Macromolecular Crystallography *Group*

Guillermo Montoya

Group Leader

Guillermo Montoya was born in Madrid (Spain) in 1967 and obtained his Bachelor degree in Biochemistry from the *Universidad del País Vasco* in 1990, and his PhD in Chemistry from the *Universidad de Zaragoza* in 1993.

He obtained both a European Molecular Biology Organisation (EMBO) and a Federation of European Biochemical Societies (FEBS) Fellowship and moved to the *Max Planck-Institut für Biophysik in Frankfurt am Main* (Germany), where he worked on membrane protein crystallisation in the group of the Nobel Laureate H. Michel.

Montoya later obtained both an EMBO long-term and a Marie Curie Fellowship and spent nine years at the European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany), working in I. Sinning's Group where he focused on the crystallisation of the cytocrome bc1 membrane protein complex and later pioneered the study of the structure of the signal recognition particle (SRP), an essential ribonucleoprotein complex involved in protein targeting.

In 1998 he was appointed as Researcher at the *Consejo Superior de Investigaciones Científicas* (CSIC) and was awarded a *Peter und Traudl Engelhorn* Foundation Research Fellowship. Since 2003 he has been an Honorary Professor in Biochemistry at the *Universidad Autónoma de Madrid* and Member of the working group in charge of the design of the biocrystallography beamline at the Spanish Synchrotron (ALBA).

Montoya has been Head of the CNIO's Macromolecular Crystallography Group since February 2002 and was acting Director of the Structural Biology and Biocomputing Programme from November 2003 to January 2006. In 2009 he was awarded the National Prizes of the *Fundación Mutua Madrileña* and the *Fundación Caja Rural de Granada-Ministerio de Sanidad*.

Summary

Macromolecules underlie all biological processes playing either dynamic roles in catalysis or signalling, static roles in scaffolding, or information storage. The focus of our Group is the molecular understanding of the role played by macromolecules involved in oncogenic processes. To achieve this we work on the structural determination of these biomolecules and their complexes.

The human genome is a sophisticated and complex coding system capable of producing thousands of different proteins in a tightly controlled manner. Proteins interact with other macromolecules forming assemblies that perform particular cellular tasks. The structural determination of these complexes will help us decipher the mechanisms that rule these processes.

Strategic Goals

- Decipher macromolecular machines involved in cell cycle dynamics and control
- Design homing endonucleases structurally for gene targeting

Staff scientists: Jasminka Boskovic, Gulnahar Mortuza, Inés G. Muñoz and Jesús Prieto. Post-doctoral fellows: María J. Marcaida, Marco Mazzorana, Rafael A. Molina and Sunita Subramanian. Graduate students: Javier Coloma (until August), Ana M. Garrote, Jaime Martínez and June Sánchez. Technicians: Elisabeth R. Bragado, Sonia Ibáñez, Pablo Mesa, Juan G. Pedrero and M. Pilar Redondo.

Highlights

S-phase and replication

DNA replication is an essential process during cell division. The identification of the DNA helicase(s) involved in eukaryotic DNA replication is still a matter of debate. Recently, the helicase activity of the hexameric MCM complex has been revealed as responsible for the unwinding of DNA during S phase in association with two partners: initiation factor Cdc45 and a four-subunit complex called GINS. In conjunction they form the CMG complex which contains ATP dependent helicase activity. We attempt to unravel the molecular mechanisms of this cellular machinery essential for eukaryotic DNA replication.

During this year we have been able to reconstitute all these components using coexpression techniques. Thus, we are now able to study them by combining X-ray crystallography and EM studies to decipher the structure of this complex and its components.

Mitotic complexes

Cellular growth and division are regulated by an integrated protein network which ensures the genomic integrity of all eukaryotic cells during mitosis. This cell cycle stage witnesses a massive reorganisation of cellular architecture. All these events need the assistance of different proteins to ensure their proper folding and functional

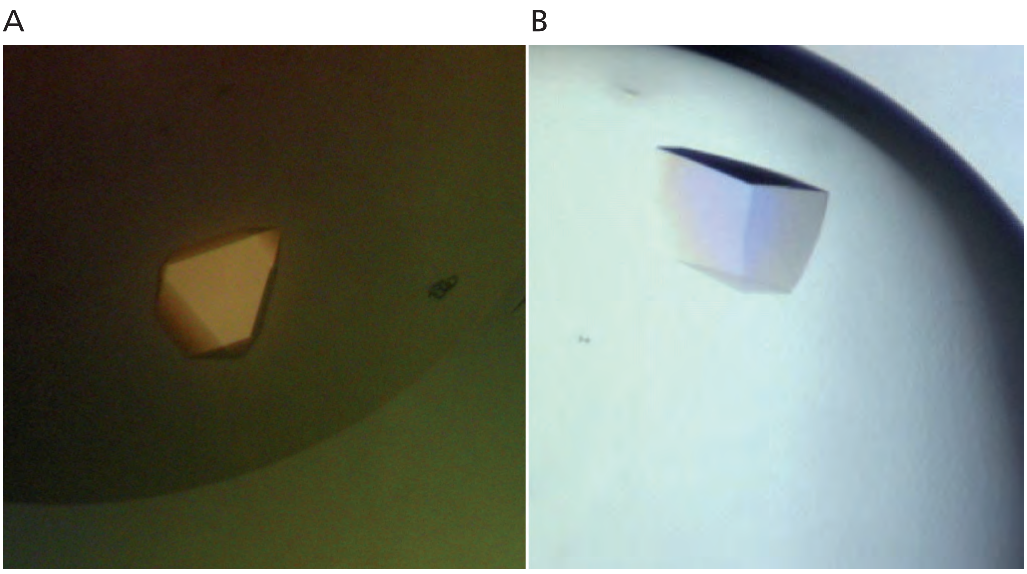

Figure 1: Crystals of a mitotic kinase (A) and a protein-DNA complex (B).

performance. One protein that performs this function is CCT (also known as TriC), a eukaryotic macromolecular complex that controls the folding of many essential mitotic regulators such as Cdc20, Cdh1, Plk1, CyclinE, tubulin and many more proteins. We have solved the structure of this macromolecular machine at 5 Å, in complex with tubulin, one of its main substrates. This finding in combination with mass spectrometry and shRNA experiments has allowed us to propose a mechanism for this macromolecular machine which involves several protein regions. Our objective is to obtain high resolution information regarding the atomic structure as well as dissect the regulation of this protein complex using site-directed mutagenesis. This work has been published in *Nature Structural Molecular Biology,* Epub December 12 2010.

Structural design of homing endonucleases for gene targeting

Homing endonucleases or meganucleases are sequence-specific enzymes which recognise large (12-45 bp) DNA target sites. These enzymes are often encoded by introns or inteins behaving as mobile genetic elements. They recognise sites that usually correspond to intron-free or intein-free genes, where they produce a DNA double-strand break (DSB). Eventually, DSB repair by homologous recombination with an intron- or intein-containing gene results in the insertion of the intron or intein where DSB occurred in specific loci in living cells.

These results present new perspectives in a wide range of applications, such as the correction of mutations linked with monogenic inherited diseases.

In 2010 our Group has participated in the development of a chimaeric enzyme that could target mutations in the RAG gene promoting its repair. We have solved the crystallographic structures of these variants revealing the molecular composition of the new target DNA recognition domain. In addition we have shown that the repair of the gene can be done in its locus in human cells, opening avenues to possible therapeutic applications.

The crystal structure of monomeric meganuclease I-DmoI in complex with its target DNA has allowed us to turn this endonuclease into a nicking enzyme, providing us with a new tool to repair genes preferentially using DSB homologous recombination. DNA nicks

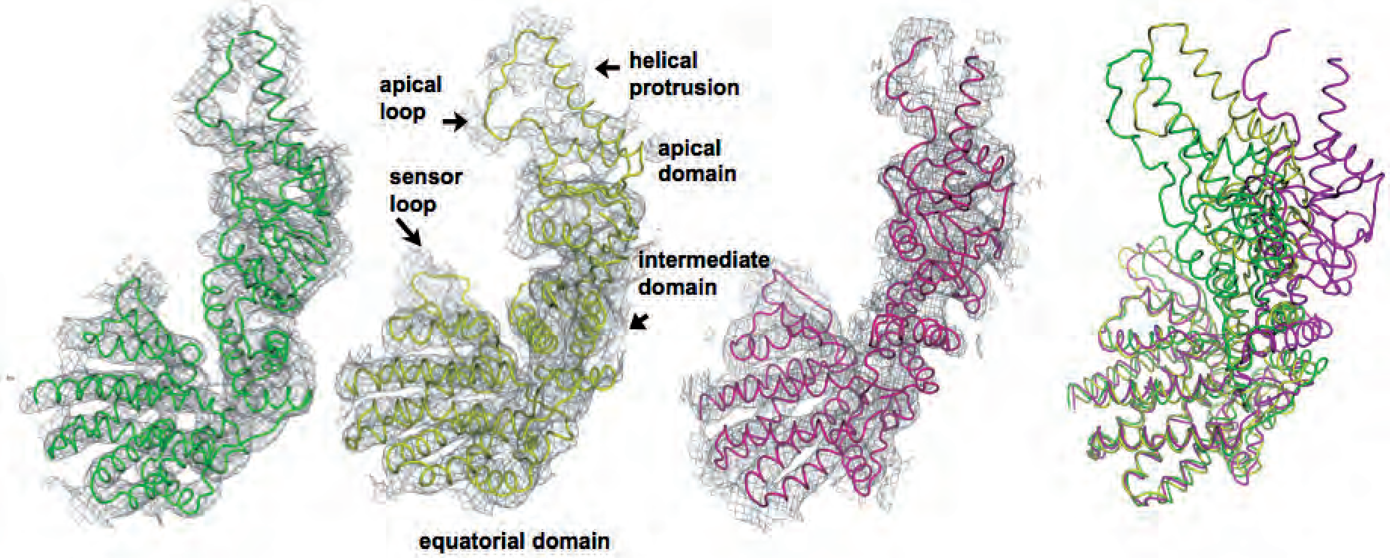

Figure 2: Comparison of the conformation of different CCT complex subunits after superimposition using the equatorial domain. The experimental electron density map is contoured at 1σ. *Nature Structural Molecular Biology,* Epub December 12 2010.

are favourably repaired using this route to avoid the unsafe non-homologous end joining (NHEJ) pathway that promotes loss of genetic information. This work has been published in *Nucleic Acids Research,* Epub September 16 2010.

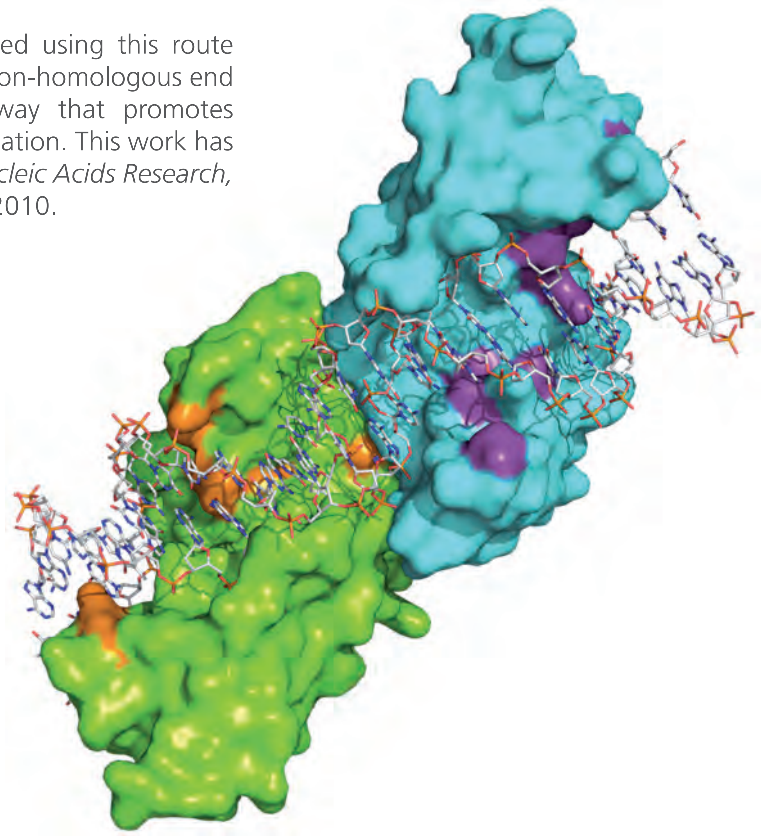

Figure 3: Crystal structure of an engineered meganuclease variant in complex with its target DNA. *Nucleic Acids Research,* Epub September 16 2010.

Publications

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Patent

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