Defining polyubiquitin chain topology

Nei-Li Chan and Christopher P. Hill

Recent crystal structures suggest models for how an asymmetric E2/E2-like protein complex synthesizes a polyubiquitin chain that functions in DNA repair and NF-κB activation pathways.

Ubiquitin is a small (8.5 kDa) protein that becomes posttranslationally conjugated to other proteins. Ubiquitination rivals phosphorylation in the extent to which it mediates and regulates a host of fundamental cellular processes, including cell cycle progression[1], oncogenesis[2], endocytosis[3] and the production of antigenic peptides[4].

Ubiquitin acts by attaching its C-terminus to lysine side chains of substrate proteins (see refs 5,6 for recent reviews of the ubiquitin pathway). Whereas endocytosis is usually mediated by addition of a single ubiquitin[3], it seems that, in most other cases, the ubiquitin ligated to a target protein is itself ubiquitinated to form polymeric chains[7]. Although all seven of the lysines in ubiquitin are potential sites of attachment to other ubiquitin molecules, the topologies of polyubiquitin chains and their functional roles appear to be strictly defined. For example, chains in which the C-terminus of one ubiquitin is ligated to the Lys 48 side chain of the neighboring ubiquitin target substrates for degradation by the 26S proteasome, whereas chains linked through Lys 63 can serve very different functions, including DNA repair[8], activation of NF-κB[9], polysome stability[10] and endocytosis[11].

The biochemical mechanism by which Lys 63-linked chains mediate these cellular processes is not known, but in all cases it seems to be independent of proteolysis by the proteasome[12]. A significant advance in understanding how a ubiquitin chain linked through Lys 63 is synthesized is now provided by Moraes *et al.*[13] on page 669 of this issue of *Nature Structural Biology*, and by VanDemark *et al.*[14] in *Cell*, who have independently determined the structure of an enzyme complex that specifically catalyzes this reaction. This complex, which consists of two protein subunits, Mms2 and Ubc13, synthesizes chains that function in DNA repair[8] and NF-kB signaling pathways[9]. Surface features of the complex, together with other biochemical data, suggest models for how ubiquitin and substrate bind to the complex, and how the enzyme synthesizes chains of the appropriate topology.

The ubiquitin pathway

The ubiquitin pathway is comprised of many enzymes and proteins that process, activate and attach ubiquitin to substrates[5,6] (box 1). The activated C-terminus of ubiquitin is carried as a thiol ester intermediate by the active site cysteine side chain of an E2 enzyme (also known as ubiquitin-conjugating protein or Ubc). E2s usually function in association with an E3 protein, which often exists as a multisubunit complex and performs the primary role in substrate selection. In this way, an E2 enzyme could participate in regulating multiple cellular processes; its exact target is determined by the associated partner protein. There are two main classes of E3 enzymes; HECT domain proteins that participate directly in catalysis by forming a thiol ester intermediate with ubiquitin, and RING domain proteins that function as molecular scaffolds to promote ubiquitination by appropriately orienting the E2–ubiquitin and substrate for catalysis.

Most purified E2s appear to be monomeric, and the available crystal structures of E3 complexes reveal association with a single E2 molecule[15,16]. Nevertheless, there is evidence that some E2s form functional homodimeric[17] or heterodimeric[18] interactions. Recent work has shown that the E2 enzyme Ubc13 functions in complex with a ubiquitin E2 variant (UEV) protein called Mms2 (ref. 8). (UEVs share significant sequence similarity with E2s but lack the active site cysteine of authentic E2 enzymes[19,20]). The Ubc13–Mms2 complex from yeast participates in a DNA repair pathway by catalyzing the synthesis of Lys 63-linked polyubiquitin[8]. The homologous human complex likely — but is currently unproven — also functions in DNA repair, while it has been established that the biochemically equivalent complex of human Ubc13 and Uev1a (a close homolog of Mms2) functions in a NF-κB activation pathway[9]. Interestingly, the ability of yeast Ubc13–Mms2 to synthesize Lys 63-linked polyubiquitin chains *in vitro* does not require an E3 (ref. 8), although the efficiency of chain assembly by the human Ubc13–Uev1a complex is greatly enhanced by the associated RING E3 protein Traf6 (ref. 9).

Ubc13–Mms2 structure

Efforts to understand the basis for Ubc13–Mms2 activity have received a major boost in the form of two crystal structure determinations. VanDemark *et al.*[14] recently reported the 1.6 Å resolution structure of the yeast Ubc13–Mms2 complex, while in this issue of *Nature Structural Biology*, Moraes *et al.*[13] describe the structure of the homologous complex from human at 1.85 Å resolution. As expected from the high (~60%) sequence similarity between yeast and human proteins, and the ~100% conservation of interface residues, the structures of the yeast and human complexes are essentially identical. Furthermore, Ubc13 closely resembles other known E2 structures, and Mms2 also adopts an E2-like fold but with some differences toward the N and C-termini. In addition to the complexes, structures are also reported for the isolated Ubc13 (ref. 14) and Mms2 (ref. 13) proteins at similarly high resolution. A comparison of these structures reveals that conformational changes induced by complex formation do not have obvious functional significance, and are limited to an N-terminal segment of Mms2 that becomes buried in the dimer interface[13].

The vast majority of heterodimeric structures in which the two protomers adopt closely related folds possess approximate two-fold rotational symmetry (examples include the transcription factors fos–jun[21] and NF-κB p65–p50 (ref. 22)). This is not, however, the case for Ubc13–Mms2, which forms a T-shaped complex in which one end of Mms2 is packed against the side of Ubc13 to bury ~1,500 Å^2 of solvent accessible surface area. This is indeed the relevant interaction because, as shown by VanDemark *et al.*[14], mutation of residues buried at the interface (Fig. 2*a*) destabilizes complex formation and elicits a phenotype equivalent to deletion of Ubc13 and Mms2 genes in yeast. An implication of the structure is that Mms2 will not bind to other active E2 enzymes, since, although residues