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The U box is a modified RING finger – a common domain in ubiquitination

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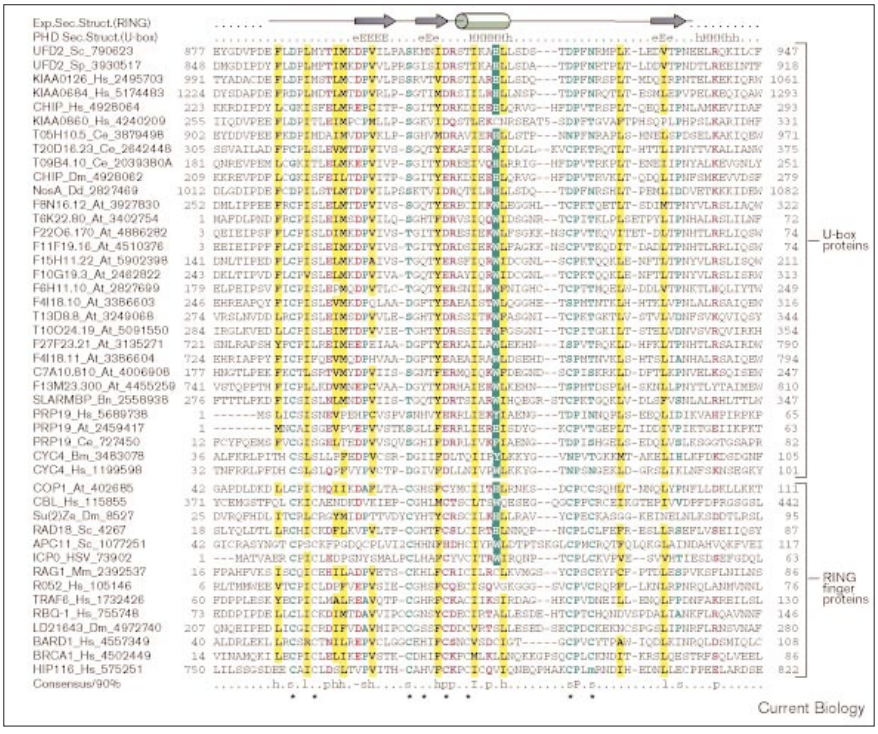
Eukaryotes use small polypeptides of the ubiquitin [1,2] and Apg12 [3] families as tags to target proteins for degradation by the proteasome. Ubiquitin ligation is a multi-step process that involves at least three classes of enzymes [1]. The E1 enzymes first charge ubiquitin in an ATP-dependent manner to form an E1–ubiquitin thioester intermediate. This activated ubiquitin is transferred to the active cysteine of an E2 enzyme and finally to an E3 enzyme,

ubiquitin transfer to the amino group of a lysine. Recently, a new class of ubiquitination enzymes, E4, the prototype of which is the yeast protein UFD2, has been identified. E4 is required for the multi-ubiquitination of ubiquitin-conjugated proteins that makes them preferred substrates for degradation [4]. The UFD2 protein and its homologs in other eukaryotes share a conserved domain designated the 'U box'. The U box mediates the interaction of UFD2 with ubiquitin conjugated proteins and therefore seems to be an essential functional unit of the E4 enzymes [4].

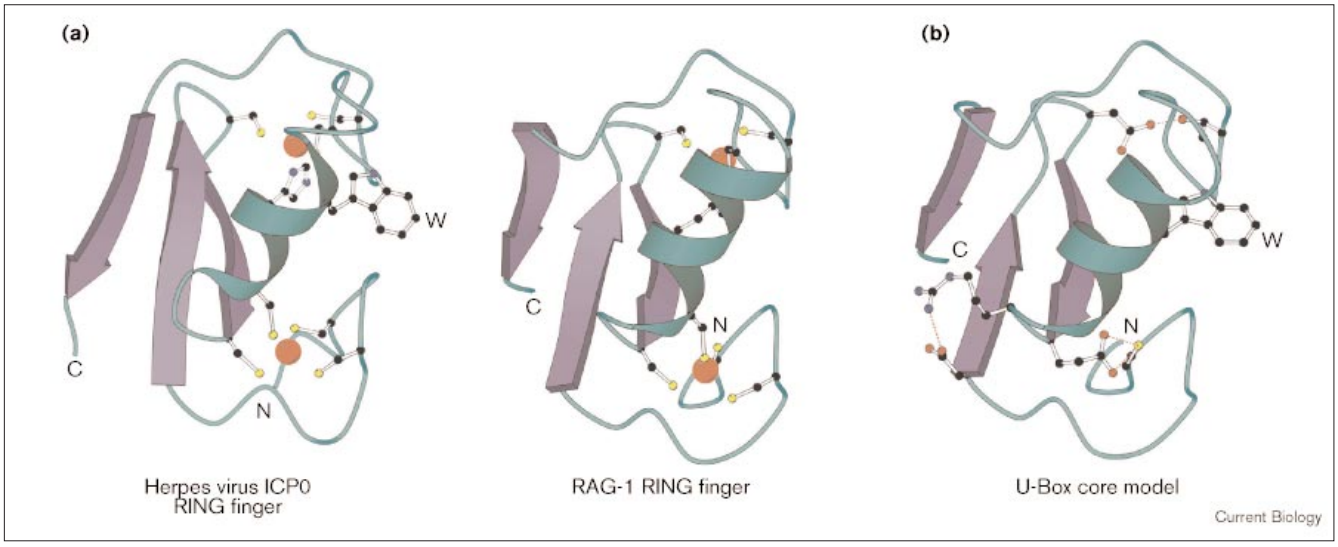
Here, we show by means of sequence-profile analysis that the U box is a derived version of the RING-finger domain that lacks the hallmark metal-chelating residues of the latter [5,6] but is likely to function similarly to the RING-finger in mediating ubiquitin-conjugation of protein substrates. A PSI-BLAST search [7] initiated with the U box of UFD2 recovered, in addition to other U-box proteins, several *bona fide* RING fingers, such as TRAF-6, RAG1 and COP1, within three iterations, with statistically significant expectation (*E*) values in the range 10^{-3}–10^{-5}. Surprisingly, however, most of the signature cysteines of the RING finger are not conserved in the U box. A reverse search with a position-specific weight matrix for the RING fingers against the complete protein sets of *Saccharomyces cerevisiae* and *Caenorhabditis elegans* and partial protein sets of *Schizosaccharomyces pombe* and *Arabidopsis thaliana*

Figure 1



Multiple alignment of U boxes and a selection of RING fingers. The coloring of the conserved positions is based on the 90% aliphatic; s (GASNSTCP), small; p (STNREQHD), polar; minus sign (DE), negatively charged. The colors are: yellow background for hydrophobic, pink for polar and green for small residues. Asterisks indicate the metal-chelating residues that are conserved in the RING-finger domains but partially or completely replaced in the U-box domains. The conserved aromatic position that is predicted to participate in the interactions of the RING finger is indicated with a green background and corresponds to Trp408 in the CBL RING finger. The consensus secondary structure of the structurally characterized RING-finger domains from ICP0 and RAG-1, along with the PHD prediction for the U box are shown above the alignment; H, h or a cylinder denotes α helix and E, e or an arrow indicates β strand;upper case E and H indicate the most confident prediction. The numbers indicate the positions of the aligned regions in the identifier. Species abbreviations: Hs, *Homo sapiens*; Ce, *C. elegans*; Dm, *Drosophila melanogaster*; Dd, *Dictyostelium discoideum*; Sc, *S. cerevisiae*; Sp, *S. pombe*; At, *A. thaliana*; Bn, *Brassica napus*; Bm, *Brugia malayi*; Mm, *Mus musculus*; HSV, Herpes simplex virus. Numbers at the start and end show the position of the domain in the whole protein sequence. UFD2 is the E4 protein, CHIP is a Hsp70-interacting protein, NosA is a protein involved in ubiquitination in *Dictyostelium*, SLARMBP is an ARM-repeat-containing protein, PRP19 is a pre-mRNA splicing factor that, in addition to the U box, contains WD40 β-propeller repeats and CYC4 is a cyclophilin-like peptidyl–prolyl isomerase; the remaining U-box-containing sequences are from functionally uncharacterized proteins.

Figure 2



The structural scaffold of the RING domain. **(a)** Known structures of the indicated protein domains. **(b)** Model of the U-box structure constructed using PROMODII from the structures in (a,b) and the U-box sequence from UFD2. W, Trp residue conserved in RING fingers; N, amino terminus; C, carboxyl terminus.

specifically detected two groups of proteins, the regular RING fingers and the U-box proteins, with significant *E* values and without any false positives. A multiple alignment of the U-box core that showed similarity to RING fingers was generated using the ClustalW program [8] (Figure 1) and used to predict the secondary structure of the U box with the PHD program [9]. The resulting prediction was compatible with the known three-dimensional structures of the RING fingers from RAG1 and herpes virus ICP0 (Figure 1). Two independent methods of structural threading, namely a secondary-structure-based approach implemented in PHD [10] and a profile-based approach implemented in GenomeThreader [11], identified the RING fingers as the best, and statistically significant, hits in a search of the PDB database for structural similarity to the U box (confidence levels of 0.99 and 0.98 for ICP0 and RAG1, respectively). These results strongly support the identification of the U box as a degenerate version of the RING-finger domain.

Examination of the multiple alignment of the U box with a selection of RING fingers shows that, except for the loss of the hallmark cysteines and a histidine, the U box retains the same pattern of amino acid residue conservation (Figure 1). When superimposed upon the structures of the RING fingers, the sequence conservation suggests a similar arrangement of three strands and a helix, as well as characteristic long loops, in both classes of domains (Figures 1,2). A crude homology model for the U box core was constructed using the PROMODII program [12], with the target (the consensus for the U box) aligned with the ICP0 (Protein Databank code 1CHC [13]) and RAG1 (1RMD [14]) structural templates (Figure 2). A striking feature that can be inferred from this model is that, unlike the classic RING finger that is stabilized by Zn^{2+} ions coordinated by the cysteines and a histidine, the U-box scaffold is probably stabilized by a system of salt-bridges and hydrogen bonds (Figure 2). The charged and polar residues that participate in this network of bonds are more strongly conserved in the U-box proteins than in classic RING fingers (Figure 1), which supports their role in maintaining the stability of the U box. Thus, the U box is a remarkable case of appropriation of a new set of residues to stabilize a domain, along with the loss of the original, metal-chelating residues.

The RING-finger domains of two distinct groups of E3 proteins, the HRT1-related proteins [15,16] and CBL [17], mediate specific, E2-dependent ubiquitination of a range of substrates in cell cycle control and signaling. Furthermore, RING fingers from otherwise unrelated proteins, such as BRCA1, SIAH-1, TRC8 and Praja1, activate E2-dependent ubiquitination, which suggests a general role for the RING-finger domain in the ubiquitin system [18]. The E3 proteins containing the RING-finger domain seem not to form thiol conjugates with ubiquitin, but rather allosterically activate E2 to transfer ubiquitin to the substrate lysines [16]. Mutagenesis of Trp408 in CBL (Figure 1) has implicated this residue

in E2 binding [17]. The equivalent position typically contains an aromatic or hydrophobic residue in the RING fingers and particularly in the U box, where it is almost invariably aromatic (Figure 1). The structural models show that this aromatic residue in the RING fingers and in the U-box domain is exposed (Figure 2) and could directly contribute to E2–ubiquitin binding via hydrophobic or aromatic stacking interactions. Thus, the RING finger and the U box in E4 proteins are likely to activate ubiquitination and multi-ubiquitination, respectively, in a similar fashion, namely by facilitating the interaction between E2 proteins and their substrates.

In addition to the previously reported combinations of the U box with other interaction domains [4], we detected proteins with fusions of the U box with the WD40 β-propellers in the splicing factor PRP19 and with a cyclophilin-like peptidyl–prolyl isomerase (Figure 1). In these proteins, the U-box domain could recruit E2 proteins for ubiquitination of pre-mRNA splicing complexes and unfolded proteins associated with the proline-isomerase chaperone, respectively. This latter role is consistent with the association of UFD2 with the AAA ATPase CDC48, which possesses chaperone activity [4], and with the presence of a U box in the HSP70-binding protein CHIP [19] (Figure 1).

These observations show that the RING-finger fold can be maintained even as its hallmark pattern of metal-chelating residues is abolished and that the RING fold is the common structural determinant of both E2-dependent ubiquitination and multi-ubiquitination of proteins. Determination of the U-box structure and analysis of its interaction with E2 will put these predictions to test.

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Binocular rivalry and perceptual coherence

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Different images presented simultaneously, one to each eye, result in an alternating perception of each image, rather than their combination [1]. It has been suggested that such binocular rivalry is mediated by reciprocal inhibition of neurones in separate monocular channels [2]. However, recent single-unit [3,4] (see also reviews [5,6]), psychophysical [7,8] and functional magnetic resonance imaging [9,10] studies suggest that binocular rivalry is resolved high in the visual pathway. Despite this evidence, there is ongoing debate over whether it is the eyes or stimulus representations that rival during binocular rivalry [7,11].

With human observers, Logothetis *et al.* [7] rapidly swapped each eye's presented image at a rate of 3 Hz and demonstrated that this does not induce rapidly changing perceptual alternations but rather, smooth and slow alternations indistinguishable from normal rivalry. This finding challenges eye or monocular-channel interpretations, leading the authors to postulate that it is the stimulus