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Ubiquitin-like proteins and their roles in archaea

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Abstract

This review highlights the finding that ubiquitin-like (Ubl) proteins of archaea (termed SAMPs) function not only as sulfur carriers but also as protein modifiers. UbaA (an E1 ubiquitin-activating enzyme homolog of archaea) is required for the SAMPs to be covalently attached to proteins. The SAMPs and UbaA are also needed to form sulfur-containing biomolecules (*e.g.*, thiolated tRNA and molybdenum cofactor). These findings provide a new perspective on how Ubl-proteins can serve as both sulfur carriers and protein modifiers in the absence of canonical E2 ubiquitin conjugating or E3 ubiquitin ligase enzyme homologs.

Keywords

protein modification; sulfur carrier; molybdenum; tRNA; proteasomes

Ubiquitin and ubiquitin-like proteins

Ubiquitin (Ub) is a small protein of 76 amino acids that acts as a post-translational modifier in eukaryotes [1]. The covalent attachment of one or more molecules of Ub to selected proteins, by a process termed ubiquitylation, regulates a wide variety of cellular activities. The best known of these functions is in proteasome-mediated degradation, where the attachment of chains of Ub can trigger the destruction of native and dysfunctional proteins [2]. Modification by Ub is also linked to non-proteolytic processes such as membrane trafficking, transcriptional control, protein kinase activation, DNA repair and chromatin dynamics [1, 3, 4].

While the highly conserved amino acid sequence of Ub is restricted to eukaryotes, a superfamily of ubiquitin-like (Ubl) proteins is now known to be present in all domains of life. In general, Ubl proteins do not share much sequence identity with Ub [4, 5]. However, Ubl proteins do have a similar three-dimensional fold (known as the β -grasp fold) and often have a C-terminal diglycine motif (exposed after translation or proteolytic cleavage) that is needed for activity [6, 7]. Similarly to Ub, many Ubl proteins are covalently attached to protein targets [5, 8]. Ubl proteins can also be covalently attached to lipids, non-covalently associated with specific proteins, or used for sulfur incorporation into biomolecules, such as the pterin-based molybdenum cofactor (MoCo) and thiolated tRNA (Table 1).

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While Ubl proteins are quite diverse in function, these proteins are often used only for a single type of mechanism (*e.g.*, only protein modification) and are typically reserved for sulfur transfer in prokaryotes. Examples of Ubl proteins that are covalently linked to target proteins include the eukaryotic Nedd8, FAT10, ISG15, SUMO, Ufm1, Atg12 and MNSF β [5]. Likewise, the eukaryotic Ubl proteins of the Atg8 family are linked via an amide bond to the lipid phosphatidylethanolamine [9]. Two of the most widely studied bacterial Ubl proteins, MoaD and ThiS, transfer sulfur in the formation of MoCo [10] and thiamine (vitamin B1) [11], respectively. The human homolog of MoaD termed MOCS2A also functions in sulfur transfer to MoCo and tRNA [12]. Other bacterial Ubl proteins that function in sulfur transfer include CysO used in the biosynthesis of cysteine (from O-acetylserine) [13], PdtH used in formation of the siderophore pyridine-2,6-bis(thiocarboxylic acid) [14], and QbsE used in formation of thioquinolobactin (a precursor of the siderophore quinolobactin) [15].

This review highlights the unexpected finding that archaeal Ubl proteins can function as protein modifiers and sulfur carriers. This finding provides a new and simplified perspective on how Ubl-proteins are activated to serve multiple functions in the cell.

Ub/Ubl conjugation and sulfur transfer: common themes

Ub/Ubl conjugation involves a series of enzyme-catalyzed steps that activate the C-terminal carboxyl group of the Ub/Ubl protein and transfer the activated form of this protein to its target (protein or lipid) [4]. To initiate this process, an E1 Ub/Ubl activating enzyme adenylates the C-terminal carboxyl group of the Ub/Ubl (Ub/Ubl-AMP) by an ATP-dependent mechanism that releases pyrophosphate (PPi) [16]. Next, a thioester linkage is formed between the C-terminal glycine of Ub/Ubl and the active site cysteine of the E1 enzyme. The Ub/Ubl moiety is transferred from E1 to an E2 Ub/Ubl conjugating enzyme to form a second thioester linkage. Finally, the Ub/Ubl protein is covalently bound to its target, often with assistance from an E3 Ub/Ubl ligase, which can also form thioester intermediate linkages with the Ub/Ubl (depending on the type of E3 Ub/Ubl ligase). Deubiquitylases (DUBs) and other related hydrolytic enzymes remove molecules linked to the last residue of the Ub/Ubl and, thus, render the system reversible [17, 18].

In protein modification, the C-terminal α -carboxyl group of the Ub/Ubl protein is typically attached to the ϵ -amino group of lysine residues of substrate proteins, resulting in the formation of a covalent amide (isopeptide) bond [4]. While less frequent, Ub has also been found attached to the N-terminal α -amino group of itself or other proteins to form linear peptide bonds [19–21]. Ubiquitin is also esterified to cysteine, serine or threonine residues of proteins [22, 23]. Following ligation of a single moiety of Ub/Ubl to the protein target, further molecules of Ub/Ubl can be added to the attached Ub/Ubl moiety forming diverse poly-Ub/Ubl chains (*e.g.*, all seven lysine residues of Ub can be modified and mixed chains of Ub and Ubl proteins can form) [24, 25].

Ubl proteins that function as sulfur carriers are activated similarly to those that modify proteins and lipids. In sulfur transfer, the C-terminal glycine of the Ubl protein is adenylated by an Ub-activating E1 enzyme homolog (*e.g.*, MoeB and ThiF for sulfur transfer to MoCo and thiamine, respectively) [26–28]. Once the Ubl protein is activated, sulfur is transferred to its C-terminal glycine residue to form a thiocarboxylate (Ubl-OSH) [28–30]. Cysteine desulfurase (CDS) and rhodanese domains (RHDs) are thought to function by a sulfur relay mechanism to thiocarboxylate Ubl proteins. Both CDS and RHDs harbor an active site cysteine residue (Cys-SH) that can react with sulfur-containing compounds [*e.g.*, cysteine and thiosulfate ($S_2O_3^{2-}$), respectively] to form a cysteine persulfide (Cys-SSH) for sulfur transfer to the Ubl protein [31, 32]. The sulfur atom incorporated into the C-terminus of the

Ubl protein can then be transferred to biosynthetic intermediates to form sulfur-containing molecules in the cell.

Ubl proteins that function in sulfur transfer and protein modification

Recently, the boundary between Ubl proteins that function in sulfur transfer and protein modification has become blurred. In particular, Ubl proteins that function as protein modifiers and sulfur carriers are now identified in organisms from each domain of life. The eukaryotic ubiquitin related modifier 1 (Urm1) provided the first example of an Ubl protein with this type of multifunctional activity [8, 33, 34]. After oxidative stress, Urm1 is found conjugated to proteins by a mechanism that requires the lysine residues of the protein target and formation of a thioester intermediate with the E1 homolog Uba4 (MOCS3) [35–37]. Urm1 can also act as a sulfur carrier in the 2-thiolation of tRNA with Uba4 required to generate a thiocarboxylated form of Urm1 for this sulfur transfer [8, 32, 38–43]. Following the discovery of Urm1, the two Ubl proteins SAMP1/2 (named for their function as small archaeal modifier proteins) were found to be bound to proteins by isopeptide bonds in the archaeon *Haloferax volcanii* [33]. The SAMPs also function as sulfur carriers for MoCo biosynthesis and tRNA thiolation [44] (details of archaeal SAMPs are highlighted in this review). TtuB of *T. thermophilus* provides the most recent example of an Ubl protein that functions in sulfur transfer [45, 46] and protein conjugation [34] with protein substrates of TtuB limited compared to the archaeal SAMPs and eukaryotic Ub/Ubl proteins. TtuB can transfer sulfur to tRNA [45, 46] and can form isopeptide bonds with lysine residues of TtuA (a PP-loop ATPase that appears to activate the tRNA target nucleoside for transfer of sulfur from TtuB) [34]. The discovery of Ubl proteins, serving as both sulfur carriers and protein modifiers, is anticipated to provide insight into how these systems can be streamlined to catalyze multiple Ubl protein activities.

Sampylation: an archaeal form of ubiquitylation

Archaea mediate a form of post-translational modification named sampylation that resembles the ubiquitylation process of eukaryotic cells. Sampylation was first identified in the archaeon *Hfx. volcanii* based on the finding that Ubl proteins termed SAMPs are covalently attached to protein targets [33] and that an Ub-activating E1 enzyme homolog (termed UbaA) is required for this protein modification [44]. SAMP and UbaA homologs are common to archaea, suggesting sampylation is universal within this domain of life. Additional components of sampylation may include homologs of eukaryotic DUBs of the JAMM/MPN+ metalloprotease subfamily, which could remove SAMPs from their protein targets [33]. Homologs of canonical E2 Ub-conjugating and E3 Ub ligase enzymes are not predicted for most archaea (including *Hfx. volcanii*) [47]. Thus, sampylation is thought to be a streamlined form of ubiquitylation. In contrast, a variation of ubiquitylation that includes simplified forms of E2 and E3 homologs is predicted for the archaeon *Candidatus Caldiarchaeum subterraneum* [48] and some bacteria [49]. However, this latter system is not anticipated to be widespread among prokaryote and is currently based only on metagenome and genome sequence.

Prior to study of *Hfx. volcanii*, the Ubl and E1-like homologs of archaea were not characterized and were presumed to function only in sulfur transfer (in analogy to what was known for bacteria). The halophile *Hfx. volcanii* provided an ideal model to examine whether or not a simplified form of ubiquitylation existed in archaea. *Hfx. volcanii* has an established genetic system [50] and a completed genome sequence [51], which enabled identification of a single E1 Ub activating enzyme homolog (UbaA) and multiple Ubl proteins with a β -grasp fold and a C-terminal diglycine motif. The Ubl proteins were expressed in this organism with N-terminal Flag tag fusions (Flag-) to monitor protein

conjugate formation by anti-Flag immunoblot. With this approach, two of the three Ubl proteins (SAMP1/2) of *Hfx. volcanii* were found to be covalently attached to target proteins through non-thiol linkages [33]. Formation of these protein conjugates required *ubaA* gene function and the C-terminal diglycine motif of SAMP1/2 [44]. Thus, an E1-like enzyme (UbaA) was presumed to be used for the activation and attachment of the Ubl C-termini to protein targets in analogy to eukaryotes.

Isopeptide bonds are formed between the C-terminal carboxyl group of archaeal Ubl proteins and the ϵ -amino group of lysine residues on target proteins. To establish formation of these bonds, SAMP1/2 conjugates were purified from *Hfx. volcanii* and cleaved with trypsin [33, 52]. Modification sites were mapped by liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based collision-induced dissociation of the tryptic peptides [33, 52]. Trypsin is a well-known protease that cleaves bonds C-terminal to lysine and arginine residues but not when these residues are modified by isopeptide linkages. Thus, tryptic peptides with lysine residues of 'missed cleavage' and +114 Da of additional molecular mass are indicative of isopeptide bonds. The added mass is derived from the C-terminal diglycine tail of the Ubl protein retained on the target protein, but released from the Ubl protein after tryptic cleavage ($\downarrow$) of the bond joining a basic residue (R/K) to the GG motif (*i.e.*, -K $\downarrow$ GG of SAMP2 and SAMP1, with the latter generated by site directed mutagenesis of serine at position 85 to a lysine residue) [33, 52].

While SAMP conjugates have only been identified as isopeptide linkages to lysine residues, the types of proteins modified and residues surrounding the modification site are diverse. Unlike SUMO, which modifies the lysine residues within a conserved minimal core ψ KxE/D motif (where ψ represents a large hydrophobic residue) [53], a consensus sequence for SAMP modification has yet to be defined. To date, 11 sites of samp2ylation have been mapped to 9 proteins including those of predicted function in metabolism, transcription, stress response and the biosynthesis of sulfur-containing biomolecules [33]. Interestingly, Lys58 of SAMP2 is samp2ylated, revealing that either free or anchored chains of poly-SAMP2 are formed in the cell [33]. Sites of samp1ylation have been mapped to two lysine residues (Lys240 and Lys247) on HVO_1864 (a homolog of MobB and MoaE proteins that function in MoCo biosynthesis in bacteria)[52]. In *Hfx. volcanii*, HVO_1864 is essential for the activity of the MoCo-containing dimethyl sulfoxide (DMSO) reductase [44]. The sampylated lysine residues of HVO_1864 are analogous to the proposed active site residues of the large (MoaE) subunit of molybdopterin (MPT) synthase in *Escherichia coli* [30]. Thus, samp1ylation of HVO_1864 may inactivate the MoaE domain of MPT synthase and reduce the rate of MoCo biosynthesis in *Hfx. volcanii*.

When SAMP conjugates are enriched from *Hfx. volcanii*, at least 34 proteins are found uniquely associated and/or modified by sampylation [33]. Growth conditions (*e.g.*, different media) alter the levels of protein conjugates and types of proteins interacting with or modified by SAMP1/2, suggesting this type of post-translational modification is regulated [33, 44]. When proteasome genes are deleted, the levels of SAMP1 conjugates are increased, suggesting at least samp1ylation may target proteins for destruction by proteasomes [33]. Interestingly, a number of proteins associated with sulfur chemistry are commonly associated with or modified by SAMP1/2, including UbaA, MsrA (a homolog of methionine-S-sulfoxide reductase that reduces methionine-S-sulfoxide to methionine, one of two sulfur-containing amino acids), and a homolog of the tandem RHD protein Yor251cp that associates with Urm1 (a Ubl sulfur carrier and protein modifier in eukaryotes) [33]. Since SAMP1/2 also appear to serve as sulfur carriers in biosynthetic pathways and require the E1-like UbaA for function (see below for details), sampylation may serve an autoregulatory role in controlling sulfur metabolism and protein modification.

Sampylation is reversible

Archaea are predicted to encode homologs of eukaryotic DUBs and other related isopeptidases that remove molecules linked to the last residue of Ub/Ubl proteins [17, 18]. DUBs are now appreciated as important regulators of ubiquitylation and cell signaling in eukaryotic cells [17, 18]. In archaea, DUB homologs are of the JAMM/MPN/MOV34 metalloenzyme (JAMM/MPN+) subfamily and not of the cysteine-type common to eukaryotes. A DUB homolog of the archaeon *Archaeoglobus fulgidus* (AfJAMM) has been characterized at the structural level and has provided a framework for modeling the structure of eukaryotic JAMM/MPN+ isopeptidases, including the Rpn11 (Poh1) subunit of 26S proteasomes and Csn5 (Jab1) subunit of the COP9 signalosome (CSN) [54, 55]. However, an enzymatic activity for AfJAMM was not reported.

Another archaeal member of the JAMM/MPN+ subfamily, HvJAMM1 of *Hfx. volcanii*, was recently characterized and found to cleave SAMP-modified proteins [52]. HvJAMM1 purified as a monomer and catalyzed the removal of SAMP1/2 from isopeptide- and linear-linked proteins. HvJAMM1 did not hydrolyze free SAMP1/2, unmodified proteins, or the amide bond that links the fluorescent reporter 7-amino-4-methylcoumarin (AMC) to Ub or diglycine. Thus, HvJAMM1 is not a general protease and instead cleaves isopeptide and linear peptide bonds that link SAMP1/2 to proteins. Site-directed mutagenesis, metal content analysis, and 3D-modeling suggest a catalytic mechanism for HvJAMM1 that is somewhat analogous to AMSH and AMSH-LP, human DUBs of the JAMM/MPN+ subfamily (Figure 1) [52]. Central to this model is a catalytic Zn^{2+} that is tetrahedrally coordinated to HvJAMM1 by the side chains of His88, His90 and Asp101 and a water molecule. The side chain of Glu31 is predicted to function by general base mechanism to polarize the water molecule (coordinated to zinc) for nucleophilic attack of the carbonyl carbon of the scissile bond of the sampylated protein. A negatively charged tetrahedral intermediate would then form that is stabilized by hydrogen bonding to the side chain of Ser98. The water molecule coordinated to the side chain of Glu31 would complete the acylation step by donating protons to the nitrogen of the isopeptide bond undergoing hydrolysis. After SAMP1/2 release, a hydrogen bond would form between Glu31 and the protein target that would release the 'desampylated' product upon collapse. Thus, HvJAMM1 may provide a means for the cell to reverse the action of UbaA and, thus, regulate sampylation, recycle the SAMPs, and maintain cellular homeostasis.

Archaeal Ubl proteins as sulfur carriers

SAMP1/2 are thought to serve as both protein modifiers and sulfur carriers, similar to eukaryotic Urm1 [8] and bacterial TtuB [34]. The E1-like UbaA is required for SAMP1/2-mediated protein conjugation and appears important in sulfur mobilization. Current evidence to support the role of SAMP1/2 and UbaA in sulfur transfer pathways is indirect. SAMP2 and UbaA are required for the thiolation of $\text{tRNA}^{\text{Lys}}_{\text{UUU}}$ based on the finding that deletion of *samp2* and *ubaA* genes causes $\text{tRNA}^{\text{Lys}}_{\text{UUU}}$ -specific RNA to migrate faster in (N-acryloylamino)phenyl mercuric chloride (APM) gels compared to parent and *trans*-complemented strains [44] (where the mercuric compound within the gel interacts with the thiocarbonyl group of tRNA and retards its migration). Likewise, SAMP1 and UbaA appear to be required for sulfur transfer to form MoCo based on the inability of *samp1* and *ubaA* deletion strains to respire on DMSO and catalyze DMSO reductase activity, compared to parent and *trans*-complemented strains [44]. As DMSO reductase requires MoCo for activity, SAMP1 and UbaA are thought to be involved in sulfur transfer for MoCo biosynthesis. In contrast to UbaA, which appears important in sulfur transfer to both MoCo and tRNA, evidence suggests the SAMPs are specialized for a particular sulfur transfer

pathway (e.g., the *samp1* gene cannot complement a Δ *samp2* strain for tRNA thiolation and *vice versa* for DMSO reductase activity) [44].

SAMPs are not essential

Although proteasomes are essential in *Eukarya* and *Archaea* (at least *Hfx. volcanii*), the Ub/Ubl systems of these two domains of life differ their impact on cell function [44, 56–58]. Sampylation is dispensable, while ubiquitylation is required for cell division [44, 57, 58]. The basis for understanding how sampylation and proteasomes impact archaeal cells is based on genetic studies of *Hfx. volcanii* [44, 56]. Proteasomes were found to be essential in this archaeon based on the inability of cells to grow after conditional deletion of the genes encoding subunits of the catalytic 20S core particles using a tryptophan-dependent promoter [56]. In contrast, *Hfx. volcanii* strains lacking components of the sampylation pathway [*i.e.*, deletion of *ubaA* (encoding the single E1 Ub activating enzyme homolog) or triple deletion of *samp1 samp2 hvo_2177* (encoding the three Ubl-proteins)] can still grow under standard laboratory conditions [44].

The finding that sampylation is dispensable and proteasomes are essential in *Hfx. volcanii* can be interpreted several ways. First, sampylation may not target proteins to the proteasome for degradation. A direct link between sampylation and proteasomes has yet to be established. However, deletions in proteasomal genes increase the levels of SAMP-conjugates in *Hfx. volcanii* [33], and protein conjugation systems regulate proteasome-mediated proteolysis in the other domains of life (pupylation in bacteria and ubiquitylation in eukaryotes) [59]. Thus, proteasomes are thought to degrade sampylated proteins and other types of degrons, with the latter stimulating the turnover of proteins that need to be removed for archaeal cells to divide. Consistent with this possibility, archaeal proteasomes degrade proteins in the absence of sampylation [*e.g.*, C-terminal hydrophobic tails stimulate the degradation of green fluorescent protein (GFP) *in vitro*] [60], and phosphoproteins (including a Cdc6/Orc1 homolog) are found to accumulate in proteasome deficient cells [61]. It is also possible that proteasomes have non-proteolytic roles that are essential in archaea. Eukaryotic proteasomes mediate non-proteolytic functions in multiple facets of the transcription process, including initiation, elongation, mRNA processing and chromatin dynamics [62].

SAMP tertiary structure

Tertiary structures and 3D models (Figure 2) support a common β -grasp fold structure and provide insight into putative functions for certain amino acid residues of SAMP1/2. Structural analyses have included a crystal structure of SAMP1 determined at 1.55Å resolution [63], an NMR structure of PF1061 (a SAMP2 homolog from the hyperthermophilic archaeon *Pyrococcus furiosus*) derived largely on the use of residual dipolar couplings (RDCs) [64], an NMR structure of MA4086 (a SAMP1 homolog from the methanogen *Methanosarcina acetivorans*) [65], and a 3D model of SAMP2 which relies upon PF1061 as a best fit template [63]. In general, SAMP1 is closely related in tertiary structure to MoaD and has extra α -helical segments (α 1 and α 3) not conserved in Ub or SAMP2. Much like the Ile44-centered hydrophobic patch of Ub [66], hydrophobic regions surrounding SAMP1 Leu60 and SAMP2 Leu40 are predicted to function in protein–protein interactions [63]. While only Lys58-linked SAMP2 chains have been detected to date [33], the orientation of Lys4 in the SAMP1 crystal structure suggests this residue may also form poly-SAMP chains [63].

Model for archaeal protein conjugation and sulfur transfer

Archaeal SAMPs function in protein conjugation and sulfur transfer, for the biosynthesis of MoCo and thiolated tRNA, by a mechanism that requires the non-canonical Ub activating E1 enzyme homolog, UbaA. While yet to be established, UbaA is thought to adenylate the C-terminal glycine of the SAMPs to activate these proteins for sulfur transfer and protein conjugation (Figure 3). In support of this possibility, UbaA and SAMP1/2 are required for sulfur transfer and protein conjugation [44], Elsa (a UbaA homolog of *M. acetivorans*) can adenylate SAMP1/2 homologs *in vitro* [65], and similar adenylation mechanisms activate Ub-fold proteins in other domains of life. In particular, E1 enzymes adenylate Ub/Ubl proteins prior to protein conjugation in eukaryotes [67]. Likewise, Ub-fold proteins (*e.g.*, MoaD, ThiS, and TtuB) are adenylated by E1 enzyme homologs (*e.g.*, MoeB, ThiF, and TtuC) in sulfur transfer pathways (MoaD–MoeB in MoCo, ThiS–ThiF in thiamine and TtuB–TtuC in thiolated tRNA) [47].

The pathways of protein conjugation and sulfur transfer are proposed to diverge after the SAMPs are adenylated. Molecular detail on where these two pathways branch is speculative. In protein conjugation, a thioester enzyme intermediate is thought to form between a conserved active site cysteine of UbaA (C188) and the C-terminal carboxyl group of the SAMP, which readies the SAMP for transfer to lysine residues on protein targets. This mechanism would be analogous to the eukaryotic Ub/Ubl-E1 thioester intermediate known to form during protein modification [67]. Sulfur transfer, in contrast, may occur by converting the C-terminal adenylated SAMP into a thiocarboxylated form. In this latter pathway, a thioester intermediate between UbaA and SAMP1/2 is not predicted. Instead, in analogy to bacterial and eukaryotic systems, the adenylated SAMPs may receive sulfur from persulfide intermediates (-Cys-SSH) of cysteine desulfurases and/or rhodanases.

The protein partners that provide specificity to the SAMP1/2 pathways are best understood for sulfur transfer. In sulfur transfer, SAMP1 and MoaE are thought to form a MPT synthase complex for MoCo biosynthesis in analogy to eukaryotes and bacteria [10]. Consistent with this possibility, SAMP1 and MoaE are required for respiration on DMSO and DMSO reductase activity [44]. Similarly, Hvo_0580 (a P-loop NTPase homolog) is thought to associate with SAMP2 in the formation of 2-thiouridine tRNA. This model is analogous to the P-loop NTPases that associate with Ubl proteins to mediate tRNA thiolation in eukaryotes (Ncs6/2–Urm1) and *T. thermophilus* (TtuA–TtuB) [8, 32, 40, 46]. Whether or not enzymes downstream of UbaA (the E1 Ub activating enzyme homolog of archaea) are needed to provide specificity to SAMP1/2 protein modification remains to be determined.

Concluding remarks and future directions

Ub/Ubl proteins are continuing to provide new insight into how a relatively simple protein of less than 100 amino acids can have a major impact on cell function and proteome composition. The E1 Ub-activating enzyme homolog UbaA and the multiple Ubl SAMPs of archaea function in protein modification as well as sulfur transfer to form important biomolecules (MoCo and thiolated tRNA) for the cell. While sampylation can alter proteome composition and is reversible, the roles of this newly discovered post-translational modification in regulating archaeal cell function are yet to be discovered (Box 1).

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Box 1. Outstanding questions

- Are UbaA and SAMP proteins sufficient to mediate protein conjugation? Or are additional proteins required downstream to facilitate the conjugation of the SAMPs to the appropriate protein targets?
- What is the molecular mechanism(s) that distinguishes Ubl protein modification from sulfur transfer in archaea? Is a thioester intermediate formed between UbaA and SAMP prior to protein conjugation, which is not formed during sulfur transfer? If so, how are these distinct intermediates regulated? What provides the source of sulfur for thiocarboxylation of the Ubl proteins in archaea (*e.g.* cysteine desulfurase or rhodanese)?
- Are there other types of protein modifiers that have yet to be discovered in archaea?

Figure 1.

Schematic representation of the catalytic mechanism proposed for HvJAMM1 in cleaving SAMP1/2 (red) from a protein target (green). Amino acid residues required for HvJAMM1 activity are indicated. Central to this model is a catalytic Zn^{2+} tetrahedrally coordinated to the side chains of His88, His90 and Asp101 and a water molecule (blue). The side chain of Glu31 is proposed to function by general base mechanism to polarize the water for nucleophilic attack of the carbonyl carbon of the scissile bond (left). The side chain of Ser98 may stabilize the presumed negatively-charged tetrahedral intermediate through hydrogen bonds (center). The water molecule coordinated to the side chain of Glu31 may complete the acylation step by donating protons to the nitrogen of the scissile bond. After SAMP1/2 release, a hydrogen bond formed between Glu31 and the protein target (right) would collapse and release the protein target in its unmodified form [Figure from [52], with permission].

Figure 2.

Structural comparison of SAMP1/2 and ubiquitin. The structures are oriented with their hydrophobic patches front and center. Ub Ile44 needed for protein–protein interaction, along with the structurally analogous SAMP1 Leu60 and SAMP2 Leu40, are highlighted in pink as stick models. The C-terminal diglycine motif, including the α-carboxyl group of Ub and SAMP1/2 that forms an isopeptide bond with lysine residues of proteins, is highlighted in blue. The additional α1 and α3 helical segments of SAMP1 are highlighted in red. Lysine residues are also shown as stick models (with exception of SAMP2 Lys64) [Figure from [63], with permission]. Abbreviations: Nt, N terminus; Ct, C terminus.

Figure 3. Working model of SAMP1/2 pathways linked to sulfur transfer and protein modification. See text for detailed discussion of the proposed molecular mechanisms.

Table 1

Demonstrated and predicted ubiquitin and ubiquitin-like systems^a

β-grasp fold protein	Activating enzyme	Target / Modification			Substrates	Function/comments	Refs
		Lipid	Sulfur	Protein			
Archaea (e.g., <i>Haloferax volcanii</i> with homologs widely distributed among archaea)							
SAMP1 (HVO_2619)	UbaA	–	+	+	Proteins (100s based on SDS-PAGE, sites mapped to 2 lysine residues of MoeA), molybdopterin precursor?	Protein modification, MoCo biosynthesis proposed	[33, 44]
SAMP2 (HVO_0202)	UbaA	–	+	+	Proteins (100s based on SDS-PAGE, sites mapped to 11 lysine residues of 9 proteins), pre-thiolated tRNA	Protein modification, tRNA thiolation	[33, 44]
Select archaea (i.e., only <i>Candidatus Caldiarchaeum subterraneum</i> to date)							
Ub-related (CSUB_C1474)	E11 (CSUB_C1476)	?	?	?	?	Predicted to modify proteins using E21 (CSUB_C1475) & Zn finger (CSUB_C1477)	[48]
Thermophilic bacteria (e.g., <i>Thermus thermophilus</i>)							
TtuB (TTC0105)	TtuC	–	+	+	Proteins (TtuC, TtuA), pre-2-thiouridine tRNA	Protein modification, tRNA thiolation	[34]
ThiS (TTC0316)	TtuC	–	+	–	Thiamine precursor?	Thiamine biosynthesis?	[46]
MoaD-1 (TTC1947)	TtuC	–	+	–	MoCo precursor?	MoCo biosynthesis?	[46]
MoaD-2 (TTC1835)	TtuC	–	+	–	WCo precursor?	WCo biosynthesis?	[46]
Mesophilic bacteria							
MoaD, MoaD1/2	MoeB, MoeBR, MoeZR	–	+	–	Molybdopterin precursor	MoCo biosynthesis	[10, 68]
CysO	MoeZ, MoeZR	–	+	–	Sulfur transferred from CysO to <i>O</i> -acetylserine by CysM	Cysteine biosynthesis	[13, 68]
ThiS	ThiF	–	+	–	Thiamine precursor	Thiamine biosynthesis	[11]
PdtH	MoeZ	–	+	–	Pyridine-2,6-bis(thiocarboxylic acid) precursor	Siderophore biosynthesis	[14, 69]
QbsE	QbsC	–	+	–	Thioquinolobactin precursor	Siderophore biosynthesis	[15]
Eukaryotes							
Ubiquitin	Uba1, Uba6	–	–	+	Proteins (1000s)	Targets to proteasomes and other functions	[5]
FAT10	Uba6	–	–	+	Use2	Proteasome mediated degradation, immunoregulation?	[70]
Nedd8 (Rub1)	Uba3–NAE1 *	–	–	+	Cullin-RING E3 Ub ligases, tumor suppressors, oncoproteins	Regulates cell cycle	[71]
ISG15	Ube1L	–	–	+	Viral and host proteins	Antiviral immune defense	[72]

β -grasp fold protein	Activating enzyme	Target / Modification			Substrates	Function/comments	Refs
		Lipid	Sulfur	Protein			
SUMO family (SUMO1/2/3)	SAE1-SAE2 ^a	–	–	+	Proteins (100s)	Modulate protein interactions, localization and conformation	[73]
Ufm1	Uba5	–	–	+	C20orf116	ER-stress induced apoptosis, erythroid development	[74, 75]
Hub1 (Ubl5)	–	–	–	–	Non-covalent association with Snu66 and Prp38	RNA splicing, Hub1 has YY vs. GG motif	[76]
Atg12	Atg7	–	–	+	Atg5, Atg3	Autophagy	[77, 78]
MNRF2 (Fub1, Fau)	?	–	–	+	Bcl-2, endophilin II	Immunoregulation?	[79, 80]
Atg8 family (e.g., Atg8, LC3, GATE-16, GABARAP)	Atg7	+	–	–	Phosphatidyl-ethanolamine	Autophagy	[9]
Urm1	Hs MOCS3 (ScUba4)	–	+	+	Proteins (MOCS3, ATPBD3, UPF0432, CAS), pre-2-thiouridine tRNA	tRNA thiolation, protein modification stimulated by oxidative stress	[8, 12, 81]
MOCS2A	MOCS3	–	+	–	Molybdopterin precursor	MoCo biosynthesis	[10]

^a Abbreviations: +, detected; –, not detected; ?, unidentified to date; *, multisubunit complex; Hs, *Homo sapiens*; Sc, *Saccharomyces cerevisiae*; ER, endoplasmic reticulum; WCo, tungsten cofactor.