The Tail of a Ubiquitin-conjugating Enzyme Redirects Multi-ubiquitin Chain Synthesis from the Lysine 48-linked Configuration to a Novel Nonlysine-linked Form*

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The UBC1 ubiquitin-conjugating enzyme from Saccharomyces cerevisiae has an overlapping function with the UBC4 and UBC5 enzymes in the yeast stress response and an important role in the G0 to G1 transition that accompanies spore germination (Seufert, W., McGrath, J. P., and Jentsch, S. (1990) EMBO J. 9, 4573–4541). In the present work we report that the UBC1 enzyme assembles onto itself a multi-ubiquitin chain in vitro whose linkage configuration is dependent on the unconserved carboxyl-terminal extension or tail that is appended to its catalytic domain. Using chemical cleavage and site-specific mutagenesis, we have mapped the location of the chain to lysine 93 which lies near the active site within the catalytic domain. The ubiquitin molecule that anchors the chain is transferred to this lysine from the active site of the same UBC1 molecule. When the tail of UBC1 is deleted, the catalytic domain synthesizes a chain that consists of ubiquitin molecules uniformly linked to one another via lysine 48. In the presence of the tail, however, a chain is assembled that is composed of linkages that are stable to alkali but which do not utilize lysines. Furthermore, when the amino terminus of ubiquitin is blocked by an appended peptide tag, chain assembly reverts from this alternative configuration to the canonical lysine 48 variety. Taken together, these results suggest that the alternative chain is composed of linkages in which one ubiquitin molecule forms a peptide bond with the α-amino terminus of another, thereby supporting the emerging view that Ub can be attached to itself or other proteins in a variety of ways.

Unlike most modifications that occur to proteins post-translationally, protein ubiquitination is complicated by the fact that ubiquitin (Ub) can be covalently linked to itself forming multi-Ub chains. Unquestionably the most prevalent and best characterized example of this phenomenon is the lysine 48 chain configuration in which each Ub molecule within the chain is tandemly linked to the next by an isopeptide bond that connects the carboxyl terminus of one Ub molecule with Lys-48 of another. This chain was first discovered, characterized, and shown to facilitate Ub-dependent proteolysis by using damaged or artificial protein substrates (Chau et al., 1988; Gonda et al., 1989; Gregori et al., 1990; Hershko et al., 1980) but was later shown to facilitate the degradation of naturally short-lived proteins as well (see for example, Hochstrasser et al. (1991)). The Lys-48 chain apparently functions in this pathway by increasing the avidity of the targeted protein for the 26 S proteolytic complex (Deveraux et al., 1994).

More recently, alternative chain configurations have been identified that use other lysines as linkage points. In earlier work, we reported that one Ub molecule can be linked to another at either lysine 29 (Lys-29) or lysine 63 (Lys-63) in yeast (Arnason and Ellison, 1994). The formation of either linkage is dependent on the stress related Ub-conjugating enzymes, UBC4 and UBC5. Furthermore, failure to form the Lys-63 linkage is correlated with extreme stress sensitivity. Taken together, these results suggested a role for Lys-63 linked Ub in stress resistance. In other work Spence et al. (1995) have pointed to a role for the Lys-63 chain configuration in the process of DNA repair. Johnson et al. (1995) have recently reported that the Lys-29 configuration is responsible for the rapid turnover of the Ub-pro-β-galactosidase test substrate in yeast. Finally, recent in vitro evidence suggests that multi-Ub chains can be formed in which Ub moieties are linked through either lysines 6 or Lys-11 (Baboshina and Haas, 1996). These examples illustrate that the multi-Ub chain can take on a number of structurally and functionally distinct guises.

Here we report that the yeast Ub-conjugating enzyme, UBC1 exhibits several curious characteristics with respect to multi-Ub chain assembly. UBC1 normally functions in conferring viability and stress resistance to yeast in combination with UBC4 and UBC5 (Seufert et al., 1990; Seufert and Jentsch, 1990). In addition, it has a unique role at the early stages of spore germination. UBC1 consists of the conserved catalytic domain that is characteristic of other Ub-conjugating enzymes and an unconserved COOH-terminal tail that is 75 residues long (Seufert et al., 1990). In the present work we show that this tail serves to switch the mode of multi-Ub chain assembly from the Lys-48 type to a structure that utilizes a nonlysine linkage.

EXPERIMENTAL PROCEDURES

Plasmids—All Escherichia coli expression plasmids carrying the yeast Ub and UBC1 derivatives shown in Fig. 1 are based on the pET-3a expression system (Studier et al., 1990). UBC1 and UBC1a expression plasmids are identical to the CDC34 expression plasmid previously described (Ptak et al., 1994) except that the appropriate coding se-
sequence for each E2 replaces the coding sequence of CDC34. This replacement is carried out in the context of a construct containing the coding sequence of all UBC1 derivatives described here. This substitution has no detectable effect on the function of UBC1 or any of its derivatives as judged by their ability to substitute for the stress functions of UBC4 and UBC5 in a strain of yeast deleted for the UBC4 and UBC5 genes (results not shown). The UBC1Δ expression plasmid is identical to the UBC1 plasmid except that codons 151 to 215 encoding the unconserved carboxyl-terminal tail have been deleted. UBC1R93 and UBC1R93 expression plasmids each contain the single Lys to Arg codon (AGG) substitution at position 93 of the UBC1 peptide sequence. Coding sequence DNA used in the construction of the expression plasmids described above was generated from yeast genomic DNA using the polymerase chain reaction and appropriately selected synthetic DNA primers.

Expression plasmids for each of the Ub derivatives illustrated in Fig. 1 are identical in sequence to the CDC34 expression plasmid described elsewhere (Ptak et al., 1994) with two exceptions. First, the coding sequence for each Ub derivative precisely replaces the entire coding sequence of CDC34. Second, the parental pET3a plasmid in which their construction lacks the BglII site upstream of the T7 promoter which was eliminated by filling in the BglII generated ends followed by blunt-end ligation. The coding portion of each Ub gene derivative is identical to yeast plasmid counterparts that have been described elsewhere (Hodgins et al., 1992). For the production of unlabeled and [35S]Met-labeled protein, cell extracts were precipitated using the chloroform-methanol extraction procedure of Wessel and Flügge (1984). For cyagen bromide cleavage, precipitated samples were resuspended in 150 μl of a freshly prepared solution containing 120 mM cyagen bromide (Eastman Kodak) in 70% formic acid. Samples were then agitated for 16 h at room temperature, dried down using a Savant Speed-Vac, resuspended in 150 μl of water and dried again to remove residual cyagen bromide. Samples were resuspended in SDS-loading buffer followed by SDS-PAGE and autoradiography as described above.

For BNPS-Skatole cleavage, precipitated samples were solubilized in 100 μl of freshly prepared solution containing 50 mM BNPS-Skatole (Sigma) in 75% glacial acetic acid, followed by incubation at room temperature for 24 h. After incubation, samples were processed for SDS-PAGE as described above.

For 2-nitro-5-thiocyanobenzoic acid cleavage (NTCB), precipitated samples were solubilized in 100 μl of a buffer containing 0.6 mM guanidine-HCl, 0.2 mM Tris acetate, pH 8.0, and 5 mM DTT, followed by a 2-h incubation at 37°C. 10 μl of NTCB was then added to each sample as described above. Following incubation for 2 h, samples were precipitated with cold trifluoroacetic acid (15% final) and resuspended in 50 μl of a freshly prepared solution containing 100 μM NTCB (Sigma) in 100% ethanol. Samples were incubated for 15 min at 37°C. Following the addition of 2 volumes of glacial acetic acid on ice, samples were dried down using a Savant Speed-Vac, resuspended in 150 μl of water and dried again to remove residual cyagen bromide. Samples were then resuspended in SDS-‐loading buffer followed by SDS-PAGE and autoradiography as described above.

RESULTS

Ub-conjugating enzymes, or E2s, are primarily recognized by their ability to transfer Ub from the Ub activating enzyme, E1, to the lysine residues of other proteins. From Fig. 2, however, it is apparent that radiolabeled UBC1, and a derivative radiolabeled of UBC1 that lacks the COOH-terminal tail (UBC1Δ) are capable of multi-ubiquitinating themselves in the presence of purified E1 and Ub. In principle, a Ub-conjugating enzyme (E2) that multi-ubiquitinates itself in vitro presents a simple model for mechanistic studies since it requires neither a target protein or Ub-protein ligase (E3). From a mechanistic standpoint, the auto-ubiquitination of the UBC1-conjugating enzyme and its tail deleted derivative is of particular interest since the pattern of multi-ubiquitination observed for either protein is dependent on the presence or absence of the tail. UBC1Δ is ubiquitinated with wild-type Ub (wt), for example (Fig. 7). UBC1Δ and UBC1ΔR93 eluted from the column with an apparent molecular mass of 20 kDa. Multi-ubiquitin Chains

Ubiquitination Reactions—Ub-conjugating enzymes, or E2s, are primarily recognized by their ability to transfer Ub from the Ub activating enzyme, E1, to the lysine residues of other proteins. From Fig. 2, however, it is apparent that radiolabeled UBC1, and a derivative radiolabeled of UBC1 that lacks the COOH-terminal tail (UBC1Δ) are capable of multi-ubiquitinating themselves in the presence of purified E1 and Ub. In principle, a Ub-conjugating enzyme (E2) that multi-ubiquitinates itself in vitro presents a simple model for mechanistic studies since it requires neither a target protein or Ub-protein ligase (E3). From a mechanistic standpoint, the auto-ubiquitination of the UBC1-conjugating enzyme and its tail deleted derivative is of particular interest since the pattern of multi-ubiquitination observed for either protein is dependent on the presence or absence of the tail. UBC1Δ is ubiquitinated with wild-type Ub (wt), for example (Fig. 7). UBC1Δ and UBC1ΔR93 eluted from the column with an apparent molecular mass of 20 kDa.
formation of multi-Ub chains in which successive Ub molecules
within the chain are tandemly linked to one another at Lys-48
(Chen and Pickart, 1990; Haas et al., 1991). By comparison,
full-length UBC1 ubiquitinated with wild-type Ub exists as an
irregular spaced distribution of bands that terminate abruptly
with the addition of either three or four Ub molecules (Fig. 2,
UBC1 panel, wt lane). Thus, the pattern of multi-ubiquitina-
tion differs between UBC1 and UBC1Δ and is therefore
strongly influenced by the UBC1 tail.

In structural terms, the multi-ubiquitination patterns ob-
served for UBC1 and UBC1Δ are likely to reflect one or a
combination of three processes: 1) multi-Ub chain assembly of
the Lys-48 variety onto a single lysine of UBC1 or UBC1Δ. 2) Multi-Ub chain assembly involving alternative types of Ub-Ub
linkages or 3) ubiquitination of multiple lysines scattered
throughout UBC1 or UBC1Δ. To discriminate between these
possibilities, five bacterially expressed Ub derivatives (Fig. 1B)
were used in conjugation reactions with either UBC1 or
UBC1Δ. UbR48 for example, lacks the linkage lysine necessary
for Lys-48 multi-Ub chain assembly. When UbR48 is used to
ubiquitinate UBC1Δ, the regular ladder of UBC1Δ conjugates
observed for wt Ub disappears and is replaced with an irregu-
larly spaced pattern of conjugates corresponding to the addi-
tion of one to several Ub molecules (Fig. 2, UBC1Δ panel,
R48 lane). Furthermore, this pattern of conjugates persists even
when a Ub derivative lacking all seven lysines (UbK0) is used in
UBC1Δ conjugation reactions (Fig. 2, UBC1Δ panel, K48 lane)
whereas reintroduction of a single lysine at position 48
(UbK48) completely restores the conjugation pattern observed
using wt Ub. Three of the four multimeric UBC1Δ-Ub conju-
gate bands that are found in UbR48 and UbK0 lanes are absent
when Lys-48 is made available for conjugation (wt Ub and
UbK48 lanes). From these results it can be concluded that the
linkage of Ub to Ub at Lys-48 occurs at the expense of these
other UBC1Δ conjugates and that therefore the UBC1Δ conju-
gates observed in wt Ub and UbK48 lanes result predominantly
and probably exclusively from multi-Ub chain formation using
a Lys-48-linked configuration.

Given the possibility that the Lys-48 independent pattern of

**FIG. 1.** UBC1 and ubiquitin constructs. Shown are diagrammatic
representations of the peptide sequences for the bacterially expressed
derivatives of UBC1 (A) and Ub (B). Lysine to arginine substitutions
and cysteine to alanine substitutions are indicated by amino acid posi-
tion. For UBC1, the catalytic domain is represented as a black box while
the carboxyl-terminal tail is represented as a white box. Similarly, the
peptide sequence of Ub is indicated in black and the amino-terminal
myc tag (m) is indicated in white. wt refers to the wild-type sequence of
yeast Ub.

**FIG. 2.** Multi-ubiquitination of UBC1 and UBC1Δ. Purified [35S]Met-labeled UBC1 or UBC1Δ were incubated with E1 and the various
purified Ub derivatives described in the legend to Fig. 1 (see also “Experimental Procedures”). Following incubation, aliquots of each reaction were
electrophoresed and gels were visualized by autoradiography. UBC and UBCΔ mark the positions of unconjugated UBC1 and UBC1Δ, respectively.
Arrows highlight the distribution of multi-ubiquitinated UBC1 and UBC1Δ conjugates.
The fact that the alternative chain is not linked at a lysine or the catalytic domain at a single position. Furthermore, the strong requirement of this alternative chain for the native NH2 terminus of Ub suggests that at least some of these species involve Ub molecules linked to another through the NH2 terminus. The multi-Ub chains of UBC1 and UBC1Δ are anchored at a common lysine. The single attachment site of Ub to either UBC1 or UBC1Δ was inferred from the above argument was mapped using three peptide cleavage reagents: CNBr (cleavage at Met), BNPS-Skatole (cleavage at Trp), and NTCB (cleavage at Cys). Fig. 3A shows the expected fragments of UBC1 or UBC1Δ produced by cleavage with each reagent and the distribution of lysines within each fragment. To simplify the electrophoretic analysis, conjugates of UBC1 or UBC1Δ are indicated at the extreme left. UBC1 or UBC1Δ multi-ubiquitination arises all or in part from the linkage of Ub to Ub, then it is clear that the UbK9 example that these alternative linkages cannot occur at any of the seven lysines contained within Ub. Furthermore, all of these conjugates are completely stable to alkaline hydrolysis, thereby ruling out the involvement of Ub-Ub linkages formed at any of the other nucleophilic side chains found in Ub, including threonine, serine, tyrosine, or histidine (results not shown). Neither of these experiments, however, address the possibility of linking Ub molecules by peptide bond formation at the α-amino terminus.

To test the possibility that this alternative pattern of multi-ubiquitination may reflect the linkage of Ub to the α-amino group of Ub, we predicted that blockage of the native NH2 terminus of Ub by the addition of an epitope tag (the myc epitope) should eliminate the alternative pattern of multi-ubiquitination observed for UBC1Δ. Using myc-Ub in a UBC1Δ conjugation reaction results in the regularly spaced ladder of bands that is characteristic of Lys-48 chain assembly, whereas mycUbR48 conjugation results in the complete disappearance of all multi-ubiquitinated species leaving only the singly ubiquitinated form of UBC1.

From these and the UBC1Δ results, we conclude that the tail of UBC1 interferes with Lys-48 chain assembly and that this interference is circumvented by the addition of the amino-terminal tag to Ub. It is also clear from these results that the irregular pattern of UBC1 multi-ubiquitination holds several properties in common with the irregular pattern produced with UBC1Δ when Ub derivatives are used that lack Lys-48. First, the irregular pattern persists for both UBC1 and UBC1Δ using a Ub derivative devoid of lysines (UbK0), second, multi-ubiquitination of either UBC1 or UBC1Δ is eliminated in the presence of mycUbR48. Taken together, these two observations argue that the irregular pattern reflects the assembly of an alternatively linked multi-Ub chain that is linked to the UBC1 catalytic domain at a single position. Furthermore, the strong requirement of this alternative chain for the native NH2 terminus of Ub suggests that at least some of these species involve Ub molecules linked to another through the NH2 terminus. The multi-Ub chains of UBC1 and UBC1Δ are anchored at a common lysine. The single attachment site of Ub to either UBC1 or UBC1Δ that was inferred from the above argument was mapped using three peptide cleavage reagents: CNBr (cleavage at Met), BNPS-Skatole (cleavage at Trp), and NTCB (cleavage at Cys).
were produced using radiolabeled UbR48 and unlabeled E2 under conditions that limited conjugate formation to predominantly the mono-ubiquitinated form (“Experimental Procedures”). Since neither UBC1 nor UBC1Δ are radiolabeled, only fragments coupled to Ub will be detected following autoradiography. Furthermore, since Ub is not significantly cleaved by any of the reagents used here, each detectable fragment will have a molecular mass that includes the molecular mass of Ub (8.3 kDa).

The cleavage patterns of UBC1 and UBC1Δ are shown in Fig. 3B. Cleavage of mono-ubiquitinated UBC1 or UBC1Δ with CNBr produces a major ubiquitinated fragment in each case. The estimated size of these fragments (24 kDa for UBC1 and 16 kDa for UBC1Δ) indicates that in each case, Ub resides on the large COOH-terminal fragment of either UBC1 or UBC1Δ. This observation immediately eliminates the first seven lysines in the UBC1 sequence as candidates for ubiquitination.

Cleavage of UBC1 and UBC1Δ with BNPS-Skatole is incomplete; however, the presence of a ubiquitinated fragment estimated at 20 kDa that is common to both UBC1 and UBC1Δ indicates that ubiquitination is confined to the NH2-terminal fragment. This observation coupled with the CNBr results localizes the site of ubiquitination in either UBC1 or UBC1Δ within a 27-residue stretch containing lysines 74 and 93.

The active site cysteine of UBC1 (C88) is situated between Lys-74 and Lys-93, therefore cleavage at this position with NTCB can be used to distinguish between the possibility that Ub resides on either of these two lysines. Cleavage of UBC1Δ with NTCB gives rise to a labeled major product with an estimated molecular mass of 22 kDa, whereas cleavage of UBC1Δ with NTCB gives rise to a major labeled product of approximately 14 kDa. This result combined with the previous cleavage results strongly suggests that Lys-93 is the Ub attachment point for both UBC1 and UBC1Δ.

To demonstrate that Lys-93 serves as the sole attachment point of chain assembly for UBC1 and UBC1Δ, derivatives of UBC1 and UBC1Δ in which Lys-93 was mutated to Arg were constructed. These derivatives were radiolabeled and tested for ubiquitination using either wild-type Ub or UbR48 (Fig. 4). For UBC1Δ, the K93R substitution entirely eliminates conjugation to wild-type Ub or UbR48. In the case of UBC1, the K93R substitution eliminates the conjugation pattern produced with either wild-type Ub or UbR48 but results in the appearance of a minor mono-ubiquitination species. Based on the absence of this species in UBC1ΔR93 lanes, we suspect that this species reflects ubiquitination at a site within the UBC1 tail that becomes more favored in the absence of Lys-93 ubiquitination. These results in combination with the chemical mapping analysis lead to the conclusion that the multi-Ub chains formed on either UBC1 or UBC1Δ are anchored to Lys-93 regardless of linkage type.

An examination of the crystallographic structure of the yeast UBC4-conjugating enzyme (Cook et al., 1993), suggested to us that the Ub molecule that anchors the chain is conjugated to Lys-93 directly from the active site of the same UBC1 molecule. Although the structure of UBC1 has not yet been determined its catalytic domain is 48% identical to UBC4 and can substitute for all UBC4 related functions (Seufert et al., 1990). In addition, UBC4 contains the structural analogue of Lys-93 (Lys-91). From Fig. 5 it is evident that this lysine is proximal to the active site cysteine, thereby raising the possibility that a Ub molecule that is linked via a thiol ester to this cysteine can be directly transferred to the ε-amino group of Lys-93 through nucleophilic attack. However, an alternative possibility is that the Ub is transferred intermolecularly to Lys-93 from the active site of another UBC1 molecule. To distinguish between these two possibilities we first constructed UBC1 and UBC1Δ derivatives in which the active site cysteine was replaced with alanine (UBC1A88 and UBC1ΔA88). We then tested whether or not [35S]Met-labeled UBC1A88 or UBC1ΔA88 could be ubiquitinated in the presence of their unlabeled wild-type counterparts. If ubiquitination of Lys-93 occurred via an intermolecular reaction with another UBC1 molecule, then the formation of radiolabeled conjugate would be dependent on the addition of unlabeled wild-type E2. If on the other hand Ub was transferred from the active site to Lys-93 of the same molecule, then radiolabeled conjugate would be absent under any circumstances. The results of Fig. 6 argue in favor of this last prediction. Therefore for both UBC1 and UBC1Δ the Ub molecule attached to Lys-93 is derived from the active site of the same molecule.

DISCUSSION

Prior to the present study there have been several reports that the assembly of Lys-48 type multi-Ub chains can be achieved in vitro with as few as three components: E1, E2, and Ub (Banerjee et al., 1993; Chen and Pickart, 1990; Haas et al., 1991; van Nocker and Vierstra, 1991). Thus, while E3s and target substrates may well facilitate chain assembly, they are not an absolute requirement in every situation. 

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2 C. Gwozd and M. J. Ellison, unpublished data.
By comparison to these earlier examples, the tail-dependent behavior of UBC1 is unique. Here, the COOH-terminal tail of UBC1 directs the assembly of an alternative chain that reverts to the Lys-48 variety upon its removal. In general, the tails of E2s are highly variable in sequence and have been connected to a host of different functions that include target recognition (Jentsch et al., 1987), E3 recognition (Dohmen et al., 1991), facilitators of E2 dimerization (Ptak et al., 1994; Silver et al., 1992), membrane anchors (Sommer and Jentsch, 1993), and determinants in autoregulation (Banerjee et al., 1993). The observation that an E2 tail can redirect the configuration of the multi-Ub chain provides yet another function for these domains and underscores their diverse evolutionary origins.

The behavior of UBC1 is best explained in terms of a competition between two mechanistically distinct pathways for chain assembly. Removal of the UBC1 tail favors the pathway that gives rise to the Lys-48 configuration. Under these circumstances the alternative pathway only emerges when linkage at lysine 48 is prevented (Fig. 2). Conversely, the presence of the UBC1 tail favors the alternative pathway. In this situation, the Lys-48 pathway only emerges when the native amino terminus of Ub is blocked. Based on these observations, we conclude that the mechanistic potential for either pathway resides within the catalytic domain of UBC1 and that the tail of UBC1 functions subtly to give the alternative pathway the competitive edge.

Notably, multi-Ub chain assembly (regardless of configuration) is not a universal property of E2s, despite the fact that the catalytic domain is characteristic of all E2s and exhibits strong structural conservation between proteins of unrelated function (Cook et al., 1992, 1993). The catalytic domain of UBC1, for example, is 48% identical to the yeast UBC4 enzyme and can substitute for loss of UBC4 function (Seufert et al., 1990). Like UBC1, UBC4 undergoes auto-ubiquitination in vitro at a lysine that is structurally equivalent to Lys-93 of UBC1 (results not shown). Unlike UBC1, however, UBC4 is incapable of multi-Ub chain assembly in vitro. Based on the level of similarity exhibited between the catalytic domains of UBC1 and UBC4 at the sequence level, there is ample evidence that their main chain structures will be largely superimposable (Cook et al., 1993). It is reasonable therefore to expect that the structural attributes that distinguish an E2 capable of chain assembly from one that is not will be subtle.

The alternative chain configuration catalyzed by UBC1 is uncharacteristic of any post-translationally created multi-Ub chain reported thus far. The fact that this configuration does not utilize lysine residues as points of linkage coupled with the fact that it requires Ub with a native amino terminus, implies that Ub moieties are linked tandemly through peptide bonds. The post-translational coupling of Ub to the α-amino group of another protein to produce a peptide bond is probably not unprecedented. Hershko and Heller (1985) observed that l-lysine, in which its lysines had been blocked chemically, nonetheless served as a substrate for polyubiquitination. While not a direct proof, this result implied that a multi-Ub chain was linked to lysine via its amino terminus (Hershko and Heller, 1985). Chemically, the tandem linkage of Ub moieties through peptide bonds is also not unprecedented as attested by the translational synthesis of polyubiquitin (Finley et al., 1987). In light of these two examples, the unique aspect of UBC1’s activity is that it affords a mechanism for the synthesis of poly-Ub post-translationally.

The evolutionary forces that have shaped and preserved the translational synthesis of poly-Ub in all eukaryotic organisms are far from clear. A commonly held view is that the translation of poly-Ub provides a rapid means of increasing the intracellular concentration of monomeric Ub through the action of specific processing proteases. The existence of a post-translational route for poly-Ub synthesis, however, raises the intriguing possibility that like other multi-Ub configurations (Arnason and Ellison, 1994; Johnson et al., 1995; Spence et al., 1995), poly-Ub functions to modify proteins.

The likelihood that this novel UBC1 directed configuration is composed of Ub molecules that are linked through peptide bonds underscores the idea that Ub can be attached either to itself or other proteins in a variety of different ways. It would be surprising if this repertoire of structures were not reflected in a range of different functions intracellularly.

Finally, UBC1 offers considerable promise as a model system for the elucidation of protein ubiquitination and multi-Ub chain assembly. A major advantage of this system is its simplicity. UBC1 targets either of two types of multi-Ub chains to a specific structurally defined lysine residue depending on circumstances that can be controlled. Thus UBC1 fulfills many of the basic requirements of a complete conjugation system without the requirement for either E3 or a target substrate.

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