MACROMOLECULAR COMPLEXES IN DNA DAMAGE RESPONSE GROUP

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OVERVIEW

Activation and assembly of many protein complexes implicated in cancer, such as kinases and polymerases, require the assistance of HSP90, a molecular chaperone. Thus, HSP90 inhibitors are being evaluated as anticancer agents.

HSP90 is needed for the activation and stability of the PI3-kinase-like kinases (PIKKs), including mTOR, ATM and ATR that regulate the DNA damage response and cell growth. Surprisingly, these kinases require the action of HSP90 but working in concert with the R2TP/Prefoldin-like (R2TP/PFDL) complex. R2TP/PFDL is the most complex HSP90 co-chaperone yet described. R2TP/PFDL contains multiple subunits and growing evidence links this complex to cancer.

Yet, how all these processes work is largely unknown. We are using cryo-electron microscopy (cryo-EM) to fully understand the molecular mechanisms of R2TP/PFDL and to bring us a step closer to designing strategies to interfere with PIKK assembly and activation.

"How kinase complexes implicated in cancer are assembled by HSP90 and R2TP is unclear. The structure of R2TP brings us a step closer to mechanistic understanding and the design of anticancer strategies."

RESEARCH HIGHLIGHTS

Cryo-EM and structure of macromolecular complexes in cancer

A defining feature of our Group is our interest in understanding the structural and molecular mechanisms of macromolecular complexes involved in the DNA damage response. For this, we use mostly biochemical and molecular biology tools in combination with cryo-electron microscopy (cryo-EM). Cryo-EM is used to visualise large macromolecular complexes, to observe their flexibility and motions, and to build atomic models. Cryo-EM is especially helpful for complex and flexible assemblies, which are typically difficult to crystallise. The structural and functional information provides mechanistic details to help understand the DNA damage response, and it is an input for the design of new strategies to interfere with these processes.

The Group is currently working on several complexes implicated in the response to DNA damage, but this year our main area of focus was the characterisation and understanding of how HSP90 and the R2TP co-chaperone function to assemble large macromolecular complexes of relevance in cancer.

How cells build protein interactions in protein kinase complexes

Assembly, activation and cellular stability of a growing list of macromolecular complexes, many of which are relevant in cancer, require the assistance of molecular chaperones. Among these, the kinases of the PI3-kinase-like family (PIKKs) function as part of large multi-subunit complexes that require HSP90 for assembly.

The PIKK family comprises proteins such as ATM, ATR and DNA-PKcs, implicated in DNA repair and DNA damage signalling, and mTOR, which controls cell growth. These kinases interact with other proteins in order to function properly and be active, as in the mTOR complex 1 (mTORC1) or ATR-ATRIP. Building these protein interactions needs the concerted action of the HSP90 chaperone and the R2TP/Prefoldin-like (R2TP/PFDL) co-chaperone. Interestingly, cells control the level of activation for some of these kinases, such as mTOR, by regulating the building of their functionally active complexes. How all this happens, the molecules involved, the mechanistic details and the implications in cancer remain poorly understood.

Our current aim is to improve our molecular understanding of the structural basis of R2TP-mediated protein complex assembly. In 2018, we reported the 3D structure of the human R2TP complex at a resolution of 3.6Å as part of a collaborative effort between our group and the group of Laurence H. Pearl at the Genome Damage and Stability Centre in the University of Sussex (UK).

Cryo-EM reveals that a C-terminal domain in RPAP3 (RNA Polymerase Associated Protein 3), one of the components of the human R2TP complex, binds to one hexameric ring of the RUVBL1 and RUVBL2 ATPases. This interaction provides a tight anchor that frees the N-terminal regions of RPAP3 involved in HSP90 binding. Cryo-EM images of R2TP show that HSP90 binding regions are extremely flexible, moving around the core provided by the RUVBL1-RUVBL2 hexameric ring. We propose that such flexible attachment is essential for placing HSP90 in the proximity of the clients, while providing sufficient conformational freedom to interact with a diversity of clients.

Together, our findings provide the first structural view of human R2TP, an essential complex for the HSP90-mediated assembly of mTORC1, ATR-ATRIP and other complexes of the PIKK family. Our structures also highlight important differences between the human complex and the much simpler homologs found in yeast. We have discovered an intricate architecture of the human R2TP complex, providing a flexible tether for HSP90, needed to cope with the assembly of multiple and diverse macromolecular complexes. A structural view of how HSP90 and its co-chaperone assists the assembly of proteins involved in cancer will bring us a step closer to the potential design of new anticancer strategies. ■

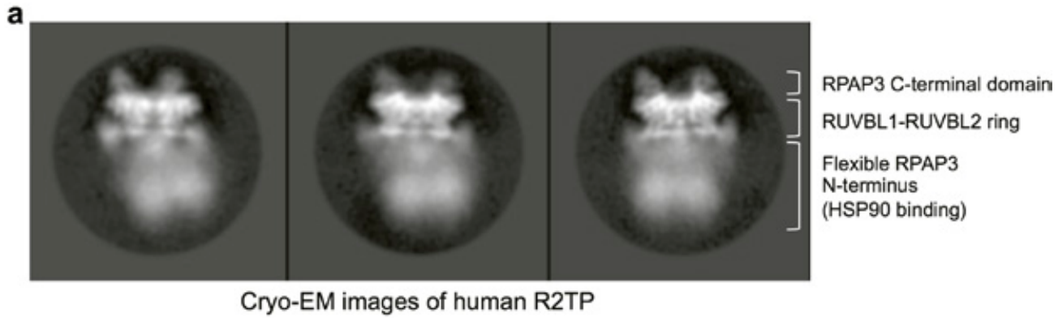

Figure 1 Selected views of the human R2TP complex as observed by cryo-EM. (**a**) Several domains can be localised, and the flexibility of the HSP90-binding regions in RPAP3 is detected.

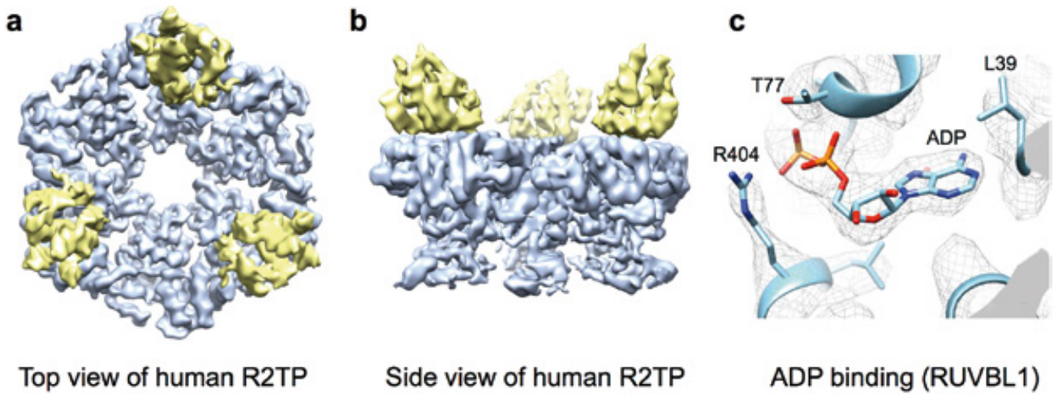

Figure 2 Cryo-EM map of the RUVBL1-RUVBL2 hexamer (blue colour) bound to the C-terminal domain of RPAP3 (yellow colour), as seen from the top (**a**) and side (**b**). (**c**) Detail of the ADP binding site. The quality of the cryo-EM density, represented in mesh, is sufficient to detect the ADP and side chains of residues in the binding site for nucleotides.

PUBLICATIONS

- Martino F, Pal M, Muñoz-Hernández H, Rodríguez CF, Núñez-Ramírez R, Gil-Carton D, Degliesposti G, Skehel JM, Roe SM, Prodromou C, Pearl LH, Llorca O (2018). RPAP3 provides a flexible scaffold for coupling HSP90 to the human R2TP co-chaperone complex. *Nat Commun* 9, 1501.
- de Jorge EG, Yebenes H, Serna M, Tortajada A, Llorca O, de Córdoba SR (2018). How novel structures inform understanding of complement function. *Semin Immunopathol*. 40, 3-14.

Book Chapter

- Muñoz-Hernández H, Pal M, Rodríguez CF, Prodromou C, Pearl LH, Llorca O (2018). Advances on the structure of the R2TP/Prefoldin-like complex. *Adv Exp Med Biol* 1106, 73-83.