STRUCTURAL BASES OF GENOME INTEGRITY JUNIOR GROUP

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OVERVIEW

Safeguarding genome integrity is essential for correct cell functioning and to prevent cancer. Our Group is interested in understanding central cellular processes that affect the integrity of the genome, such as the metabolism of nucleotides, DNA recombination or the maintenance and recognition of chromatin architecture. These processes depend on the assembly of large and dynamic macromolecular complexes. We combine protein engineering, X-ray crystallography, nuclear magnetic resonance (NMR) and single-particle electron microscopy (EM), together with biochemical and functional studies, in order to decipher the structure of these protein-protein and protein-DNA complexes, as well as to understand their catalysis and regulatory mechanisms at the atomic level. This knowledge should guide the design of compounds to modulate protein activity and provide novel opportunities for fighting tumours.

"We obtained an atomic view of the ATC domain of human CAD – a metabolic gatekeeper controlling cell proliferation – bound to the anti-tumour drug PALA, and localised CAD within the cell."

Graduate Students
Francisco Del Caño, Alba Ruiz (until February)

Technicians
Araceli Grande (TS)*, Igor Yefimenko (TS)*

**Titulado Superior* (Advanced Degree)

RESEARCH HIGHLIGHTS

Unmasking CAD, a metabolic gatekeeper of cell proliferation

CAD is a 1.5 MDa multi-enzymatic complex formed by hexameric association of a ~240 kDa polypeptide with four functional domains: glutaminase (GLNase), carbamoyl phosphate synthetase (CPSase), aspartate transcarbamoylase (ATCase) and dihydroorotase (DHOase). Each domain catalyses one of the initiating steps in the *de novo* biosynthesis of pyrimidine nucleotides. CAD is tightly regulated by allosteric effectors and by phosphorylation through different signalling cascades, and its activity is key to fuel the high demand of pyrimidines during cell growth and proliferation. Despite its central role in metabolism and its potential as an anti-tumour target, there is no detailed information about the architecture of CAD or about the structure of any of its functional domains. We aim to decipher the structure of the complex and to understand its catalytic and regulatory mechanisms at the atomic level.

Structure and functioning of the ATC domain of human CAD

We resolved the crystal structure of the ATCase domain of human CAD – free or bound to carbamoyl phosphate, or to the anti-tumour drug PALA – confirming its overall similarity with bacterial homologues (Ruiz-Ramos *et al.*, 2016). Unexpectedly, we found a decreasing affinity for PALA that could help to understand tumour resistance to this drug. Mutagenic and biochemical analysis linked the lowered PALA affinity to the communication of conformational changes between the ATCase subunits. The mutation of one key residue in this mechanism was recently found by others to cause the first CAD-related human disease (Ng B.C. *et al., Hum Mol Genet*, 2015).

Using CRISPR to understand the functioning of CAD *in vivo*

We generated fluorescent recombinant chimeras and used CRISPR to introduce green fluorescent protein (GFP) into the endogenous CAD gene and to knockout CAD in human cell lines. These tools enable us to interrogate important aspects of CAD functioning *in vivo*. By tracking the subcellular localisation of CAD in mammalian cells we demonstrated that, contrary to previous reports, CAD is located exclusively at the cytosol and does not translocate into the nucleus during the cell cycle. These engineered proteins and gene edited cells are also proving to be instrumental for the identification of interacting protein partners and for the testing of the disease-causing potential of newly identified clinical mutations in CAD. ■

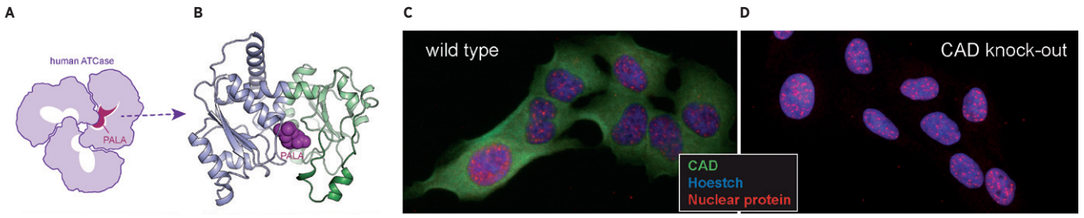

Figure (**A**) Cartoon representation of human ATCase trimer. (**B**) Crystal structure of human ATCase bound to the anti-tumour drug PALA. (**C, D**) Subcellular localisation of CAD using fluorescence microscopy in U2-OS wild-type cells (C) and in CRISPR-generated CAD knock out cells (D).

PUBLICATION

- Ruiz-Ramos A, Velázquez-Campoy A, Grande-García A, Moreno-Morcillo M, Ramón-Maiques S (2016). Structure and functional characterization of human aspartate transcarbamoylase, the target of the anti-tumoral drug PALA. *Structure* 24, 1081-1094.