2. EXECUTIVE SUMMARY

The FONDAP Center for Cell Regulation and Pathology “Joaquin V. Luco” has as main purposes to stimulate research activity and scientific training in basic areas of modern Biomedical Sciences. The Center is inserted on the grounds of the Facultad de Ciencias Biológicas, in the Central Campus of the Pontificia Universidad Católica de Chile, where the university’s Medical School is also located. Its physical location together with its Project Programs and academic activities promote cooperation and interaction among FONDAP scientists and their peers in other laboratories of our Faculty and medical divisions. The Programs Project of the Center addressed during 2000-2004 two major research areas: 1) The protein traffic mechanisms that determine the composition and dynamics of the cell surface, especially in cells with polarized phenotypes, and; 2) Signaling receptors and transduction systems related with vascular physiology, muscle differentiation and neurotoxicity. These two areas include experimental model systems directly related with pathological conditions such as Alzheimer’s disease, cancer, hypertension, autoimmunity and muscular dystrophy, among others. Several aspects of the basic knowledge produced by the Center have potential applicability in the design of new strategies for therapeutics in some of these diseases.

For the period 2005-2009 eight Program Projects were executed; 1) Intracellular Protein Traffic: Molecular Mechanisms, Functional Implications and Disease. 2) Function of Proteoglycans in Myogenesis and Fibrosis. 3) Role of Plasma Membrane Nucleotide Receptors. 4) Role of PPARs in Neural Function. 5) Role of the WNT Signaling Pathway in Synaptic Function and Neurodegenerative Diseases. 6) Trafficking, Function and Regulation of the Low-Density-Lipoprotein Receptor-Family members. 7) Early Development of the Vertebrate Embryo. 8) Neurotrophin Signaling. The main scientific contributions of each Program Projects during this decade can be summarized as follow;

Intracellular protein traffic:

Novel role of cholesterol and PKA on basolateral regionalization of the HDL receptor. Recycling endosomes are the site of function of the clathrin adaptor AP1B in basolateral sorting. Discovery of a new mechanism of regulation of EGF receptor operating upon the permanency versus endocytosis of inactive receptors at the cell surface, mediated by basal PKA activity. Evidences that anti-ribosomal P antibodies associated with psychiatric lupus are neurotoxic and recognize a novel integral membrane protein on the neuronal cell surface.

Function of proteoglycans in myogenesis and fibrosis:

Identification of several proteoglycan modulating growth factor activities and its role as organizer of the extracellular matrix which is essential for the myogenesis process. The role of several of these proteoglycans was established during skeletal muscle regeneration process associated to muscular dystrophies. The discovery of novel role of decorin in TGF-β mediated
signaling involving LRP-1 receptor and the identification of several factors participating in the fibrosis process associated to skeletal muscular dystrophies.

**Plasma membrane nucleotide receptors:**
They described for the first time that the vasomotor action of nucleotides is linked to the trans-modulation of EGF receptors. Identification of the molecular mechanisms by which trace metals such as copper or zinc regulate the physiological activity of several purinergic receptor channels. Important advances to understand mechanisms of ATP release from tissues and cells in culture.

**PPARs in neural cell function:**
Identification of endogenous activators of peroxisome proliferator activated receptor (PPAR) α. These receptors, particularly PPAR γ and β are involved in oligodendrocyte function and differentiation. Moreover PPARγ is strongly involved in neuronal cell survival, and is present in axons and is retrogradely transported.

**The Wnt signaling pathway in synaptic function and neurodegenerative diseases:**
Identification of the Wnt signaling pathway as a new therapeutic target for Alzheimer’s disease. The activation of several central nervous system signaling pathways prevents Aβ-induced neurodegeneration. Evidence that Wnt signaling plays a role in synaptic maintenance and function, canonical Wnt ligands affects presynaptic region and the activation of non-canonical pathway modulates postsynaptic region, including both excitatory and inhibitory synapses.

**Traffic of LDL-Related Receptor Protein:**
Description of the sorting signals for the low-density-lipoprotein (LDL) receptor family members, including LRP1 and Megalin. The activation of PPAR α and γ and FXR regulated megalin both in vitro and in vivo. Evidence that the ApoE Receptor2 (ApoER2) interacts and affects the amyloid precursor protein (APP) trafficking and Aβ production. Discovery that megalin has several phosphorylation sites and that Wnt signaling regulates megalin phosphorylation.

**Studies on early development of Xenopus:**
Identification of several proteoglycans in early Xenopus development, this work open new avenues of research: i.e., Syndecan 4 is involved in focal adhesion turnover and it is regulated by a non-canonical Wnt pathway. Analysis of the role of syndecan 4 in mouse development and its control by the Wnt/PCP pathway. This latter work might contribute to the understanding of the basis of neural tube closure defects.

**Neurotrophin signaling:**
Discovery that neuronal endosomes, in addition to be a platform for signaling, are also a platform for g-secretase mediated processing of p75, generating a soluble C-terminal fragment with signaling capabilities. First report showing the relation between a neuronal
response (BDNF-induced dendritic branching) and the recycling pathway (Rab11-regulated) in central neurons.

During this ten years period a total of 214 ISI papers in qualified international journals were published by members of the Center. Of them, 93 publications were between the years 2000 and 2004 and 121 during the period 2005-2009; this latter corresponds to a 31% increase in the number of publications. The average impact index of the 215 publications corresponds to 5.13. The initial group of researchers that funded the Center started with an average of 4.07 in the year 2000, increasing to a value of 4.97 for the period 2000-2004. The average impact index for the period 2005-2009 rose to a value of 5.20. These values are exceptional higher for the country and the region and most of the publications are over the 85% ranking/field.

The Center has produced synergistic effects upon the quality, productivity, and impact of the scientific activity in the country and became an attractive environment for young scientists and students interested in the cellular and molecular foundations of the biomedical research. Collaborations among members of the Center have been progressively increased.

Part of these collaborations is reflected in the number of publications among the Center members were also very important; 37 publications correspond to collaborations between the senior investigators. This number is significantly elevated for national standards were collaboration is not a common practice among the researchers. The collaborative activities of the Center are not only aimed to be reflected in publications but also in the generation of an environment specially suited for interactions among scientists as well as the formation of human scientific resources. Our students recognize this as an important achievement of our Center. It is mostly through the student interactions that research collaborations are encouraged and accomplished. This has been one of the more important tasks of the Center during this project. Now students can freely move between different labs, sharing methodologies, equipments, ideas and executing experiments than can not be done in their own laboratories. This has been the product of the common effort of the Center members. Several of our PhD students spent one month or more working in foreign leader laboratories and brought new technologies and ideas to the Center. All these activities together with the encouraging environment converge in better trained students. More than 80% of our PhDs accessed to post-doctoral positions in leader laboratories abroad and several has already obtained positions as independent researches in other Universities of the country.

Regarding human resources during this ten year period the Center formed 182 students; of them 91 correspond to PhDs and 94 undergraduates. This figure increased notably in the period 2005-2009 since of the total of 182 students trained in the Center, 120 students correspond to the period 2005-2009. Our staff members coordinated core courses of two of the PhD programs of our Faculty. We also contributed to the Medical Sciences PhD program of the Faculty of Medicine of our University. Students from this program made their PhD thesis working in our laboratories. In our Department, the FONDAP Center contributed substantially to direct the
scientific training at both the undergraduate and post-graduate levels. It has also an important influence in the academic enterprise.

The Center during this ten years project housed 37 postdoctoral fellows from the country and abroad. This number is exceptionally high. In the first quinquennium the total number of Postdocs was 17, whereas in the second period their corresponded to 34. This was a special effort of the Center in order to recruit a higher number of Postdocs to execute their research at the different Center’s laboratories.

In addition, our Center has collaboratively fostered a number of initiatives that promote scientific interactions and interchange. These include the bimonthly scheduled FONDAP-seminars (total 270), of them 96 corresponded to speakers from the Center (senior scientists, Post-doc and graduate students), 108 from other Chilean universities and 66 were investigators from abroad. The Center organized a total of 14 international workshops chaired by members of the Center with an average of 6 international scientists invited to participate, with an average of attending students of around 50. In addition, the participation in national and international meetings was also remarkable; a total of 936 members of the Center attended to Congress; 42% corresponds to International Congress and Conference while 58% attended to National meetings, workshop and courses. Exchange of member of the Center was also a very important activity, 124 visits to abroad laboratories, mainly by graduate students and Postdoc. Of the total of our international visitors, 20 resulted in fruitful and ongoing collaborations. The Center also organized during these years two FONDAP meetings outside the University gathered more than 120 members of the FONDAP Center, including the undergraduate and graduate students, postdoctoral fellows and all the senior investigators. These meetings were very important to evaluate the on-going of the Center, fortify some initiatives as well as to revise others.

The Scientific Advisory Board which visited us during three opportunities during this ten year period and the FONDAP Reviewer Board with the on-site visits recommended to focus in the most relevant problems of each program projects along the years and to make corrections. These instances of evaluation and recommendation were so important for the success of the Center. Thus, we were able to open new avenues for effective and attractive collaborations that put together complementary expertise approaching these areas. However, although we have a strong commitment for collaborations, each senior investigator needs to maintain his own independent research as a capital already acknowledged by the scientific community.
A Business Unit Expertise dedicated to foster the relationship with national and pharmaceutical companies, started three-year ago. Major pharmaceutical corporations, biotechnological companies, international research institutions and other domestic research institutions became aware of the CRCP human and infrastructure capabilities to perform basic and applied biomedical research. The main goal of the Business Unit was to obtain new financial sources for the CRCP. Eventually all this information, allows us to apply and eventually won a “Base Center of Excellence in Science and Technology” called Center for Aging and Regeneration (CARE).

Concerning outreach, during these last years several activities were carried out including: “Special courses for High School Teachers of Biology”. A weekly seminar and a monthly CRCP-MIFAB seminar, attracted around 60 participants from the major Universities of Santiago, including University of Chile and Andrés Bello, Last year, We have Monthly Interviews to all seniors scientist of CRCP in the Radio Program “Foro Ciudadano” (Citizen Forum, journalist Virginia Quevedo) that is broadcast from 100 community radio stations across the country, reaching approximately 650,000 people. The main idea was to give marginalized citizens access to scientific information in simple words. Mass media interviews and articles in newspapers, magazines and electronic publications accessible via the Internet, and interviews in radio and television (total last year: 214), including EL MERCURIO, La Nación, Emol, Estrategia, El Diario Financiero, Revista Capital, Canal 13 and Chilevisión, Radios Cooperativa, Zero and ADN.

Two books were published during the CRCP time:

- **Las Incomunicaciones del Alzheimer.** Book on Alzheimer’s disease for lay people Griten by Dr. Nibaldo Inestrosa. (Agosto 2007).
- **El Peso de la Obesidad en el Siglo XXI.** Book that explains the impact of Obesity, gitten by Dr. María Paz Marzolo and Dr. Ada Cuevas (April 2010).

*International recognition to CRCP scientists:* The Pontifical Academy of Sciences distinguished Dr. Juan Larraín with the Pius XI Medal from the hands of Pope Benedict XVI. MDA distinguished Dr. Enrique Brandan works and supports his research with a Grant in the fibrosis field. Dr. Brandan also became a corresponding member of the Chilean Academy of Sciences. Dr. Nibaldo C. Inestrosa receives the 2008 National Award in Natural Sciences.
ISI IMPACT INDEX DURING REPORTED PERIOD.

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A team of 5 senior scientists highly competitive in their respective fields, with a high record of publications, presented the grant to the FONDAP. In terms of impact the 5 investigators already received Presidential Chairs by the Chilean Government, therefore they were already at the peak of their careers, therefore, to increase rapidly the impact factor of the group it was no an easy task. Two senior investigators were incorporated during the second period, plus a third one almost at the end of the Center. The last two years of the period the impact factor clearly increased a with roughly the same average number of papers published, which we think show the consolidation of the group and of the CRCP Center.
3.1 PROGRAM PROJECT 1: INTRACELLULAR PROTEIN TRAFFIC: MOLECULAR MECHANISMS, FUNCTIONAL IMPLICATIONS AND DISEASE

**Dr. Alfonso Gonzalez**

1) **Protein traffic in the exocytic and endocytic routes: molecular mechanisms, pathways and role in epithelial cell polarity.**

Major findings: 1.1) Disclose sorting mechanisms to the apical cell surface of epithelial cells that operate independently of glycosylation, thus complementing the current hypothesis of N- and O-glycans as apical sorting signals  1.2) Novel role of cholesterol and protein kinase A (PKA) on basolateral regionalization of the High density lipoproteins (HDL) receptor SR-BI  1.3) Introduction of cell-permeable peptides to compete for the sorting machinery in cells in vivo, revealing unexpected plasma membrane sorting events, both during endoplasmic reticulum-to-Golgi and trans-Golgi network-to-plasma membrane transport  1.4) Definitive evidence of recycling endosomes (RE) as the site of function of the clathrin adaptor AP1B in basolateral sorting, showed also that certain proteins follow a post-TGN trans-endosomal route through perinuclear RE, while other proteins by-passes this route. A cross-roads of post-Trans Golgi Network biosynthetic and recycling pathways explain numerous controversial observations and posits a paradigm-shift in the field of polarized protein sorting.

2) **Regulation of epidermal growth factor receptor (EGFR): Role of heterologous stimulus and trafficking.**

Major findings: 2.1) Cross-talk between EGFR and the receptors for urokinase and extracellular nucleotides (P2Y1R), which can play crucial roles in vascular physiology and certain cancers ; 2.2) A novel mechanism of regulation of EGFR operating upon the permanency versus endocytosis of inactive receptors at the cell surface, mediated by basal PKA activity and suitable for down-regulation by signalling phosphatidic acid (Norambuena et al. Mol Biol Cell. In revision). PKA activity also controls efficiency of EGF/EGFR intracellular segregation to a degradation pathway. Pharmacological intervention on such control system opens new possibilities for interfering the EGFR function with anti-tumoral purposes, complementary to those already in use.

3) **Cell surface targets and pathogenic mechanisms of autoantibodies in systemic lupus erythematosus.**

Major findings: 3.1) Evidences that a lectin called galectin-8 can play an immunologic role potentially altered in autoimmunity, as lupus patients produce function-blocking autoantibodies against Gal-8, while in turn Gal-8 induces apoptosis of activated T cells 3.2) Evidences that anti-ribosomal P antibodies associated with psychiatric lupus are neurotoxic and recognize a novel integral membrane protein of the neuronal cell surface, which we called NSPA (from Neuronal Surface P Antigen), presumably corresponding to a plasma membrane integral
ubiquitin ligase. *Paper selected by “Faculty of 1000 Biology” and highlighted in special comment in the J Exp Med.* Our more recent data (presented in international meetings in collaboration with Dr. Inestrosa) indicates that anti-P antibodies present in peripheral blood can provoke neural dysfunctions in the Central Nervous System they impair memory flexibility in mice, under conditions that open the blood brain barrier. This would be a major line of research for the next decade.

**Dr. Gonzalez’s Collaborations with FONDAP members:** 1) Dr. Gonzalez and Dr. Huidobro-Toro developed a highly synergistic collaboration that led to discover interesting relationships between the role of extracellular nucleotide receptors (P2YR), EGFR and lipid rafts. This collaboration originated a PhD thesis, a post-doctoral training and three publications in prestigious journals; 2) Dr. González and Dr. Inestrosa collaborated in defining and reviewing cell biology aspects of Prion. They also started a collaboration studying the neuropathogenic role of autoantibodies associated with neuropsychiatric lupus; 3) Dr. González and Dr. Marzolo collaborated in studies on the role of the adaptor AP1B in basolateral trafficking.

**PROGRAM PROJECT 2: FUNCTION OF PROTEOGLYCANS IN MYOGENESIS AND FIBROSIS**

*Dr. Enrique Brandan*

*Function of Proteoglycans as modulators of Growth Factors and organizer of the extracellular matrix (2000-2004):*

Growth factors are present during skeletal muscle development, differentiation and regeneration, although they are strong inhibitor of this process. (a) We demonstrated that a reduction of the proteoglycan decorin resulted in a decreased responsiveness to transforming growth factor type-β (TGF-β). These findings strongly suggested a new role for decorin during skeletal muscle terminal differentiation by activating TGF-β-dependent signaling pathways. We also found that the expression of betaglycan, a proteoglycan known as TGF-β-receptor type III, is upregulated during myogenesis by MyoD and retinoic acid by a mechanism independent of myogenin. (b) Another appealing concept evolved of these investigations is that myogenin expression is not sufficient to successfully drive skeletal muscle formation and that the extracellular matrix (ECM) is required to complete this process. The role of the ECM particularly the glycosaminoglycans is highlighted with the findings that dermatan sulfate is an enhancer of growth factor-dependent proliferation and migration, two critical processes involved in skeletal muscle formation.

These results suggest novel roles for proteoglycans modulating growth factor activity and reveal a unique function to the ECM during skeletal muscle formation.

It is worth to mention that for the first time we identified, localized and determined some functional aspects of syndecan in the nematode *C. elegans.*
Role of proteoglycans during skeletal muscle development and regeneration (2000-2004):

During development a fine regulation of signals derived from diverse growth factors and ECM take place. We evaluated the expression of myogenin and proteoglycans during limb formation. We found that myogenin was temporally and spatially coincident with the expression of syndecan-3 and Decorin. We determined heparan sulfate proteoglycans (HSPGs) expression in skeletal muscle regeneration induced by damage. Four major species –perlecan, glypican, syndecan-3 and syndecan-4- were transiently up-regulated. Grafting experiments into regenerating muscle showed that myoblasts with inhibited syndecan-3 expression showed an impaired capacity to fuse and form skeletal muscle fibers. These data constituted the first in vivo evidence suggesting the requirement of a specific HSPG for successful skeletal muscle regeneration. We also evaluated biglycan expression in skeletal muscle during muscle regeneration in mice. A transient and dramatic up-regulation of biglycan was associated with newly formed myotubes. Biglycan-null mice maintains its regenerative capacity but a delay in regenerated fiber growth and a decreased expression of embryonic myosin is observed.

We also identified endogenous extracellular ligands for muscle HSPGs. Among them, histone H1 is present in the ECM of skeletal muscle cells, where it interacts specifically with perlecan and exerts a strong proliferative effect on myoblasts, suggesting an active role for histone H1 during skeletal muscle regeneration.

Expression of proteoglycans in dystrophic muscles (2000-2004):

To understand the role of proteoglycans in several skeletal muscular dystrophies the level of expression of them was evaluated in skeletal muscle from biopsies of patients with Duchenne muscular dystrophy and the murine model mdx. These studies suggest that the amounts of specific heparan sulfate and chondroitin/dermatan sulfate proteoglycans are augmented in dystrophic skeletal muscle.


We found that lipoprotein-receptor related protein (LRP) is an endocytic receptor for decorin. Conceptually more important we discovered that LRP together with decorin are required for TGF-β signaling in a Smad-independent fashion, unveiling a new regulatory mechanism for TGF-β signaling by decorin and LRP. We also discovered that ECM proteoglycans diminish TGF-β bio-availability for its transducing receptors appearing as a feasible mechanism for the attenuation of this inhibitory growth factor during skeletal muscle formation.

Regarding the control of expression of TGF-β-receptors in skeletal muscle we found that TGF-β signaling is regulated by electrical activity in skeletal muscle cells and TGF-β type I receptor is transcriptionally regulated by myotube excitability. These findings support the possibility for a novel regulatory mechanism acting on TGF-β signaling cascade in skeletal muscle cells.
**Fibrosis: A hallmark of skeletal muscular dystrophies (2000-2004):**

Fibrotic disorders are typified by excessive connective tissue and ECM deposition that precludes normal healing processes of different tissues. Fibrosis is a hallmark in the different skeletal muscle dystrophies, being an important barrier for myoblast cell therapies. Connective Tissue Growth Factor (CTGF) has been involved in several types of fibrosis. It is expressed in myoblasts and the factor induces the synthesis of fibrotic proteins together with myoblast de-differentiation. Fibroblasts isolated from *mdx* skeletal muscles are fibrotic and express higher levels of fibronectin and collagen. The fibrotic phenotype is age dependent as well as the fibrotic feature in *mdx* muscle. Normal fibroblasts respond to TGF-β or CTGF as expected increasing fibronectin, surprisingly, both cytokine inhibits this effect in the fibrotic fibroblasts. We have also evaluated the role of CTGF and metalloproteinase activity in fibrosis. Our finding suggest that expression, regulation and activity of MMP-2 can play an important role in the initial steps of fibrosis, and shows that fibronectin levels can regulate the cellular response to CTGF.

This research plus the collaborative investigations originated thirty publications in high qualified journals. During these ten years eleven graduate students got their Ph.D. degree and five received Post-Doctoral training.

**PROGRAM PROJECT 3: ROLE OF PLASMA MEMBRANE NUCLEOTIDE RECEPTORS**

*Dr. J. Pablo Huidobro-Toro*

**Nucleotide Receptor Signaling**

Our studies centered on the molecular aspects of P2X receptor allosteric regulation, the micro regionalization of P2Y1 and P2Y2 receptors in the cell membrane of cultured cells and tissues. Lately, we also contributed to the understanding the role of lipid raft microdomains on nucleotide receptors. An important additional focus of interest has centered on the mechanisms of ATP release and metabolism from cultured cells or tissues and the role of presynaptic receptors as key modulation proteins necessary for the control of sympathetic co-transmission.

We identified the molecular mechanism by which trace metals such as copper or zinc, or neurosteroids, regulate the physiological activity of P2X2, P2X4 or P2X7 receptor channels. With the aid of site-directed mutagenesis, chimeric receptors and receptor subtypes splice variants we designed and synthesized more than 50 mutated P2X receptors. These variants were crucial in the identification of the amino acids involved in trace metal coordination. The recent availability of the crystallized P2X4 receptor from zebra fish has further highlighted our hypothesis on the role of the identified amino acids as metal coordinants allowing us to deduce structural metal binding “pockets” that modulate the P2X receptor channel.

We recently described a novel concept for receptor signaling related to the notion that redox state of the cell conditions the P2X channel gating. We further identified the critical role of
Cys\(^{430}\), the firstly described intracellular cysteine that regulates channel opening, within the P2X family of receptors. Novel interests will focus on the identification of neurosteroid modulator sites and particularly the ATP binding site(s) allowing us to establish whether the receptor gating requires two or three ATP molecules.

A foremost, and well received, contribution from our group emerged from the application of cell biology techniques to study human P2Y receptor dynamics. Based on previous findings on the physiology of P2Y\(_1\) and P2Y\(_2\) receptors in the superficial vessels of human placentae, we used sodium carbonate or OptiPrep sucrose density gradients to isolate lipid rafts from this tissue and assessed the micro regionalization of these receptors first in the absence and later to tissue exposed to selective receptor ligands. We described for the first time the variables involved in the mobilization of receptor to and from the rafts upon occupation, its internalization dynamics and its intracellular transduction mechanisms. Our studies show, for the first time, that the vasomotor action of nucleotides is linked to the transmodation of EGF receptors, through Ras/Rac protein activation to ERK phosphorylation. The functional coupling of the nucleotide receptor to the EGF receptor was paralleled by bioassays using isolated segments of biopsies from these human blood vessels.

Finally, substantial efforts were devoted to understand mechanisms of ATP release from tissues and cell in culture and to identify ATP metabolism by ectoATPases. We described that noradrenaline or CGRP, a sensory neuron peptide, are potent presynaptic inhibitors of the release of ATP, NA and ir-NPY from sympathetic neuron nerve endings; the receptors involved were dully characterized. In addition, we were the first to document that an adenosine A\(_{2A}\) receptor acts as a positive presynaptic modulator of ATP release from sympathetic nerve endings. More interestingly, we deduced that an adenosine tone regulates the release, since two selective A\(_{2A}\) receptor antagonists decreased the release and in addition blocked the agonist-evoked effect. Recently, we examined whether a mechanical stimuli elicited the release of ATP from HeLa cells in culture. Since these cells essentially lack connexins, the release of nucleotides is likely mediated by a mechanosensor inhibited by gadolinium. Interestingly, ATP acts in this model as an autocrine signal; it activates several P2 receptors, mobilizes intracellular calcium, and phosphorylates the EGF receptor presumably through nucleotide transmodulation.

As a whole, these contributions indicate that we are the first lab in Chile to actively pursue Molecular Pharmacology as a discipline, a goal that could never have been met without FONDAP funding. Among the most outstanding international collaborators, we collaborated with F. Rassendren at Montpelier, France and S. Stojiljovic at NIH, (Bethesda, MD)

In addition, these research lines allowed the training of several postdoctoral fellows (6), doctoral students (5) and undergraduates (20), key to publish our observations in top journals within this area of cellular biology.
PROGRAM PROJECT 4: ROLE OF PPARS IN NEURAL FUNCTION

Dr. Miguel Bronfman

**PPARα physiological ligands.**

Main findings in this project show that ω-Hydroxylated-eicosatrienoic acids (ω-OH acids), products of the sequential metabolism of arachidonic acid (AA) by the cytochrome P450 CYP2C epoxygenase and CYP4A ω-hydroxylase gene subfamilies, serve as endogenous PPARα activators. This is the first demonstration that P450 AA monooxygenases participate in PPARα activation. Because PPARα target genes are involved in the control of lipid levels these data suggest a new pharmacological target in the treatment of hyperlipemia. Further, evidence is provided that drugs used in the treatment of hyperlipemia, such as fibrates, most likely act by enhancing the synthesis of ω-OH fatty acids rather than by directly activating PPARα, thus, challenging the accepted view that fibrates are PPARα ligands in vivo.

**PPARs in oligodendrocyte function and differentiation. Possible involvement in glioma resistance to differentiation.**

In this project we provided evidence, for the first time, that both PPARγ and PPARβ are involved in oligodendrocyte function and differentiation. Furthermore, we demonstrated a complex cross talk between PPAR isoforms in driving C6 rat glioma cell differentiation to the oligodendrocytic lineage. PPARβ increases PPARγ and PPARα expression, which in turns induce markers of the oligodendrocytic lineage (PPARγ-dependent) and peroxisomal β-oxidation (PPARα-dependent) that is necessary for the synthesis of membrane components. Although PPARβ is expressed in C6 cells it is constitutively inactive and its overexpression is required. We have obtained comparable results in two human glioma cell lines. Most interestingly, in rat pre-oligodendrocytes, PPARβ or PPARγ agonist directly induces differentiation, which in both cases is abrogated by a PPARγ antagonist, suggesting that in normal oligodendrocytes PPARβ is active and drives PPARγ-dependent differentiation. As a whole, these results suggest that PPARs are involved in oligodendrocyte differentiation but might be constitutively repressed in glioma cells explaining the resistance to differentiation and strong proliferative properties of this tumor cells.

**PPARγ is involved in neuronal cell differentiation and survival.**

In a collaborative effort with Dr Inestrosa’s, and Dr. FC. Bronfman’s programs, we have shown that PPARγ is strongly involved in neuronal cell survival. First we found that PPARγ is a target of the NGF survival pathway and that its activation prevents β-amyloid-induced neurodegeneration in hippocampal cells. Second, we found that PPARγ supports survival in neurons through a mechanism involving increased expression of the Bcl-2 anti-apoptotic protein and resulting in stabilization of mitochondria and protection against oxidative stress. In agreement with this proposal we have shown, in collaboration with Dr. R Quintanilla and Dr. G.V. Johnson’s lab. (Department of Anesthesiology, University of Rochester, University Medical Center), that a PPARγ agonist prevents mitochondrial dysfunction in mutant huntingtin-
expressing cells. All together, this work strongly suggests PPARγ as a target in the treatment of neurodegenerative diseases.

**PPARγ is present in axons and retrogradely transported.**

In this collaborative effort with Dr. F. Court and Dr. F.C.Bronfman labs we have found that PPARγ is present in axons of different neuronal types. Furthermore, we found that PPARγ is present in the sciatic nerve, as demonstrated using immunostaining and western blot. PPARγ mRNA is also present in sciatic nerve, as assessed by in situ hybridization, strongly suggesting that PPARγ can be locally synthesized in the axon. Moreover, PPARγ is retrogradely transported in the sciatic nerve. Most interestingly, we found that PPARγ local expression in the axons is strongly increased after nerve injury, as assessed using the sciatic nerve crush technique, suggesting that PPARγ might has either a non-genomic local function in axons or that it is involved in injury signaling to the cell body. In agreement with this hypothesis we found that 4 h after sciatic nerve injury PPARγ is strongly increased in L4-L5 dorsal root ganglia. We think that these are ground breaking observations. However, they remain descriptive. In order to add functional data to these observations we have initiate a collaborative project with Prof. Mike Fainzilber, at the Weizmann Institute, Israel. Dr. Fainzilber is a recognized international expert in retrograde axonal signaling and will help us in assessing the role of PPARγ in nerve injury signaling using models of conditional lesion stimulation. J. Pablo Lezana, a graduate student will expend next 4 month in Dr. Fainzilber´s lab doing these experiments.


**Dr. Nibaldo C. Inestrosa**

*Neuroprotective role of the Wnt signaling pathway against Aβ toxicity*

We determined both in cultured hippocampal neurons and in an *in vivo* model of AD Alzheimer's disease (AD) (rats injected in the hippocampus with amyloid Aβ fibrils), that Aβ neurotoxicity resulted in a decrease of β-catenin, moreover LiCl, which mimics Wnt signaling by inhibiting glycogen synthase kinase-3β (GSK-3β) promoted neuronal survival and maintains cytoplasmic β-catenin. *In vivo,* chronic lithium treatment protected from neurodegeneration by rescuing β-catenin levels and improved the deficit in spatial learning induced by Aβ. These results lead to the proposal that a sustained loss of Wnt signaling function may be involved in the Aβ-dependent neurodegeneration observed in AD. In fact, the Wnt-3a ligand was able to overcome toxic effects induced by Aβ in hippocampal neurons by increasing neuronal survival, β-catenin levels and the expression of Wnt target genes. In addition, we recently identified Ca-calmodulin protein kinase IV (CAMKIV) as a novel Wnt target gene probably involved in protection of hippocampal neurons against Aβ.
Activation of the Wnt signaling pathway via cross-talk with other signaling pathways prevents Aβ-induced neurodegeneration.

The emerging role of Wnt signaling as a therapeutic target for AD treatment led us to evaluate potential pathways that interact with the Wnt signaling:

1. First, a bi-functional compound that includes Ibuprofen (an anti-inflammatory drug) and prostigmine (a cholinesterase inhibitor) (IBU-PO), protects hippocampal neurons from Aβ neurotoxicity. This effect is mediated by the activation of Wnt signaling, since the increase in the activity of GSK-3β induced by Aβ is down-regulated by co-treatment with the bi-functional compound.

2. Second, we studied the effect of some antioxidants on the canonical Wnt signaling pathway. Treatments with Trolox (an hydro-soluble analogue of vitamin E) and 17β-estradiol, increase β-catenin and inhibits the increase in GSK-3β observed after Aβ exposure.

3. Third, we identified α7 nicotinic acetylcholine receptors (α7-nAChR) as a target gene of the Wnt pathway. Activation of α7-nAChR with nicotine protects culture hippocampal neurons from Aβ toxicity, and at the same time increased β-catenin levels, indicating a cross-talk with the Wnt signaling. Finally, Wnt-7a regulates the presynaptic localization of APC and α7-nAChRs, being APC an intermediary in the re-localization process of the receptor.

4. Finally, we found that the activation of peroxisome proliferator activated receptors (PPAR) prevented neuronal cell death induced by the Aβ. Activation of PPARγ and PPARδ prevents reactive oxygen species production, cytoplasmic calcium increase, changes in mitochondrial viability and β-catenin degradation. We studied recently the effect of the PPARγ agonist, rosiglitazone in a double transgenic mice model of AD, we found memory improvement, a decreased Aβ burden, and Aβ oligomers and decreased astrocytic and microglia activation. Rosiglitazone activates the Wnt signaling pathway in vivo, since increases β-catenin and inhibits GSK-3β, suggesting that activation of PPARγ prevents Aβ-induced neurodegeneration by a mechanism that might involve a cross-talk with the Wnt signaling pathway.

In conclusion, the crosstalk of the Wnt signaling with other cellular pathways opened new research possibilities for AD therapy.

Physiological role of the Wnt signaling in synapse development and function.

The roles of the Wnt signalling pathway in several developmental processes, including synaptic differentiation, are well-characterised. The expression of Wnt ligands and Wnt signalling components in the mature mammalian central nervous system suggest that this pathway might also have a role in synaptic maintenance and function.
We showed that Wnt-7a acts as a canonical Wnt ligand in rat hippocampal neurons, stimulates the clustering of presynaptic proteins, and induces the recycling and exocytosis of synaptic vesicles. In addition, electrophysiological analysis on adult rat hippocampal slices indicated that Wnt-7a increases neurotransmitter release in CA3-CA1 synapses by decreasing paired pulse facilitation and increasing the miniature excitatory post-synaptic currents frequency. A presynaptic role was also determined for the canonical ligand Wnt-3a. The effects of Wnt-3a are mediated by Frizzled-1 receptor. These evidences suggest that the presynaptic function of rat hippocampal neurons is modulated by the canonical Wnt signaling.

On the other hand, the non-canonical ligand Wnt-5a, induces short term changes in the clustering of the postsynaptic scaffold protein PSD-95. Wnt-5a promotes the recruitment of PSD-95 from a diffuse dendritic cytoplasmic pool through a JNK-dependent signaling pathway. In addition, using adult rat hippocampal slices, we found that Wnt-5a modulates glutamatergic synaptic transmission through a postsynaptic mechanism. These results suggest that the Wnt/JNK pathway modulates the postsynaptic region of mammalian synapse. Moreover, we recently determined that Wnt-5a prevents Aβ oligomers-induced depression of glutamatergic transmission in hippocampal slices. More recently, we have found that Wnt-5a induces the surface expression and maintenance of the GABA_A receptor into the neuronal membrane. Electrophysiological analysis of rat hippocampal slices indicates that Wnt-5a regulates the inhibitory synapses postsynaptically. Our results also provided evidence that Wnt-5a regulates the recycling of functional GABA_A receptor in mature hippocampal neurons.

This research plus some collaboration originated 50 publications in good journals, including our recent Nature Review Neuroscience (Feb 10, 2010). During these ten years 15 graduate students got their Ph.D. degree and eight received Post-doctoral training.

PROGRAM PROJECT 6: TRAFFICKING, FUNCTION AND REGULATION OF THE LOW-DENSITY-LIPOPROTEIN (LDL) RECEPTOR-FAMILY MEMBERS

Dr. María Paz Marzolo

**LDLR members trafficking/function in cells with polarized phenotype (epithelial cells and neurons).**

LRP1 and Megalin have basolateral and apical sorting determinants respectively, in their corresponding cytoplasmic domains. We described megalin as a first example of type I membrane protein with cytoplasmic apical sorting information. We described that the sorting determinants operating in epithelial cells and neurons are the same for LRP1. The receptor has three major sorting signals based on tyrosine, but operating in different cell compartments, two during the biosynthetic traffic and at least two in the endocytic trafficking. The sorting machinery involved are AP1B, probably operating at post-TGN sorting and in the common recycling compartment, and sorting nexin 17, which recognizes a
critical Y in the context of NPxY motif. This proximal NPxY motif determines the recycling of LRP1 from the basolateral sorting endosome, via recognition by SNX17. The same tyrosine is relevant for the exit of LRP1 from the TGN, for still undetermined machinery. Regarding SNX17 we learn about the role of this cytoplasmic trafficking protein in the recycling and processing of the amyloid precursor protein (APP). We find that the suppression of SNX17 affects APP trafficking and increases its processing to Aβ. In addition the endocytic adaptor protein Dab2 is required for APP internalization, and the block of Dab2 expression/function, prevents APP endocytosis and processing to Aβ. We also work on the role of protein kinase D (PKD) in neuronal sorting of somatodendritic proteins. Also related to ApoER2 trafficking, we described that the receptor is internalized slowly, compared with other receptors of the family and by a clathrin-mediated pathway, requiring the NPxY motif (the same motif engaged in signaling and recycling). This motif is recognized in the endocytic pathway by Dab2. The receptor is also highly expressed in lipid rafts, feature that does not determine the endocytosis rate nor the pathway involved.

The role of ApoER2 in Neurodegeneration

We described that APP and APOER2 interact and that APOER2 affects APP trafficking and Aβ production. Besides APP reduction in the endocytosis rate, the expression of APOER2 increases Aβ production, mainly because it redirects APP to a lipid rafts fraction and increases, by a still unknown effect, gamma-secretase activity over APP-CTF. In relation with Neurodegeneration and apoER2, we are also studying the role of ApoER2 in the condition of cholesterol accumulation in endosomal compartments, observed in the Niemann-Pick C disease. We have found that in NPC neurons (chemically induced to the disease phenotype with the drug U 18666 A) there is a reduction in dendrite arborization and also an increase in the apoptosis. These effects can be partially reverted by the apoER2 ligand reelin, indicating that apoER2/reelin signaling system could have a protective role in this disease.

ApoER2 processing and trafficking

We described that the trafficking of the receptor is affected in the condition of cholesterol accumulation, in cells either mutant for the protein NPC1 or by treating the cells with U18666A. The expression of the receptor is reduced at the cell surface, with no change in the total protein, and the intracellular receptor is increased. This effect is produced by an increase in the endocytosis of apoER2 and a reduction in the recycling. The physiological consequences of these alterations in the traffic could be a change in the receptor processing, which is seen in the different models (cell lines and hippocampal neurons), with an increase in the substrate for the γ-secretase complex of ApoER2 (ApoCTF). In addition, we describe that ApoER2 is secreted normally in exosome particles, and this process is also affected by cholesterol accumulation. Finally, the reduction in ApoER2 cell surface expression could be part of the explanation of why we see less signaling in the NPC model neurons (see description of the results above).
The role of nuclear receptors PPARs and FXR, in the regulation of megalin expression.

We generated data showing that megalin is regulated in vitro and in vivo, by FXR and PPARα and γ receptors and their corresponding agonists. This work was started in 2004, when we studied megalin expression in human and mice gallbladder and its regulation by bile acids, FXR agonists. Related to PPARs the results can be resumed as: 1) FXR agonists induce megalin expression in vitro in different cell types, including kidney, yolk sac and human gallbladder cells in culture, and in vivo, in gallbladder and kidney. 2) FXR KO animals express less megalin in kidney and gallbladder. 3) There are three PPREs in the human megalin promoter, being at least the last one able to bind to PPARγ in an EMSA assay. 4) PPARα and γ agonists induce megalin expression at RNA and protein level, both in cell culture as well as in vivo, in mice and in rats and the effects are abolished by specific PPAR antagonists. 5) PPARα but not and γ agonists activate Luciferase expression under the control of the 1500 pb of megalin promoter (containing the first PPRE element of human megalin promoter).

To study the role of the PPPSP motif, which determines megalin phosphorylation, in megalin function/signaling.

1) Megalin has several putative phosphorylations sites. All of them where tested by site directed mutagenesis and neither of them contributes to the high phosphorylation level of megalin we discovered at least under non-stimulation conditions. 2) We found that a PPPSP motif is relevant to the phosphorylation of the receptor and that the kinase involved is the GSK3. 3) Mutation S170/A or inhibiting the kinase activity has the same consequences for megalin trafficking; that is does not modify the apical distribution of the receptor but increases the cell surface expression and the recycling of megalin to the apical cell surface.

We continued with the study of megalin phosphorylation and our recent unpublished results show that Wnt/β-catenin pathway regulates megalin phosphorylation (inhibiting, since it inhibits GSK3) and expression. We found that megalin promoter contains TCF/LEF sites in the promoter and that the expression of luciferase reporter gene is activated when in under the part of megalin promoter and the induction of Wnt-1 or Wnt-3a.

In addition, as part of FONDAP collaboration, with Dr. Enrique Brandan, we described that LRP1 is expressed in myoblasts and myotubes and that the receptor is involved in the endocytosis and degradation of the proteoglycan decorin.
PROGRAM PROJECT 7: EARLY DEVELOPMENT OF THE VERTEBRATE EMBRYO

Dr. Juan Larraín.

1.- Role of Proteoglycans in early embryonic development

We have unveiled a role for biglycan, syndecan4 and syndecan1 in early Xenopus development. These studies started with the isolation of the Xenopus homologues for each of these proteoglycans, studying their expression pattern during development, functional studies in Xenopus embryos and biochemical experiments to understand mechanism of action at the molecular level. All these work open new avenues of research and we have specially focused on studying the role of syndecan4 in neural tube closure and non-canonical Wnt signalling. Regarding this area we are specifically working on

- The effect of non-canonical Wnt signalling in regulating Syn4 and focal adhesion turnover: from this work a MS entitled “Non-canonical Wnt signalling induces ubiquitination and degradation of the focal adhesion receptor Syndecan4” by Carvallo et al., was submitted to Developmental Cell in June. The MS was rejected. During the last months we have followed some of the referees suggestions and we are preparing a new version of the MS that will be submitted soon.

- To study the role of syndecan4 in mouse development and Wnt,PCP signaling: we envision that this research line, that was started thanks to the FONDAP grant will led us to contribute to our understanding of the molecular and cellular basis of neural tube closure defects. This work corresponds to the PhD thesis of Ms. Noelia Escobedo (PhD Program in Cell and Molecular Biology) and implicates collaboration with three laboratories: Dr. Sarah Wilcox-Adelman, Boston University USA; Dr. Oliver Wessely, Louisiana State University, USA; Dr. Andrew Copp, University College London

2.- Spinal cord regeneration in Xenopus tadpoles

This research line was started during the last three years of the Center. Its long-term goal is to understand the molecular and cellular basis of spinal cord regeneration in Xenopus. We have described a role for hyaluronan in tail regeneration and its cross-talk with the Wnt pathway. In addition we are currently studying the role of sox2, a marker of neural stem cells, in spinal cord regeneration in Xenopus. This project is part of an ongoing collaboration with Dr. Felipe Court (Department of Physiological Sciences, P. Universidad Católica de Chile).

3.- Analysis of the transcriptome involved in Dorso-ventral patterning of the Xenopus embryos

A new line of research was opened in the lab. to perform global analysis of the transcriptome involved in dorso-ventral patterning of the Xenopus embryo. This research was conducted in collaboration with Dr. Francisco Melo as part of a FONDECYT grant (N° 1070357). We have identified a list of novel transcripts that are differentially expressed along the dorso-ventral axis at gastrula stage embryos. We are currently studying three of these novel
transcripts. Two of them encode novel proteins that are highly conserved from *Drosophila* to humans. Functional experiments indicate that they regulate nervous system development.

In addition we have also identified a transcript that encodes a putative transposase and the sense and antisense strands are transcribed. To identify possible small RNA generated from this double stranded RNA we performed Deep Sequencing from dorsal and ventral libraries in collaboration with Dr. John Mattick (The University of Queensland, Australia). We are currently in the process of analyzing this information.

**PROGRAM PROJECT 8: NEUROTROPHIN SIGNALING: CELL BIOLOGY, PROTEOLYTIC PROCESSING, AND ROLE IN CENTRAL NERVOUS SYSTEM REPAIR AND DISEASE**

*Dr. Francisca Bronfman*

Neurotrophins (NGF, BDNF, NT3 y NT4) bind two different king of receptors, the p75 neurotrophin receptor (p75), that binds all neurotrophins and the tyrosine kinase receptors (Trks) that specifically binds one particular neurotrophin (ex., NGF binds TrkA and BDNF binds TrkB). In 2003, we published a paper from my postdoctoral studies showing for the first time that p75 neurotrophin receptor continues signaling after internalization in recycling endosomes. This paper has been highly cited (83 times). In my own lab I have continued this research line and in 2007, we showed that endosomes, in addition to be a platform for signaling, are also a platform for γ-secretase mediated processing of p75, generating a soluble C-terminal fragment with signaling capabilities. Therefore, our results suggest that internalization regulates signaling and processing of p75 receptor. In addition, we also showed that the activation of TrkA by NGF regulates the γ-secretase cleavage of p75, showing that there is a cross talk between TrkA and p75 at the levels of γ-secretase. We are now preparing a manuscript describing that the γ-secretase involved in the regulation of p75 cleavage by TrkA is ADAM17.

We also show that activation of ERK1/2 and JNK are necessary for TrkA-induced p75 cleavage by NGF, moreover, while TrkA is able to induce p75 processing, we know that TrkB is not capable of doing so, we think it is because TrkA induces long lasting activation of ERK and JNK, necessary for ADAM17 activation, while TrkB signaling is down-regulated faster. These last experiments are now underway.

In compartmentalized sympathetic neuronal cultures that allows distinguishing between signaling in the cell body compare to the axons, we have established that in the cell body, p75 is internalized very slowly to endosomes different than early or late endosomes. Studies in PC12 cells have indicated that p75 follows an unusual pathway characterized buy the presence of GPI-anchored receptors such as the Nogo receptor. This internalization process is blocked my inhibition of JNK signaling in sympathetic neurons. By using Qdot technology we have established that p75 is retrogradely transported with a stop and go movement reminiscent
of dynein-mediated movement. In addition, we know that p75 is able to mediate an apoptotic retrograde signaling in compartmentalized neuronal cultures.

**Neurotrophin signaling and structural plasticity**

Another aspect we are interested is in neurotrophin-induced structural plasticity. Particularly, BDNF-induced dendritic branching of central neurons such as hippocampal neurons. We have investigated the role of the Rab11-regulated recycling pathways in BDNF/TrkB-induced dendritic plasticity. We have found that this endosomal system is a downstream effector of TrkB signaling pathway in such a way that down regulation of Rab11 activity inhibit BDNF-induced branching. This is the first report showing the relation between a neuronal response and the recycling pathway in central neurons.

We have also been interested in the physiology of basal forebrain cholinergic neurons they are long-projecting neurons in the central nervous system and are one of the neuronal populations affected in Alzheimer’s disease. For many years it has been thought that NGF is a retrograde survival factor for them. By using and axotomy model that disconnect cholinergic neurons from there target (the hippocampus) we have shown that axotomy-induced neurotrophic withdrawal causes the loss of phenotypic differentiation and down regulation of NGF signaling, but not death of septal cholinergic neurons paper that has been recently accepted in Molecular Neurodegeneration.

**Neurotrophin and Niemann Pick type C disease**

Another aspect that we are studying is the relationship between neurotrophin signaling in neurodegenerative diseases where there is an abnormal endosomal function. We have used as a model the Niemann Pick type C disease characterized by accumulation of cholesterol and lipids in the endocytic pathway and neurodegeneration. We have found that the neurotrophin response is abnormal in such a way that signaling molecules are up-regulated particularly NGF-mediated AKT/PLC-gamma signaling.

Along these years my lab have established collaborations with international laboratories some of them are listed below: Dr. Wim Annaert (Center for Human Genetics, Catholic University of Leuven, Belgium); Dr. Mike Fainzilber (Department of Biological Chemistry, Weizman Institute of Science, Israel) and Dr. William Mobley (Department of Neuroscience, Univ. California, San Diego, USA).
3.2 HUMAN RESOURCES.

The CRCP Center provides an almost perfect environment for a high performance in forming Human Resources for Science, as reflected in a high number of PhDs students and postdoctoral fellows, including an encouraging academia and intellectual environment, enriched with properly equipped laboratories and facilities.

From the beginning, the CRCP became the center of attractions for students from two PhD program mentions of our faculty, the Cell Biology and Molecular Biology and the Physiology programs, the impact in forming PhD was illustrated at the end of the first 5 years of the Center when our CRCP Center (5 senior researchers) formed 34% of the total graduates students of the whole faculty (with 50 professors). That percentage gave an idea what was the impact of our work.

Concerning co-tutorial thesis, well this was a more complicated story, the philosophy to share different labs, different ideas in just one PhD thesis is not so easy to implement in our discipline. For several reasons, for a long time our labs were separated of each others (different buildings), each professor is engaged in different subfields, one working on trafficking of proteins in epithelial cells and other in the function of proteoglycans during muscle differentiation, just to mention one example. This of course not the case for others areas (i.e., Ecology, where at one time one professor had 12 co-tutorial PhD thesis at the same time). Well this is simple not possible in a cell and molecular biology lab. In a few cases, two professors engaged in a specific collaboration help one student to work in two different areas, and eventually the student completes the degree, but using much more time that time for a thesis 3-4 years, (for example Dr. Sonja Buvinic, worked with Dr. JP Huidobro-Toro and Alfonso González).

The number of postdoctoral fellows trained in the CRCP Center increased slowly, mainly because the philosophy of the Chilean students was to leave the country for such endeavor, and again in contrast with other disciplines (i.e. Mathematics, Theoretical Physics or Ecology) only a few foreign students want to come to Chile for a postdoctoral work in a place with average level of complex equipment. Anyway during the years the center receives several postdoctoral fellows from Spain and Germany.

Another aspect that make a big impact in our students as well as in our scientific community were the International Workshops and Seminars organized through the years, we first started in 2000 with the first South American course on the “Basic Biology of the Nematode C. elegans”, the next year we organized an outstanding workshop and seminar on “Signal Transduction in Biomedicine”, in addition the following years we organized several symposium on Muscle Disease, Nuclear Receptor Family, Lipoprotein and Purinergic Receptors and different aspects of Developmental Biology and Stem Cells. During the 10 years period we also had the opportunity to organize two world events, the first one in Pucón in 2002 where we have the “International World Congress on Cholinesterases”, with more than 100 hundred foreign scientists and in 2005
we had the “First Chilean International Symposium on Neurodegenerative Diseases” in which the top-notch scientists of the field came to Santiago, including John Hardy, Colin Masters, Moussa Youdim, Claudio A. Cuello, Ashley Bush and Eckardt Mandelkov to mention a few of them.
3.3 NATIONAL AND INTERNATIONAL COLLABORATION:

It is very hard to summarize the national and international collaborations carried out during the 10 years period of the CRCP Center, so in the next pages or so, we will give only a glance of what happens the last years with the 5 seniors investigators that started the Center. I would like to emphasize that the network of collaboration allows the trip of students and researchers abroad, and at the same time permits real scientific collaboration that made the difference with the period before the CRCP Center. I am sure we are more complete scientists than before this experience, and for the future generation that happens to share with us this wonderful experience, it will make a whole difference.

Dr. Alfonso González

At the national level: collaborated with Dr. María Paz Marzolo on LRP trafficking, which generated a paper in *Mol Biol Cell*. He also collaborated with Dr. Juan Pablo Huidobro Toro, studying lipid rafts and P2Y1R function, which generated two nice papers one in the *Journal of Cell Science* and another in *Mol Pharmacol*. Dr. González and Dr. Nibaldo Inestrosa, produced a review on prion cell traffic published in *Curr. Pharm. Design*. More recently they are collaborating on studies regarding the neuropathogenic role of anti-P antibodies.

At the international level: Dr. González has established collaborations with Dr. David Valenzuela (Regeneron Pharmaceuticals, NY, USA), Dr. Enrique Rodriguez-Boulan, (Cornell Medical College, N.Y) with Dr. Peter Heinklein (Humbolt University, Germany) and Dr. Alberto Luini (Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy), a former PhD student Jorge Cancino, is doing his postdoc in Luine’s lab.

Dr. Enrique Brandan

At the national level: collaborated with Dr. Inestrosa on studies of proteoglycans in nerve differentiation, a manuscript was published (*Journal Cell. Physiology*, 2008). They also collaborated studying the effect of CGRP on the regulation of acetylcholinesterase at the Neuromuscular junction, a manuscript was published (*Brain Res Review, 2004*). With Dr. Marzolo published a Journal of Biological Chemistry paper in 2008 on the interaction of LRP and decorin.

At the international level: collaborate with Dr. Vincent Mouly, associated to the Association Française contre les Myopathies. With Dr. Gunnar Pejler, Swedish University of Agricultural Sciences, Biomedical Centre, Uppsala, Sweden. We have the KO mice (Serglycin -/-), and we plan to evaluate skeletal muscle regeneration in these mice as well in SerGlycin -/- mdx. With Dr. Roel Goldschmeding, Utrecht University. In a study of the role of CTGF and skeletal muscular fibrosis.

Dr. Juan Pablo Huidobro-Toro

At the national level: collaborated with Dr. González on the cell biology and molecular pharmacology of the P2Y receptors (papers published in *Journal of Cell Science* and *Molecular Pharmacology*). With Dr. Inestrosa collaborated to study whether the production of nitric oxide
(NO) by hippocampal cultured neurons are related to Wnt-5a signaling, a series of experiments showed for the first time that Wnt-5a boosted the release of NO from these neurons, a paper is in preparation for Nature.

At the international level: with Dr. Stanko Stojilkovic, NIH, USA to study the desensitization of the human P2X1 receptor, a nucleotide receptor critical for sperm migration, and animal reproduction, along the vas deferens ductus. A former PhD student Carlos Coddou is now a postdoc at NIH. With Prof. Benedito Machado, Univ. Sao Paulo, Ribeirao Preto, there is a long standing interest to examine the role of P2X receptors in the brain stem.Two doctorate students from Dr. Machado’s lab have come to our lab to learn biochemical techniques on purinergic receptors.

Dr. Miguel Bronfman

At the national level: With Dr. Inestrosa, fruitful collaboration on the effect of PKC PPARγ agonists to prevent amyloid neurotoxicity (papers in FASEB Journal 2002; Exp. Cell Research 2005; Journal of Biological Chemistry 2006) Dr. Maria Paz Marzolo on many tools related to PPARs system to study human glioblastoma. With Dr. Francisca Bronfman, studies dealing with the role of PPAR-gamma in retrograde axonal signaling.

At the international level: collaborated with Dr. G.V. Johnson, Department of Anesthesiology, University of Rochester, University Medical Center, 601 Elmwood Avenue, Rochester, NY 14642, USA. Recently (2008) a paper was published in Journal of Biological Chemistry on “Rosiglitazone treatment prevents mitochondrial dysfunction in mutant huntingtin-expressing cells”

Dr. Nibaldo C. Inestrosa

At the national level: With Dr. Christian Bonansco and Dr. Marcos Fuenzalida from the Department of Physiology, Universidad of Valparaíso, Chile, several papers are the outcome of these collaborations, on the canonical and non-canonical Wnt signalling, including Journal of Biological Chemistry (2008, 2009), J. Neuroscience (2010).

At the international level: is collaborating with Dr. Ernest Arenas, Molecular Neurobiology at the Karolinska Institute, Stockholm, Sweden, in Wnt signaling and neurogenesis, we recently published a review together in Nature Rev. Neuroscience. Dr. Enrique Toledo a former PhD student is doing his postdoc with Dr. Arenas. With Dr. Rebecca Kohn from Ursinus College, PA, USA, a Fullbright Visiting Professor we published a review on the use of Caenorhabditis elegans as a model for the human disease Inclusion Body Myositis (Rebolledo et al., Mol. Neurobiol 38: 178-198 (2008). With Dr. Andrés Barría, Department of Physiology at the University of Washington, Seattle, WA, USA in glutamatergic synapses and Wnt signaling regulation, Dr. Waldo Cerpa, a former PhD student of the CRCP Center is currently in Seattle doing his postdoctoral training.
3.4 OUTREACH

This aspect was incorporated only the last 5 years of the CRCP Center. Concerning outreach, during these last years several activities were carried out including: A weekly seminar and a monthly CRCP-MIFAB seminar, attracted around 60 participants from the major Universities of Santiago, including University of Chile and Andrés Bello, Last year, we have Monthly Interviews to all seniors scientist of CRCP in the Radio Program “Foro Ciudadano” (Citizen Forum, journalist Virginia Quevedo) that is broadcast from 100 community radio stations across the country, reaching approximately 650,000 people. The main idea was to give marginalized citizens access to scientific information in simple words. Mass media interviews and articles in newspapers, magazines and electronic publications accessible via the Internet, and interviews in radio and television (total last year: 214), including EL MERCURIO, La Nación, Emol, Estrategia, El Diario Financiero, Revista Capital, Canal 13 and Chilevisión, Radios Cooperativa, Zero and ADN.

Two books were published by the CRCP Center:

- **Las Incomunicaciones del Alzheimer.** Book on Alzheimer’s disease for lay people Griten by Dr. Nibaldo Inestrosa. (Agosto 2007).
- **El Peso de la Obesidad en el Siglo XXI.** Book that explains the impact of Obesity, griten by Dr. María Paz Marzolo and Dr. Ada Cuevas (April 2010).

We can also mention here a “Special Course for High School Teachers” organized on January of 2009. The subject was related to “Neurobiology and Neurodegenerative Diseases”. In addition some members of the CRCP Center like Drs. Huidobro-Toro and Inestrosa, teach different general courses in PENTA-UC a group of students from Public Schools coming from poor families of the South of Santiago (La Florida and Puente Alto).
4. OTHER RELEVANT ASPECTS

Several members of CRCP Center received Honors and memberships from the Academy

**Member of Editorial Board of the Journal of Biological Chemistry.** Dr. Nibaldo C. Inestrosa, became a member of the Editorial Board of the *Journal of Biological Chemistry* for a period of 5 years starting on July, 2008. Newspaper La Estrategia [www.estrategia.cl](http://www.estrategia.cl) (May 8, 2008);

**Member of the Chilean Academy of Sciences.** Dr. Enrique Brandan became a corresponding member of the Academy, was presented by Professor Deodato Radic on May 29th, 2008. ([www.bionoticias.cl](http://www.bionoticias.cl)). Newspaper La Nación ([www.lanacion.cl](http://www.lanacion.cl))

**Pio XI Award 2008 was obtained by a member of CRCP.** El Dr. Juan Larraín received the Pio XI Medal in Science from the Pontifical Academy of Sciences (August 8), the Roman Catholic Church, Pope Benedict XVI presented the award to Prof. Larraín. Newspaper El Mercurio ([www.emol.cl](http://www.emol.cl)), the first recipient of the *Pio XI Award* was the physicist Stephen Hawking, August 13th ([www.biologiachile.cl](http://www.biologiachile.cl)) ([www.universia.cl](http://www.universia.cl)).

**Chilean National Prize in Natural Sciences 2008.** El Dr. Nibaldo C. Inestrosa received the National Prize in Natural Sciences. He received the phone call from the Minister of Education Mrs. Mónica Jimenez, the same day of his birthday (August 26th). Newspaper El Mercurio ([www.emol.cl](http://www.emol.cl), [www.mineduc.cl](http://www.mineduc.cl); [www.conicyt.cl](http://www.conicyt.cl). The official ceremony takes place on December 17th, at the Claustro of the Recoleta Dominica Church, Santiago.
5. PERSPECTIVES OF THE CRCP CENTER

As we discussed previously a Business Unit Expertise dedicated to foster the relationship with national and pharmaceutical companies, started three-year before the end of the CRCP Center help us to foresee the future in relation to obtain new financial sources. This allows us to apply and eventually won a “Base Center of Excellence in Science and Technology” called Center for Aging and Regeneration (CARE). We do not think that we will be able to do it without the “training” that we had during the CRCP-FONDAP time. In this sense, we fill pretty confident that in the next future we will continue and expand our experiences, training and learning how to react to new and sometimes adverse conditions.
6. ACCOMPLISHMENT OF INSTITUTIONAL COMMITMENTS

At the beginning of the CRCP Center, we had certain problems because our Faculty did not gave all the help we thought we deserved. In particular, we expect to have a more rapid and straightforward renovation of our spaces, at the end, it takes several years to be in good shape, with renovated labs and good interaction with our authorities. At the Administration level we always have a cordial and very professional interaction.
7. ADVISORY COMMITTEE

The International Scientific Advisory Board which visited us in three opportunities during this ten year period, and the FONDAP Reviewer Board with the on-site visits recommended to focalize our work in the most relevant problems of each projects, this was carried out along the years and we made corrections accordingly.

These instances of evaluation and recommendation were so important for the success of the Center. Thus, we were able to open new avenues for effective and attractive collaborations that put together complementary expertise approaching these areas. However, although we have a strong commitment for collaborations, each senior investigator needs to maintain his own independent research as a capital already acknowledged by the scientific community.
8. DOCUMENTS GENERATED BY THE PROJECT DURING REPORTED PERIOD