SPOP Oligomerization Drives the Assembly of Multivalent Cullin3-RING Ubiquitin Ligase Complexes

Darren Yong[1], Wesley J. Errington[1], Gilbert G. Prive[1,2]
[1] Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada
[2] Ontario Cancer Institute, Princess Margaret Cancer Centre, University Health Network, Toronto, Ontario, Canada

Protein ubiquitination is an essential post-translational modification. It is responsible for regulating transcription, cellular signaling and protein degradation inside cells. There are over 600 E3 ubiquitin ligases encoded in the human genome and dysfunction in these enzymes is implicated in several human diseases, including prostate cancer. The Cullin3-RING Ligases (CRL3) are modular enzymatic complexes that use adapter proteins to target different pools of substrates for ubiquitination and proteosomal degradation. A fundamental feature of the BTB class of CRL3 substrate adaptors is the ability to self oligomerize and assemble multiple CRL3s to form multivalent complexes and achieve higher activity. Previously, we solved the crystal structure of the N-terminal domain of Cul3 in complex with the BTB domain of the adaptor protein SPOP at 2.4 Å. We have used this structure to generate a high order model of the full SPOP-CRL3 complex. Our *in vitro* biophysical experiments show that SPOP-mediated oligomerization is concentration-dependent and CRL activity is highly dependent on SPOP self-assembly. We also found that prostate cancer mutations in SPOP abolish the interaction with the ERG transcription factor, a known oncogene. This work demonstrates that the architecture and stoichiometry of CRL3 complexes is important in regulating the factors required for prostate cancer progression.