

Cell Division and Cancer Group

Summary

Mitosis is a critical process for the proper segregation of chromosomes and genomic stability. In our laboratory we are interested in understanding the vital roles of several mitotic regulators – not only in the cell cycle but also in physiological processes in different cell types. We are characterising several mouse models with genetic alterations in various mitotic regulators and microRNAs to gain insight into their *in vivo* relevance and potential use in cancer therapy.

Strategic Goals

- Understand the basic cell cycle control mechanisms in mammalian cells
- Develop animal models with genetic alterations in cell cycle regulators to study their physiological relevance as well as their putative use in cancer therapy
- Characterise the physiological role of microRNAs in cell biology and tumour development
- Understand the functional relevance of cancer stem cells and their proliferative control

Marcos Malumbres *Group Leader*



Marcos Malumbres was born in Tudela, Spain in 1965. He studied Biology at the *Universidad de Navarra* and then moved to the *Universidad de León* to characterise the genes and molecular pathways leading to amino acid biosynthesis in bacteria. He obtained his PhD in Molecular Biology at the *Universidad de León* in 1993 and moved to the New York University Medical Centre, New York, USA (1994 – 1998) for his postdoctoral training in collaboration with A. Pellicer. During this period, he focused on the effect of Ras activation in cell cycle control and cell proliferation.

Marcos returned to Spain at the end of 1998 to join M. Barbacid's lab in the then newly created CNIO. For five years his research focused on the generation of mouse models to analyse the *in vivo* role of cyclin-dependent kinases and their inhibitors in cell cycle progression and tumour development. In 2003 he obtained a Staff Scientist position at the *Consejo Superior de Investigaciones Científicas* (CSIC) and in June 2004 he decided to stay at the CNIO to lead the Cell Division and Cancer Group.

Marcos Malumbres has authored more than 70 papers including relevant contributions to understanding the *in vivo* function of key cell cycle regulators and their implication in cancer therapy. He has also contributed to deciphering the role of microRNAs in tumour development and their putative therapeutic uses in cancer. Malumbres received the Beckman-Coulter Award for Young Scientists in 2005.



Staff scientists: Guillermo de Cárcer, Ignacio Pérez. **Post-doctoral fellows:** María J. Bueno, Massimo Chiesa, Pei Pei Gan (since September), Ruth Sánchez. **Graduate students:** Cristina Aguirre, Manuel Eguren, Gonzalo Fernández-Miranda, María Guillamot, Eusebio Manchado, Víctor Quereda, Esther Rodríguez (until May), Marianna Trakala (since October), Paulina A. Wachowicz. **Technicians:** Beatriz Escobar, Marta Gómez de Cedrón, Susana Temiño.

Highlights

Modelling the function of mitotic kinases and phosphatases

We have focused on two major kinases – Aurora and Polo – that regulate progression throughout mitosis. We have identified a new regulatory motif in the Aurora kinases that modulates their function during mitosis. All three Aurora kinases, A, B and C, contain a SUMOylation motif highly conserved through evolution. We have observed that Aurora A and Aurora B are tagged by different SUMO peptides and that the interference with this post-translational modification results in defective Aurora function and genomic instability.

Genetic ablation of Aurora A or Aurora B results in embryonic lethality at different stages between embryonic days (E) 3.4 and 7.5. Aurora C may complement for loss OF Aurora B during the first embryonic cell divisions. We are currently analysing the essential roles of these proteins in the cell cycle using conditional knockout cells. Our data support a critical function for Aurora B in microtubular attachment to kinetochores and suggest new non-mitotic functions that are currently being explored in our laboratory.

Plk1 is also required for embryonic development and its genetic ablation results in multiple mitotic aberrations at

the morula stage. Partial Plk1 deficiency results in various *in vivo* phenotypes; we are currently analysing the relevance of this protein in both the cell cycle and cell cycle-independent processes. We have identified a fifth member of this family as well, Plk5, which also contains a Polo-box domain. This protein is mostly expressed in brain cells and its overexpression interferes with the entry into mitosis. Interestingly, Plk5 seems to be necessary for neuronal function and is found deregulated in brain tumours.

In addition to these kinases we are interested in the Cdc14 phosphatases and how they control the exit from mitosis and the transition from mitosis to the following cell cycle. We have recently generated conditional knock out alleles for each of these phosphatases and have uncovered an oncogenic function of Cdc14B that is not shared by Cdc14A.

The anaphase-promoting complex and protein degradation

The Anaphase Promoting Complex (APC/C, or cyclosome) is an E3-ubiquitin ligase whose activity depends on two co-activators: Cdc20 and Cdh1/Fzr1 (Figure 1). The APC/C protein complex is responsible for the tagging of many proteins during mitosis (such as cyclins, Aurora, Polo, securin, etc.) with ubiquitin molecules. Genetic ablation of Cdh1 results in embryonic lethality at mid-

gestation. This embryonic lethality is due to placental defects accompanied by lack of endoreduplication of trophoblast giant cells.

We are currently analysing the effect of acute ablation of Cdh1 in adult mice as well as its tissue-specific deletion in proliferative and non-proliferative tissues. On the other hand, ablation of Cdc20 results in embryonic lethality in the 2-cell stage of embryonic development. Cdc20-null cells arrest in metaphase in accordance with the function of this APC/C cofactor in the metaphase-to-anaphase transition. Cdc20 also appears to play an essential role in adult somatic cells since its acute genetic ablation results in mitotic arrest and proliferative defects *in vivo*. Our current efforts focus on the dissection of Cdc20-dependent functions during mitotic progression. Additionally, we are interested in validating the therapeutic potential of Cdc20 *in vivo* and the cellular consequences of its genetic ablation in different tissues.

Control of cell proliferation by microRNAs

We are studying the relevance of microRNAs (miRNAs) in the cell cycle at different levels. We have first analysed the expression of miRNAs in the early phases of the cell cycle and have identified a pattern of E2F-dependent expression of miRNAs in primary cells (Figure 2). Several of these miRNAs modulate major proliferation pathways including major cell cycle regulators such as cyclins and cyclin-dependent kinases.

We have also explored the downregulation of multiple miRNAs in specific leukemias induced in mice. Downregulation of miRNAs by genetic deletion or hypermethylation-derived transcriptional repression may participate in the upregulation of critical oncogenes such as *Myc* or *Abl*. We are currently validating other new miRNA targets in these tumour cells. Re-expression of some of these miRNAs results in decreased oncogenic levels and a dramatic arrest

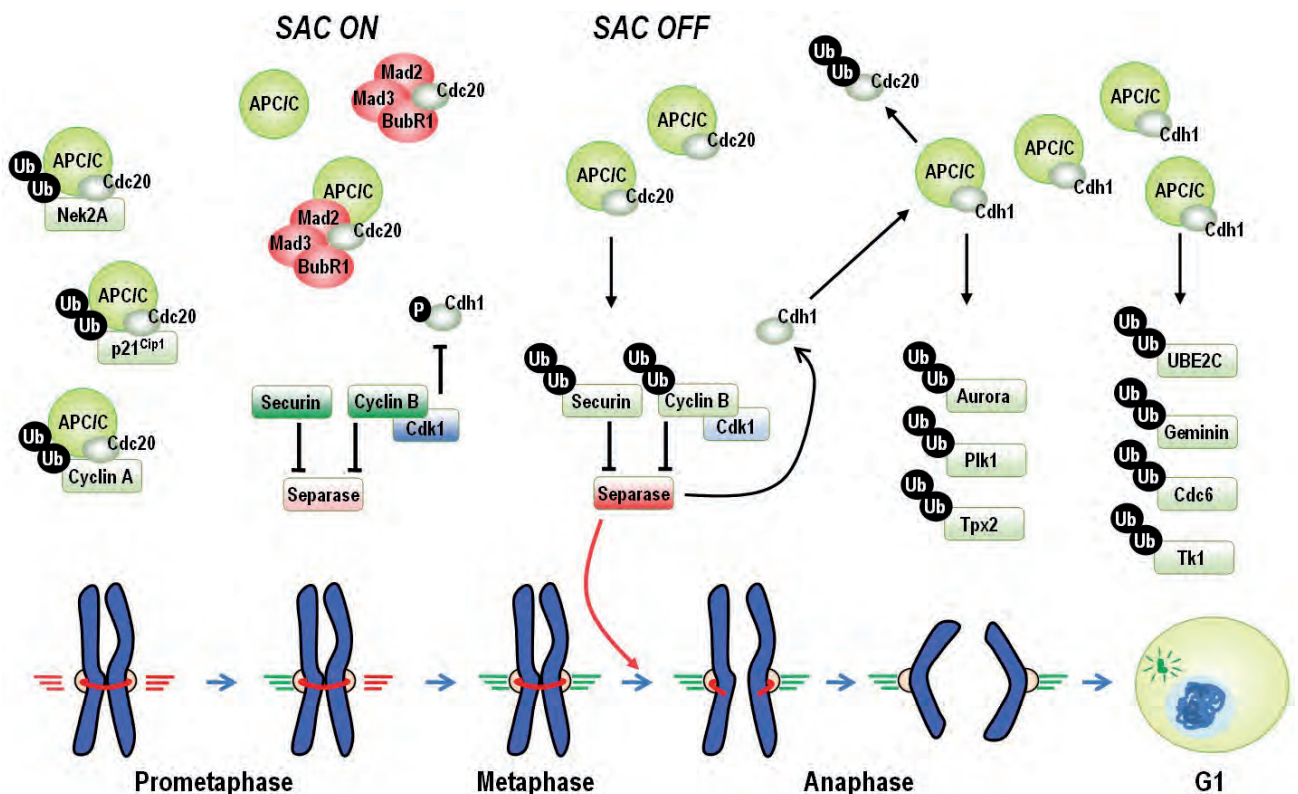


Figure 1: APC/C functions in the cell cycle. The APC/C complex along with its cofactors Cdc20 or Cdh1, target multiple cell cycle regulators for degradation at different stages during mitosis or exit from mitosis.

in proliferation, suggesting a tumour-suppressor role for these microRNAs and the putative therapeutic use of microRNA re-expression in tumours. We are currently generating some miRNA loss- and gain-of-function mouse models to address their critical roles and potential therapeutic values *in vivo*.

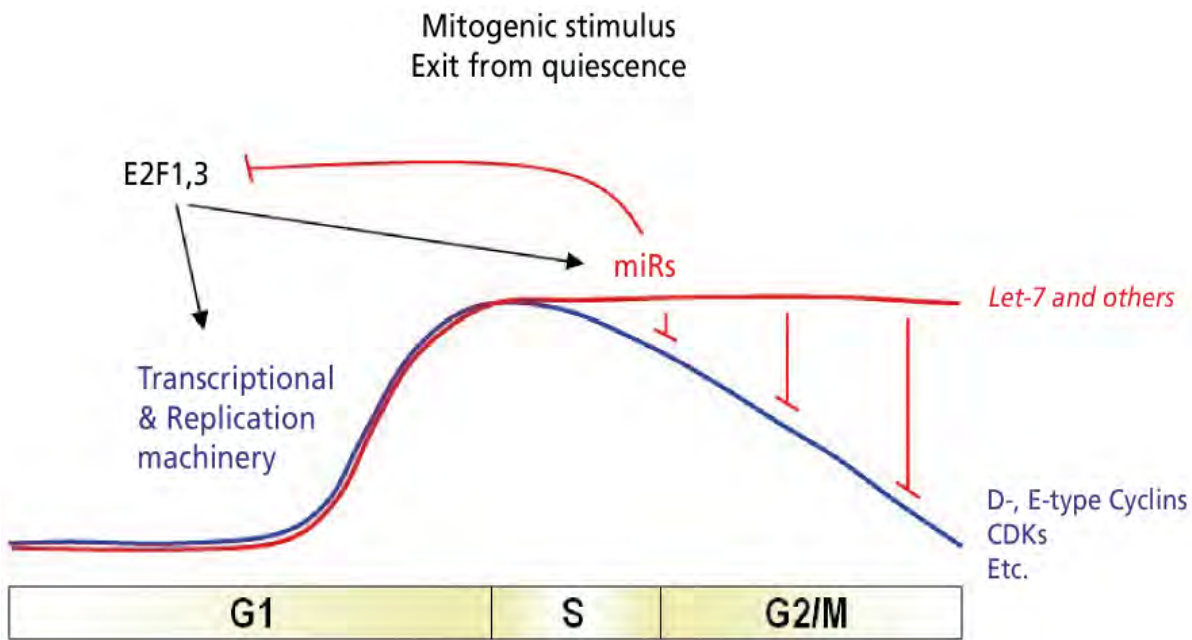


Figure 2: A model for the role of miRNAs induced by E2F transcription factors during the cell cycle. Whereas proteins induced by E2F trigger DNA replication, E2F-induced miRNAs maintain low levels of cell cycle regulators and E2F targets (e.g. Cyclin E) to limit the effect of these E2F factors.

Publications

Malumbres M, Barbacid M (2009). Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer* 9, 153-156.

Malumbres M, Harlow E, Hunt T, Hunter T, Lahti JM, Manning G, Morgan DO, Tsai LH, Wolgemuth DJ (2009). Cyclin-dependent kinases: a family portrait. *Nat Cell Biol* 11, 1275-1276.

Muñoz P, Blanco R, de Carcer G, Schoeftner S, Benetti R, Flores JM, Malumbres M, Blasco MA (2009). TRF1 controls telomere length and mitotic fidelity in epithelial homeostasis. *Mol Cell Biol* 29, 1608-1620.

Quereda V, Malumbres M (2009). Cell cycle control of pituitary development and disease. *J Mol Endocrinol* 42, 75-86.

Garcia-Lavandeira M, Quereda V, Flores I, Saez C, Diaz-Rodriguez E, Japon MA, Ryan AK, Blasco MA, Dieguez C, Malumbres M, Alvarez CV (2009). A GRFa2/Prop1/stem (GPS) cell niche in the pituitary. *PLoS ONE* 4, e4815.