

Signalling and Cell Cycle Group

26

Scientific Report 2010 **cnio**



Ángel R. Nebreda

Group Leader (until June)

Ángel R. Nebreda was born in 1961 in Benavente, Spain. He studied Biology at the *Universidad de Salamanca* (Spain) 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 the cell cycle machinery by signalling pathways, especially during the G2/M transition in *Xenopus* oocytes.

In 1995, he started his own group at the European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany) 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 function 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.

Ángel 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 *FEBS Letters* since 2004.

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



Highlights

The chromatin-remodelling complex SRCAP (SNF2-related CBP Activator Protein) regulates chromatin structure in yeast by modulating the exchange of histone H2A for the H2A.Z variant. We have investigated the contribution of H2A.Z-mediated chromatin remodelling to mammalian cell differentiation reprogramming. We found that the SRCAP subunit named ZNHIT1 or p18^{Hamlet}, which is a substrate of p38 MAPK, is recruited to the myogenin promoter at the onset of muscle differentiation, in a p38 MAPK-dependent manner. We also found that p18^{Hamlet} is required for H2A.Z accumulation into this genomic region and for subsequent muscle gene transcriptional activation. Accordingly, downregulation of several subunits or the SRCAP complex impairs muscle gene expression. Thus, SRCAP/H2A.Z-mediated chromatin remodelling is a key early event in muscle differentiation-specific gene expression. Our results also identify a mechanism by which p38 MAPK-mediated signals are converted into chromatin structural changes, thereby facilitating transcriptional activation during mammalian cell differentiation.

The CDC25B phosphatase regulates the activation of CDK1-Cyclin B at the onset of mitosis, being a key target of the checkpoint pathways activated by cellular stress and DNA damage. Previous work has reported that checkpoint activation induces the sequestration of CDC25B in the cytoplasm. We have found that in response to UV irradiation, the levels of CDC25B protein can be downregulated independently of classical checkpoints pathways such as p53, ATM/ATR and p38 MAPK. We also found that translational repression mediated by eIF2 α phosphorylation regulates CDC25B expression levels. Collectively, our results illustrate a new mechanism of CDC25B regulation in response to stress.

RINGO/Speedy proteins are direct activators of Cdk1 and Cdk2 that have no sequence homology to cyclins. We have characterised the role in cell-cycle progression of a new human member of this protein family referred to as RINGO C. We show that siRNA-mediated knockdown of RINGO C results in premature mitotic exit with misaligned chromosomes, even in the presence of microtubule poisons. Time-lapse-microscopy experiments suggest that RINGO C is involved in the spindle-assembly checkpoint (SAC). Consistent with this idea, RINGO-C-depleted cells show impaired recruitment of the SAC components Mad2, Bub1 and BubR1. As the checkpoint is overridden, cells display defective chromosome segregation, which leads to an increased number of micronuclei and binucleated structures. Intriguingly, we found that RINGO C can associate with the mitotic kinase Aurora B, and downregulation of RINGO C produces mislocalisation of the active form

of Aurora B in prometaphase. Our results indicate a role for RINGO C in the mitotic checkpoint, which might be mediated by defective recruitment of SAC components and deregulation of the activity of Aurora kinase B.

Activation of CDK1 is essential for M-phase entry both in mitosis and meiosis. G2-arrested oocytes contain a pool of CDK1/cyclin B complexes that are maintained inactive due to the phosphorylation of CDK1 on Thr14 and Tyr15 by the Wee1 family protein kinase Myt1, whose inhibition suffices to induce meiosis I entry. CDK1/XRINGO and p90Rsk can both phosphorylate and downregulate Myt1 activity *in vitro*. We have identified five p90Rsk phosphorylation sites on Myt1, which are different from the CDK1/XRINGO sites, and elucidated how both kinases synergise during oocyte maturation to inhibit Myt1, ensuring meiotic progression. We found that phosphorylation of Myt1 by CDK1/XRINGO early during oocyte maturation, not only downregulates Myt1 kinase activity but also facilitates the recruitment of p90Rsk and further phosphorylation of Myt1. Mutation of the five p90Rsk residues to alanine impairs Myt1 hyperphosphorylation during oocyte maturation and makes Myt1 resistant to the inhibition by p90Rsk. Importantly, Myt1 phosphorylated by p90Rsk does not interact with CDK1/cyclin B, ensuring that the inhibitory phosphorylations of CDK1 cannot take place after meiosis I entry and contributing to the all-or-none meiotic response.

Publications

Santamaria PG, Nebreda AR (2010). Deconstructing ERK signaling in tumorigenesis. *Mol Cell* 38, 3-5.

Ruiz EJ, Vilar M, Nebreda AR (2010). A two-step inactivation mechanism of Myt1 ensures CDK1/cyclin B activation and meiosis I entry. *Curr Biol* 20, 717-723.

Cuadrado A, Corrado N, Perdiguero E, Lafarga V, Muñoz-Canoves P, Nebreda AR (2010). Essential role of p18Hamlet/SRCAP-mediated histone H2A.Z chromatin incorporation in muscle differentiation. *EMBO J* 29, 2014-2025.

Mourón S, de Cárcer G, Seco E, Fernández-Miranda G, Malumbres M, Nebreda AR (2010). RINGO C is required to sustain the spindle-assembly checkpoint. *J Cell Sci* 123, 2586-2595.

Cuadrado A, Nebreda AR (2010). Mechanisms and functions of p38 MAPK signalling. *Biochem J* 429, 2014-2025.

Ferreiro I, Joaquin M, Islam A, Gomez-Lopez G, Barragan M, Lombardía L, Domínguez O, Pisano DG, Lopez-Bigas N, Nebreda AR, Posas F (2010). Whole genome analysis of p38 SAPK-mediated gene expression upon stress. *BMC Genomics* 11, 144.

Lemaire M, Ducommun B, Nebreda AR (2010). UV-induced downregulation of the CDC25B protein in human cells. *FEBS Lett* 584, 1199-1204.