



Cell signalling: Regulation and function

We study the mechanisms that regulate signal transduction and their role in physiology and pathology, with the aim to improve and/or develop new therapeutic tools. We focus on two major research lines, the negative cross-

talk between the nuclear receptor (NR)-stress activated protein kinase (SAPK) pathway, and the Nek9/Nek6/7 NIMA-family signalling cassette. In relation to the former, we center on a subset of NRs, namely the glucocorticoid receptor (GR) and the members of the peroxisome proliferator-activated receptor (PPAR) and liver X receptor (LXR) subfamilies, which share the capacity to down-regulate inflammatory responses. We have shown that, upon ligand binding, all these NRs inhibit the activation of the SAPK c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways, all crucial mediators of pro-inflammatory signals. Therefore, inhibition of SAPK pathways by these NRs may be a mechanism by which to exert anti-inflammatory action, but also other relevant pharmacological activities, such as anti-diabetic and anti-atherosclerotic activity. In relation to the second research line, we address how phosphorylation regulates the execution of mitosis. In this regard, we study the signalling module composed of the NIMA-family kinases NERCC1/Nek9, Nek6 and Nek7, with the aim to elucidate its regulation, its relationship with other mitotic signalling components and its function, and to determine whether these kinases could be used as targets of pharmacological drugs.

NR-SAPK pathway cross talk: mechanisms and actions

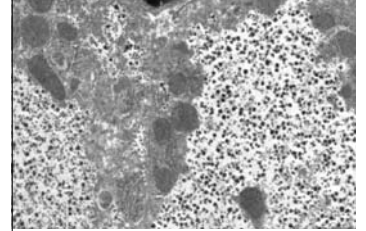
Inflammation is a body response triggered by pathogens and noxious stimuli, such as chemicals or physical injury, which damage tissues and cells. Inflammatory responses underlie a wide variety of physiological and pathological processes. The classical events that trigger short-term or acute inflammation are infection and tissue injury, and considerable progress has been made to unravel this response at the molecular and cellular level. However, stress and dysfunction in tissues similarly induce a process characterised by being a systemic-chronic-low grade inflammatory response. This process is probably responsible for the chronic inflammatory conditions associated with many diseases that are highly prevalent in modern societies and that include classical chronic inflammatory diseases, such as rheumatoid and psoriatic arthritis, inflammatory bowel disease, chronic obstructive pulmonary disease, but also metabolic diseases, for instance, obesity, type 2 diabetes (T2D), atherosclerosis, and cancer (Medzhitov, 2008). In the last decade it has become evident that inflammation is a crucial factor at the origin and/or for the progression of all these chronic diseases, a circumstance that may explain the beneficial effects of anti-inflammatory therapy in these diseases. Therefore efforts and resources are being dedicated to the search, development, and improvement of anti-inflammatory drugs (Karin, 2005). Furthermore, supporting this strong link between inflammation and metabolism, NRs initially identified for their role in the regulation of glucose and

lipid metabolism, such as PPARs and LXRs, were found to have anti-inflammatory properties and are expressed in cells of the immune system, such as macrophages (Bensinger and Tontonoz, 2008). In addition, the protein kinases of the same pathways that orchestrate the inflammatory response, JNK and IKK (I κ B kinase), were found to contribute to promoting insulin resistance (in addition to their involvement in the production of pro-inflammatory and diabetogenic cytokines) by an inhibitory phosphorylation of the insulin receptor substrate (IRS)-1 (Hotamisligil, 2005). In this context, our studies have shown that the inhibition of the JNK pathway by the glucocorticoid receptor (GR) is responsible for the interference of these hormones with the AP-1 complex (Caelles *et al*, 1997; Caelles *et al*, 2002).

More recently, we have shown that thiazolidinediones (TZDs), which are synthetic ligands for PPAR γ with insulin-sensitising activity, also have the capacity to inhibit the JNK pathway. Moreover, our results indicate that the inhibition of this pathway by TZDs mediates the anti-diabetic action of these drugs, and consistently, genetic ablation of *jnk1* abrogates the hypoglycemic action of TZD in mice (Díaz-Delfín *et al*, 2007). This study expanded the scope of pharmacological actions (that is to say anti-inflammatory and anti-diabetic activities) mediated by the negative interference of NRs on SAPK pathways. Other members of the PPAR family, as well as LXRs inhibit LPS-induced activation of the JNK and p38MAPK pathways upon ligand activation in primary macrophages (peritoneal- and bone marrow-derived).

Given that the kinetics of this inhibitory action is compatible with the requirement of gene transcription, we are performing transcriptomic analyses to identify potential candidates to mediate the interference of these NRs on SAPK pathways. Potential candidate genes were identified as those encoding known inhibitors of SAPKs, such as the cell cycle regulator p21*waf-1*, which in addition is a transcriptional target of all these NRs, and the dual specificity phosphatase MKP-1, which mediates MAPK inhibition by the GR. Although we have observed that *waf-1* expression is increased specifically in white and brown adipose tissue in various mouse models of obesity, this potential candidate has been discarded, as *waf-1*-deficient mice are resistant to the development of diet-induced obesity and insulin resistance. MKP-1 involvement in the inhibition of SAPK pathways by PPARs and LXRs has also been discarded, as we have found no evidence that this gene is a transcriptional target of any of these receptors. In summary, the inhibition of SAPK pathways by NRs is exerted at different levels along the signal transduction cascade and is mediated by distinct mechanisms depending on the cell type and the NR involved.

The observation that the NR-SAPK pathway cross-talk is negative and mutual implies that activation of SAPK pathways may alter the functionality of these NRs. This notion was previously proposed as a mechanism to account for the resistance to GCs found in the clinic (Adcock and Lane, 2003). In addition, JNK is activated in diseases that are treated with ligands of the NRs we are currently studying. In this context, we generated a transgenic mouse model (GFP-MKK7D) in which JNK activation depends on the Cre recombinase-dependent expression of a constitutively-activated mutant of MKK7 (MKK7D). We have already floxed/activated the transgene in pancreatic β -islets (by crossing these mice with the strain B6.Cg-Tg(*Ins2-cre*)25Mgn/J), and mice became glucose-intolerant as a result of increased insulinemia in response to hyperglycemia. In this particular model, we are characterising at molecular level the mechanism by which activation of the JNK pathway leads to pancreatic dysfunction and that, unexpectedly, is not caused by the induction of pancreatic cell death or by major structural abnormalities of the islets, as determined by morphometric analysis (Figure 1).



Research Group Members

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

Nek9 (also known as Nercc1) is a member of the NIMA-family of protein kinases. Nek9 in collaboration with the related Nek6 and Nek7 has a crucial although not well understood role in the control of mitotic progression. Nek9 is activated on the centrosomes and spindle poles during mitotic entry. Once active, Nek9 interacts with Nek6 and Nek7, two highly similar kinases of the NIMA family that can be directly phosphorylated and activated by Nek9. A number of studies have shown that Nek9, Nek6 and Nek7 are required for normal spindle formation, chromosome segregation, mitotic progression and cytokinesis, although the molecular basis for these observations is currently unknown.

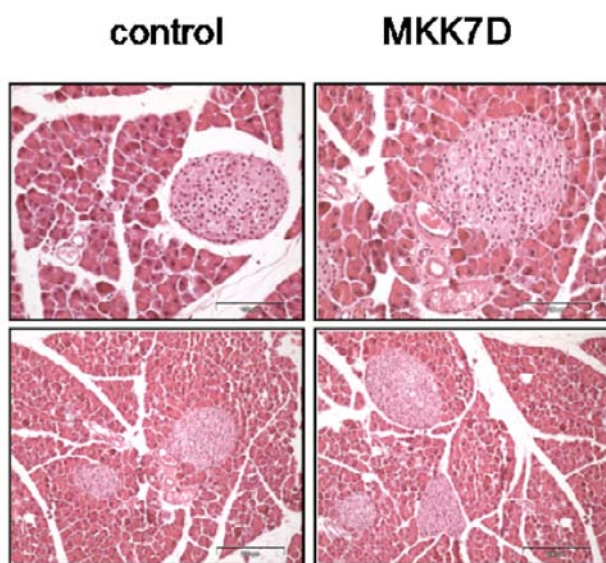


Figure 1. *In vivo* JNK activation in insulin-producing cells does not produce significant changes in the number or morphology of pancreatic β -islets. Pancreata of unfloxed (control) and floxed (MKK7D) GFP-MKK7D transgenic mice stained by eosine-hematoxylin.

Our group studies the mitotic function of the Nek9/6/7 cassette as well as the regulation of its upstream kinase, Nek9. For this purpose, we have performed various experiments aimed to identify proteins that interact with the protein kinases. One of the proteins identified as a Nek9 interactor, LC8 (also known as DYNLL), was previously found to be a component of several macromolecular complexes, such as the motor dynein. We have characterised LC8-Nek9 binding and produced different point mutants of these proteins that are not able to interact. Using these and other tools, we have shown that LC8 does not act as an adaptor between Nek9 and dynein, and that although LC8 is not necessary for Nek9 dimerization it is involved in Nek9 multimerisation and collaborates in its activation mechanism, most probably by amplifying Nek9 activity during *in vivo* activation. More importantly, we have shown that LC8 binding to Nek9 is regulated by phosphorylation, as we have identified a Nek9 resi-

due which, once phosphorylated, impedes LC8 binding to the kinase. This residue is autophosphorylated in response to Nek9 activation, thus inducing LC8 release from active Nek9. Using a range of approaches, such as RNAi, we have demonstrated that LC8 allows Nek6/7 interaction with Nek9, and thus their subsequent activation. We propose that LC8 is a key controller of signal transduction through the Nek9/6/7 module and show for the first time that LC8 binding to other proteins can be regulated through partner phosphorylation.

Following our interest in unravelling how phosphorylation controls the execution of mitosis, we have started a project in collaboration with Jens Lüders' group (Cell and Developmental Biology Programme) to study the regulation through phosphorylation of the γ -tubulin ring complex (γ -TURC), a multiprotein complex formed by γ -tubulin and a number of associated proteins that is indispensable for microtubule nucleation (and thus spindle formation), and that we have previously shown to interact with Nek9 in various systems. To this end, we have developed a method based on the expression of tagged γ -tubulin that allows the purification of significant amounts of γ -TURC from both exponentially growing and mitotic cells; we expect that this approach will allow us to analyse both the protein content and phosphorylation of the complex and to study its spatiotemporal regulation during the cell cycle.

Regarding the study of the function of the Nek9/6/7 module, we are currently addressing its relationship with Eg5, a mitotic kinesin that we previously demonstrated to be an *in vivo* substrate of Nek6. We are also in the process of producing Nek9 $-/-$ knock out mice, and have generated heterozygous Nek9 $+/-$ mice. This has allowed us to determine that Nek6 is required for normal development as Nek6 $-/-$ knock out mice die at early stages of embryogenesis. At present we are characterising this mice model, and we anticipate that the culture of Nek6 $-/-$ MEF cells will be a valuable tool to better understand the molecular role of this kinase.

Finally, we have also made significant advances in the study of the activation mechanism of Nek9 and identified the proteins responsible for the regulation of this kinase. Other ongoing projects in our group address the following: the determination of the structure of an inactive form of Nercc1; the binding of Nek6/7 and LC8 to this kinase (in collaboration with David Reverter, Institute of Biotechnology and Biomedicine, Autonomous University of Barcelona, Spain); and the Nek9/6/7 signalling module in *Xenopus* mitotic egg extract (in collaboration with Isabelle Vernos, Center for Genomic Regulation, Spain).

Scientific output

Publications

Casals-Casas C, Álvarez E, Serra M, de la Torre C, Farrera C, Sánchez-Tilló E, Caelles C, Lloberas J and Celada A. CREB and AP-1 activation regulates MKP-1 induction by LPS or M-CSF and their kinetics correlate with macrophage activation versus proliferation. *Eur J Immunol*, **39**(7), 1902-13 (2009)

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Research networks and grants

Ajuts a grups de recerca reconeguts
Generalitat de Catalunya, 2009-SGR-163 (2009-2013)
Principal investigator/Researcher: Carme Caelles, Joan Roig

El módulo de señalización Nercc1/Nek6/7; regulación y funciones
Spanish Ministry of Science and Innovation, BFU2008-03441/BMC (2009-2011)
Researcher: 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 (2007-2009)
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
'Marcelino Botin' Foundation, IO-FMBotin (2008-2010)
Principal investigator: Carme Caelles

Collaborations

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

LXR-MAPK pathways cross talk
Annabel F Valledor, University of Barcelona (Barcelona, Spain)

Regulation of MAPK pathways in macrophage
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, Autonomous University of Barcelona (Barcelona, Spain)

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

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