MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

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OVERVIEW

Our Group uses cryo-electron microscopy (cryoEM) to determine the 3D structure of large macromolecular complexes of relevance in cancer at high resolution. Structural information, in combination with molecular and cell biology and biochemistry, is then used to propose how these molecules work and increase our understanding of the molecular basis of cancer. Most of our efforts are currently focused on 2 major areas of research: the study of i) chaperones essential for the activation of several macromolecular complexes relevant in cancer and ii) complexes implicated in the repair of DNA damage and in genomic instability. In collaboration with other groups, we are also studying the structure and mechanisms of several amino acid transporters.

"We have improved our understanding of the molecular mechanisms involved in spliceosome maturation, and cytosolic DNA sensing by the DNA repair protein Ku70/Ku80 and its subversion by some poxviruses."

RESEARCH HIGHLIGHTS

Understanding the maturation of the spliceosome, a cellular process involved in some types of cancer

Splicing is a cellular mechanism that facilitates the reading of DNA and multiplies the number of potential protein sequences in a cell by allowing the synthesis of several different proteins from a single gene. Alternative splicing is an extraordinarily complex process that requires the coordinated action of multiple proteins, each specialised in very specific functions. These proteins are assembled and matured, forming large macromolecular complexes, a process that is tightly controlled, and any failure can result in genetic diseases (FIGURE 1A). Several types of cancer present failures in the splicing processes, which is an advantage for tumour cells since these failures improve their rate of survival.

We have investigated some of the factors that enable the assembly and maturation of the spliceosome, particularly PRPF8, one of U5 snRNP's main components. We used biochemistry, interaction mapping, mass spectrometry and cryoEM to study the role of RUVBL1 and RUVBL2 ATPases and the ZNHIT2 protein in the biogenesis of PRPF8. We found that ZNHIT2 forms a network of contacts between several assembly factors required for PRPF8 biogenesis including ECD and AAR2, and that ZNHIT2 connects PRPF8 with the R2TP-HSP90 chaperone machinery, which is required for PRPF8 maturation. In addition, cryoEM showed how ZNHIT2 binds RUVBL1-RUVBL2 and affects the conformation of RUVBL2 (FIGURE 1B), which regulates RUVBL1-RUVBL2 ATPase activity.

Taken together, our results reveal part of the complex mechanisms that regulate the maturation of the splicing machinery, an essential process for the cell that can cause diseases such as cancer when perturbed.

Mechanism that helps some poxviruses to evade our cellular defence system

The Ku70-Ku80 complex is an essential component of the non-homologous end-joining (NHEJ) machinery that repairs DNA double strand breaks. Its structure shows that the protein comprises a preformed ring that can encircle duplex DNA. Ku70-Ku80 is the first protein to detect the presence of a break in the DNA thanks to this capacity to bind DNA like a ring encircles a finger.

Interestingly, Ku70-Ku80 is also present in the cytoplasm of cells, but its role there is not to detect and repair broken DNA but to alert the cell of the presence of viruses and activate cellular defences. The capacity of Ku70-Ku80 to encircle a linear dsDNA is used in the cytoplasm to detect viral DNA and initiate an inflammatory and innate immunity response. But some of these viruses have evolved countermeasures against these DNA sensors to attempt to block or delay the host immune response and allow the proliferation and spread of the disease. Vaccinia virus (used in the development of the smallpox vaccine and belonging to the poxvirus family) produces 2 proteins, C4 and C16, that bind to Ku70-Ku80 and inactivate its downstream signalling to the cellular immune response; however, the mechanism has not been well understood.

Using cryoEM, we have determined the 3-dimensional structure of C16 and its complex with Ku70-Ku80 (FIGURE 2A). In collaboration with L. H. Pearl's group (University of Sussex) and the Institute of Cancer Research in UK, we discovered that C16 and C4 proteins produced by the virus act as plugs that insert into the central hole of Ku70-Ku80, which it uses to thread itself into DNA, inhibiting Ku70-Ku80's ability to recognise viral DNA (FIGURE 2B). The structure of the C16 – Ku70-Ku80 complex was determined at high resolution, which allowed us to identify atomic details of how C16 binds and inactivates Ku70-Ku80, identifying key residues. Interestingly, by comparing the protein sequences of the C4 and C16 homologues in other viruses of the same family, we found that the regions involved in Ku inactivation are conserved in several orthopoxviruses, including smallpox and monkeypox. ■

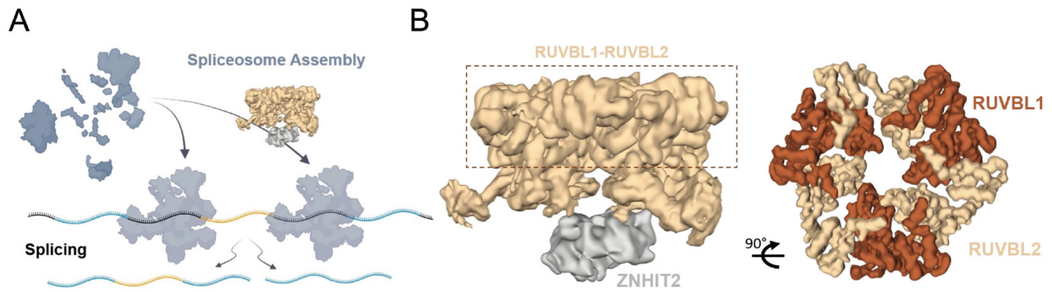

FIGURE 1 RUVBL1, RUVBL2 and ZNHIT2 form a complex required for the maturation of PRPF8 and the spliceosome. (**A**) Drawing representing the need of several assembly factors during the assembly of the spliceosome. (**B**) CryoEM map of the RUVBL1-RUVBL2-ZNHIT2 complex. Right panel highlights RUVBL1-RUVBL2 in the complex.

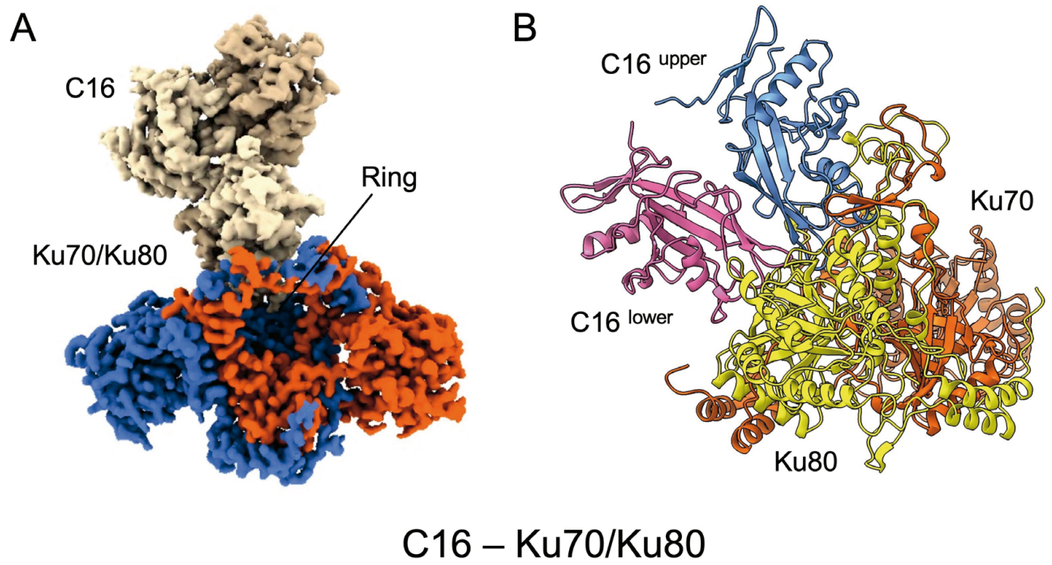

FIGURE 2 Structure of C16 — Ku70-Ku80 complex. (**A**) CryoEM map of the C16 protein from vaccinia bound to Ku70-Ku80. (**B**) One view of the atomic model of C16 — Ku70-Ku80, showing 2 copies of C16 C-terminal domain binding to Ku70-Ku80.

PUBLICATIONS

- Rivera-Calzada A, Arribas-Bosacoma R, Ruiz-Ramos A, Escudero-Bravo P, Boskovic J, Fernandez-Leiro R, Oliver AW, Pearl LH, Llorca O (2022). Structural basis for the inactivation of cytosolic DNA sensing by the vaccinia virus. *Nat Commun* 13, 7062.
- Rullo-Tubau J, Bartoccioni P, Llorca O, Errasti-Murugarren E, Palacín M (2022). HATs meet structural biology. *Curr Opin Struct Biol* 74, 102389.
- Serna M, González-Corpas A, Cabezudo S, López-Perrote A, Degliesposti G, Zarzuela E, Skehel JM, Muñoz J, Llorca O (2022). CryoEM of RUVBL1-RUVBL2-ZNHIT2, a complex that interacts with pre-mRNA-processing-splicing factor 8. *Nucleic Acids Res* 50, 1128-1146.

AWARDS AND RECOGNITION

- Marina Serna: *Premio Josep Tormo* Award for Structural Biology 2022, the Spanish Biochemical and Molecular Biology Society (*SEBBM*).
- María Ibarra Dauden: "*Premios Fundación Merck Salud-ASEICA por el Impulso de las Vocaciones Científicas – Investigadoras*", *Merck Salud* Foundation and the Spanish Association for Cancer Research (*ASEICA*).