Structural biology of proteins, nucleic acids and their complexes

Our research focuses on the 3D structure of proteins, nucleic acids and their complexes with the aim to further our understanding of several essential mechanisms in the cell. We use a number of molecular biology and structural biology techniques, with a focus on X-ray diffraction crystallography. We examine systems related to horizontal gene transfer which involve DNA translocation across the cell membrane. In addition, we address the regulatory mechanisms of gene expression and the control mechanisms of DNA replication. We also study unique DNA structures, like DNA junctions, and novel drugs that target DNA.

Horizontal gene transfer
Conjugation is the main route for horizontal gene transfer in bacteria and is responsible for the spread of antibiotic resistance. During conjugation, plasmid DNA is processed and then transported across cell membranes between cells (Rüth and Coll, 2006). DNA processing involves a key protein called relaxase, which recognises an extruded DNA hairpin at the Origin of Transfer, nicks the DNA and separates one of the strands, which will be subsequently transferred. After our studies leading to the resolution of the first structure of a relaxase-DNA complex, we have deepened our analysis of the molecular mechanism involved in the relaxase activity by solving a protein-DNA complex (Figure 1) that includes the scissile phosphate. This study shows that a metal ion is required for phosphate polarisation or reaction intermediate stabilisation to facilitate the nucleophilic attack of the catalytic tyrosine of the relaxase (Boer et al, 2006).

Transcription regulation
We study several transcription factors and their complexes with other proteins and DNA promoter regions. In the bacteriophage Φ29, we have examined the transcriptional regulator p4, which functions as a switch between early and late gene expression during the infection cycle. By solving the structure of the p4 dimer bound to a 41 bp-long promoter DNA sequence, we have revealed how this is achieved (Badia et al, 2006). A delicate interaction, involving only one base-specific recognition contact at each tip of the elongated p4 dimer, is mediated by a novel DNA binding motif that we have termed N-hook (Figure 2).

In another study we have addressed the E. coli PhoB transcriptional activator, a response regulator of the two-component signal transduction system that con-

Figure 1. TwC relaxase nicks the DNA after binding to an extruded DNA hairpin at the plasmid Origin of Transfer during bacterial conjugation (Boer et al, 2006).
controls the expression of more than 40 genes related to phosphate assimilation. Constitutively active mutants of the protein have been structurally characterised in order to examine the mechanism leading to PhoB activation (Solà et al., 2006; Arribas-Bosacoma et al., 2006). Finally, a ternary complex including the transcription factor, the DNA binding sequence and the C-term, a subunit of the RNA polymerase, has been solved, showing how the polymerase is recruited to the promoter region (Blanco et al., in preparation).

DNA structure and drug-DNA interactions
We are currently analysing unique DNA structures, such as four-way and three-way junctions related to DNA recombination and other processes. A novel cytotoxic drug consisting of a supramolecular helicate has been shown to bind to a three-way junction DNA with a perfect fit in the central trigonal cavity of the junction (Oleksi et al., 2006). Electrostatic and stacking interactions stabilise the interaction in a binding mode that has not been described previously (Figure 3).

Structural genomics
Considerable research effort is devoted to setting up medium/high throughput technologies for the expression and crystallisation of proteins and complexes (Fogg et al., 2006). In relation to these activities, our group participates in several National and European Structural Genomics consortia: VIZIER (www.vizier-europe.org), 3D-REPERTOIRE (www.3drepertoire.org), GENES (ub.cbm.uam.es/genes) and SPINE 2-Complexes (www.spine2.eu).

Figure 3. A novel cytotoxic drug, a supramolecular helicate, recognises a three-way junction DNA (Oleksi et al., 2006).
PUBLICATIONS


RESEARCH NETWORKS AND GRANTS

Estructura de proteínas de unión al DNA

Proyecto Plan Nacional de I+D+I BIFU2005-06758/BMC - Estructura genómica y proteómica de genómica y proteómica


Principal Investigator and Coordinator: Miquel Coll

Comparative structural genomics of virulenzymes involved in replication (VIZIER)

Integrated Project VI PM LSHG-CT-2005-512028

European Union: 2005-2009

Principal Investigator: Miquel Coll

A multidisciplinary approach to determine the structures of protein complexes in a model organism (3D-REPERO)

European Union: 2005-2009

Principal Investigator: Miquel Coll

Structural effects arising from major groove DNA recognition by metallo-supramolecular cylinders (MARCHY)

European Union: 2006-2010

Principal Investigator: Miquel Coll

Consorcio para el descubrimiento y desarrollo de nuevos fármacos (GENIUS PHARMA)

European Union: 2002-2006

Principal Investigator: Miquel Coll

Proyecto CENIT

CDTI (Ministerio de Industria, Turismo y Comercio): 2006-2009

Principal Investigator: Miquel Coll

Genómica estructural: aplicación a proteínas y complejos proteicos relacionados con el cáncer

Proyecto Integrado GEN2003-20642. Acción estratégica
**CENTROSOMA 3D: Hacia la comprensión estructural y funcional del centrosoma**

**Proyecto CONSOLIDER-INGENIO 2010**

Ministerio de Educación y Ciencia: 2006-2011

Principal Investigador: Miquel Coll

**COLLABORATIONS**

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<td>Plasmid replication</td>
<td>Manuel Espinosa and Gloria del Solar (Centro de Investigaciones Biológicas, CSIC, Spain)</td>
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<td>Bacterial conjugation</td>
<td>Fernando de la Cruz (Universidad de Cantabria, Spain)</td>
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<td>DNA packaging</td>
<td>José L Carrascosa and José María Valpuesta (Centro Nacional de Biotecnología, CSIC, Spain)</td>
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<td>Transcription regulation</td>
<td>Margarita Salas (Centro de Biología Molecular, CSIC, Spain)</td>
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<tr>
<td>Transcription regulation</td>
<td>Antonia Herrero (Instituto Bioquímica Vegetal y Fotosíntesis, CSIC, Spain)</td>
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<td>DNA-drugs</td>
<td>Mike Hannon (University of Birmingham, UK)</td>
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<td>Juan Carlos Zabala (Universidad de Cantabria, Spain)</td>
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<td>Ernest Giralt (IRB Barcelona, Spain)</td>
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<td>Chromatin-modifying proteins</td>
<td>Ferran Azorín (IRB Barcelona, Spain)</td>
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**Transcription regulation**

Juan Aguilar (Universitat de Barcelona, Spain)

Barry L. Wanner (Purdue University, USA)

**HTP protein expression**

Darren J. Hart (EMBL Heidelberg, Germany)

**Miquel Coll’s group, March 2006.**