Our group seeks to structurally characterise proteins and nucleic acids, and their complexes with the aim to further our understanding of several essential mechanisms in the cell. Using a number of molecular and structural biology techniques, with an emphasis on X-ray crystallography, we study the regulatory mechanisms of gene expression and the control mechanisms of DNA replication. In addition, we address molecular machines for DNA translocation, such as those involved in horizontal gene transfer in bacteria and DNA packaging in viruses. We also study unique DNA structures, such as DNA junctions.

**Transcription regulation**

In order to elucidate how transcription is regulated, we have structurally characterised several transcription factors, their complexes with other regulatory proteins, and their DNA binding regions (Badia et al., 2006). In one study, we addressed the *E. coli* PhoB transcriptional activator, a response regulator of the two-component signal transduction system that controls the expression of more than 40 genes related to phosphate assimilation.

A transcription initiation quaternary sub-complex was prepared and crystallised and its structure determined. This sub-complex includes the transcriptional regulator PhoB, the $\sigma_4$ domain of the $\sigma^{\text{E}}$ subunit of the RNA polymerase, the $\beta$-flap tip-helix of the RNA polymerase and the pho box promoter DNA region. The structure unveils how the RNA polymerase is recruited to the promoter region and provides clues on how transcription initiates (Gomez-Blanco et al., 2009, in preparation; Figure 1). In another study, we analysed proteins of the L-ascorbate *ula* regulon (Garcés et al., 2008a, b).

**Horizontal gene transfer**

Whatever the route used, the horizontal transfer of DNA, a phenomenon that contributes to the rapid evolution of micro-organisms, requires sophisticated multi-protein machinery to enable the long and charged nucleic acid molecule to cross the cell envelope barriers. In bacteria, the main route for cell-to-cell DNA transfer is conjugation, a mechanism responsible for the spread of antibiotic resistance. We have been studying the DNA processing machinery for conjugation in Gram-negative bacteria (Gomis-Rüth and Coll, 2006; Russi et al., 2008). Recently, we have extended the analysis to a Gram-positive bacteria conjugative system. The structure of the streptococcal plasmid relaxase MobM has been the first component of this system to be characterised (Russi et al., 2009, in preparation). The N-terminal nuclease domain has been determined in complex with a DNA hairpin, showing how the origin of transfer is recognised by the relaxase (Figure 2).

**DNA packaging in herpesviruses**

Another system for DNA translocation that we have addressed is the DNA packaging machinery of human cytomegalovirus (HCMV). Like all herpesviruses, HCMV replicates its genomic DNA...
into high molecular mass head-to-tail concatemers by a rolling-circle mechanism. The long DNA molecule is then cut into unit-length genomes and each genome is packaged into one viral procapsid. The DNA endonuclease and packaging activities are performed by a complex of proteins called terminase, which, in HCMV, includes UL56 and UL89. In collaboration with Darren Hart (EMBL, Grenoble) and using an ultra-high throughput method, we have screened 18,000 constructs and found a soluble domain that corresponds to the nuclease domain of the UL80 terminase subunit. Its 3D structure has been solved and shows an RNase H fold with a 2 metal-containing active site.

DNA replication control

DNA replication is a key cell event performed by diverse mechanisms in different organisms. Among these mechanisms, rolling circle replication (RCR) is a rapid one, leading to the generation of single or multiple copies of circular DNA or RNA.
molecules. RCR is always initiated by a triggering reaction that consists of the site-specific cleavage of the parental nucleic acid within the origin of replication region. This cleavage is catalysed by initiator proteins, which thus provide a primer for the DNA or RNA polymerases to start synthesis. We have solved the 3D structure of one of these initiator proteins, the plasmid pmv158 initiator RepB, and have unveiled that, like ring-helicases, it oligomerises as an hexamer with a central channel. The protein presumably encircles one of the DNA strands to confer processivity to the replisome complex (Figure 3; Boer et al., 2009).

DNA structure and drug-DNA interactions

We have analysed unique DNA structures, such as the four-way and three-way junctions related to DNA recombination and other processes. Three-way DNA junctions were proved to be the structural targets of novel cytotoxic drugs consisting of supramolecular helicates that perfectly fit in the central cavity of the junction (Oleksy et al., 2006). During 2008 we have determined several DNA-helicate complexes (Figure 4) that show both similar and new drug-nucleic acid interactions.

Publications


Other references


Research networks and grants

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Principal investigator: Miquel Coll

Ajuts a grups reconeguts
Principal investigator: Miquel Coll
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Principal investigator: Miquel Coll

Collaborations
Centrosomal proteins
Cayetano González, IRB Barcelona (Barcelona, Spain)

Chromatin-modifying proteins
Ferran Azorín, IRB Barcelona (Barcelona, Spain)

DNA drugs
Cristina Vicent, Instituto de Química Orgánica General-CSIC (Madrid, Spain)

DNA drugs
Mike Hannon, University of Birmingham (Birmingham, UK)

DNA packaging
José Carrascosa and José María Valpuesta, Centro Nacional de Biotecnología-CSIC (Madrid, Spain)

Histone methyl transferases
Xavier Barril, University of Barcelona (Barcelona, Spain)

HTP protein expression
Darren Hart, European Molecular Biology Laboratory (Grenoble, France)

Plasmid replication and transfer
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Prolyl oligopeptidases
Ernest Giralt, IRB Barcelona (Barcelona, Spain)