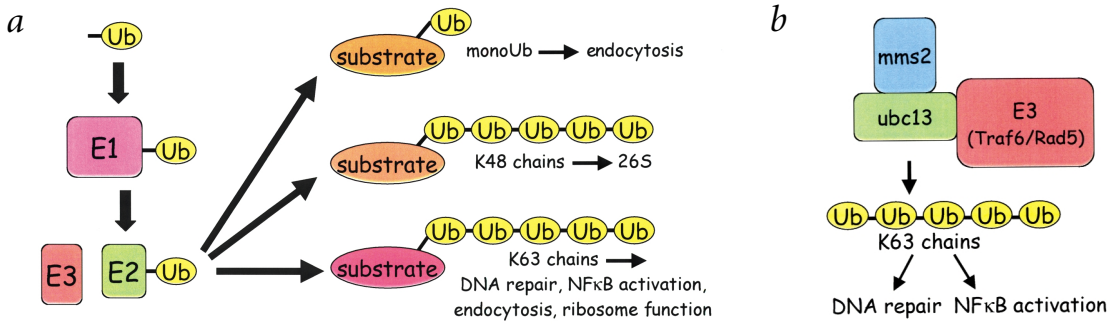
Box 1 Ubiquitin pathway of posttranslational modification

a, Following activation by an ATP-dependent E1 enzyme, the C-terminus of ubiquitin is attached by a thiol ester bond to the active site cysteine residue of an E2 enzyme. In a step that usually requires the assistance of an E3 enzyme, the ubiquitin is then ligated to a target protein by an isopeptide bond between its C-terminus and a lysine side chain of the substrate. Monoubiquitination can target substrate proteins, such as ligand-bound receptors in the plasma membrane, for endocytosis and eventual degradation in lysozomes/vacuoles[3]. Elaboration of a polyubiquitin chain that is linked through Lys 48 residues serves to target substrates to the 26S proteasome for degradation, a process that mediates many of the best characterized roles of the ubiquitin system[7]. In contrast, polyubiquitin chains linked through Lys 63 residues can serve as modifiers of function by mechanisms whose biochemistry is not understood but do not result in an increased rate of proteasomal degradation[8–12]. The scheme outlined here is highly simplified. In yeast, 11 E2s and potentially >50 E3s have been identified. Further complexity and regulation is provided by the presence of chain elongation factors[31], ubiquitin binding proteins[32,33], and 17 deubiquitinating enzymes[34].



b, The E2 enzyme Ubc13 forms a complex with Mms2 (or the homolog Uev1a) that synthesizes Lys 63-linked polyubiquitin chains that function, without proteolysis, in DNA repair[8] and NF-κB activation pathways[9]. The ability of Ubc13-Mms2 to synthesize Lys 63-linked chains can be enhanced by association with a RING E3 enzyme[9] and, in at least one instance, the associated E3 protein is itself the substrate for conjugation to ubiquitin[26].

buried at the interface are conserved between yeast and human Ubc13, they are not conserved in other E2s.

Interactions with ubiquitin

In order to catalyze synthesis of polyubiquitin chains, the Ubc13–Mms2 complex must bind two different ubiquitin molecules in distinct ways. The C-terminus of the donor ubiquitin must attach *via* a thiol ester to the Ubc13 cysteine, while the acceptor ubiquitin must be oriented with its Lys 63 side chain in close proximity. Three channels on the surface of the protein complex lead toward the active site cysteine of Ubc13 and appear to be topologically suitable for binding to ubiquitin (Fig. 2*b*). Both Moraes *et al.*[13] and VanDemark *et al.*[14] propose that channel 1, which is distant from Mms2 and is comprised exclusively of Ubc13 residues, forms the donor-binding site (Fig. 2*c*). Moraes *et al.*[13] base this proposal on published NMR experiments with other E2 enzymes[23,24], which showed that ubiquitin induced chemical shift changes in residues that are either in channel 1 or immediately adjacent to the active site cysteine. VanDemark *et al.*[14] provide further support for this model by characterizing an Ala to Arg mutation that was designed to disrupt contacts in channel 1. This mutation results in a four-fold reduction in the rate of isopeptide bond formation but no alteration in the rate of thiol ester bond formation, heterodimer stability, or K_m for the acceptor ubiquitin. These observations imply that this mutation impedes optimal orientation of the donor ubiquitin for reaction with Lys 63 of the acceptor ubiquitin, although the retention of significant activity indicates that interactions with this residue are not absolutely critical. Indeed, given the inherent flexibility of ubiquitin C-terminal residues and the high mobility observed for ubiquitin when bound in a covalently linked E2 ester complex[23], it seems possible that restrictions on the orientation of the folded domain of the donor ubiquitin may be relatively lax.

VanDemark *et al.*[14] and Moraes *et al.*[13] note that channels 2 and 3 are formed by residues from both Ubc13 and Mms2 (Fig. 2*b*), and therefore appear suited to defining a function, such as binding to the acceptor ubiquitin, that is specific to this complex. VanDemark *et al.*[14] conclude that the acceptor ubiquitin binds in channel 2 (Fig. 2*c*), because they find that mutations of residues in channel 2 result in impaired chain assembly without any measurable effect on heterodimer formation or E2–ubiquitin thiol ester formation. In contrast, mutation of a residue in channel 3 has no measurable effect on steady state chain assembly. The site of channel 2 for acceptor binding is further supported by competition binding studies using the mutant proteins[14].

It had been previously established that association with the human E3 protein Traf6, which belongs to the RING family of E3 enzymes, greatly enhances the activity of the closely related human Ubc13–Uev1a complex to catalyze formation of Lys 63-linked polyubiquitin[9]. Yeast two-hybrid analysis[25] indicated that Rad5 (yeast homolog of Traf6) binds directly to Ubc13 rather than Mms2, and a model for the interaction between Ubc13 and the RING domain of its associated E3 is provided by comparison with the known structure of an analogous E2-RING complex[15]. As shown in Fig. 2*c*, these observations indicate that the E3 protein RING domain will bind close to channel 3. Moraes *et al.*[13] therefore cautiously favor channel 3 as the binding site for the acceptor ubiquitin, although in light of the mutagenesis data from VanDemark *et al.*[14], this proposal now seems less likely. VanDemark *et al.*[14] alternatively suggested that channel 3 may be the binding site for the protein substrate to which Lys 63 polyubiquitin chains are attached. This speculation seems even more attractive in light of a very recent publication, which indicates that the Traf6 E3 protein is itself the primary substrate of ubiquitination by Ubc13–Uev1a (ref. 26).

The work discussed here contributes to an emerging view of ubiquitin pathways in which the classical distinction between E2, E3, and substrate proteins is becoming blurred. For example, Traf6, like a number of other RING domain E3 proteins[2], is also a substrate that becomes conjugated to ubiquitin, and one could argue that the E2-like protein Mms2 is really an E3 enzyme since it is required for the E2 protein Ubc13 to ubiquitinate its substrate. The important point is that ubiquitination reactions are often performed by multisubunit complexes in which each component plays an integral role.