crowding" effect, resulting in pentagonal wires, as previously predicted by theorists (*2*, *9*). Finally, nanowires of magnetic metals like nickel could exhibit an interesting interplay between structure, magnetism, and conductance. All in all, there could be, hanging impalpably between two fine needle tips, a whole new nanoworld to be explored.

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PERSPECTIVES: BIOCHEMISTRY

All in the Ubiquitin Family

Mark Hochstrasser

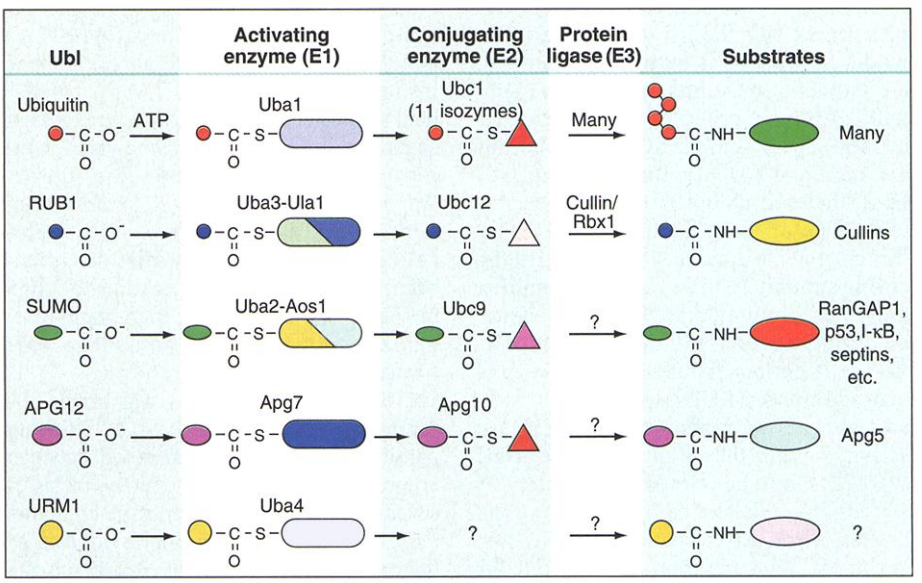
The function of a protein can be altered by changing its conformation, localization, or interaction with other molecules. An efficient way to do this is to modify the protein after it has been synthesized (posttranslational modification) through the addition of other molecules. For example, the addition of a ubiquitin molecule to a protein (called ubiquitination) often tags that protein for degradation.

In the case of ubiquitination, the modifying group, ubiquitin, is itself a protein. Recently, a surprisingly diverse set of additional ubiquitin-like modifier proteins (Ubls) have come to light. These Ubls all resemble ubiquitin in their amino acid sequences and often have similar means of attachment to their target proteins, but they also have unique and unanticipated functions (*1*, *2*). In addition, there are ubiquitin-related proteins in which a ubiquitin domain (UbD) is built into a larger polypeptide and is not excised or attached to other proteins. Such UbDs may impart properties to a protein similar to those conferred by a transferable Ubl, but the UbD is irreversibly tied to a single protein.

A series of enzymes are responsible for attaching ubiquitin and its cousins to other proteins (see the figure) (*1*). For all Ubls, the carboxyl group of the last amino acid of the Ubl (ubiquitin and most Ubls terminate in a glycine-glycine dipeptide) is first activated by adenylation. A thiol group in the activating enzyme (E1), which initiates the ubiquitination enzyme cascade, then attacks the Ubl–carboxyl–adenosine 5′-monophosphate to yield an E1-Ubl thiolester. Then, E1 transfers its activated Ubl to a cysteine on another protein (the conjugating enzyme, E2). From E2, the Ubl is transferred to a lysine residue in the target protein with the help of an E3 ligase.

From direct biochemical analysis or, more tentatively, from sequence comparisons, we now know of at least a dozen distinct Ubl-ligation pathways. Two of the most intensively studied Ubls are SUMO (small ubiquitin-related modifier) and RUB1 (related-to-ubiquitin 1). Several of SUMO's target proteins have been identified, and more can be anticipated. In at least some instances, addition of SUMO to a target protein (sumoylation) changes the localization of this protein or its interactions with other proteins. Indeed, the first identified target of SUMO, the trafficking protein RanGAP1 (which helps to shuttle proteins from the cytoplasm to the nucleus) requires the addition of SUMO before it can be localized to the nuclear pores. Localization of several proteins to discrete sites within the nucleus (called PML nuclear bodies) also depends on their sumoylation (*3*).

Recent genetic data support the view that protein sumoylation is required for the import of at least some proteins into the nucleus. In *Drosophila* embryos, most of the morphogen called Bicoid is imported into the nucleus, where it regulates transcription. This import requires an operational SUMO-conjugation system (*4*). Potentially, sumoylation of RanGAP1 (or another transport machinery component) is required for Bicoid import. Alternatively, a SUMO-conjugated form of Bicoid may be more efficiently imported (or less efficiently exported). Whether Bicoid is directly modified by SUMO is not yet known, but the *Drosophila* Dorsal protein (a member of the NF-κB rel family of transcription factors) is sumoylated in vivo, and elevated levels of SUMO pathway components stimulate its nuclear import (*5*). In some situations, different sumoylation events may have opposite functional effects in the same system. The mammalian I-κB protein—



Wrestling with target proteins. The pathways for conjugation of ubiquitin or members of the related ubiquitin-like modifier family (such as SUMO and RUB1) to their target proteins. Three steps are involved: activation of ubiquitin or a related modifier molecule by the E1 activating enzyme, transfer of the activated moiety to the E2 conjugating enzyme, and transfer of the modifier to its protein target through the activity of an E3 ligase. SUMO refers to a family of Ubls that has three known members in vertebrates, the founding member being SUMO-1 (ortholog of yeast Smt3). In mammalian cells, RUB1 is called NEDD8.

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which negatively regulates NF-κB by sequestering it in the cytoplasm—is also sumoylated in vivo (*6*). Various extracellular signals lead to the ubiquitination and degradation of I-κB, thereby enabling active NF-κB to move into the nucleus. Sites of I-κB ubiquitination have been mapped, and surprisingly, the same lysine residues are targets for sumoylation. Upon conjugation with SUMO, I-κB cannot be ubiquitinated or degraded, and NF-κB cannot be mobilized. Thus, there appears to be a complex regulatory interplay between ubiquitination and sumoylation of I-κB and sumoylation of NF-κB/rel.

Other transcription factors are also sumoylated, including the mammalian p53 tumor suppressor protein, the c-Jun protooncoprotein, and the *Drosophila* Tramtrack protein; all are also targets for ubiquitination (*7*). For p53, the SUMO attachment site is distinct from sites linked to ubiquitin, and sumoylation can change p53 transcriptional activity without altering p53 expression. How this happens is an open question, but altering the conformation of p53 or its subcellular relocalization are possibilities. Recently, the E3 ligase responsible for p53 ubiquitination, Mdm2, was also found to be efficiently sumoylated (*7*). Addition of SUMO to the Ring finger motif of Mdm2 prevents its self-ubiquitination and down-regulation by modifying the same lysine that Mdm2 uses to ubiquitinate itself. Mdm2 sumoylation is reduced by DNA damage, and this correlates with an increase in p53 in vivo.

In contrast to the diverse array of targets for SUMO, the list of known RUB1-modified proteins is extremely short—all are cullins (*8*). Cullins are common subunits of a large group of multisubunit E3 ligases—the so-called SCF (Skp1/cullin-1/F-box) and CBC (cullin-2/elongin BC) E3s—that add ubiquitin to its target proteins. One of these E3 ligases, $SCF^{\beta TrCP}$, directs the ubiquitination of I-κB; the cullin subunit (Cull-1) of this complex must be conjugated to RUB1 to ensure the ubiquitination and degradation of I-κB (*8*). A curious feature of this system is that addition of RUB1 to the cullin subunit appears to depend on RUB1 ligase activity within the ubiquitin ligase itself (*9*). This might be related to a property of a second protein shared by SCF and CBC ligases, Rbx1. Rbx1 contains a RING finger motif, and this motif is required for the ubiquitination activity of Rbx1 (and of other RING finger ubiquitin ligases). In many cases, RING-finger E3 ligases exhibit strong self-ubiquitination activity in vitro. The RUB1 E2, Ubc12, might be able to bind to the ligase just as the normal ubiquitin E2 does and to transfer its activated RUB1 to the cullin subunit. Conceivably, the RUB1 pathway may have evolved as a regulator of the ubiquitin system proper—perhaps to limit E3 self-ubiquitination, which may down-regulate E3 activity, or to help trigger a conformational switch in the subsequently formed ubiquitin E2-E3 complex that stimulates addition of multiple ubiquitin molecules to target proteins.

Among the most remarkable of the Ubls are the modifiers Apg12 and Urm1, two polypeptides that, at best, have only weak sequence similarity to ubiquitin (*10*, *11*). Although these Ubls are not closely related in sequence to ubiquitin, their activation depends on E1-like activating enzymes. All of these enzymes share a similar nucleotide-binding motif and other sequence similarities, including a putative cysteine active site downstream of the nucleotide-binding element.

The autophagy pathway in yeast (induced under starvation conditions) results in engulfment and digestion of cytoplasm and organelles. Ubl Apg12 is a component of this pathway, and its orthologs exist in many organisms (*10*). Mizushima *et al.* discovered that Apg12—1 of at least 16 yeast autophagy factors identified so far—is covalently linked in vivo to another factor, Apg5. A third factor, Apg7, required for this ligation is similar to E1-like enzymes. The carboxyl-terminal glycine of Apg12 forms an amide linkage with a specific lysine of Apg5, analogous to the linkage of ubiquitin to its protein targets. Another autophagy factor, Apg10, is also required for the conjugation of Apg12 to Apg5. Apg10 behaves like an E2 enzyme insofar as it forms an E1 (Apg7)–dependent thiolester with Apg12, despite lacking sequence similarity to any known E2 conjugating enzyme (*12*). How the Apg12-Apg5 conjugate contributes to autophagy is unknown, although the observed relocalization of Apg12 to a distinct membrane fraction upon conjugation to Apg5 may be a key event in the membrane reorganization required for autophagosome formation.

The discovery of Urm1 was rather different from that of Apg12 (*11*). Striking parallels had already been noted between ubiquitin activation and the biosynthesis of several sulfur-containing enzyme cofactors (*11*). Specifically, microbial synthesis of thiamin and molybdopterin each requires a specific sulfurtransferase, ThiF or MoeB, respectively. These enzymes bear unmistakable sequence similarity to the E1-like enzyme superfamily. Remarkably, the sulfur-transfer chemistry in both cases involves addition of a sulfur atom to the carboxyl-terminal carboxyl group of a short polypeptide, ThiS or MoaD, each of which terminates in a glycine-glycine dipeptide. As with ubiquitin, the carboxyl termini of ThiS and MoaD are first activated through adenylation by respective E1-like enzymes. Furukawa *et al.* used the ThiS and MoaD sequences to search the completed yeast genome sequence for potential relatives. They detected a distantly related sequence, Urm1 (ubiquitin-related modifier 1). They subsequently looked for proteins that could bind to Urm1 and isolated Uba4, a protein related to the E1-like enzyme superfamily, particularly ThiF and MoeB. Rather than being a sulfurtransferase, however, Uba4 forms a thiolester with Urm1, which is then transferred to one or more cellular proteins. The molecular function of the Urm1 pathway is not known, but it is required for normal growth, particularly at high temperature.

From these and other results, it is reasonable to suggest that ubiquitin and Ubls, together with their activating enzymes, may have evolved from a protein-based sulfide donor system similar to ThiS-ThiF or MoaD-MoeB. Coevolution of Ubl-cleaving proteases would have allowed the Ubl-coding sequences to become genetically mobile, much like self-splicing protein introns (inteins). A chance recombination event that fuses a Ubl-coding sequence in-frame and upstream of another open reading frame would generally be functionally invisible if the Ubl were efficiently excised from the protein product. In some cases, such fusions may have provided a selective advantage, as in the fusion of the cleavable ribosomal protein with ubiquitin (the ubiquitin enhances incorporation into ribosomes of the transiently linked ribosomal peptide). Genetic mobility of Ubl-coding elements may have led to the proliferation of Ubls as well as proteins with UbDs—there are already 30 or so known classes of Ubl/UbD proteins. Most UbDs are near the amino termini of proteins, which would be expected if an initially cleavable Ubl became locked on by mutation of the fusion joint or of the cleaving protease.

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