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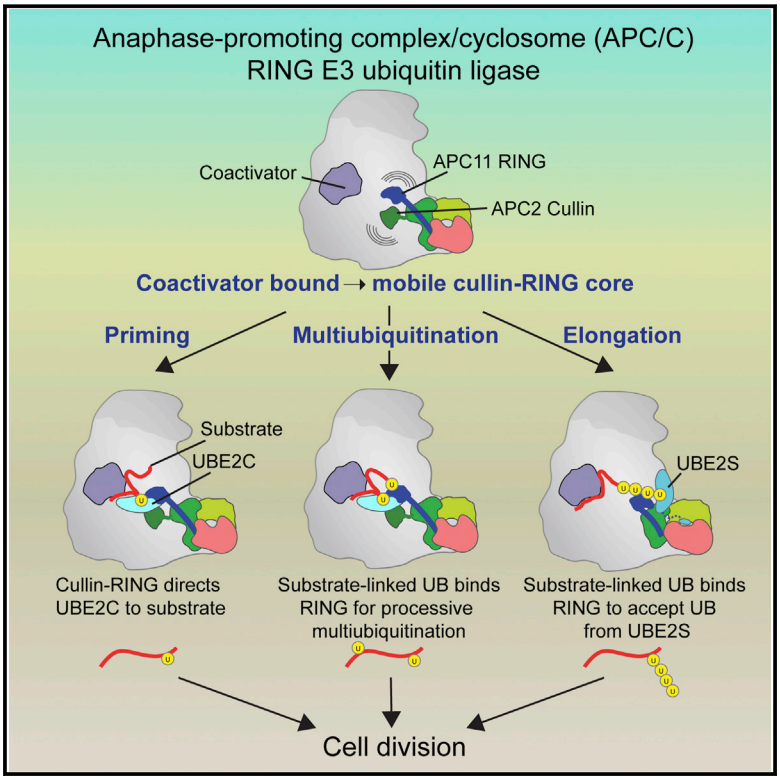
Dual RING E3 Architectures Regulate Multiubiquitination and Ubiquitin Chain Elongation by APC/C

Graphical Abstract



Highlights

- Cryo-EM shows mechanisms of polyubiquitination by cell-cycle regulator APC/C
- Distinct cullin-RING-E2 architectures for multiubiquitination and chain elongation
- RING activates UBE2C and binds substrate-linked ubiquitin to amplify processivity
- RING delivers ubiquitin for K11-linked chain elongation by UBE2S placed by cullin

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In Brief

Alternate functions call for alternate architectures: proteins responsible for ubiquitination arrange themselves differently depending on the kind of ubiquitin modifications their substrate will receive.

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Dual RING E3 Architectures Regulate Multiubiquitination and Ubiquitin Chain Elongation by APC/C

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SUMMARY

Protein ubiquitination involves E1, E2, and E3 trienzyme cascades. E2 and RING E3 enzymes often collaborate to first prime a substrate with a single ubiquitin (UB) and then achieve different forms of polyubiquitination: multiubiquitination of several sites and elongation of linkage-specific UB chains. Here, cryo-EM and biochemistry show that the human E3 anaphase-promoting complex/cyclosome (APC/C) and its two partner E2s, UBE2C (aka UBCH10) and UBE2S, adopt specialized catalytic architectures for these two distinct forms of polyubiquitination. The APC/C RING constrains UBE2C proximal to a substrate and simultaneously binds a substrate-linked UB to drive processive multiubiquitination. Alternatively, during UB chain elongation, the RING does not bind UBE2S but rather lures an evolving substrate-linked UB to UBE2S positioned through a cullin interaction to generate a Lys11-linked chain. Our findings define mechanisms of APC/C regulation, and establish principles by which specialized E3–E2–substrate-UB architectures control different forms of polyubiquitination.

INTRODUCTION

Posttranslational modification by multiple ubiquitins (UBs) or UB chains is a predominant eukaryotic mechanism regulating protein half-life, location, interactions, or other functions. After an E1 enzyme links UB to the catalytic Cys of an E2 enzyme (~30 in humans), the thioester-bonded E2~UB intermediate is employed by an E3 enzyme (~600 in humans) (Deshaies and Joazeiro, 2009). Most E3s display a domain that recruits a substrate's degron motif and a hallmark RING domain thought to bind a cognate E2~UB intermediate that determines acceptor Lys properties (Metzger et al., 2014; Streich and Lima, 2014). Some E2s promiscuously modify lysines irrespective of context, while others generate linkage-specific UB chains (Christensen et al., 2007; Mattiroli and Sixma, 2014). Our current understanding is based on a limited number of landmark structures showing how RING domains align E2~UB active sites for nucleophilic attack, how a RING E3–E2 complex can target a preferred acceptor Lys, and how one RING forms homologous complexes with different E2~UB intermediates (Branigan et al., 2015; Dou et al., 2012; McGinty et al., 2014; Plechanovová et al., 2012; Pruneda et al., 2012; Reverter and Lima, 2005; Scott et al., 2014). However, structural models for dynamic polyubiquitination of substrates remain elusive.

Visualizing substrate polyubiquitination is challenging because proteins are modified on assorted sites, and UB chains are often elongated in a linkage-specific manner where each distal UB progressively added to a growing chain is successively presented to the catalytic center to accept another UB. The multiple ubiquitination sites are essentially moving targets for a catalytic RING–E2~UB assembly. Furthermore, E3 RING and degron-binding domains are often flexibly tethered, raising the question of how catalytic encounter could be achieved (Berndsen and Wolberger, 2014; Streich and Lima, 2014). Here, we addressed how mobile RING E3–E2 assemblies and UB-linked substrates are positioned for modification of multiple substrate lysines and evolving UBs by the essential human E3, the 1.2 MDa multisubunit