Near-atomic cryo-EM structure of the chaperonin CCT in complex with its substrate mLST8, a key component of the mTOR complex

Cuellar, J.[1]

[1] National Centre for Biotechnology (CNB-CSIC), Madrid, Spain

The mechanistic target of rapamycin (mTOR) Ser/Thr kinase (289 KDa) forms two multi-protein signaling complexes, identified as mTORC1 and mTORC2, which are master regulators of cell growth, metabolism, survival and autophagy in response to growth factors, nutrients, oxygen and stress [1]. As a result, they are involved in many pathological processes including cancer, type II diabetes and neuro-degeneration, and both represent high-value therapeutic targets. mLST8 is a 37 kDa β-propeller protein which, together with mTOR, is a core component of both mTOR complexes [2,3].

In a previous work we used Cryo-EM and XL-MS to characterize the folding pathway of another remarkable β-propeller protein, G protein β-subunit, that is assisted by CCT and its cochaperone PhLP1 [4]. Given that β-propeller proteins are the largest class of CCT substrates, we sought to investigate whether they share a common folding mechanism. The structural characterization of CCT-mLST8 complex by cryo-EM at ≈4 Å resolution shows near-native mLST8 bound deep within the CCT cavity interacting with the equatorial domains of CCT α, γ and θ subunits. XL-MS experiments confirm the interaction with at least two of these subunits. Surprisingly, the comparison of these results with those obtained with Gβ evidence a different folding mechanism between the two β-propeller proteins and rules out the existence of a general folding mechanism for this family of proteins.

References

[1] Laplante, M. and Sabatini, D. M. (2012). mTOR signaling in growth control and disease. Cell 149, 274-293"

[2] Kim, Y. C. and Guan, K. L. (2015). mTOR: a pharmacologic target for autophagy regulation. J Clin Invest 125, 25-32"

[3] Yang, H., Rudge, D. G., et al. (2013). mTOR kinase structure, mechanism and regulation. Nature 497, 217-224."

[4] Plimpton, R. L., Cuéllar, J., et al. (2015). Structures of the Gβ-CCT and PhLP1 - Gβ-CCT complexes reveal a mechanism for G-protein β-subunit folding and Gβγ dimer assembly. Proc. Natl. Acad. Sci. USA 112, 2413-2418