COMMENTARY

Ubiquitin in disguise unveils a cryptic binding site in 1.2-MDa anaphase-promoting complex/cyclosome

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Ubiquitin serves as a protein modifier and pervasive signaling molecule in eukaryotes, regulating major events throughout the lifetime of a cell, including pathways used for synthesis, repair, and degradation. Aptly named for its ubiquitous presence in human cells, proteomics studies have revealed tens of thousands of sites in ~5,000 substrates, suggesting that most proteins are ubiquitinated during their cellular lifetime (1). Ubiquitin can be added as an individual block to substrate sites (monoubiquitination) or expanded in diverse ways to form chains through 8 possible linkage points; this Lego-like feature of ubiquitination enables binding to >150 receptors and fuels its ability to signal with both versatility and specificity (2–4). Identifying receptor sites for ubiquitin de novo is challenging, as ubiquitin-binding domains are diverse in structure and binding mechanism, resulting in no globally shared consensus sequence (5). In PNAS, Watson et al. (6) present a workflow that can be applied broadly to discover new ubiquitin-binding sites. They apply this method to the 1.2-MDa E3 ligase anaphase-promoting complex/cyclosome (APC/C), which promotes ubiquitin-dependent turnover of cell cycle regulators, including cyclins, to control cell division. This approach yields a specific protein inhibitor for the APC/C complex and unveils a cryptic binding site for K48-linked ubiquitin chains.

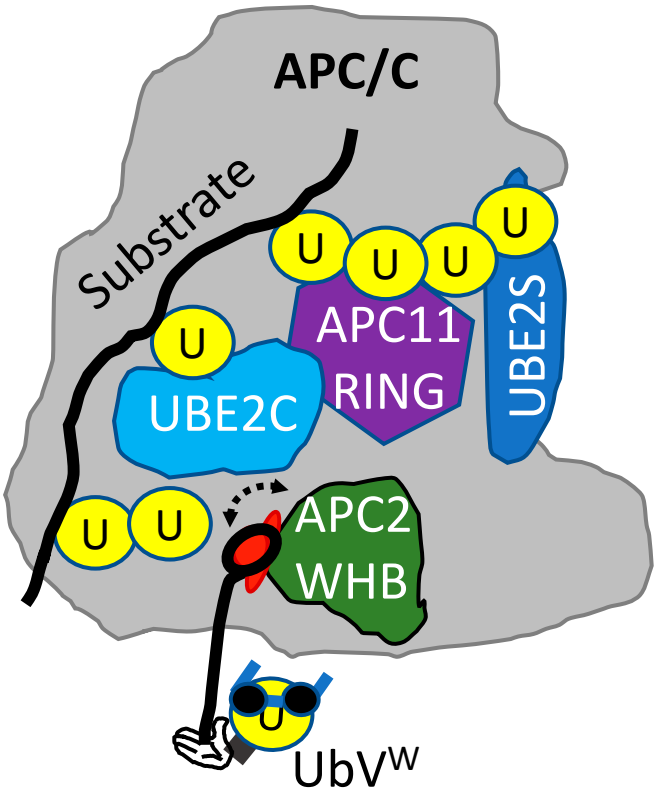

Fig. 1. Cartoon illustrating the APC/C cullin-RING catalytic core with ubiquitin variant UbV^{W} hijacking the ubiquitin-binding exosite of the APC2 WHB domain. A ubiquitinated substrate (black with yellow ubiquitin moieties) is depicted engaging the catalytic site of the priming E2 UBE2C (blue) and the APC11 RING domain (purple) exosite, which supports catalysis by both UBE2C and the elongating E2 UBE2S (indigo). The APC2 WHB domain (green) exosite (red) for ubiquitin overlaps with the surface used to recruit UBE2C and is targeted by UbV^{W} (symbolized with a black lasso).

Where and how ubiquitin is added to substrate sites or built into chains is dictated by >700 proteins, ~90 of which hydrolyze ubiquitin from substrates or disassemble ubiquitin chains. Ubiquitin is activated by an adenosine 5′-triphosphate–dependent reaction for covalent trafficking via thiolester bonds through an E1-E2-E3 enzymatic cascade. In addition to carrying ubiquitin at their catalytic sites, E2 and E3 enzymes can bind ubiquitin noncovalently at exosites remote from catalytic surfaces (7). APC/C belongs to the RING family of E3 ligases and, as such, does not covalently interact with ubiquitin but rather promotes ubiquitin transfer to substrates directly from a bound E2. It functions with 2 E2 enzymes to achieve distinct catalytic outcomes, and their different combinations of activity yield substrates with differential efficiency for degradation by the proteasome (8). UBE2C primes APC/C substrates by attaching short ubiquitin chains, including one or multiple single ubiquitin moieties, while UBE2S extends ubiquitin chains on APC/C substrates through K11 linkages. UBE2C is recruited to the APC/C through interactions with the RING domain of APC11 and the

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winged-helix B (WHB) domain of APC2 (9, 10). In a previous study, phage-displayed libraries were used to generate ubiquitin variants (UbVs) that target the APC11 RING domain for inhibition of multiubiquitination by UBE2C and ubiquitin chain elongation by UBE2S (11). In a new study, Watson et al. (6) identify a UbV that selectively inhibits UBE2C and not UBE2S activity. By combining protein engineering, biochemistry assays, and NMR spectroscopy, Watson et al. (6) find this UbV inhibitor of APC/C to block UBE2C substrate priming by occupying the UBE2C-binding site in the APC2 WHB domain and hence name it UbV^{W}. The UBE2C-binding site in APC2 is remote from where the elongating E2 UBE2S binds (9) and is apparently disposable for UBE2S-catalyzed polyubiquitination, as UbV^{W} did not affect this activity.

It is noteworthy that a similar competitive binding mechanism is used endogenously by the mitotic checkpoint complex (MCC) to suppress APC/C activity in response to unattached kinetochores. In this case, the MCC BUBR1 subunit sterically occludes both UBE2C recruitment sites in APC/C, namely that provided by APC11 and APC2 (12, 13). BUBR1 also blocks the APC/C substrate-binding site (12, 13).

Derived from ubiquitin, UbVs generally retain the β-grasp fold, but can do so in funky ways. A crystal structure of UbV^{W} revealed a domain-swapped dimer with an extended β1 strand that forms an antiparallel β-sheet with the opposite subunit (6). Watson et al. (6) note that such swapped dimeric structures have been observed in other UbVs (14, 15) and collectively display flexibility across the dimer interface. In UbV^{W}, Gly10 is replaced with tryptophan and situated at the center of the extended β1 strand (6). By using amino acid substitution, Watson et al. (6) find this tryptophan to be critical for oligomerization and to play a role in APC2 binding.

To study UbV^{W} interaction with APC2 WHB domain further, a tenacious feat of protein engineering was applied to optimize the complex for study by NMR spectroscopy (6). A further mutation was introduced into UbV^{W} by replacing I44 with aspartic acid, and an asymmetric dimer was formed by tandem affinity chromatography with unmodified UbV^{W}. The resulting UbV^{W_dim} was dimeric without higher-order self-association and bound APC2 at the unmodified UbV^{W} moiety. The NMR structure revealed overlap between APC2 amino acids involved in binding to UbV^{W} and those previously determined to function in UBE2C recruitment, demonstrating that UbV^{W} inhibits UBE2C catalysis by occluding the UBE2C-binding surface contributed by APC2.

Since the authors had previously targeted a known E3 ubiquitin-binding site with a UbV (11), they hypothesized that UbV^{W} could conversely be acting as ubiquitin in disguise and thus unveiling a new ubiquitin-binding site in APC/C contributed by APC2 (6). Indeed, many UbV^{W} amino acids that interact with APC2 are unmodified from ubiquitin, including L8, I44, V70, and L73, and mutation of I44, which is typically bound by ubiquitin receptors, abrogated APC2 binding. An exosite for ubiquitin was already identified in the APC/C contributed by the APC11 RING domain and was found to facilitate additional rounds of UBE2C-catalyzed ubiquitination by amplifying affinity for ubiquitin-modified substrates and to recruit ubiquitinated substrates for K11-linked chain elongation by UBE2S (11). It was this APC11 exosite that was targeted previously for APC/C inhibition by a UbV (11).

Watson et al. (6) tested their hypothesis that UbV^{W} binds to a cryptic ubiquitin-binding site in the WHB domain by using NMR. Sites of moderate or high affinity for ubiquitin chains typically retain weak affinity for monoubiquitin; NMR readily detects weak interactions, and ubiquitin has ideal spectral properties (16), making NMR a powerful tool to assay ubiquitin binding. The authors find the APC2 WHB domain to bind monoubiquitin, motivating further experiments that revealed preference for K48-linked diubiquitin over K11- and K63-linked chains (6). NMR analyses indicated a binding site for ubiquitin on the APC2 WHB that mimics UbV^{W} interactions. UbV^{W} is able to provide an expanded interaction surface and higher affinity by inclusion of tryptophan substituted for Gly10. In a stunning example of complementarity, crystallization yielded a placement of ubiquitin at another site, unique from where the NMR analyses indicated ubiquitin and UbV^{W} to bind. Dual placement of the 2 ubiquitin monomers situated the C terminus of ubiquitin from the X-ray diffraction structure proximal to K48 of the UbV^{W} structure, leading to a model structure and a mechanistic rationale for APC2 preference for K48-linked ubiquitin chains.

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Altogether, with this new study (6), the APC2-APC11 cullin-RING catalytic core is now known to have 2 exosites for ubiquitin (Fig. 1). Competitive binding of a UbV to the APC11 exosite at the exclusion of ubiquitin inhibits both UBE2C and UBE2S activity on substrates (11), and this binding site is not known to participate in recruitment of other APC/C factors. By contrast, dissecting the ubiquitin-binding function of the APC2 exosite is complicated by its overlapping requirement for UBE2C recruitment and positioning at the APC/C. In contrast to the APC11 exosite, that in APC2 is not needed for UBE2S activity, as UbV^{W} does not affect chain elongation catalyzed by this E2 (6). It is possible that binding at this site to K48-linked ubiquitin chains helps modulate the positioning or release of UBE2C from APC2. BUBR1 from the MCC also binds and blocks this exosite in APC2 (12, 13), demonstrating common features between the UbV^{W} and a natural inhibition mechanism. The identification of the APC2 exosite paves the way for future studies that offer much promise in uncovering the nuances of chain assembly on APC/C substrates.

UbVs substitute amino acids at the surface of the ubiquitin fold to sacrifice the broad targeting capacity of ubiquitin for higher-affinity, more selective targeting of specific ubiquitin receptors or processing enzymes (17). This strategy of specialized interactions through substitutions at the ubiquitin structural domain surface has evolved naturally in cells by ubiquitin shuttle factors to bind ubiquitin receptor sites in the proteasome with higher affinity than ubiquitin itself (18). Artificial UbVs have been developed to provide tools for perturbing specific interactions within ubiquitin pathways, including by inhibiting or enhancing the enzymatic activity of E2s, E3s, and deubiquitinating enzymes (11, 17, 19, 20). UbV^{W} could be used in future studies to further interrogate UBE2C function with APC/C. This study also highlights an approach to identify new ubiquitin-binding sites. New receptors and binding motifs for noncovalent interaction with ubiquitin continue to be discovered, with >25 known ubiquitin-binding domains (5). The surface properties of ubiquitin are thus evolved for large-scale targeting rather than for specific interactions with a sole cellular binding partner. The unexpected utility of a UbV to reveal a novel ubiquitin-binding site in the APC/C indicates that UbVs could be used generally to identify ubiquitin-binding domains in other complexes.

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