. Hypotheses*, 57 (3), 389-392, 2001

(Services cités : Biochimie Médicale, UMR 8602)

Mental retardation is progressive in Down syndrome: individuals are born with normal intelligence which starts to decline linearly within the first year. This phenomenon can be observed with phenylalanine in patients with phenylketonuria, therefore it is compatible with metabolic intoxication. The toxic compound could be hydrogen sulfide. The amount of the compound is probably increased in Down syndrome by increasing active cystathionine beta synthase. This heuristic hypothesis requires further investigation. (C) 2001 Harcourt Publishers Ltd. [References: 33]

#### **LOPES C., GASSANOVA S., DELABAR J.M., RACHIDI M.**

The cask/lin-2 drosophila homologue, camguk, could play a role in epithelial patterning and in neuronal targeting.

*Biochem. Biophys. Res. Commun.*, 284 (4), 1004-1010, 2001

(Services cités : UMR 8602)

Drosophila Camguk (Cmg) is a member of the CAMGUK subfamily of the MAGUK. family of proteins which are localized at cell junction and other plasma membrane specialized regions, from worms to mammals, The protein structure of Cmg, as the other CAMGUK proteins, is characterized by only one PDZ domain and an additional CaM kinase domain, similar to CaMKII. While the mammalian ortholog CASKs play an important role in synaptic protein targeting and in synaptic plasticity, the Drosophila Cmg role is unknown. To study its potential role, we reported a detailed analysis of mRNA distribution of the Drosophila cmg gene at cellular and developmental level, during embryonic, larval, pupal and adult stages. The transient: cmg transcription in midgut and Malpighian tubules may suggest a potential function in cell junction

formation and in epithelial tissue patterning. Interestingly, cmg transcription increases substantially during embryonic neuroblast proliferation, becoming predominant in the developing central nervous system (CNS) during embryonic and postembryonic development stages and in the mature brain. In addition, a high transcriptional level was detected in the eye imaginal discs and in the adult retina, demonstrating a specific and continuous expression of cmg in neuroblasts and photoreceptor neurons, from the onset of cytodifferentiation. Our findings suggest that Cmg could play a potential role in transmembrane protein targeting, particularly in synapses. These observations suggest the existence of a common highly conserved mechanism involved in forming and maintaining proper synaptic protein targeting, which are fundamental features of synaptic plasticity, learning and memory. Through its function, the CaM kinase domain-containing Cmg may be involved in signal transduction cascade. Its potential relation to Calmodulin and CaMKII is discussed. (C) 2001 Academic Press. [References: 46]

**SANTIARD-BARON D., LACOSTE A., ELLOUK-ACHARD S., SOULIE C., NICOLE A., SARASIN A., CEBALLOS-PICOT I.**

The amyloid peptide induces early genotoxic damage in human preneuron nt2.  
*Mutat. Res.-Fundam. Mol. Mech. Mut.*, 479 (1-2 Special Issue SI), 113-120, 2001  
(Services cités : U383, UMR 8602)

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the extracellular deposition of amyloid beta-peptide (Abeta) in the brain. Abeta is involved in the pathogenesis of AD but the molecular mechanisms of its neurotoxicity are unknown. Here, we report that Abeta exposure on human preneuronal NT2 cells provoked a strong and early up-regulation of growth arrest and DNA damage inducible gene (Gadd45 mRNA), an indicator of DNA damage and DNA excision-repair processes, strongly suggesting that Abeta causes an early DNA strand breakage leading to a cellular DNA repair response. Comet assay clearly demonstrated that both full-length Abeta (1-42), and its minimal cytotoxic fragment Abeta (25-35), caused DNA breakage as early as 3h after the start of Abeta exposure. This extensive DNA damage provoked by Abeta constitutes an early event in the pathogenic cascade leading to neuronal death which could contribute to the neuropathogenesis of AD.

**2000**

**GUIPPONI M., BRUNSCHWIG K., CHAMOUN Z., SCOTT H.S., SHIBUYA K., KUDOH J., DELEZOIDE A.L., EL SAMADI S., CHETTOUH Z., ROSSIER C., SHIMIZU N., MUELLER F., DELABAR J.M., ANTONARAKIS S.E.**

C21orf5, a novel human chromosome 21 gene, has a caenorhabditis elegans ortholog (pad-1) required for embryonic patterning.

*Genomics*, 68 (1), 30-40, 2000

(Services cités : UMR 8602, Histo-Embryologie & Cytogénétique)

To contribute to the development of the transcription map of human chromosome 21 (HC21), we isolated a new transcript, C21orf5 (chromosome 21 open reading frame 5), encoding a predicted 2298-amino-acid protein. Analysis of the genomic DNA sequence revealed that C21orf5 consists of 37 exons that extend over 130 kb and maps between the CBR3 (carbonyl reductase 3) and the KIAA0136 genes. Northern blot analyses showed a ubiquitously expressed RNA species of 8.5 kb. RNA in situ hybridization on brain sections of normal human embryos revealed a strong labeling in restricted areas of the cerebral cortex. In silico analysis of the deduced C21orf5 protein revealed several highly probable transmembrane segments but no known protein domains or homology with known proteins. However, there were significant homologies to several

hypothetical *Caenorhabditis elegans* proteins and *Drosophila melanogaster* genomic sequences. To investigate the function of C21orf5, we isolated the cDNA of the *C. elegans* ortholog and performed double-stranded RNA-mediated genetic interference experiments. The major phenotype observed in the progeny of injected animals was embryonic lethality. Most of the tissues of the embryo failed to undergo proper patterning during gastrulation, and morphogenesis did not occur; thus we termed the ortholog pad-1, for patterning defective 1. These results indicated that pad-1 is essential for the development and the survival of *C. elegans*. This study provides the first example of the use of *C. elegans* as a model to study the function of genes on human chromosome 21 that might be involved in Down syndrome. (C) 2000 Academic Press. [References: 29]

**JAARSMA D., HAASDIJK E.D., GRASHORN J.A.C., HAWKINS R., VAN DUIJN W., VERSPAGET H.W., LONDON J., HOLSTEGE J.C.**

Human cu/zn superoxide dismutase (sod1) overexpression in mice causes mitochondrial vacuolization, axonal degeneration, and premature motoneuron death and accelerates motoneuron disease in mice expressing a familial amyotrophic lateral sclerosis mutant sod1.

*Neurobiol. Dis.*, 7 (6), 623-643, 2000

(Services cités : UMR 8602)

Cytosolic Cu/Zn superoxide dismutase (SOD1) is a ubiquitous small cytosolic metalloenzyme that catalyzes the conversion of superoxide anion to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Mutations in the SOD1 gene cause a familial form of amyotrophic lateral sclerosis (fALS). The mechanism by which mutant SOD1s causes ALS is not understood. Transgenic mice expressing multiple copies of fALS-mutant SOD1s develop an ALS-like motoneuron disease resembling ALS. Here we report that transgenic mice expressing a high concentration of wild-type human SOD1 (hSOD1(WT)) develop an array of neurodegenerative changes consisting of (1) swelling and vacuolization of mitochondria, predominantly in axons in the spinal cord, brain stem, and subiculum; (2) axonal degeneration in a number of long fiber tracts, predominantly the spinocerebellar tracts; and (3) at 2 years of age, a moderate loss of spinal motoneurons. Parallel to the development of neurodegenerative changes, hSOD1(WT) mice also develop mild motor abnormalities. Interestingly, mitochondrial vacuolization was associated with accumulation of hSOD1 immunoreactivity, suggesting that the development of mitochondrial pathology is associated with disturbed SOD1 turnover. In this study we also crossed hSOD1(WT) mice with a line of fALS-mutant SOD1 mice (hSOD1(G93A)) to generate "double" transgenic mice that express high levels of both wild-type and G93A mutant hSOD1. The "double" transgenic mice show accelerated motoneuron death, earlier onset of paresis, and earlier death as compared with hSOD1(G93A) littermates. Thus in vivo expression of high levels of wild-type hSOD1 is not only harmful to neurons in itself, but also increases or facilitates the deleterious action of a fALS-mutant SOD1. Our data indicate that it is important for motoneurons to control the SOD1 concentration throughout their processes, and that events that lead to improper synthesis, transport, or breakdown of SOD1 causing its accumulation are potentially dangerous. (C) 2000 Academic Press. [References: 69]

**ORTI R., RACHIDI M., VIALARD F., TOYAMA K., LOPES C., TAUDIEN S., ROSENTHAL A., YASPO M.L., SINET P.M., DELABAR J.M.**

Characterization of a novel gene, C21orf6, mapping to a critical region of chromosome 21q22.1 involved in the monosomy 21 phenotype and of its murine ortholog, orf5.

*Genomics*, 64 (2), 203-210, 2000

(Services cités : UMR 8602)

Phenotypic and molecular analyses of patients with partial chromosome 21 monosomy enabled us to define a region, spanning 2.4 Mb between D21S190 and D21S226, associated with arthrogryposis, mental retardation, hypertonia, and several facial anomalies. The markers of the region were used to screen a total human PAC library (Ioannou, RZPD). We isolated 57 PACs, which formed primary contigs. EST clusters (UNIGENE collection) located in a 6-Mb interval, between D21S260 and D21S263, were mapped in individual bacterial clones. We mapped the WI-17843 cluster to the PAC clone J12100, which contains the two anchor markers LB10T and LA329. The open reading frame extends over 960 bp, with three putative start codons. The 1695-bp cDNA containing a polyadenylation signal should correspond to the full-length cDNA. From the genomic sequence, we deduced that the gene contained five exons and that there was a putative promoter sequence upstream from exon 1. In silico screening of DNA databases revealed similarity with a murine EST. The corresponding cDNA (1757 bp) sequence was very similar (>85%) to the human cDNA and had an open reading frame of 876 nucleotides. Somatic hybrid mapping localized the cDNA to mouse chromosome 16. EST analyses and RT-PCR indicated that the third exon in the human gene (exon 2 in the mouse) undergoes alternative splicing. Northern blot hybridization showed that the gene was ubiquitously expressed in humans and mice. The longest mouse clone was used to generate riboprobes, which were hybridized to murine embryos at stages E-9.5, E-10.5, E-12.5, E-13.5, and E-14.5-15, to study the pattern of expression during development. Ubiquitous labeling was observed, with strong signals restricted to limited areas of the telencephalon, the mesencephalon, and the interrhombomeric regions in the central nervous system, and other regions of the body such as the limb buds, branchial arches, and somites. Copyright 2000 Academic Press.

**RACHIDI M., LOPES C., GASSANOVA S., SINET P.M., VEKEMANS M., ATTIE T., DELEZOIDE A.L., DELABAR J.M.**

Regional and cellular specificity of the expression of tprd, the tetratricopeptide down syndrome gene, during human embryonic development.

*Mech. Develop.*, 93 (1-2), 189-193, 2000

(Services cités : UMR 8602, Génétique Médicale Pédiatrique)

The TPRD gene (tetratricopeptide (TPR) containing Down syndrome gene) is one of the candidate genes in the Down syndrome chromosomal region-1. Duplication of this gene may be the cause of major phenotypic features of Down syndrome. Here we show that the TPRD expression is developmentally regulated during human embryogenesis. At the earliest stages of development (Carnegie 8-12) TPRD expression is ubiquitous. At later developmental stages (Carnegie stages 14, 16 and 18), it becomes restricted to the nervous system, as is the case for the mtprd gene during mouse development. We extended our analysis of TPRD expression during fetal development of the human nervous system (13, 22 and 24 weeks). A new oblique illumination technique was used to compare signal intensity and cell density. Some regions of the nervous system such as the external cortical layers of the brain, and the inner neuroblastic layer of the eye, strongly express the TPRD gene. (C) 2000 Elsevier Science Ireland Ltd. All rights reserved. [References: 12]

**THIERY E., GOSSET P., DAMOTTE D., DELEZOIDE A.L., de SAINT SAUVEUR N., VAYSSETTES C., CREAU N.**

Developmentally regulated expression of the murine ortholog of the potassium channel kir4.2 (kcnj15).

*Mech. Develop.*, 95 (1-2), 313-316, 2000

(Services cités : UMR 8602, Anatomopathologie)

The gene KIR4.2 (K<sup>+</sup> inwardly rectifying channel 4.2) has been recently identified in the Down syndrome Chromosome Region 1. We have cloned the mouse ortholog of KIR4.2 and characterized its expression pattern. In situ hybridization showed a restricted and developmentally regulated pattern of expression. The expression is starting at E12.5 and expands at E14.5 in different tissues and organs, which may be affected in Down syndrome: heart, thymus, thyroid gland, and perichondrium. At E17.5, additional epithelia (kidney, bladder, stomach, lung) expressed also strongly the gene. (C) 2000 Elsevier Science Ireland Ltd All rights reserved. [References: 11]

**VIALARD F., TOYAMA K., VERNOUX S., CARLSON E.J., EPSTEIN C.J., SINET P.M., RAHMANI Z.**

Overexpression of *msim2* gene in the zona limitans of the diencephalon of segmental trisomy 16 *ts1cje* fetuses, a mouse model for trisomy 21: a novel whole-mount based rna hybridization study.

*Brain Res. Dev. Brain Res.*, 121 (1), 73-78, 2000

(Services cités : UMR 8602)

Trisomy 21 (Down syndrome) is the most common chromosomal abnormality associated with mental retardation in humans. *Sim2*, a human homologue of *Drosophila sim* gene, which acts as a master regulator of the early development of the fly central nervous system midline, is located on chromosome 21, in the Down syndrome critical region, and might therefore be involved in the pathogenesis of some of the morphological features and brain anomalies observed in Down syndrome. We report here the detailed expression pattern of murine *mSim2* gene in *Ts1Cje* mice fetuses, a segmental trisomy 16 mouse model for trisomy 21, and its overexpression in the zona limitans of the diencephalon using a new quantitative method based on the whole-mount RNA hybridization technique. (C) 2000 Elsevier Science B.V. All rights reserved. [References: 18]

**1999**

**GOSSET P., AIT-GHEZALA G., SINET P.M., CREAM N.**

Isolation and analysis of chromosome 21 genes potentially involved in Down Syndrome.

*J. Neural Transm.- Supp.*, (57), 197-209, 1999

(Services cités : UMR 8602, Histo-Embryologie & Cytogénétique)

**GUEGAN C., CEBALLOS-PICOT I., CHEVALIER E., NICOLE A., ONTENIENTE B., SOLA B.**

Reduction of ischemic damage in NGF-transgenic mice: Correlation with enhancement of antioxidant enzyme activities.

*Neurobiol. Dis.*, 6 (3), 180-189, 1999

(Services cités : UMR 8602)

If permanent focal ischemia is induced by middle cerebral artery occlusion (MCAO), neurons within the infarcted territory die by necrosis and apoptosis (or programmed cell death). We have previously shown, using a mouse strain transgenic (tg) for the nerve growth factor (NGF) gene, that tg mice have consistently smaller infarcted areas than wild-type (wt) animals, correlated with upregulated NGF synthesis and impaired apoptotic cell death. We studied, in wt and tg mice subjected to MCAO, the activities of several antioxidant enzymes and the synthesis of the proteins of the Bcl-2 family. Our results show that the antiapoptotic Bcl-2 protein and glutathione

peroxidase are recruited after MCAO. NGF-tg mice also had an intrinsic resistance to oxidative stress because their basal copper zinc superoxide dismutase (SOD) and glutathione transferase activities were high. Additionally, manganese SOD activity increased in NGF-tg mice after MCAO, correlating strongly with the resistance of these mice to apoptosis. (C) 1999 Academic Press. [References: 36]

**LOPES C., RACHIDI M., GASSANOVA S., SINET P.M., DELABAR J.M.**

Developmentally regulated expression of mtpd, the murine ortholog of tprd, a gene from the Down syndrome chromosomal region 1.

*Mech. Develop.*, *84* (1-2), 189-193, 1999

(Services cités : UMR 8602)

The gene tprd, which contains three tetratricopeptide domains, has been recently localized in the Down syndrome (DS) chromosomal region 1. We have cloned a cDNA encoding part of the murine ortholog of tprd and used it to characterize the expression pattern of this gene during development and at the adult stage. At E8.5 the expression is uniform. In the later stages of embryogenesis, although expression remains ubiquitous, a pattern of tissues with particularly high expression develops: the strong expression is restricted to non proliferating zones of the nervous system such as the external layer of the cortex, the spinal cord, the cranial and root ganglia and the nerves. In the brain of adult mouse the strongest signals are observed in layers II-III and V-VI of the cortex, in the hippocampus and in the cerebellum, which correspond to the abnormal brain regions seen in DS patients. (C) 1999 Elsevier Science Ireland Ltd. All rights reserved. [References: 15]

**MERAD-SAIDOUNE M., BOITIER E., NICOLE A., MARSAC C., MARTINOU J.C., SOLA B., SINET P.M., CEBALLOS-PICOT I.**

Overproduction of Cu/Zn-superoxide dismutase or Bcl-2 prevents the brain mitochondrial respiratory dysfunction induced by glutathione depletion.

*Exp. Neurol.*, *158* (2), 428-436, 1999

(Services cités : UMR 8602)

Recent work has focused attention on the role of oxidative stress in various acute and chronic neurodegenerative diseases. Low concentrations of the powerful antioxidant glutathione (GSH) and impaired brain energy metabolism, particularly in the substantia nigra, are key features of Parkinson's disease (PD). The main goal of this study was to better characterize the deleterious effects of brain GSH depletion on mitochondrial function. We depleted GSH in the brains of newborn wild-type (WT) and transgenic (Tg) mice overproducing either human Cu/Zn-superoxide dismutase (h-CuZnSOD) or human Bcl2 (h-Bcl-2), by subcutaneous injection of L-buthionine sulfoximine (BSO), a specific inhibitor of gamma-glutamylcysteine synthetase. GSH was 97% depleted in brain homogenates and 90% depleted in brain mitochondria for both WT and Tg mice. This depletion of brain GSH led to a decrease in the activity of the GSH-dependent antioxidant enzyme glutathione peroxidase, both in WT and in Tg animals. BSO treatment decreased the activities of respiratory complexes I, II, and IV in the brain homogenates of WT mice. BSO-treated h-CuZnSOD or h-Bcl-2 Tg mice had no respiratory chain deficiencies. Thus, brain GSH depletion leads to the impairment of mitochondrial respiratory chain activity. The protection of mitochondrial respiratory function by overproduction of Bcl-2 may result from a decrease in the generation of reactive oxygen species (ROS) or lipid peroxidation. The protection of mitochondria by overproduction of CuZn-SOD is consistent with the involvement of superoxide or superoxide-derived ROS in the mitochondrial dysfunction caused by brain GSH

depletion. This study demonstrates that the antioxidant balance is critical for maintenance of brain mitochondrial function, and its disruption may contribute to the pathogenesis of PD. (C) 1999 Academic Press. [References: 67]

**QUERE I., PAUL V., ROUILLAC C., JANBON C., LONDON J., DEMAILLE J., KAMOUN P., DUFIER J.L., ABITBOL M., CHASSE J.F.**

Spatial and temporal expression of the cystathionine beta-synthase gene during early human development.

*Biochem. Biophys. Res. Commun.*, 254 (1), 127-137, 1999

(Services cités : UMR 8602)

We report the cystathionine-beta synthase (CBS) gene expression pattern during early human embryogenesis (3 to 6 weeks post conception) by in situ hybridization and in fetal and adult tissue by Northern Blot analysis. Probes were chosen to recognize either the common sequence to all known CBS mRNAs or the sequences of two different major exons 1 issued of we have previously identified. We demonstrate by in situ hybridization that CBS is continuously expressed from the earliest stages studied (22 days post conception) during embryogenesis in the tissues of developing embryos which will after birth present clinical abnormalities in homocystinuria patients. It is expressed at an especially high level in the neural and cardiac systems until the liver primordium appears. In embryonic central nervous system, the whole neural tube and primary brain vesicles are labeled. Secondary brain vesicles labeling are dependent on the neuroepithelium differentiation. The ventricular layer of the rhombencephalon, cranial nerve nuclei and then after cerebellar cortex derived from rhombencephalon ventricular layer are strongly labeled. Thalamus and other derivatives of the diencephalon plate, the neuroblastic layer of the retina, lens and dorsal root ganglia are labeled. After 35 days post conception, CBS mRNAs was detected in endocardial cells and in cells derived from the neural crest of the heart and in particular developing mesodermic regions such as the primitive hepatocytes of the liver, mesonephros vesicles, various endocrine glands and developing bones. We could not detect tissue specificity of different probes at this embryonic stage. Northern blot analysis consistently detected mRNA species in fetal 25 weeks post conception brain, liver and kidney. The common cDNA probe revealed the 2.5 and 3.7 kb mRNA species from brain, liver and kidney. The exon Ib probe detected only the 2.5 kb mRNA and the exon Ic probe the 3.7 kb mRNA in these three tissues. In adult tissue, the Ib probe detected only the 2.5 kb mRNA and the Ic probe only the 3.7 kb mRNA in the liver. (C) 1999 Academic Press. [References: 25]

**RACHIDI M., LOPES C., TAKAMATSU Y., OHSAKO S., BENICHOU J.C., DELABAR J.M.**

Dynamic expression pattern of Ca<sup>2+</sup>/calmodulin-dependent protein kinase II gene in the central nervous system of *Drosophila* throughout development.

*Biochem. Biophys. Res. Commun.*, 260 (3), 707-711, 1999

(Services cités : UMR 8602)

Calcium/calmodulin-dependent protein kinase II (CaM KII) is thought to be involved in the majority of the neuronal functions mediated by intracellular free Ca<sup>2+</sup>, and has been implicated in long-term potentiation, learning, and memory. In this work, we have examined in detail the RNA expression pattern for the *Drosophila* CaM KII gene by in situ hybridization, during embryonic, larval, pupal, and adult stages. Our results indicate that expression of CaM KII was homogeneous in early embryos, but that during development the gene transcription rapidly became restricted to neuroblasts and their progeny in the nervous system. This predominant

expression in the nervous system is maintained during late embryogenesis and post-embryonic development. A signal compartmentalization appeared in the larval central nervous system, where the CaM KII expression became progressively concentrated in the anterior ganglia. In the adult brain, a specific expression was more abundant in a subset of neurons around the central brain, particularly the mushroom bodies and the central complex, structures that play an important role in learning and memory. (C) 1999 Academic Press. [References: 35]

**SAILLE C., MARIN P., MARTINOU J.C., NICOLE A., LONDON J., CEBALLOS-PICOT I.**

Transgenic murine cortical neurons expressing human Bcl-2 exhibit increased resistance to amyloid beta-peptide neurotoxicity.

*Neuroscience*, 92 (4), 1455-1463, 1999

(Services cités : UMR 8602)

The generation of reactive oxygen species has been implicated in the neurotoxicity of amyloid beta-peptide, the main constituent of the senile plaques that accumulates in the brain of Alzheimer's disease victims. In this study, we have compared the toxicity of amyloid beta-peptide on cultured cortical neurons from control mice and transgenic mice expressing either human copper-zinc superoxide dismutase or human Bcl-2, two proteins that protect cells against oxidative damage. Copper-zinc superoxide dismutase overexpression failed to protect cortical neurons against the toxicity of amyloid beta-peptide(25-35) [the minimal cytotoxic fragment of amyloid beta-peptide(1-42)] as assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction and an enzyme-linked immunosorbent assay using an antibody directed against microtubule-associated protein-2 (a specific neuronal protein), ruling out a role for superoxide anion and peroxynitrite in amyloid beta-peptide-evoked neurotoxicity. On the contrary, cortical neurons expressing human copper-zinc superoxide dismutase exhibited increased apoptotic nuclei in both untreated and amyloid beta-peptide(25-35)-exposed neurons. Transgenic neurons expressing human Bcl-2 were partially protected against amyloid beta-peptide-induced neuronal death. This neuroprotection appears to be related to the complete inhibition of apoptosis induced by both amyloid beta-peptide(25-35) and amyloid beta-peptide(1-42).

**SANTIARD-BARON D., GOSSET P., NICOLE A., SINET P.M., CHRISTEN Y., CEBALLOS-PICOT I.**

Identification of beta-amyloid-responsive genes by RNA differential display: early induction of a DNA damage-inducible gene, gadd45.

*Exp. Neurol.*, 158 (1), 206-213, 1999

(Services cités : UMR 8602)

Alzheimer's disease is a neurodegenerative disorder characterized by the extracellular deposition in the brain of amyloid beta-peptide (A beta), presumed to play a pathogenic role. However, the precise molecular mechanisms of its neurotoxicity are not fully understood. Recent studies have suggested that it may exert its toxic effect via activation of transcription factors. We investigated A beta-responsive genes in human preneuron NT2 cells, at early stages of A beta(25-35) exposure, by RNA differential display. A beta induced the expression of (i) the growth arrest and DNA damage-inducible gene (gadd45) implicated in the DNA excision-repair process; (ii) a stress-signaling kinase gene encoding the mitogen-activated protein kinase/Erk kinase kinase-1 (MEKK1); (iii) a new growth factor-inducible immediate-early gene, CYR61, the product of which functions as an extracellular matrix signaling molecule; (iv) other immediate-early genes,

such as c-jun and c-fos; (v) the gene encoding the basic fibroblast growth factor (bFGF); (vi) a gene encoding a constituent of the mitochondrial pyruvate dehydrogenase complex, the dihydrolipoamide dehydrogenase-binding protein (E3-BP); and (vii) an unidentified human gene (KIAA0099). A beta not only activates but also represses genes: (i) the gene encoding "hinge" protein, a subunit of the mitochondrial cytochrome-c reductase and (ii) the SRp55 gene encoding a splicing factor involved in constitutive pre-mRNA splicing and alternative splice site selection. Our results underscored A beta-responsive genes that play key roles in the response (damage/recovery) of neuron cells to A $\beta$  exposure. In particular, the strong upregulation of gadd45, indicating DNA damage, was detected early in A beta cytotoxicity. This suggests that DNA strand breaks occurred rapidly in cells exposed to A beta, which may be a critical event in A beta neurotoxicity. (C) 1999 Academic Press. [References: 53]