

Genomic Instability Group

32

Scientific Report 2010 *crío*



Óscar Fernández-Capetillo

Group Leader

Óscar Fernández-Capetillo was born in Bilbao (Spain) in 1974. He studied biochemistry and subsequently obtained his PhD in 2001 from the *Universidad del País Vasco* under the supervision of A. Zubiaga. His PhD research focused on the role of E2F transcription factors on cell-cycle control and the development of the immune system. He then joined the laboratory of A. Nussenzweig at the National Cancer Institute in Bethesda (USA) for his postdoctoral training (2001-2004), where he studied how cells respond to DNA damage. His main contributions from this period focused on elucidating the role of the histone variant H2AX on genome maintenance.

In 2004 Óscar joined the CNIO to lead the Genomic Instability Group where he developed an interest in understanding how cells respond to replicative stress and the impact of this type of DNA damage on cancer and ageing. During this period his studies revealed a synthetic lethal interaction of ATR hypomorphism with p53 loss and that an intrauterine exposure to DNA damage can accelerate ageing later in life.

Óscar Fernández-Capetillo's research has been recognised through several national and international awards/honours including the Eppendorf-*Nature* Award for Young Investigators (2009), the election to EMBO Young Investigator (2008), an ERC Starting Grant (2007), the membership in the EPIGENOME Network of Excellence (2006) and the Swiss Bridge Award (2005).

Summary

DNA damage is a common initiator of cancer and ageing. However, the actual nature of the damage which arises endogenously is still poorly understood. Our research aims at understanding how cells respond to "replicative stress" (RS), a specific type of DNA damage that unavoidably arises every time a cell replicates its DNA, and which is mainly prevented by the ATR and Chk1 kinases.

We have now generated systems in which we can activate these kinases at will and mutant mice with very low levels of ATR. Both systems have allowed us to investigate the physiological role of an appropriate response to RS. In addition, we are currently investigating the actual processes that lead to the generation of DNA damage during replication. We now want to exploit this knowledge to explore strategies to fight cancer and extend mammalian lifespan.

Strategic Goals

- Investigate potential synthetic lethal interactions between cancer-associated mutations and ATR/Chk1 kinases
- Develop chemical inhibitors of the ATR kinase that can be explored for cancer chemotherapy
- Explore whether keeping the genome integer for longer is sufficient to extend lifespan and/or protect from carcinogenesis
- Investigate the role of SUMOylation-based genome maintenance pathways in cancer prevention



Staff scientists: M. Alexandra Bras and Matilde Murga. **Post-doctoral fellows:** Ariana Jacome and Andrés J. López. **Graduate students:** Paula Gutiérrez, Bárbara Martínez (until June), Ángela Monasor, María Nieto (since September), Juan L. Rodríguez, Enrico Tenaglia and Luis I. Toledo. **Technicians:** Sara Rodrigo (since October), Rebeca Soria and Rafal Zur (until August).

Highlights

Understanding the role of ATR in the response to oncogenes

One of the most recent developments discussed in cancer research is the finding that oncogenic activation leads to the generation of DNA damage. We now have evidence to show that oncogene-induced DNA damage is linked to replication and is thus prevented by ATR and Chk1 kinases. We have now explored whether

we can exploit the damage generated by oncogenes as a property to target cancer cells.

With the use of the ATR-Seckel mouse strain developed in our lab, we are now evaluating how the presence of an oncogene (i.e. c-Myc) is tolerated by ATR hypomorphic cells. Our preliminary data suggests that targeting the cellular response to replicative stress (RS) might

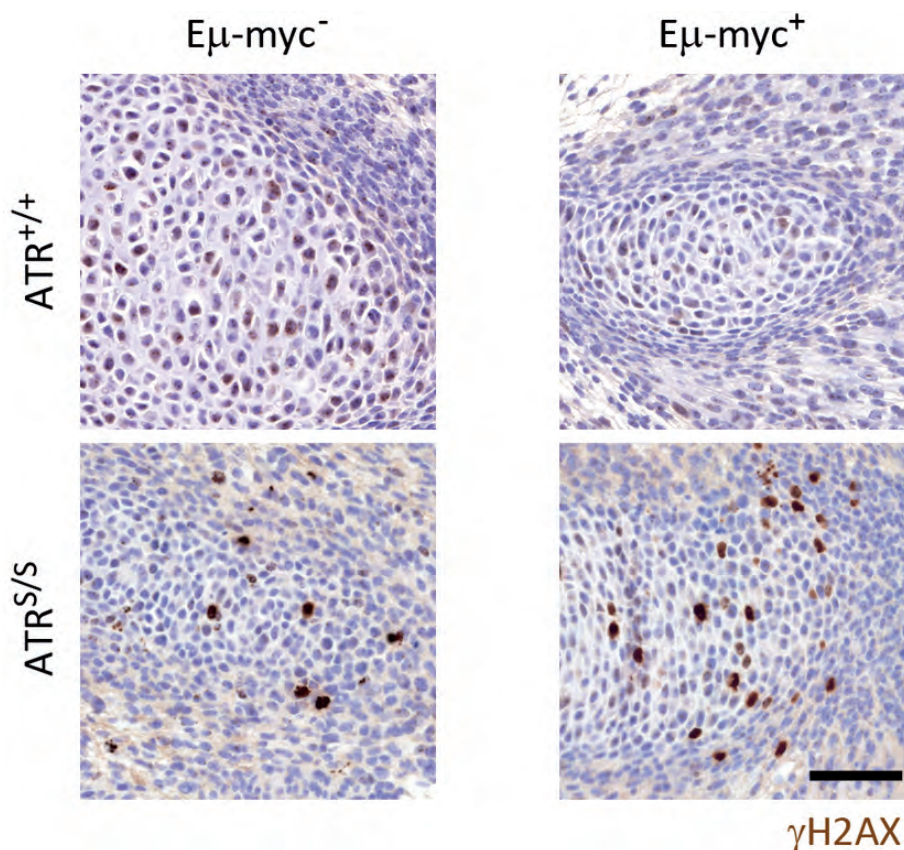


Figure 1: Myc overexpression increases RS on ATR mutant mice. Immunohistochemistry (IHC) of γ H2AX on the developing bone of 13.5 dpc embryos of the indicated genotypes. Scale bar indicates 50 μ m. Pan-nuclear γ H2AX staining is indicative of RS (in contrast to the foci-like pattern associated with DNA breaks).

indeed be particularly toxic for cancer cells that present high levels of RS (Figure 1). Noteworthy, part of this work has led us to discover that the DNA damage generated by an oncogene can also accelerate ageing in certain conditions. We are now extending this research to several other oncogenes and/or tumour-types to evaluate the generality of this finding.

Development of chemical inhibitors of ATR

DNA damage-less replication does not exist, and a certain degree of RS is generated in every S phase. Hence, cells cannot live without the enzymes that protect our genome from RS. Due to the essential nature of these proteins, genetic studies are limited by cell-lethality. In contrast, inhibiting their activity with chemical compounds allows for the study of their function. Unfortunately no potent inhibitors of ATR exist. One of the limitations for the discovery of ATR inhibitors is that the activity of the kinase is restricted to S/G2. This hinders cell-based screenings due to the large number of false positives that would derive from an

indirect effect of the tested compound on the cell cycle. To overcome this limitation we previously developed a cellular system in which ATR activity can be unleashed at will throughout the cell cycle and in the absence of any actual DNA damage. After adapting this system for its use in a High-Throughput Imaging pipeline, and with the help of the CNIO's Experimental Therapeutics Programme, we have now identified several compounds that can inhibit ATR in the nanomolar range (Figure 2). We are now at the early stages of characterising these inhibitors and their potential applications.

SUMOylation as a genome caretaker mechanism

Most studies on DNA damage response have focused on analysing the phosphorylation-based signalling cascades that are triggered by genomic lesions. However, it is becoming increasingly clear that signalling pathways based on other post-translational modifications such as Ubiquitinylation or SUMOylation might play an equally important role in this response. Part of our research has been dedicated to

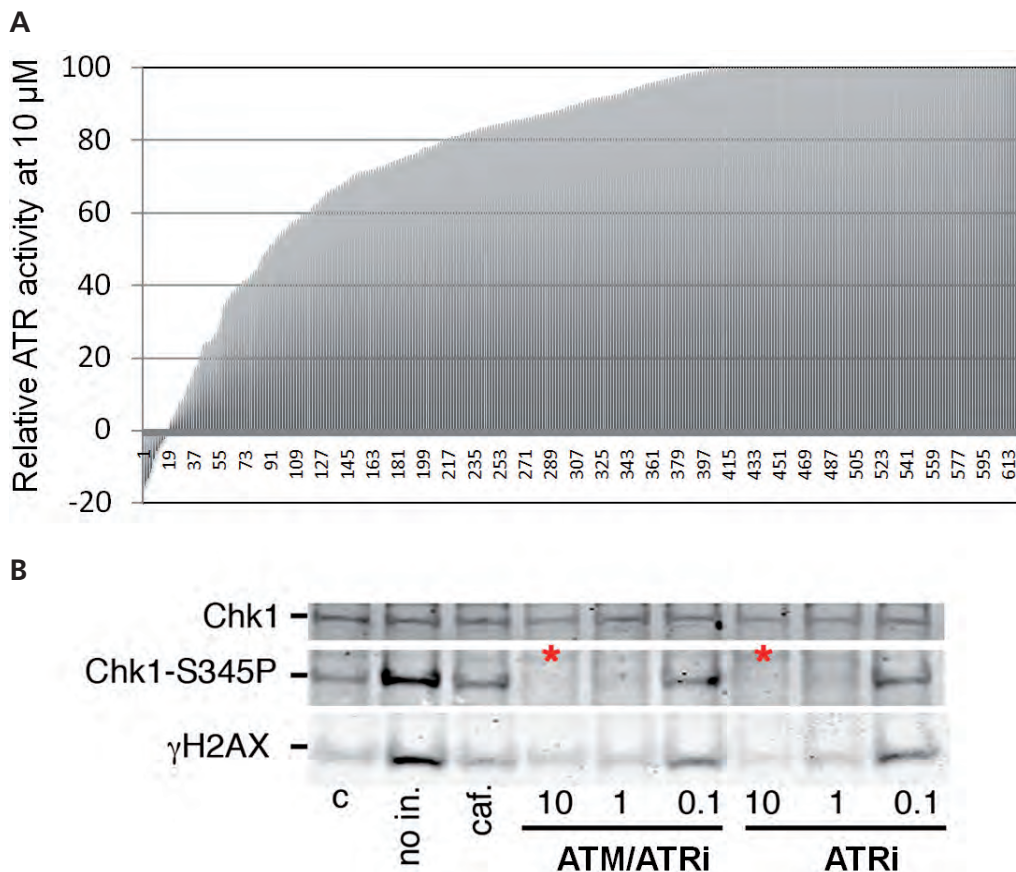
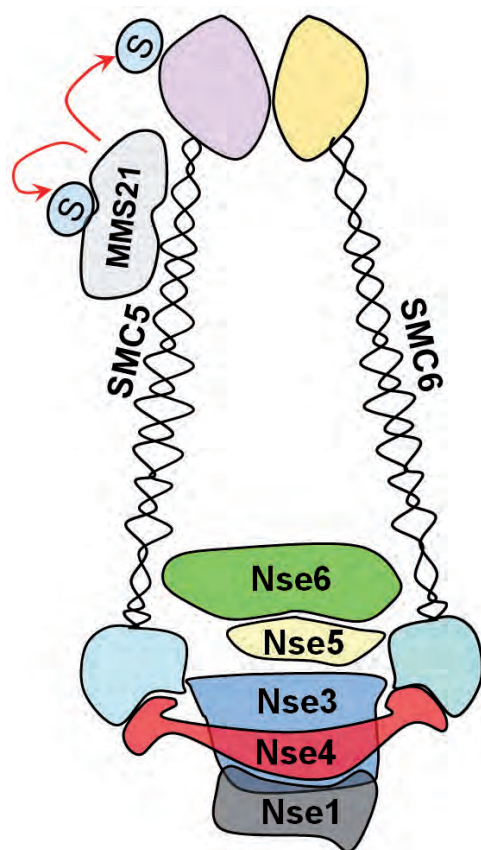


Figure 2: Identification of chemical inhibitors of ATR. (A) The figure illustrates the results of the screening for 650 candidate drugs tested (in terms of percentage of ATR inhibition vs. control). (B) 2 of these inhibitors were able to inhibit ATR mediated phosphorylation events (i.e. Chk1 and H2AX) in the nanomolar range. [Conditions: Untreated, +4-OHT, and plus decreasing concentrations of the 2 cmpds - μ M-].

studying a SUMO E3 ligase named Mms21 which was shown to be important for genomic maintenance in yeast. The protein is an essential component of the SMC5/6 complex, which is analogous to cohesins



and condensins, but for which little is known about its true function (Figure 3).

We have now generated a battery of reagents including several Mms21 mouse models and are starting to understand what its function might be in a mammalian organism. All our current experimental data supports an important role of this complex in dealing with inter-sister chromatid junctions, which will lead to anaphase bridges and breakage in mitosis if not properly resolved. The analysis of this complex is still in its initial stages and the relevant targets remain unknown. Hence, how this activity is linked to Mms21-mediated SUMOylation has now become an important part of our research. Notably our current data shows that Mms21 mutant mice are cancer-prone, providing important information on the relevance of this pathway for cancer protection.

Figure 3: Mms21 as part of the SMC5/6 complex. The figure represents the known structure of the SMC5/6 complex, which resembles condensins and cohesins. Six additional Non-SMC elements (Nse) form a complex in yeast, although 2 of these proteins are not identified in mammals (Nse5 and Nse6). Nse2/Mms21 is an essential E3 SUMO ligase that is tightly bound to the coiled-coil region of SMC5. Besides itself and the members of the complex, the SUMOylation targets of Mms21 remain largely unknown.

Publications

Bunting SF, Callén E, Wong N, Chen HT, Polato F, Gunn A, Bothmer A, Feldhahn N, Fernandez-Capetillo O, Cao L, Xu X, Deng CX, Finkel T, Nussenzweig M, Stark JM, Nussenzweig A (2010). 53BP1 inhibits homologous recombination in *Brca1*-deficient cells by blocking resection of DNA breaks. *Cell* 141, 243-254.

Campaner S, Doni M, Hydbring P, Verrecchia A, Bianchi L, Sardella D, Schleker T, Perna D, Tronnersjö S, Murga M, Fernandez-Capetillo O, Barbacid M, Larsson LG, Amati B (2010). Cdk2 suppresses cellular senescence induced by the c-myc oncogene. *Nat Cell Biol* 12, 54-59.

Santos MA, Huen MS, Jankovic M, Chen HT, López-Contreras AJ, Klein IA, Wong N, Barbancho JL, Fernandez-Capetillo O, Nussenzweig MC, Chen J, Nussenzweig A (2010). Class switching and meiotic defects in mice lacking the E3 ubiquitin ligase RNF8. *J Exp Med* 207, 973-981.

McNees CJ, Tejera AM, Martínez P, Murga M, Mulero F, Fernandez-Capetillo O, Blasco MA (2010). ATR suppresses telomere fragility and recombination but is dispensable for elongation of short telomeres by telomerase. *J Cell Biol* 188, 639-652.

Vogler C, Huber C, Waldmann T, Ettig R, Braun L, Izzo A, Daujat S, Chassignet I, Lopez-Contreras AJ, Fernandez-Capetillo O, Dunder M, Rippe K, Längst G, Schneider R (2010). Histone H2A C-terminus regulates chromatin dynamics, remodeling, and histone H1 binding. *PLoS Genet* 6, e1001234.

Kumar A, Fernandez-Capetillo O, Carrera AC (2010). Nuclear phosphoinositide 3-kinase beta controls double-strand break DNA repair. *Proc Natl Acad Sci USA* 107, 7491-7496.

Fernandez-Capetillo O. (2010). Intrauterine programming of ageing. *EMBO Rep* 11, 32-36.

López-Contreras AJ, Fernandez-Capetillo O (2010). The ATR barrier to replication-born DNA damage. *DNA Repair* 9, 1249-1255.

Herranz D, Muñoz-Martin M, Cañamero M, Mulero F, Martínez-Pastor B, Fernandez-Capetillo O, Serrano M (2010). Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. *Nat Communications* 1, 1-8.

Awards and Recognition

Beckman-Coulter Award, Spanish Society of Biochemistry and Molecular Biology (SEBBM)

IMPULSA Award, Prince of Girona Foundation, Spain