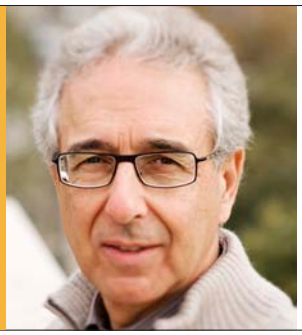
JOAN J GUINOVART



Study of the regulatory mechanisms of glycogen metabolism and characterisation of therapeutic targets

Our research group specialises in the study of the regulatory mechanisms of glycogen metabolism and the alterations of these processes in disease. We have a long tradition of research into glycogen synthase, the key enzyme in the regulation of glycogen synthesis. To this end, to address biological issues, we combine our knowledge of biochemistry and metabolism and follow a multidisciplinary approach that includes a wide variety of techniques from molecular biology, cell biology, proteomics, RNA silencing, gene transfer, mutant mouse generation and structural biology. Our work has contributed to revitalising this field by making a series of discoveries that have shown that, against general belief, there is still much ground to be covered. Our main achievements during 2008 are summarised below.

Determination of the key phosphorylation sites involved in the modulation of glycogen deposition in liver

Glycogen synthase (GS), the key enzyme that catalyses glycogen synthesis, is regulated by reversible phosphorylation at multiple sites. While much research effort has focused on the identification and functional consequences of the phosphorylation of the almost ubiquitous muscle isoform of GS (MGS), little has been devoted to the liver isoform (LGS). Dephosphorylation is correlated with the activation of GS, but in the particular case of LGS, the key sites involved in its activation have not been identified. In this regard, we have analysed the effect of dephosphorylation at the sites of LGS homologous to those described for MGS. Serine residues at these sites were replaced by non-phosphorylatable alanine residues, singly or in pairs, and the resultant LGS variants were expressed in cultured cells using adenoviral vectors. The sole mutation at site 2 (Ser7) yielded an enzyme that was almost fully active and able to induce glycogen deposition in primary hepatocytes incubated in the absence of glucose, as well as in FTO2B cells, a cell line that does not normally synthesise glycogen. Mutation at site 2 was also sufficient to trigger the aggregation and translocation of LGS from the cytoplasm to the hepatocyte cell cortex in the absence of glucose. However, this redistribution was not observed in hepatocytes incubated without glucose when an additional mutation (E509A), which renders the enzyme inactive, was introduced. This result strongly suggests that LGS translocation is strictly dependent on glycogen synthesis.

In conclusion, site 2 of LGS is the most potent regulatory site of the activity of the enzyme. Given that the LGS activation state is reduced in diabetes mellitus, the development of strategies aiming to increase the phosphorylation of this site may improve the accumulation of glycogen in liver, and thereby contribute to reducing hyperglycemia (Ros *et al*, in press, 2008).

Study of the adaptative regulation of glycogen metabolism in the embryonic liver

Mammalian embryonic livers accumulate glycogen in the absence of glucokinase expression. Glucokinase (hexokinase type IV) is required for the accumulation of glycogen in adult liver, and in embryonic livers hexokinases I and II are the only glucose-phosphorylating enzymes expressed. In adult liver, these two hexokinases would not normally have the capacity to build up enough levels of glucose-6-phosphate to activate LGS. Our results show that embryonic livers express massive levels of both hexokinases I and II, thus allowing the synthesis of sufficient amounts of glucose-6-phosphate to activate LGS and consequently hepatic glycogen synthesis.

Our results provide an explanation for the reorganisation of hexokinase expression in liver during fetal life and after birth. Glycogen plays a key role during embryonic development as it ensures pup survival in the period between birth and first receiving its mother's milk. Pups use glycogen deposits as a ready source of energy at the moment of birth and need to build their glycogen reserves during development in such a way that they do not depend on their mother's feeding state. By a considerable increase in the expression of the high affinity hexokinases I and II, embryos not only ensure

Principal Investigator Joan J Guinovart **Research Associates** Jorge Domínguez, Mª del Mar García **Postdoctoral Fellows** Adelaida Díaz, Jordi Duran, Carlos Rodríguez, David Vilchez, Delia Zafra **PhD Students** Romina Bertinat, Óscar Blanco, Mireia Díaz, Carles Martínez, Laura Nocito, Susana Ros, Isabel Saez, Felipe Slebe, Carlos Spichiger, Jordi Vallès **Research Assistant** Anna Adrover **Lab Technicians** Lydie Babin, Emma Veza **Administrative Assistant** Carolina Sánchez **Visiting Scientist** Jacques Borg (France)

their capacity to use glucose in all circumstances, but at the same time they produce enough glucose-6-phosphate to ensure hepatic glycogen accumulation even when the mother is fasting and blood glucose levels decrease. By means of this mechanism, embryos safeguard liver glycogen stores, thereby providing a crucial advantage at the moment of birth before their first ingestion of milk, which dramatically induces the expression of glucokinase (Cifuentes *et al*, 2008).

Study of the mechanisms of action of the anti-diabetic and anti-obesity agent sodium tungstate

Our group discovered that tungstate is an oral glucose-lowering and anti-obesity agent This compound has completed Phase I and II of clinical trials. Tungstate normalises carbohydrate metabolism in liver, stimulates insulin secretion, and regenerates pancreatic beta-cells in neonatally streptozotocin-treated diabetic rats. This compound is an efficient anti-diabetic agent in ZDF rats, a genetic model of type 2 diabetes. Our group, in collaboration with those headed by Ramon Gomis (IDIBAPS-Hospital Clínic de Barcelona), Rafael Salto (University of Granada) and Joan Enric Rodríguez Gil (Autonomous University of Barcelona), has devoted much research effort to the study of the effects of tungstate at both the physiological and molecular level.

During 2008, in collaboration with the group led by Joana Mª Planas (University of Barcelona), we have addressed the effects of tungstate treatment on the transport of monosaccharides in the intestine of diabetic animals. This is a crucial aspect to study in diabetes since the first step in the control of glycemia is the regulation of the transit of dietetic sugars from the intestinal lumen through the enterocytes to the bloodstream. To this end, we have analysed the action of this compound on the intestinal expression of Na^+/D-glucose cotransporter (SGLT1) and brush-border membrane

disaccharidase activities. In mammal intestine, D-glucose and D-galactose enter enterocytes through the brush-border membrane, mainly via the Na^+-dependent, high-affinity, low-capacity SGLT1. In diabetic rats, up-regulation of SGLT1 increases the capacity of the intestine to absorb monosaccharides. Our results indicate that tungstate restores the activity of brush-border disaccharidases and the expression and activity of SGLT1 in rat jejunum. These effects limit the entry of sugars into the body, thereby contributing to the anti-diabetic action of tungstate (Miro-Queralt *et al*, 2008).

A second study done in collaboration with Rafael Salto's group (University of Granada) is an extension of joint work between our groups aimed to dissect the action of tungstate on glucose transport in muscle myotubes at the molecular level. Our results show that tungstate treatment enhances glucose uptake in myotubes through an increase in the total amount and translocation of GLUT4 transporter. The effects on glucose uptake were additive to those of insulin. Our results indicate that tungstate exerts its actions through an increase in the transcription of GLUT4 mediated by the myocyte enhancer factor-2 (MEF2). This transcriptional activation is dependent on one of the key molecular actions of tungstate, the activation of the extracellular signal-regulated kinases 1 and 2 (ERK1/2). This is the first study to report the activation of GLUT4 transcription by a glucose-lowering compound through an ERK1/2-dependent increase in MEF2 levels (Girón *et al*, 2008).

Study of the regulatory mechanisms of glycogen metabolism through the laforin-malin complex and the consequences of the accumulation of glycogen in neurons

Our group has discovered that neurons have the enzymatic machinery and the capacity to synthesise glycogen but not to degrade it (Vilchez *et al*, 2007). In addition, we have shown that glycogen accumulation in these cells is pro-apoptotic. Neurons keep the glycogen synthesising machinery inactive by a series of well coordinated intracellular mechanisms: (i) confinement of GS (the key enzyme for glycogen synthesis) in the nucleus; (ii) inactivation of GS by phosphorylation; and (iii) controlled degradation of GS and Protein Targeting to Glycogen (PTG), a regulatory subunit of protein phosphatase-1, by a novel regulatory mechanism involving a complex formed by two new players, laforin and malin, and the ubiquitin-proteasome system. Failure to keep GS under control, which results in glycogen synthesis, damages neurons by triggering apoptotic signalling. We are currently dissecting the apoptotic signalling cascade induced by the accumulation of glycogen in neurons and we have made several ultrastructural studies on the effects in neurons. In addition, we are currently generating animal models of gain- and loss-of-function of GS and the associated regulatory proteins in order to provide further insight into the physio-pathological implications of abnormal glycogen accumulation *in vivo*.

Our studies, in collaboration with the groups headed by Pascual Sanz (Instituto de Biomedicina de Valencia, CSIC) and Santiago Rodríguez de Córdoba (Centro de Investigaciones Biológicas, CSIC), have gone further into the characterisation of the mechanisms that regulate the laforin-malin complex. We have shown that the interaction between laforin and malin is a regulated process that is modulated by the AMP-activated protein kinase (AMPK). We provide evidence that the formation of the laforin-malin complex is positively regulated by AMPK. We show that laforin, but not malin, has the capacity to interact physically with the catalytic subunit of AMPK and that AMPK phosphorylates laforin. These data provide evidence of an additional function of AMPK in glycogen metabolism, where its activation is known to lead to an increase in the phosphorylation and inactivation of GS and also to an increase in glucose uptake (Solaz-Fuster *et al*, 2008).

SCIENTIFIC OUTPUT

Publications

Cifuentes D, Martínez-Pons C, García-Rocha M, Galina A, de Pouplana LR and Guinovart JJ. Hepatic glycogen synthesis in the absence of glucokinase: the case of embryonic liver. *J Biol Chem*, **283**(9), 5642-49 (2008)

Girón MD, Sevillano N, Vargas AM, Domínguez J, Guinovart JJ and Salto R. The glucose-lowering agent sodium tungstate increases the levels and translocation of GLUT4 in L6 myotubes through a mechanism associated with ERK1/2 and MEF2D. *Diabetologia*, **51**(7), 1285-95 (2008)

Guinovart JJ. IUBMB and the Mediterranean. *IUBMB Life*, **60**(5), 249 (2008)

Miró-Queralt M, Guinovart JJ and Planas JM. Sodium tungstate decreases sucrase and Na^+/D-glucose cotransporter in the jejunum of diabetic rats. *Am J Physiol Gastrointest Liver Physiol*, **295**(3), G479-84 (2008)

Solaz-Fuster MC, Gimeno-Alcañiz JV, Ros S, Fernandez-Sanchez ME, Garcia-Fojeda B, Criado Garcia O, Vilchez D, Domínguez J, García-Rocha M, Sánchez-Piris M, Aguado C, Knecht E, Serratosa J, Guinovart JJ, Sanz P and Rodríguez de Córdoba S. Regulation of glycogen synthesis by the laforin-malin complex is modulated by the AMP-activated protein kinase pathway. *Hum Mol Genet*, **17**(5), 667-78 (2008)

Research networks and grants

Ayudas para potenciar y dar soporte a los grupos de investigación
Agency for Administration of University and Research Grants (AGAUR), 2005-SGR057 (2005-2008)
Principal investigator: Joan J Guinovart

Enfermedad de Lafora: papel de laforina y malina
'La Caixa' Foundation, BM06-340-02 (2007-2009)
Principal investigator: Joan J Guinovart

Estudio de las alteraciones en la homeostasis iónica e implicación de las proteínas G en el mecanismo de acción del agente antidiabético tungstato de sodio
Spanish Ministry of Science and Innovation, SAF2007-64722 (2007-2008)
Principal investigator: Joan J Guinovart

Estudio de un nuevo mecanismo de regulación del metabolismo del glucógeno. Análisis de las implicaciones patológicas de la acumulación anómala de polímeros de glucosa
Spanish Ministry of Science and Innovation, BFU2008-00769 (2009-2011)
Principal investigator: Joan J Guinovart

Mejora de la predicción traslacional de los ensayos de seguridad no clínica al hombre
Spanish Ministry of Science and Innovation, Noscira (former Neuropharma), Consorcio Melius, CENIT project (2007-2010)
Principal investigator: Joan J Guinovart

Molecular basis of progressive myoclonus epilepsy of the Lafora type
'MTV3' Foundation, 061930 (2007-2009)
Principal investigator: Joan J Guinovart

Nuevos fármacos y dianas para el tratamiento de la diabetes mellitus
'Marcelino Botín' Foundation (2006-2010)
Principal investigator: Joan J Guinovart

Regulación del metabolismo del glucógeno hepático, muscular y neuronal. Alteraciones en situaciones patológicas
Spanish Ministry of Science and Innovation, BFU2005-2253/BMC (2005-2008)
Principal investigator: Joan J Guinovart

Relación del síndrome diabético con la expresión y localización celular de la fructosa 1,6-Bifosfatasa y la glucógeno sintasa, enzimas claves en la homeostasis de la glucosa
Spanish Agency for International Cooperation, A/6647/06 (2007-2008)
Principal investigator: Joan J Guinovart

Collaborations

Analysis of the 3D structure of glycogen synthase
Joan C Ferrer, University of Barcelona (Barcelona, Spain)

Characterisation of glycogen metabolism in reproductive tissue: analysis of alterations in pathological situations
Joan E Rodríguez-Gil, Autonomous University of Barcelona (Barcelona, Spain)

Characterisation of the anti-diabetic and anti-obesity actions of tungstate
Ramon Gomis, IDIBAPS-Hospital Clínic (Barcelona, Spain)

Determination of the 3D structure of the glycogen synthases
Ignasi Fita, IRB Barcelona (Barcelona, Spain)

Glycogen-induced dysfunctions in pancreas and retina and their involvement in the ethiogenesis of diabetes mellitus
Ramon Gomis, IDIBAPS-Hospital Clínic (Barcelona, Spain) and Rafael Simó, Institut de Recerca Hospital Vall d'Hebrón (Barcelona, Spain)

Histological analysis of the alterations in the neuronal glycogen metabolism in neurological diseases
Teresa Ribalta, Hospital Clínic (Barcelona, Spain)

In silico design of modulators of the glycogen synthase activity
Modesto Orozco, IRB Barcelona (Barcelona, Spain)

Laser-induced forward transfer: a direct writing technique for biosensors preparation
José L Morenza, University of Barcelona (Barcelona, Spain)

Mechanism of action of anti-hyperglycaemic compounds and development of in vitro methods for screening their mode of action
Loranne Agius, School of Clinical Medical Sciences-Diabetes, The Medical School (Newcastle upon Tyne, UK)

Molecular basis of Lafora disease
Santiago Rodríguez de Córdoba, Centro de Investigaciones Biológicas-CSIC (Madrid, Spain) and Pascual Sanz, Institute of Biomedicine of Valencia-CSIC (Valencia, Spain)

Molecular dissection of the mechanisms of action of the anti-diabetic agent sodium tungstate in skeletal muscle
Rafael Salto and Mª Dolores Girón, University of Granada (Granada, Spain)

Study of hypoxia and glycogen accumulation
Luis del Peso, Instituto de Investigaciones Biomédicas-CSIC (Madrid, Spain)

Study of the actions of sodium tungstate on the ionic homeostasis
Miguel A Valverde, Pompeu Fabra University (Barcelona, Spain)

Study of the alterations in glycogen metabolism associated with colon cancer
Santiago Ramón y Cajal, Institut de Recerca Hospital Vall d'Hebrón (Barcelona, Spain)

Study of the alterations of glycogen metabolism in animal models with neurological diseases
Martí Pumarola, Autonomous University of Barcelona (Barcelona, Spain)

Study of the molecular targets and biological actions of sodium tungstate
José Ramón Murguia, Universidad Politécnica de Valencia (Valencia, Spain)

Study of the proteomic alterations induced by tungstate treatment of diabetic animals
Carmen Cámara, Universidad Complutense de Madrid (Madrid, Spain)

The use of Drosophila melanogaster as a model system for the study of Lafora disease
Marco Milán, IRB Barcelona (Barcelona, Spain)

Awards and honours

Josep Trueta Prize for the best research manuscript, Academy of Medical and Health Sciences of Catalonia and the Balearic islands (2008)
Awardee: Joan J Guinovart

Young Scientific Award (accessit), Promega Biotech Ibérica, XXXI Congress of the Spanish Society for Biochemistry and Molecular Biology (2008)
Awardee: David Vílchez