

Structural Bases of Genome Integrity

Junior Group



Santiago Ramón-Maiques

Junior Group Leader

Santiago Ramón-Maiques was born in Valencia in 1973 and obtained his degree in Biology from the *Universidad de Valencia* in 1996. From 1997-2003 he worked with V. Rubio at the *Instituto de Biomedicina de Valencia* (CSIC), obtaining his PhD in Biology in 2001 for the functional and structural characterisation of a hyperthermophilic kinase. He completed his training in Protein Crystallography with I. Fita at the *Centro de Investigación y Desarrollo* (CSIC-Barcelona).

After a 1.5-year postdoctoral stay with V. Rubio, during which time they identified and characterised a new structural family of enzymes, Santiago obtained a Human Frontiers Science Program Fellowship to work with W. Yang at the National Institute of Health (NIH, Bethesda). His work focused on the structural characterisation of the V(D)J recombinase. The challenges of deciphering the architecture of this macromolecular complex led him to join A. Steven's laboratory at the NIH to learn single-particle electron microscopy (EM). Additionally, he determined the crystal structure of the regulatory PHD finger of RAG2 in complex with the epigenetically modified histone H3, and the structure of the human translesion DNA polymerase *eta*.

After 5 years at the NIH he returned to Spain to apply his expertise to other studies of large macromolecular complexes. In January 2010 Santiago joined the CNIO as a Junior Group Leader. His research efforts combine X-ray crystallography and single-particle EM with protein engineering and functional assays to solve the structure of large macromolecular complexes and understand their molecular mechanisms involved in cancer processes.

During his career Santiago has been awarded with the *Premios Extraordinarios de Fin de Carrera y de Doctorado* and received fellowships from EMBO, Human Frontier Science Program and the Spanish Ministries. In 2003, he received the *Josep Tormo* Award in Structural Biology for the structural determination of acetyl glutamate kinase.

Summary

Our group is interested in the structural and functional characterisation of the V(D)J recombinase and other large macromolecular assemblies that, in addition to playing central roles in the cell, are implicated in genome integrity and/or cancer.

Using a hybrid structural approach that combines X-ray crystallography and single-particle EM, we aim to decipher the architecture, catalysis and regulatory mechanisms of these macromolecular complexes to better understand why their malfunction favours tumour development as well as provide new strategies for the development of possible anti-cancer treatments.

Strategic Goals

- To determine the structure and function of large macromolecular complexes implicated in cancer
- Decipher the structure of the V(D)J recombinase formed by RAG1 and RAG2 proteins with the aim of understanding its architecture, catalysis and regulatory mechanisms
- Elucidate the structure, functioning and regulation of the antitumoural target CAD, a 250 kDa multi-enzymatic protein that controls the initial steps of the *de novo* synthesis of pyrimidines



Highlights

The majority of processes in the cell depend critically on the transient assembly of macromolecular complexes. These assemblies are generally large and dynamic and therefore difficult targets for structural characterisation. However, solving the structure of these complexes is required to understand cell function. We aim to combine protein engineering, X-ray crystallography and single-particle electron microscopy (EM) to determine the structure of two large macromolecular complexes that have central functions in the cell and are implicated in tumour development: the recombinase RAG1/2 and CAD.

The recombinase formed by the proteins RAG1 (1040 aa) and RAG2 (530 aa) initiates V(D)J recombination, the specialised DNA rearrangement used by the immune system to assemble immunoglobulin and T-cell receptor genes from a large number of gene segments. Mutations on either RAG1 or RAG2 cause defects in B and T cell maturation, leading to severe combined immunodeficiencies. Moreover, the activity of RAG has been related to the appearance of chromosomal rearrangements in lymphomas. Despite its importance for the development of the immune system and its implication in cancer, there is no structural information available for the catalytic machinery of RAG1/2. While still working in W. Yang's group, Santiago purified a 500 kDa complex of the recombinase and two DNA molecules. In collaboration with M. Gellert and A. Steven's groups they tackled the structural characterisation of the complex, describing the general architecture and composition of the RAG-DNA assembly. Santiago also solved the crystal structure of the complex between K4 trimethylated histone H3 and the regulatory PHD finger of

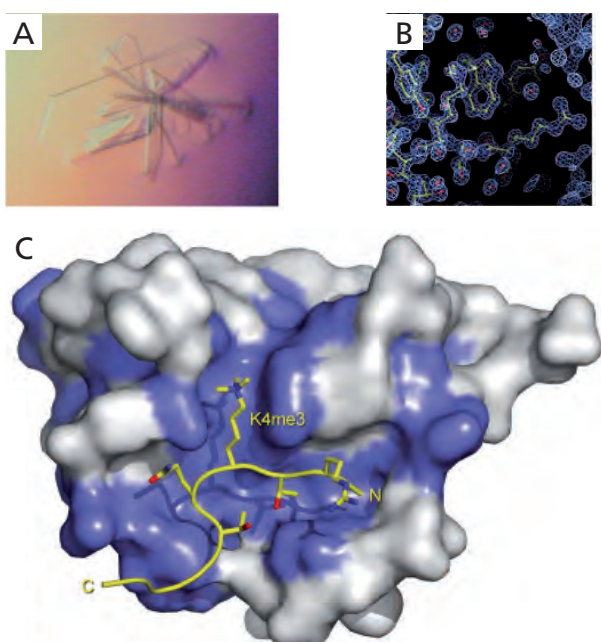


Figure: Crystal structure of the RAG2 PHD finger in complex with H3K4me3. (A) Crystals of the complex. (B) Detailed electron density map at 1.1 Å resolution. (C) Surface representation of the PHD with the H3K4me3 peptide bound. The protein surface that corresponds to highly conserved residues is coloured in blue.



Post-doctoral fellow: Nada Lallous (since May). **Graduate student:** Marija Dramicanin (since September). **Technician:** Araceli Grande (since February).

RAG2, demonstrating that the interaction with chromatin is crucial for the activity of the recombinase.

Santiago's Group at the CNIO continues with the structural characterisation of the recombinase RAG1/2 in order to understand its catalytic and regulatory mechanisms. This should provide new strategies for targeting the complex in the development of possible anti-tumoural treatments.

CAD is a 1.5 MegaDa macromolecular complex that controls the initial steps of the *de novo* biosynthesis of pyrimidines and plays an important role in cancer. Many tumours overexpress CAD to allow the fast synthesis of pyrimidines required for tumour replication and the expression level of CAD is a parameter of tumourigenic potential. CAD is therefore a target for the design of anti-tumoural drugs, although only one, PALA, has been marketed and used for cancer treatment. CAD is a homohexamer formed by a 256 kDa multifunctional polypeptide containing three enzymatic activities: Carbamyl phosphate synthetase II (CPSII), Aspartate transcarbamylase (ATC) and Dihydroorotase (DHO). The activity and allosteric regulation of CAD is well-characterised biochemically. It is also known that CAD is regulated through sequential phosphorylation by MAPK and PKA signalling cascades during the cell cycle. However, no structural information about this protein or any of its domains is available. Our Group aims to decipher CAD's structure to understand its catalysis and channelling of substrates and products, as well as its regulation, both allosterically and through phosphorylation. Understanding this anti-tumoural target will be of use for the rational design of compounds that modulate its activity.

Published at other institutions

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