How Ubiquitin Takes Its Cue

Proteins often just cannot get away from ubiquitin, a versatile protein tag that might mark them for destruction, sorting, or enlistment into gene repair or cell division. Now researchers have determined how a ubiquitin-binding component of several proteins, called CUE, recognizes that tag. They solved the crystal structure of CUE and found that it groups in pairs capable of binding single ubiquitins, and that multiple pairs can team up to form a series of pockets for binding multiple ubiquitin molecules.

Researchers originally discovered ubiquitin, named for its nearly identical form in cells from yeast to human, as a tag marking other proteins for destruction. Some-times the tag is a single ubiquitin; other times it is a string of them. They have since learned that these tags help regulate a range of basic cellular processes by attracting different proteins that recognize and bind to ubiquitin. The discovery of new ubiquitin-binding modules brought to light additional functions for ubiquitin in the recent past, but researchers still know little about how such modules work. One mystery is how they can tell a single ubiquitin molecule from a string of them. Hoping to shed light on that ability, a group from the National Institutes of Health and the Mayo Clinic crystallized and solved the structure of a yeast protein's CUE domain, which recognizes single ubiquitin tags.

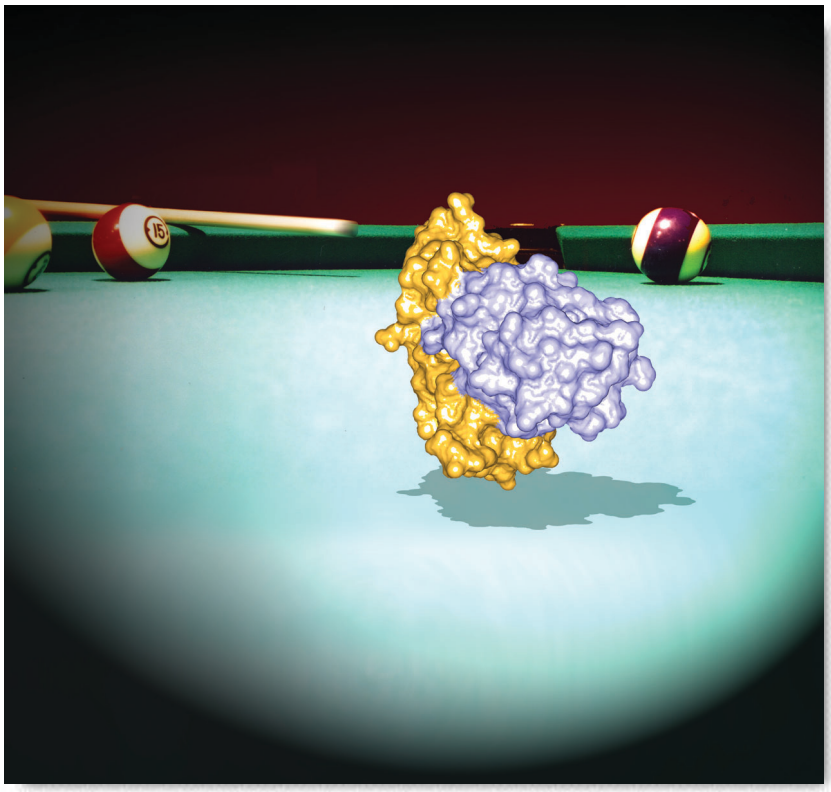

Fig. 1. The CUE domain (gold) joins in pairs to form a basket-like pocket that cups the ubiquitin protein (blue) [Image was created by G. Prag].

Based on x-ray diffraction data obtained at the SBC-CAT 19-ID beamline and the SER-CAT 22-ID beamline at the APS, the group found that CUE has a short, rod-like structure consisting of three helices, very similar to another ubiquitin-binding domain called UBA. They were surprised to find that CUE domains pair up to bind ubiquitin. Individual CUE molecules, or monomers, join together by swapping one helix for the identical one from their partner. The researchers found that such a pair, or dimer, undergoes a dramatic structural shift upon binding, flexing into a basket shape that cups the protein tag (Fig. 1). CUE can also dimerize in real cellular conditions as part of a full-length protein, they found.

To understand why the dimer is responsible for binding ubiquitin, the group constructed mutant proteins whose key binding amino acids were swapped for amino acids that interfere with binding. The first and last helices of the CUE monomer nestle against ubiquitin, whereas the middle helix faces away. In the dimer, however, the middle helix of each monomer snuggles closer to ubiquitin. The group mutated either the middle helix or the flanking helices of the dimer and found that both kinds of mutation made CUE much less sticky to ubiquitin, indicating that the middle and flanking helices both take part in binding. Because the monomer can bind only with the flanking helices, the group conclude that this interaction is not enough to cause tight binding.

Surprisingly, each dimer was joined to two ubiquitin molecules in the crystal structure, despite forming a basket designed to hold just one. Because of the way the dimer forms, the same amino acids in one monomer that bind to ubiquitin on the inner, concave surface of the basket are also present in the other monomer, but on the outer, convex surface. If two baskets are side by side, their outer surfaces complement each other and form essentially the same binding pocket as the inner basket does. Two CUE dimers would therefore have three basket-like pockets and be able to bind three ubiquitin molecules. The researchers say that the existence of such a binding pattern could allow domains like CUE to bind individual ubiquitin molecules or strings of them, depending on its state—monomer or dimer. ❍

See: G. Prag[1], S. Misra[1], E.A. Jones[1], R. Ghirlando[1], B.A. Davies[2], B.F. Horazdovsky[2], and J.H. Hurley[1], "Mechanism of Ubiquitin Recognition by the CUE Domain of Vps9p," Cell **113**, 609–620 (30 May 2003).

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