

Cell signalling: Regulation and function



Our group studies the regulatory and cross-talk mechanisms that underlie signal transduction, with the aim to gain a better understanding of physiological and pathological processes and to improve and develop therapeutic tools. We focus on two research lines, the nuclear receptor-MAPK negative cross-talk and the Nercc1/Nek6/7 NIMA-family signalling cassette. In relation to the former, we address a subset of nuclear receptors (NRs), namely the glucocorticoid receptor (GR) and the members of the peroxisome proliferator-activated receptor (PPAR) and liver X receptor (LXR) subfamilies, and the c-Jun N-terminal kinase (JNK) pathway. We are exploring the mechanisms responsible for the inhibition of the JNK pathway by these NRs, and analysing the involvement of this inhibition in mediating the pharmacological actions of the ligands of these NRs, namely anti-inflammatory and/or anti-diabetic activities. In relation to the second research line, we study the regulation and function of the signalling module formed by the NIMA-family kinases Nercc1 (also known as Nek9), Nek6 and Nek7. Previous data showed that this module is activated during mitosis and has a central role in spindle formation and mitotic progression. Our goals are to unravel the mechanism of Nercc1 activation, to identify Nercc1 and Nek6/7 substrates, and to validate these kinases as drug targets and/or prognosis markers in diseases related to cell cycle dysfunction.

Nuclear receptor-MAPK pathway cross-talk: mechanisms and actions

In this research line, we focus on the down-regulation of the JNK pathway by a subset of NRs whose ligands exhibit anti-diabetic and/or anti-inflammatory properties. These are the PPAR and LXR subfamilies, which are involved in the control of glucose and lipid homeostasis, and the GR, respectively.

Compelling evidence has shown that chronic inflammation promotes the development of several pathological conditions such as obesity and type 2 diabetes. The role of pro-inflammatory cytokines, such as tumour necrosis factor (TNF)- α , in promoting insulin resistance in obesity, a condition linked to the development of type 2 diabetes, was reported many years ago. In recent years, it has been demonstrated that pro-inflammatory signal transduction pathways, in particular JNK, IKK/NF κ B and PKC θ , are involved in the obesity-induced progressive loss of insulin sensitivity (reviewed in Wellen and Hotamisligil, 2005). In particular, JNK phosphorylates the insulin receptor substrate (IRS), abrogating its interaction with the insulin receptor (IR), a mechanism that constitutes a negative feedback loop on insulin signalling. However, under pathological conditions, JNK phosphorylation of the IRS promotes insulin resistance (reviewed in Hotamisligil, 2005). In addition to obesity and type 2 diabetes,

recent studies have implicated JNK in other pathological conditions, such as cancer, cardiac hypertrophy and failure, arthritis, asthma and neurodegeneration. Thus, given their potential therapeutic applications, JNK inhibitors have received much research attention (reviewed in Manning and Davis, 2003).

JNK inhibition by PPARs and LXRs. Thiazolidinediones (TZDs) are synthetic PPAR γ ligands with insulin-sensitising activity and are thus used in the treatment of type 2 diabetes. In spite of the relevance of these drugs, little is known about their mechanism of action or their molecular target. This knowledge gap thus blunts the rational development of new molecules with improved pharmacological profiles. We have demonstrated that TZDs, through interaction with PPAR γ , inhibit JNK pathway activation. Moreover, we have shown that the JNK inhibition by TZD/PPAR γ is crucial for the anti-diabetic properties of these drugs since their hypoglycemic activity is abrogated in JNK1-deficient mice (Díaz-Delfín *et al*, 2007).

JNK inhibition by TZDs also occurs in macrophages and correlates with their capacity to repress pro-inflammatory gene expression. In this context, this inhibitory action on the JNK pathway is related to the anti-inflammatory properties of these molecules. In addition, the current state of the art, which agrees that a low-grade chronic inflammatory process is at the origin

Principal Investigator Carme Caelles Research Associate Joan Roig PhD Students M^a Teresa Bertran, Kader Cavusoglu, Rodrigo Gatica, Jordi Lanuza, Laura Regue, Mariana Vargas Lab Technician Cristina Vila Visiting Student Giuseppe Pulice



and/or promotes a series of diseases including type 2 diabetes, supports the notion that the anti-inflammatory activity exerted by TZDs has a relevant role in the anti-diabetic action of these compounds. In agreement with this, Mercedes Ricote's group (Hevener *et al*, 2007) has demonstrated that macrophage-specific PPAR γ -deficient mice are glucose-intolerant as a result of exacerbated secretion of pro-inflammatory mediators by macrophages. Also, the anti-diabetic action of TZDs is almost abrogated in these animals. In addition to TZDs, we have demonstrated that ligands for other members of the PPAR and LXR subfamilies, which also show anti-inflammatory, and in some cases anti-diabetic activity, also inhibit the activation of the JNK pathway in macrophages.

In our search for a mechanism responsible for the inhibition of JNK by TZDs, we observed an increased expression of the cell cycle regulator p21waf-1 in the adipose tissue of obese mice.

This finding led us to study the phenotype of the waf-1 knock-out (KO) mouse in the context of obesity and insulin resistance. Waf-1 KO mice are protected from the development of adiposity and insulin resistance when fed a high-fat high-carbohydrate diet (HFD; Figure 1).

JNK inhibition in the anti-inflammatory action of glucocorticoids. We previously reported the inhibition of JNK by glucocorticoids (GCs) and proposed the relevance of this signaling pathway for the anti-inflammatory actions of these drugs. We also described a molecular mechanism responsible for this inhibition (Caelles *et al*, 1997; Bruna *et al*, 2003). In addition to JNK, the IKK/NF κ B pathway is also relevant for the inflammatory response, and it is also known to be target of GC action. According to our data, JNK is required for NF κ B activation since it increases the SCF ^{β TRCP} complex, by enhancing β TRCP mRNA stability, which is responsible for I κ B ubiquitination and, hence,

degradation. We have demonstrated that JNK inhibition by GCs through this mechanism negatively regulates NF κ B activation.

Generation of a transgenic murine model that allows the conditional activation of JNK and its validation as a model to study pancreatic failure. We have generated a transgenic mouse with the aim to study the contribution of JNK activation to the development of insulin resistance and pancreatic β -cell failure. This mouse model harbours a Cre-conditional expression transgene encoding a constitutively activated mutant form

of MKK7, a highly specific JNK MAP2K. We have activated the transgene in pancreatic β -islets by crossing parental transgenic mice with a mouse strain that expresses the Cre recombinase specifically in pancreatic β -cells. Cre-mediated recombination of the transgene leads to JNK activation in pancreatic β -cells concomitantly with the development of glucose intolerance and a failure to increase insulinemia in response to hyperglycemia.

The signalling module formed by the NIMA family of kinases Nerc1/Nek9-Nek6/7: regulation and function

The NIMA family of protein kinases is named after the product of the *nimA* gene, a protein kinase from *Aspergillus nidulans* that has a central role in G2/M transition and mitotic progression (O'Connell *et al*, 2003). During evolution, the NIMA family of kinases (Nek1-11 in mammals) has acquired several functions related to the control of centrosome/microtubule systems, including crucial roles during mitotic progression (Quarby and Mahjoub, 2005; Roig and Avruch, 2006; O'Regan *et al*, 2007). We have previously described that Nerc1 (also known as Nek9) binds and activates two other highly homologous members of the NIMA family, Nek6 and Nek7, and have proposed that they form a signalling module activated at the centrosomes and spindle poles during mitosis (Roig *et al*, 2002; Belham *et al*, 2003; Roig *et al*, 2005). Nerc1, like Nek6 and Nek7, is required for correct spindle formation, chromosome segregation and mitotic progression (Roig *et al*, 2002; Roig *et al*, 2005), although the molecular bases for this action are currently unknown.

To elucidate the cellular role of the Nerc1/Nek6/7 module during mitosis, relate it to other mitotic signalling networks, and identify its signalling targets, our group aims to (i) study how the activity of the upstream kinase, Nerc1/Nek9, is regulated; (ii) determine the structural basis for the mechanism of Nerc1 inhibition/activation; (iii) identify and study Nerc1 and Nek6/7 substrates; (iv) use different systems to interfere with Nerc1 and Nek6/7, in order to gain a broad view of their function at the cellular and organismic level, relating it to the results of point 3; and (v) validate Nerc1 as a drug target and identify chemical inhibitors of the protein kinase with therapeutic value. We are now pursuing the above goals in collaboration with several research groups and a biotechnology company. Our final objective is to produce a complete picture of the position of the Nerc1/Nek6/7 module in relation to the myriad of signalling events that control mitosis and the cell cycle.

During 2008, we have advanced in both our understanding of the activation mechanism of Nerc1 during mitosis, as well as the identification of substrates of the Nerc1/Nek6 module. In addition, we are collaborating with David Reverter (Institut de Biotechnologia i Biomedicina, Universitat Autònoma de Barcelona) to produce, crystallise and determine the structure of an inactive form of Nerc1; with Jens Lüders (IRB Barcelona) to elucidate how phosphorylation controls microtubule nucleation in mitosis; and with Isabelle Vernos (CRG, Barcelona) to study the signalling module in the *Xenopus* mitotic egg extract.

We have performed a number of yeast two-hybrid assays using distinct forms of Nerc1/Nek9 as bait, and now have several

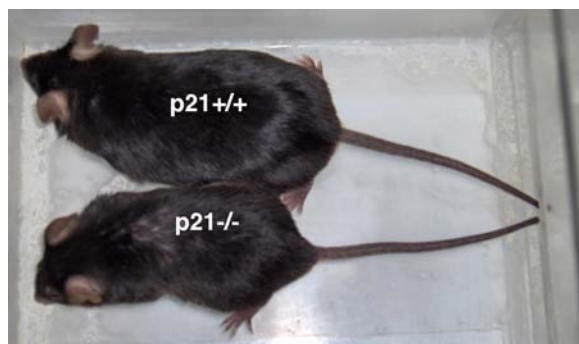


Figure 1. *p21* deficiency protects from HFD-induced adiposity. Picture shows 16-week-old WT (*p21*^{+/+}) and *p21*-deficient (*p21*^{-/-}) mice which had been fed a HFD since they were 4 weeks old.

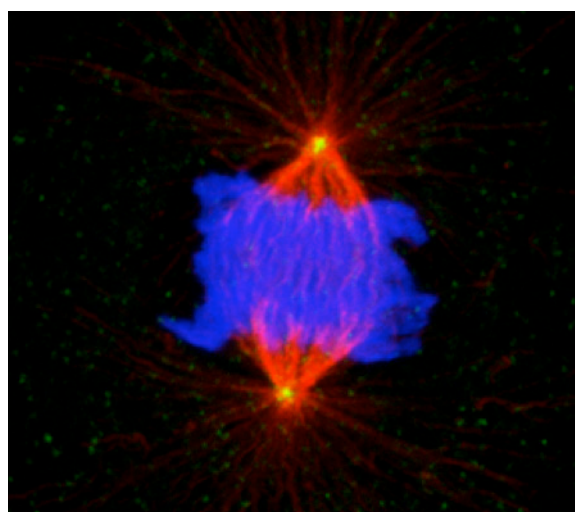


Figure 2. Phospho-Eg5 immunolocalisation in mitosis. Mitotic XL177 cell fixed and stained with antibodies to XLEg5[Ser1046P] (green), and β -tubulin (red). DNA is stained with DAPI (blue).

candidates for Nercc1 regulators and/or Nercc1/Nek6/7 substrates, 12 of them proteins with a known function during mitosis. At present, we are validating the interactions identified and starting to analyse a selected group of these proteins. Regarding Nercc1/Nek6 substrates, we have identified one of the most important mitotic motors, the kinesin Eg5 (also known as Kif11 and Kinesin-5), as a Nercc1/Nek6-interacting protein, and demonstrated that it is a Nek6 substrate both *in vitro* and *in vivo* (Rapley *et al*, 2008). Thus, Nek6 is constitutively associated with the C-terminal tail of Eg5 and has the capacity to phosphorylate the kinesin at Ser1033 (in human Eg5), a site that is phosphorylated *in vivo* during mitosis. We have produced several antibodies that recognise both human and *Xenopus* Eg5 phosphorylated at the Nek6 site, and have shown that, while during mitosis total

Eg5 resides in the cytoplasm and the length of spindle microtubules, the phosphorylated motor is localised at spindle poles and the proximal region of spindle microtubules (but not in astral microtubules, Figure 2). This subcellular location overlaps in part with the activation pattern of Nercc1/Nek6. Our results show for the first time that cells have differentially modified and spatially localised pools of Eg5. This finding may help us to explain how Eg5 performs its multiple cellular roles. We have also demonstrated that phosphorylation of the novel Nek6 site is physiologically important for normal Eg5 function, as mutant forms of the kinesin lacking a phosphorylatable Nek6 site rescue Eg5 depletion with only 50% of the efficiency shown by wild-type kinesin. We are currently studying the molecular basis behind these observations.

SCIENTIFIC OUTPUT

Publications

Rapley J, Nicolàs M, Groen A, Regué L, Bertran MT, Caelles C, Avruch J and Roig J. The NIMA-family kinase Nek6 phosphorylates the kinesin Eg5 at a novel site necessary for mitotic spindle formation. *J Cell Sci*, 121, 3912-21 (2008)

Vallador AF, Arpa L, Sánchez-Tilló E, Comalada M, Casals C, Xaus J, Caelles C, Lloberas J and Celada A. IFN- γ -mediated inhibition of MAPK phosphatase expression results in prolonged MAPK activity in response to M-CSF and inhibition of proliferation. *Blood*, 112, 3274-82 (2008)

Vallador AF, Sánchez-Tilo E, Arpa L, Park JM, Caelles C, Lloberas J and Celada A. Selective role of MAPKs during the macrophage response to IFN- γ . *J Immunol*, 180, 4523-29 (2008)

Research networks and grants

El módulo de señalización Nercc1/Nek6/7; regulación y funciones
Spanish Ministry of Science and Innovation, BFU2008-03441/BMC (2008-2011)

Principal investigator: Joan Roig

Estudio de una nueva vía de señalización mitótica compuesta por las NIMA quinasas Nercc1, Nek6 y Nek7. Regulación y funciones
Spanish Ministry of Science and Innovation, BFU2005-05812 (2006-2008)

Principal investigator: Joan Roig

Papel de la c-Jun N-terminal kinase en las acciones fisiológicas y farmacológicas de los glucocorticoides y los ligandos de PPARs y LXRs
Spanish Ministry of Science and Innovation, BFU2007-62087/BMC (2007-2010)

Principal investigator: Carme Caelles

Relación de la expresión del receptor de insulina y la activación de la vía PI3K/AKT con la expresión de enzimas glicogénicas y gluconeogénicas en células tubulares de riñón de ratas normales y diabéticas

Fundación Marcelino Botín (2008-2009)

Principal investigator: Carme Caelles

Collaborations

Functional analysis of JNK activation in pancreatic β -cells
Ramón Gomis, IDIBAPS (Barcelona, Spain)

Identification of inhibitors of mitosis
Mercury Therapeutics Inc (Woburn, MA, USA)

MAPK pathways in macrophages
Antonio Celada, IRB Barcelona (Barcelona, Spain)

Regulation of microtubule nucleation through phosphorylation
Jens Lüders, IRB Barcelona (Barcelona, Spain)

Structural basis for the mechanism of Nercc1 autoinhibition
David Reverter, Institute of Biotechnology and Biomedicine, Autonomous University of Barcelona (Barcelona, Spain)

*Study of the regulation and function of the Nercc1/Nek6/7 signalling module in the *Xenopus* egg extract system*
Isabelle Vernos, Centre for Genomic Regulation (Barcelona, Spain)

The role of JNK in myogenesis
Pura Muñoz-Cánoves, Pompeu Fabra University (Barcelona, Spain)