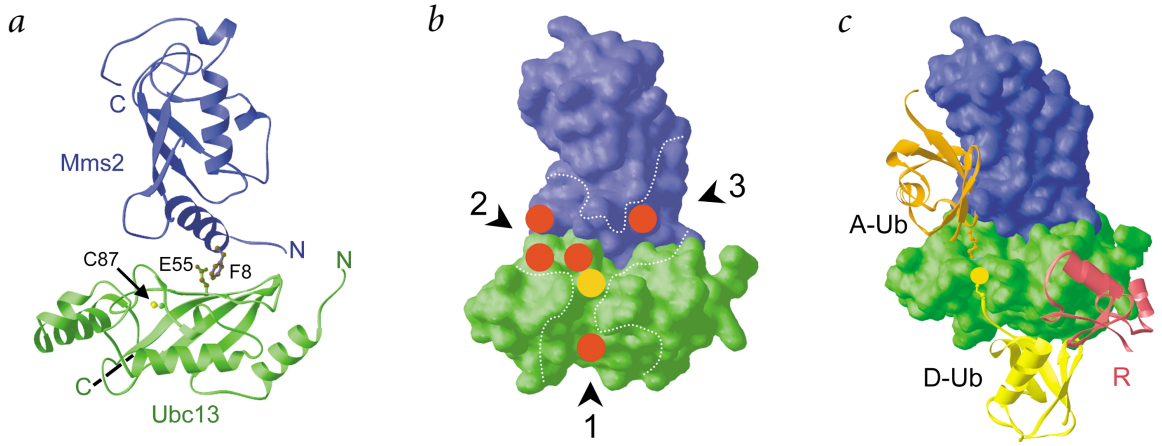

Fig. 2 Structure of the Ubc13–Mms2 complex. This figure was made using coordinates and approximate color scheme of the yeast structure[14]. The view orientation is similar to that shown for the human complex[13]. ***a***, Ribbon diagram. The active site cysteine (Cys 87) of Ubc13 is drawn with a yellow sulfur atom and indicated with an arrow. Importance of the crystallographic interface was demonstrated by mutation of Mms2 Phe 8 and Ubc13 Glu 55[14]. ***b***, Molecular surface representation. Ubc13 Cys 87 is indicated with a yellow dot. Three channels that lead towards Cys 87 are delineated with white dotted lines and are indicated with arrowheads and numbered according to VanDemark *et al.*[14]. Mutants used to test the binding surfaces for donor and acceptor ubiquitin are indicated with red dots[14]. The only channel 2 mutant reported in the original publication[14] is indicated by the dot closest to Ubc13 Cys 87. In subsequent work, similar effects have been observed for the other two mutants (Mms2 S33A and Ubc13 D124A) indicated on this figure (C. Pickart, pers. comm.). ***c***, Proposed location of binding partners. VanDemark *et al.*[14] proposed the approximate location of donor and acceptor ubiquitins (D-Ub and A-Ub) on the basis of mutagenesis and modeling. The donor ubiquitin C-terminus is bound in a thiol ester with the active site cysteine residue of Ubc13, while Lys 63 of the acceptor ubiquitin is extended toward the active site. The binding site for the RING domain E3 protein (R) was inferred from analogy to the structure of a complex between c-Cbl and UbcH7[15]. Moraes *et al.*[13] agree with the placement of the donor ubiquitin and the RING domain, but are less specific about the acceptor ubiquitin site. This figure was prepared using the program RIBBONS[35].

Concluding remarks

The Ubc13–Mms2 crystal structures[13,14] provide a framework for deciphering the various interactions that define specificity for synthesis of Lys 63-linked polyubiquitin and ubiquitination of the RING protein substrate. There are still, however, some major unanswered questions. For example, although models are being developed for how ubiquitin and the RING domain protein bind to Ubc13–Mms2, reliable and precise models will require determination of relevant complex structures. Other outstanding questions concern the mechanism by which binding of the RING protein enhances the efficiency of chain formation and how relevant portions of Traf6 are orientated for ubiquitin conjugation. Even more fundamentally, the basic mechanism of catalysis still remains a mystery, since the E2 active site seems to lack a base to deprotonate the attacking lysine amine and groups that might stabilize the developing negative charge on the C-terminal carbonyl oxygen of ubiquitin[6]. Additional interesting subjects for further study include other UEV proteins, such as Vps23/Tsg101, which has been implicated in a variety of cellular functions including intracellular trafficking[27], and the assembly and release of retrovirus particles[28]. It has been suggested that Vps23/Tsg101 serves as a ubiquitin-binding module[29,30], although it is not known if the mode of this association resembles that of Mms2, or if Vps23/Tsg101 binds to an active E2 partner. Finally, although the E2 protein Cdc34 is dimeric[17] and the E2s Ubc6 and Ubc7 can associate with each other[18], it is not generally known if homo- or heterodimerization of E2 proteins is important for their function, or if these interactions resemble the unusual asymmetric structure of Ubc13–Mms2. Thus, although progress is being made, much more genetic, biochemical, cell biological, and structural work is needed before we can really understand the important and fascinating pathways of posttranslational modification by ubiquitination.

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