



Structural biology of protein-nucleic acid complexes and molecular machines

Our research effort focuses on the structural characterisation of proteins and nucleic acids, and their complexes in order to elucidate their mechanism of action. Using X-ray crystallography and other structural and molecular biology methods, we address the following: i) the initiation of DNA replication in plasmids by analysing the initiator protein RepB; ii) the termination of transcription in prokaryotes, which is mediated by the Rho factor; and iii) DNA packaging in herpesvirus, in which a molecular machine called terminase plays a key role. In addition, we analyse a number of unique DNA structures formed by DNA three-way junctions and supramolecular drugs.

Replication initiation

DNA replication is a key biological event performed by diverse mechanisms that deal with the incapacity of DNA polymerases to start *de novo* DNA synthesis. Among these mechanisms, rolling circle replication (RCR) is used by a variety of genetic entities (transposons, plasmids, bacteriophages and viruses) that replicate autonomously. RCR is initiated by a triggering reaction that consists of the site-specific cleavage of one of the strands of the DNA duplex at the origin of replication. This endonuclease reaction is catalysed by initiator proteins, which provide the primer for the polymerase to start DNA synthesis. Initiators also participate in the termination of DNA replication in a strand-transfer event.

The structure of the full-length RepB, the replication initiator of a streptococcal plasmid, has been solved by X-ray crystallography and electron microscopy (Boer *et al*, 2009), revealing a hexameric ring molecule where each protomer has two domains (Figure 1). The origin-binding and catalytic domains show an α/β plate fold and are highly mobile, which would account for recognition of two distinct DNA sites. The oligomerisation domains are all-helical, and form a compact ring with a central channel, a feature found in ring helicases and, in particular, in the initiator proteins of oncogenic viruses, such as papillomavirus and SV40. This observation suggests that, in a similar way, RepB encircles one of the DNA strands during replication to confer high processivity to the replisome complex.

Transcription termination

The Rho factor is a ring-shaped ATP-dependent helicase that mediates transcription termination in most prokaryotic cells by disengaging the transcription elongation complex formed by the RNA polymerase, DNA and the nascent RNA transcript. We have solved the structure of the early RNA-free state of Rho from *Thermotoga maritima* to 2.3 Å resolution (Canals *et al*, 2009;

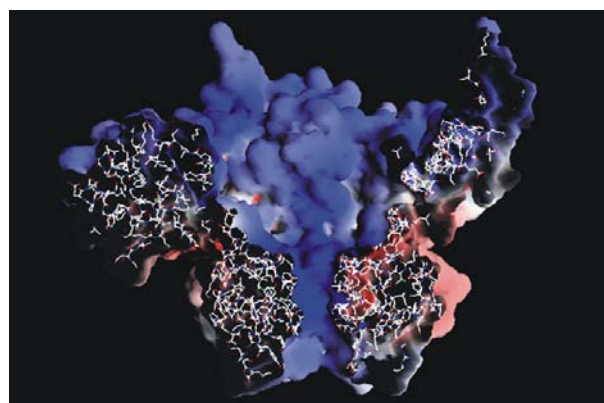


Figure 1. The replication initiator protein RepB.

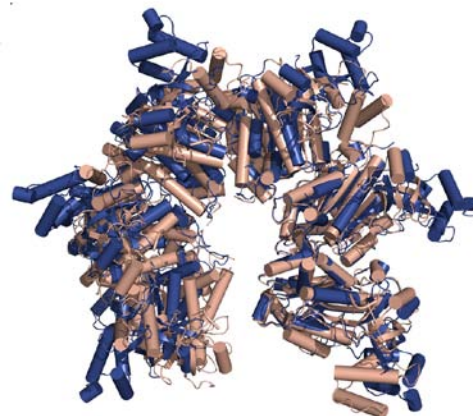
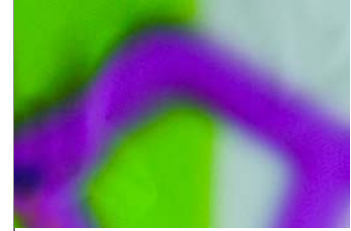


Figure 2. Superposition of the generated *T. maritima* Rho ring (blue) and the crystallographic ring from *E. coli* (green).



Canals *et al.*, in press). This RNA-free structure had eluded crystallisation for many years but now completes previous studies. The structure allows the characterisation of the apo-form of Rho and reveals an RNA-recruiting site that becomes hidden after occupancy of the adjacent specific primary RNA-binding site. These findings suggest an enriched model for mRNA capture that is consistent with previous data (Figure 2).

Herpesvirus DNA packaging

During viral replication herpesviruses package their DNA into the procapsid by means of the terminase molecular machine. In human cytomegalovirus (herpesvirus 5), subunit UL89 of the terminase cleaves the long DNA concatemers into unit-length genomes for encapsidation. We used an ultra-high throughput screening method to identify a soluble and purifiable fragment (UL69-C) from 18,432 randomly truncated constructs (Figure 3). We crystallised this fragment and solved its 3D structure to 2.15 Å resolution (Nadal *et al.*, in preparation). The structure reveals that UL89-C belongs to the RNase H/integrase superfamily, a vast group of nucleases and polynucleotidyl transferases that includes resolvases, integrases, transposases, RNA slicers, spliceosomal proteins and Okazaki-fragment cleaving RNases. On the basis of the structural similarities, we tested the inhibitory effect of HIV integrase inhibitors on the nuclease activity of the terminase and found that one of them is a potent inhibitor of UL89, a result that could facilitate the development of novel antiherpes molecules.

Complexes of DNA three-way junctions and supramolecular nanocylinders

Metallo-supramolecular cylinders are a class of helicate coordination compounds displaying three-fold symmetry and exhibiting high affinity for DNA. We previously showed (Oleksy *et al.*, 2006) that one of these cylinders has the capacity to induce three-way junctions in palindromic DNA by occupying the central junction cavity with extraordinary shape complementarity. We have solved a number of additional DNA-cylinder complex structures (Figure 4), all revealing a similar drug-DNA binding mode (Boer *et al.*, in press). These structures also indicate that non-covalent cylinder-DNA interactions may be used for the directed assembly of DNA-based nanomaterials.

Research Group Members

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Scientific output

Publications

Boer DR, Ruíz-Masó JA, López-Blanco JR, Blanco AG, Vives-Llàcer M, Chacón P, Usón I, Gomis-Rüth FX, Espinosa M, Llorca O, del Solar G and Coll M. Plasmid replication initiator RepB forms a hexamer reminiscent of ring helicases and has mobile nuclease domains. *EMBO J*, **28**(11), 1666-78 (2009)

Boer DR, Canals A and Coll M. DNA-binding drugs caught in action: the latest 3D pictures of drug-DNA complexes. *Dalton Trans*, **3**, 399-414 (2009)

Canals A and Coll M. Cloning, expression, purification and crystallization of the Rho transcription termination factor from *Thermotoga maritima*. *Protein Expr Purif*, **65**(2), 174-78 (2009)

Other references

Boer DR, Kerckhoffs JM, Parajo Y, Pascu M, Usón I, Lincoln P, Hannon MJ and Coll M. Self-assembly of functionalizable two-component 3D DNA arrays through the induced formation of DNA three-way-junction branch points by supramolecular cylinders. *Angew Chem Int Ed Engl* (in press)

Canals A, Usón I and Coll M. The structure of RNA-free Rho termination factor indicates a dynamic mechanism of transcript capture. *J Mol Biol* (in press)

Oleksy A, Blanco AG, Boer R, Usón I, Aymamí J, Rodger A, Hannon MJ and Coll M. Molecular recognition of a three-way DNA junction by a metallo-supramolecular helicate. *Angew Chem Int Ed Engl*, **45**, 1227-31 (2006)

Research networks and grants

A multidisciplinary approach to determine the structures of protein complexes in a model organism (3D-Repertoire)
European Commission, LSHG-CT-2005-512028 (2005-2010)
Principal investigator: Miquel Coll

Ajuts a grups de recerca reconeguts
Agency for Administration of University and Research Grants (AGAUR), 2009 SGR 1309 (2009-2012)
Principal investigator: Miquel Coll

Ayuda complementaria al proyecto europeo 'Spine2-complexes'
Spanish Ministry of Science and Innovation, BFU2007-29798-E (2008-2009)
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Ayuda complementaria al proyecto 'Genómica estructural comparativa para enzimas víricos'
Spanish Ministry of Science and Innovation, BFU2005-24122-E (2006-2010)
Principal investigator: Miquel Coll

Ayuda complementaria al proyecto 'Una aproximación multidisciplinaria para determinar las estructuras de los complejos proteicos en un organismo modelo'
Spanish Ministry of Science and Innovation, BFU2005-24123-E (2006-2009)
Principal investigator: Miquel Coll

Caracterización estructural de los inhibidores de metiltransferasas
Spanish Ministry of Science and Innovation, PETRI PET2007-0319-03 (2008-2010)
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Centrosoma 3D: Hacia la comprensión estructural y funcional del centrosoma
Spanish Ministry of Science and Innovation, Consolider CSD2006-23 (2006-2010)
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Comparative structural genomics of viral enzymes involved in replication (VIZIER)
European Commission, LSHG-CT-2004-511960 (2004-2009)
Principal investigator: Miquel Coll

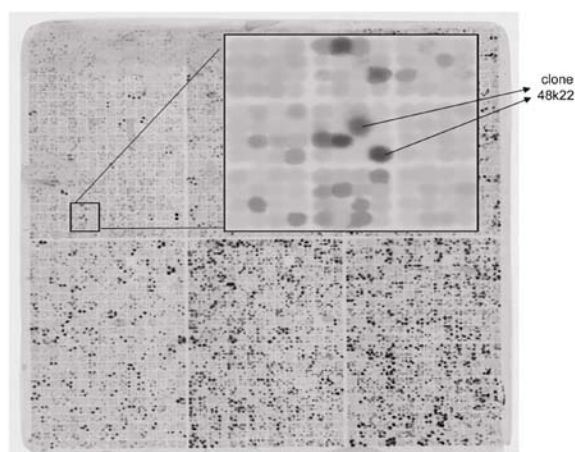


Figure 3. Blot of protein expression screen of 18,432 random deletion constructs of ul89.



Figure 4. Herpesvirus terminase UL89-C structure with modelled DNA bound.

Estructura de proteínas y complejos de unión al ADN
Spanish Ministry of Science and Innovation, BFU2008-02372/BMC (2009-2011)
Principal investigator: Miquel Coll

Spine2-complexes
European Commission, LSHG-2006-031220 (2006-2010)
Principal investigator: Miquel Coll

TEACH-SG: Advanced strategies for expression of protein complexes in yeast
Agency for Administration of University and Research Grants (AGAUR), ARCS1 00152-TEACH-SG (2009)
Principal investigator: Miquel Coll

Training and education in high volume and high value structural genomics
European Commission, LSSG-CT-2007-037198 (2008-2009)
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Collaborations

Centrosomal proteins

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Transcription regulation

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