

Signalling and Cell Cycle Group

Summary

We are interested in understanding basic mechanisms of cell proliferation and differentiation, especially in relation to how external signals are interpreted by cells to elaborate the appropriate responses. Our studies focus on two main areas: (1) Mechanisms of signal transduction by the stress-activated MAP kinases p38 α and p38 β and their role in carcinogenesis and (2) Regulation and role of the RINGO/Speedy proteins, a new family of CDK activators.

Strategic Goals

- Understand signal integration by p38 MAP kinases and their role in carcinogenesis
- Characterise the regulation and function of the CDK activators RINGO/Speedy
- Investigate how signalling pathways control the cell cycle machinery
- Generate mouse models to study *in vivo* roles of p38 MAP kinases and RINGO proteins

Ángel R. Nebreda *Group Leader*

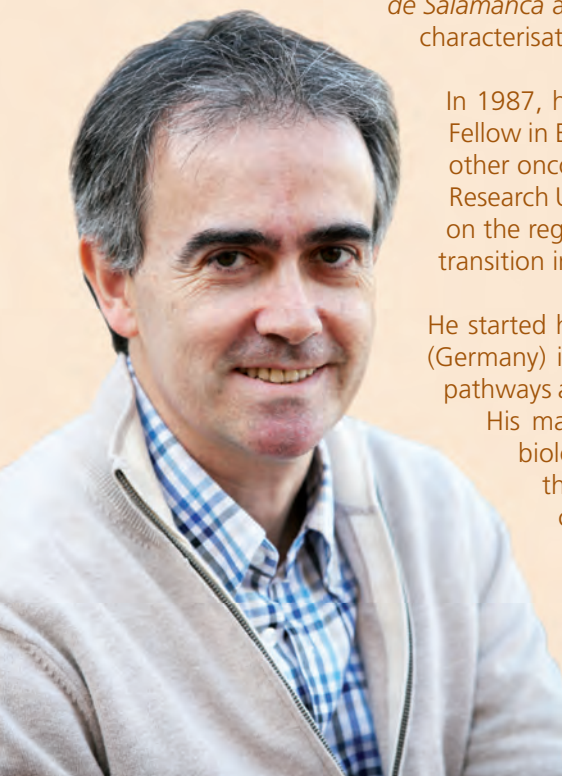
Ángel R. Nebreda was born in 1961 in Benavente, Spain. He studied Biology at the *Universidad de Salamanca* and received his PhD in 1986 from the same university working on the cloning and characterisation of yeast β -glucanase genes.

In 1987, he moved to the National Institutes of Health, Bethesda (USA), as a Post-doctoral Fellow in E. Santos' Laboratory, where he studied signal transduction mechanisms by Ras and other oncogenes. He then returned to Europe in 1992 to join T. Hunt's Group at the Cancer Research UK Clare Hall Laboratories, South Mimms (UK). During this period, his work focused on the regulation of cell cycle machinery by signalling pathways, especially during the G2/M transition in *Xenopus* oocytes.

He started his own group at the European Molecular Biology Laboratory (EMBL), Heidelberg (Germany) in 1995, where he and his team worked on signal transduction by MAP kinase pathways and the mechanisms that control CDK activation during oocyte meiotic maturation.

His main contributions during this period relate to the mechanisms of activation and biological functions of p38 MAP kinases, and the signalling pathways that orchestrate the meiotic cell cycle, using the *Xenopus* system. His Group also cloned and characterised a new family of proteins named RINGO/Speedy that can directly activate Cdk1 and Cdk2.

Nebreda joined the CNIO as a Group Leader of the Signalling and Cell Cycle Group in 2004. He was elected EMBO Member in 2003 and has been an Editor of the journal *FEBS Letters* since 2004.





Staff scientists: Ana Cuadrado, Iván del Barco, Patricia González, Ignacio Torrecilla (June - October), Marcial Vilar (until October). **Post-doctoral fellow:** Bastien Mathieu Lemaire. **Graduate students:** Jalaj Gupta, Vanesa Lafarga (until April), Carmen L. Pereira, Edgar J. Ruiz, Aneta M. Swat. **Technicians:** Soraya Ardila, Juan M. Coya (since February), Laura E. Doglio, Esther Seco.

Highlights

Signal integration by p38 MAP kinases

The cellular response to DNA damage involves the activation of checkpoint pathways that impose a delay in cell-cycle progression and control DNA repair and replication. Activation of p38 α MAPK is known to play an important role in the G2/M cell cycle arrest induced by DNA damage, however,

little is known about the potential role of this signalling pathway in the G1/S transition.

Up-regulation of the cyclin-dependent kinase inhibitor p21^{Cip1} is thought to contribute in a major way to the G1/S cell cycle arrest induced by γ -radiation.

We have found that inhibition of p38 α MAPK impairs p21^{Cip1} accumulation

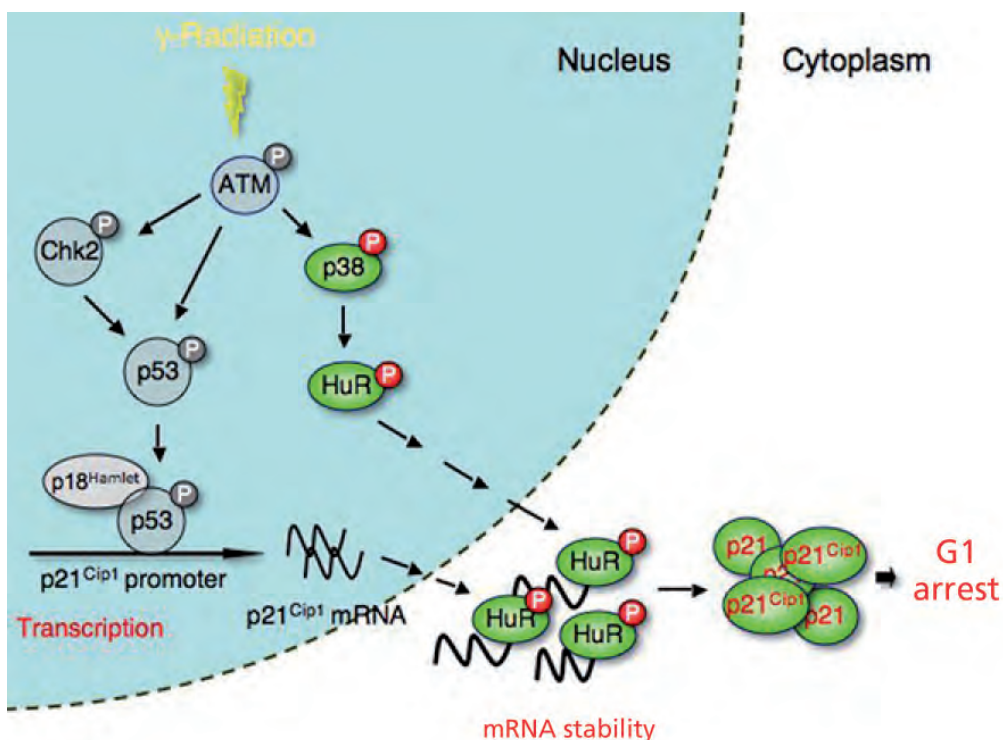


Figure 1: A model for the implication of p38 MAPK in p21^{Cip1} up-regulation induced by γ -radiation that complements p53-dependent transcriptional activation. p38 α phosphorylates HuR on Thr118, which in turn results in enhanced binding of HuR to p21^{Cip1} mRNA, mRNA stabilisation, and the accumulation of p21^{Cip1} protein, leading to G1 cell cycle arrest.

and, as a result, the ability of cells to arrest in G1 in response to γ -radiation. We investigated the underlying molecular mechanism and also found that p38 α induces p21Cip1 mRNA stability, without affecting its transcription or stabilisation of the protein. In particular, p38 α phosphorylates the mRNA binding protein HuR on Thr118, which results in cytoplasmic accumulation of HuR and an enhanced binding to p21Cip1 mRNA.

Our findings help explain the emerging role of p38 α MAPK in the cellular response to DNA damage and reveal p53-independent networks that cooperate in modulating p21Cip1 levels at the G1/S checkpoint (Figure 1).

The majority of tumour suppressor mechanisms coordinated by p38 α MAPK occur at the post-translational level, despite the important role of p38 α in the regulation of gene transcription and the profound changes in gene expression that are associated with cancer progression. We have analysed whole genome expression profiles of H-Ras^{G12V}-transformed wild-type and p38 α -deficient cells and have identified more than 200 genes that are regulated by p38 α throughout malignant transformation. Expression and functional analysis have validated several of these genes as possible transcriptional mediators of the tumour suppressor effect of p38 α against oncogenic Ras-induced malignant transformation.

Interestingly, about 10% of the genes that are downregulated by p38 α are associated with EGF receptor (EGFR) function, suggesting that transcriptional inhibition of EGFR signalling is an important function of p38 α to suppress the initiation of tumourigenesis.

We are using genetically modified mouse models to investigate the *in vivo* functions of p38 α and its closely related family member p38 β , as well as the contribution of this signalling pathway to inflammation-associated tumour development. Our initial results have identified non-redundant roles for both p38 MAPKs in embryogenesis.

Cell cycle regulation by RINGO proteins

Cell cycle progression is regulated by cyclin-dependent kinases (CDKs), whose activation is usually associated with the binding of regulatory subunits called cyclins. CDK1 and CDK2 can also be activated by a family of proteins named RINGO or Speedy, which have no amino acid sequence homology with cyclins. Five different mammalian RINGO proteins have been identified and all of them share a conserved central core of about 75 residues (Figure 2).

We have found that mammalian RINGO A is a highly unstable protein whose expression and phosphorylation are periodically regulated during the cell cycle. Expression of a RINGO A mutant with enhanced stability results in the

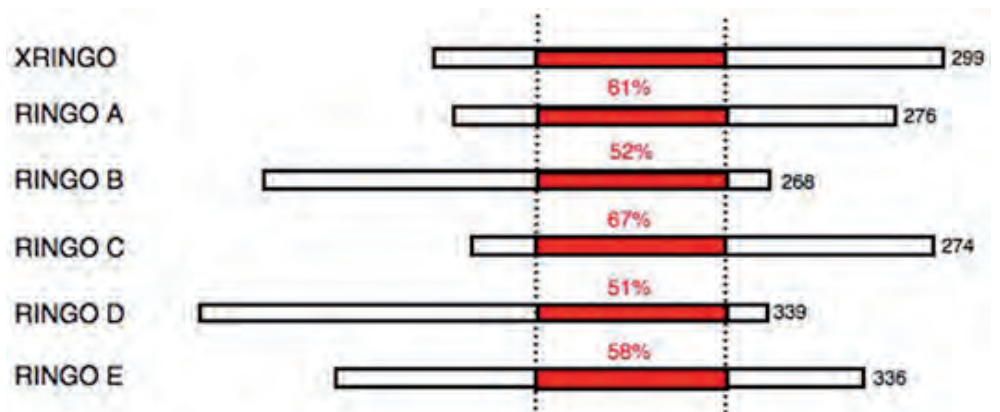


Figure 2: A family of mammalian RINGO/Speedy proteins. The percentage of identity in the most conserved region, referred to as the core (in red), is indicated in comparison with the prototypic *Xenopus* protein.

accumulation of high levels of RINGO A at late stages of mitosis, which interfere with cytokinesis and chromosome decondensation (Figure 3). These data indicate that tight regulation of RINGO A is important for the somatic cell cycle.

We have also investigated the role of mammalian RINGO C in cell cycle progression and found that siRNA-mediated knockdown of RINGO C prevents cellular arrest at prometaphase. Time-lapse microscopy experiments suggest the implication of RINGO C in the spindle assembly checkpoint (SAC). Consistent with this idea, RINGO C-depleted cells show impaired recruitment of the mitotic checkpoint components Mad2, Bub1 and BubR1. Concurring with checkpoint override, cells also display chromosome misalignments as well increased number of micronuclei and binucleated structures.

Intriguingly, RINGO C can associate with the mitotic kinase Aurora B, and RINGO C downregulation produces mislocalisation of the active form of Aurora B in asynchronous cell cultures in prometaphase. These results indicate a role for RINGO C in the mitotic checkpoint, which may be mediated via Aurora B regulation.

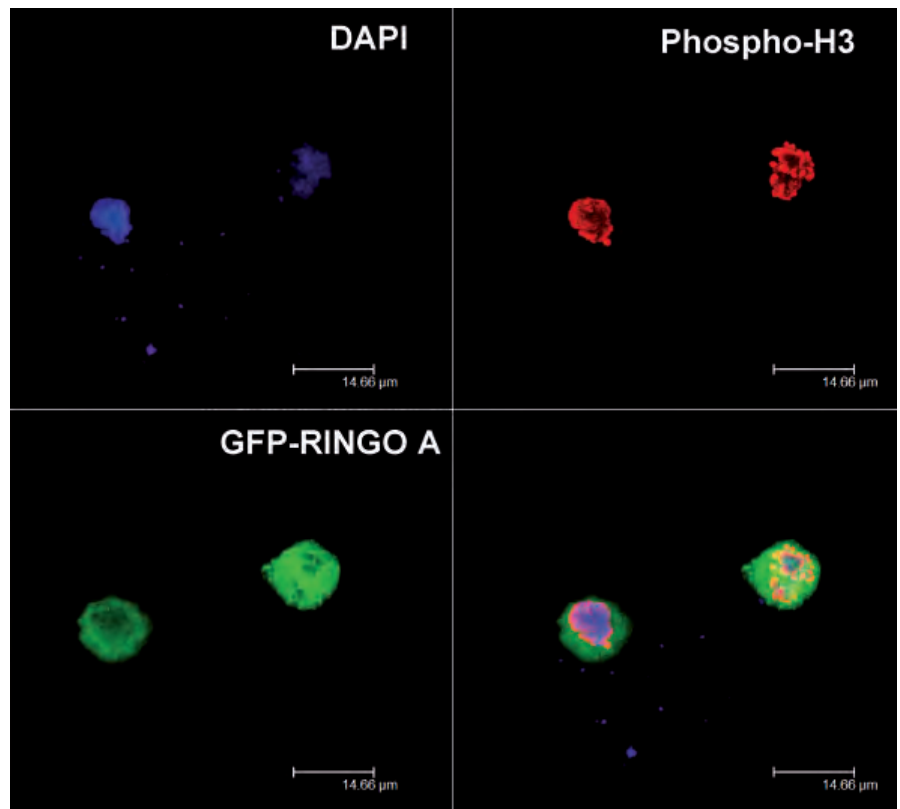


Figure 3: GFP-RINGO A expression impairs cell cycle progression.

Publications

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