ELECTRON MICROSCOPY UNIT

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**Plan de Empleo Joven-Licenciado* (Youth Employment Plan-Graduate)

OVERVIEW

The Electron Microscopy (EM) Unit is a central core facility and a research laboratory that provides CNIO researchers, as well as the broader research community, with access to Transmission Electron Microscopy and also provides expertise in EM image analysis. As a core facility, we offer standard specimen preparation techniques for proteins, protein complexes and vesicles, data collection and data processing tailored to the particular needs of the users. We also offer training for regular users on the use of equipment, as well as guidance regarding specimen preparation.

"We have used single-particle electron microscopy to elucidate the molecular architecture of full-length TRF1 and to demonstrate how it assists its interaction with other proteins and telomeric DNA."

RESEARCH HIGHLIGHTS

The Electron Microscopy Unit is a research facility that supports biological scientific projects ranging from the cellular to the macromolecular level. The EM Unit performs sample preparation protocols, negative staining, cryo-EM, and data collection methods, as well as 2D and 3D data processing.

In collaboration with CNIO's Telomeres and Telomerase Group (Molecular Oncology Programme) and the Crystallography and Protein Engineering Unit (Structural Biology and Biocomputing Programme), we used the single-particle electron microscopy technique to obtain the first low resolution structures of full-length TRF1 dimer (shelterin component) and its structure in complex with telomeric DNA. We contributed to the understanding of the molecular mechanism that protects the ends of chromosomes: our results demonstrate that full-length TRF1 presents a molecular architecture that assists its interaction with telomeric DNA and at the same time makes TRFH domains accessible to other TRF1 binding partners. Furthermore, our studies suggest hypothetical models on how other proteins such as TIN2 and tankyrase contribute to regulate TRF1 function.

In collaboration with Iván Ventoso, from the *Centro de Biología Molecular* 'Severo Ochoa' (CSIC-UAM) and the *Departamento de Biología Molecular, Universidad Autónoma de Madrid* (UAM), the EM Unit participated in the novel findings that illustrate how viral mRNA is threaded into the 40S subunit during the scanning process. Based on structural and functional data, we generated new insights into the scanning process, describing how a stem-loop in the proximal region of viral mRNA can promote a Eukaryotic Initiation Factor 2 (eIF2)-less translation initiation by trapping in RNA extensions of the ribosomal 40S subunit.

We continued our collaboration with the CNIO Cell Signalling and Adhesion Group (Structural Biology and Biocomputing Programme) on PI(4,5)P_2-mediated induction of Focal Adhesion Kinase (FAK) clustering at the cell membrane, applying 2D electron crystallography. ■

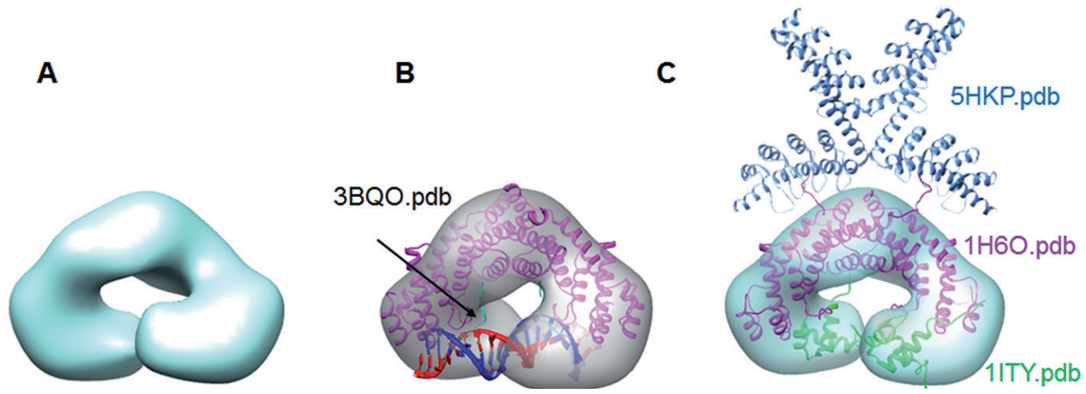

Figure TRF1 structure (A) and model for TRF1 DNA binding (B) and release (C). The interaction with TIN2 stabilises the complex with DNA through direct interaction of TIN2 with DNA. Tankyrase 1 engages the TRF1 dimer on two opposite sides of the molecule, introducing the PARylation and the release of TRF1.

PUBLICATIONS

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- Boskovic J, Martinez-Gago J, Mendez-Pertuz M, Buscato A, Martinez-Torrecuadrada JL, Blasco MA (2016). Molecular Architecture of Full Length TRF1 Favors its Interaction with DNA. *J Biol Chem* 291, 21829-21835.
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