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Weighing up the possibilities: Controlling translation by ubiquitylation and sumoylation

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Abstract

Regulation of protein synthesis is of fundamental importance to cells. It has a critical role in the control of gene expression, and consequently cell growth and proliferation. The importance of this control is supported by the fact that protein synthesis is frequently upregulated in tumor cells. The major point at which regulation occurs is the initiation stage. Initiation of translation involves the interaction of several proteins to form the eIF4F complex, the recognition of the mRNA by this complex, and the subsequent recruitment of the 40S ribosomal subunit to the mRNA. This results in the formation of the 48S complex that then scans the mRNA for the start codon, engages the methionyl-tRNA and eventually forms the mature 80S ribosome which is elongation-competent. Formation of the 48S complex is regulated by the availability of individual initiation factors and through specific protein-protein interactions. Both of these events can be regulated by post-translational modification by ubiquitin or Ubls (ubiquitin-like modifiers) such as SUMO or ISG15. We provide here a summary of translation initiation factors that are modified by ubiquitin or Ubls and, where they have been studied in detail, describe the role of these modifications and their effects on regulating protein synthesis.

Introduction

Initiation of protein synthesis

Protein synthesis is of fundamental importance in cells and its regulation is crucial for the continued viability of organisms. The process comprises 3 stages: initiation, elongation and termination. Of these, initiation is generally considered to be one of the major regulatory steps of gene expression in mammalian cells. Initiation requires the function of a number of translation initiation factors (Fig. 1), several of which have key roles in cell survival and oncogenesis. These proteins modulate the binding of mRNA to the ribosome, a process facilitated by the assembly of the cap binding protein (eIF4E), a helicase (eIF4A) and a scaffold protein (eIF4G), to form the eIF4F complex (eIF4E/eIF4A/ eIF4G).[1-3] The eIF4G scaffold protein possesses domains that interact with eIF4E, eIF4A, eIF3 and the poly(A) binding protein, PABP.[1-4] PABP itself is regulated by interaction with other proteins; binding of Paip1 to PABP stimulates protein synthesis while interaction with Paip2 is inhibitory to translation.[5,6] The activity of the eIF4F complex is regulated by a family of proteins, the eIF4E binding proteins (4E-BPs). Using a conserved motif, 4E-BPs compete with eIF4G for a common surface on eIF4E and inhibit eIF4F assembly. In mammalian cells, activation of the mechanistic target of rapamycin (mTORC1) leads to phosphorylation of 4E-BP1 in a hierarchical manner. This promotes protein synthesis by releasing eIF4E and enabling eIF4F complex assembly on the m^7GTP cap of mRNA, mediating 40S ribosomal subunit binding by a bridging interaction between eIF4G and eIF3.[1-3]

FIGURE 1

In most organisms there is more than one isoform of most of these translation initiation factors. For example, there are 3 isoforms of eIF4A, eIF4G and PABP.[7-9] In some cases the functions of the isoforms are indistinguishable, in others there are indications that the different isoforms display mRNA-specific regulation.[7-9] Further work will be required to uncover the full range of functions and specificities of these isoforms.

Ubiquitin like proteins

Ubiquitin-like proteins (Ubls) comprise a family of structurally related proteins. The different members of the family share sequence similarities, and in particular the proteins contain a conserved β-grasp fold consisting of 5 β sheets and one a helix.[10] Ubiquitin is a 76 amino acid protein and is the most highly conserved member of the Ubl family, with 96% identity between yeast and human ubiquitin. SUMO (small ubiquitin-like modifier) is less conserved between species and contains a longer, more variable N-terminal extension than ubiquitin being around 100–110 amino acids in total length.[11] ISG15, between 155– 165 amino acids in length, contains 2 ubiquitin-like domains.[12] It was the first member of the family to be identified and, unlike ubiquitin and SUMO, is present only in vertebrates. The gene was so named because it was observed to be an interferon stimulated gene encoding a 15 kDa protein.[13] Most members of the Ubl family are synthesized as precursor proteins that need to be processed to a mature form to reveal a di-glycine motif at the C-terminus that is required for activation and subsequent conjugation of the Ubl to target proteins. The exception to this is ISG15 in fish and bovine species where the protein is synthesized in the mature form.[14] Ubls are attached to one or more lysine residues in target proteins. There are no known consensus sequences for conjugation sites for ubiquitin and ISG15. However SUMO is

frequently, although not always, attached to lysine residues present within the consensus sequence ψKxE, where ψ = a hydrophobic amino acid and x is any amino acid.[11]
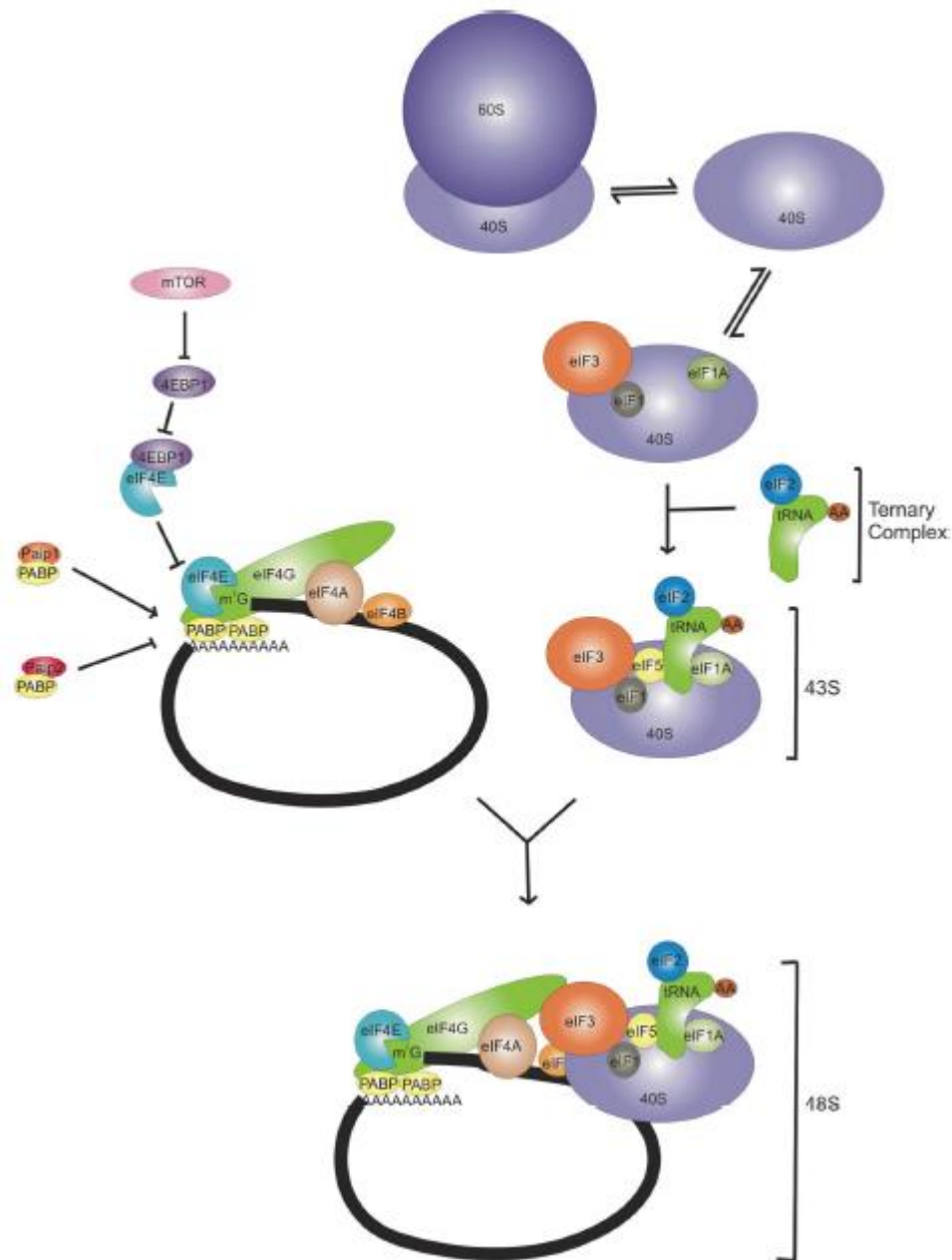
Figure 1 Formation of the 48S preinitiation complex. eIF1, 1A and 3 interact with the 40S ribosomal subunit. This then interacts with eIF5 and the ternary complex (eIF2-GTP-Met-tRNA) to form the 43S complex. In parallel, eIF4E and eIF4A are recruited by eIF4G to form the eIF4F complex. The availability of eIF4E is controlled by 4E-BP1, which in turn is regulated by phosphorylation by mTOR. The eIF4F complex binds to the cap on mRNA along with Poly(A)-binding protein (PABP) and eIF4B. PABP is regulated via interactions with 2 PABP proteins, PAIP1 and PAIP2. The 43S complex then binds close to the cap from where it can scan the mRNA for the start codon.

Ubiquitylation

Ubiquitin can be covalently attached to lysine residues in target proteins as a monomer or in the form of chains. This occurs via the activity of a number of proteins, the E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugating enzyme) and E3 (ubiquitin ligase) proteins (Fig. 2). In most organisms there is a single E1, around 40 E2s and hundreds of E3s (reviewed in [15,16]). Ubiquitin is produced as a precursor protein that is processed to the mature form by one of a small number of specific ubiquitin proteases, to reveal a diglycine motif at the C-terminus. Ubiquitin is then activated in an ATP-dependent manner, by the formation of a thioester bond between the C-terminal glycine residue and a cysteine residue on the E1 activating enzyme. From here it is passed to an E2 ubiquitin conjugating enzyme, again, via the formation of a thioester bond between the C-terminal glycine residue and a cysteine residue. Attachment of ubiquitin requires one of a large number of E3 ubiquitin ligases, which in many cases interact directly with target proteins, but which in some instances interact with targets via an adaptor protein. In the main, the E3s provide the specificity for the modification. Ubiquitin chain formation occurs via lysine residues within ubiquitin itself, and also requires the activities of the E1, E2 and E3 enzymes. The most common linkages are via K11, K48 and K63.[17,18] Ubiquitin can be removed from targets by the actions of deubiquitinating enzymes (DUBs). Ubiquitylation has 2 main roles: targeting of proteins for proteolysis and modification of protein function. The best studied role of ubiquitylation is its targeting of proteins for proteasome-mediated degradation. This involves the recognition of K11- and K48- linked ubiquitin chains by the 26S proteasome.[19] However, there is a rapidly expanding literature on other roles for ubiquitylation. For example ubiquitylation of PCNA is required for the recruitment of an error-prone polymerase to undertake translesion DNA synthesis e.g,[20] while ubiquitylation of membrane proteins is required for endocytosis and ubiquitylation of PIN2 is required for vacuolar sorting (reviewed in [21]). In these cases the modification involves a single ubiquitin or K63-linked chains.

Sumoylation

The process of sumoylation is very similar to that of ubiquitylation, involving SUMO-specific E1 (SUMO activating enzyme), E2 (SUMO conjugating enzyme) and E3 (SUMO ligase) proteins.[11] There is a single E1 (a heterodimer), a single E2 (Ubc9) and to date around 12 E3s have been identified. Unlike ubiquitylation, an E3 is not always required for modification, as the E2 is in some cases sufficient, and can provide a degree of target specificity.[22] Like ubiquitin, SUMO can be attached to proteins either as a monomer or in the form of poly-SUMO chains.[11] Sumoylation affects protein-protein interactions,[23,24] protein activity[25] and protein localization.[26] In addition, SUMO chains interact with STUbLs (SUMO-targeted ubiquitin ligases) that bring about ubiquitylation of the target protein and associated SUMO chains, resulting in proteasome–mediated proteolysis.[27]

ISGylation

ISG15 is conjugated to target proteins in a manner similar to that of ubiquitin and SUMO.[28] ISG15 expression and modification (ISGylation) are activated by Type I interferon (IFN), which is one of a number of critical cytokines in the innate immune response. As is the case for ubiquitin and SUMO, there are proteases that are specific for processing ISG15 and deconjugating it from target proteins

(e.g., USP43,[29]) and a specific E1 enzyme for ISG15.[29] However, some of the E2s (e.g., UbcH8) and E3s (e.g., Efp—the partner of UbcH8, and HHARI—the human homolog of Drosophila ariadne) involved in ISGylation also appear to be involved in ubiquitylation.[30,31]
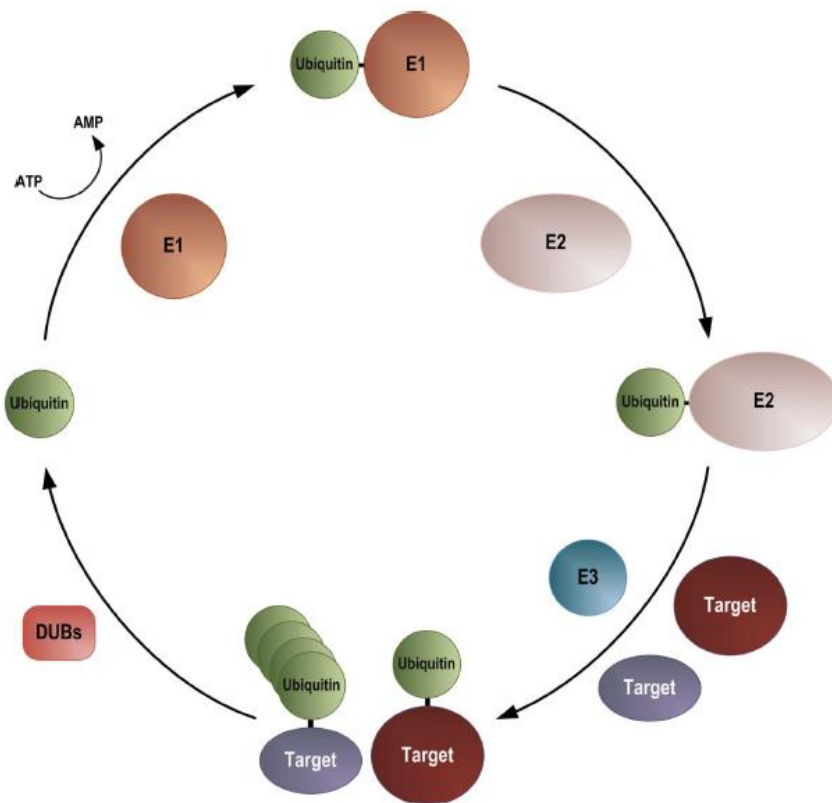

Figure 2 Ubiquitylation pathway. E1 = Ubiquitin activating enzyme, E2 = ubiquitin conjugating enzyme, E3 = ubiquitin ligase, DUB D deubiquitylating enzyme. Ubiquitin is activated by the formation of a ubiquitin-adenylate before forming a thioester bond with a cysteine residue in the E1 ubiquitin activating enzyme. Ubiquitin is passed to an E2 ubiquitin conjugating enzyme, again forming a thioester bond. Target proteins are recognized by E3 ubiquitin ligases, either directly or via an adaptor, and ubiquitin is attached via the formation of an ε-amino bond. Ubiquitin can be attached to target proteins either as a monomer, or in the form of ubiquitin chains. Ubiquitin can be removed from target proteins by the action of one of a number of DUBs.

Identification of Ubl Attachment Sites and the Roles of Modification

Early methods for the identification of modified sites involved site-directed mutagenesis of individual lysine residues in target proteins, followed by analysis in vitro or in vivo to determine whether modification still occurred. While this has been successful in some cases (e.g.,[32]) in many cases it has been problematic since other lysine residues are frequently used instead of the normal sites in the mutant proteins. More recently, mass spectrometry has been used successfully for site identification (e.g.,[33]). This involves the cleavage of modified proteins by trypsin or other suitable

protease to release peptides from the target. This method is facilitated by having a protease cleavage site close to the C-terminal diglycine motif attached to the target, so that only a few extra amino acids remain attached to the modified site. Modification sites are thus detected by the identification of peptides that are increased in Mr by an amount dependent on the position of the cleavage site within the Ubl.

Analysis of the role of the modifications is hampered by the fact that frequently, only low levels of modified forms are observed in cells. The reason for this could be that the modifications are transient, are labile, or as in the case of poly-ubiquitylation and poly-sumoylation, are targeting the protein for proteasome-mediated destruction. It is also possible that modification may be confined to target molecules in a particular cellular location. Additionally, it is proposed that this form of post-translational modification is not like modifications such as phosphorylation—i.e., an on/off switch. For example, in the case of SUMO, it is proposed that in some cases modification results in a change in conformation of the target protein that is maintained even after desumoylation occurs. Thus analysis of the roles of these modifications has lagged behind analysis of the function of other types of modifications.

Identification of the roles of the modifications has been undertaken, in the main using in vitro assays to look at relative binding abilities of wild type and unsumoylatable mutant proteins for their binding partners e.g.,[32] or by introduction of mutant coding sequences into cells to determine the effect of inability to modify a particular protein. This is relatively straightforward in yeast where a mutant copy can be integrated in the genome as the sole copy of the coding sequence e.g.,[34] In mammalian cells, the mutant sequence can be introduced by transfection, but is dependent on having cells where the gene has been knocked out or where siRNA depletion is efficient. Depletion of the any of the enzymes in the conjugation pathway would be likely to affect multiple targets and would not be appropriate.

Role of Modification by Ubiquitin or Ubls in Translation Initiation Factors

A series of recent proteomic screens have identified numerous translation initiation factors that are modified by either ubiquitin or SUMO, or in many cases, by both (Table 1). Additionally, some of the screens have identified the lysine residues required for the modification. Early studies involved the overexpression of the modifier, but recently more refined methods using diGly capture techniques have been used to identify sites when the modifier is expressed at endogenous levels e.g.[35,36] These studies use mass spectrometry to identify diGly-modified peptides obtained by trypsin digestion of cellular proteins. A list of modified sites can be found at PhosphoSitePlus[37] (http://www. phosphosite.org/home). In many cases, individual lysine residues are identified as a single 'hit', making them less likely target sites than lysine residues that are highly represented, as for example is observed in eIF4A and eIF4G proteins.

Table 1 Proteins identified in proteomic screens as being modified by ubiquitin or SUMO

Initiation factor	Ubiquitin	SUMO	Reference
eIF1A	Hs Ubiquitin*Mm Ubiquitin	Rn SUMO-3	35,36,58,59
eIF2A	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-2*At SUMO	35,55,59,80
eIF2α	Hs Ubiquitin*Mm Ubiquitin	Dm SUMO	36,56,59
eIF2B-β	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-1/2	36,59,81
eIF2β	Hs Ubiquitin*Mm Ubiquitin	At SUMO*Sc SUMO-1	36,59,80,82
eIF2 subunit 1	Hs Ubiquitin*Mm Ubiquitin	Rn SUMO-3	35,58,59
eIF2γ	Hs Ubiquitin*Mm Ubiquitin	Dm SUMO*Hs SUMO-1*Hs SUMO-2/3*Sc SUMO	35,36,56,57,59,82,83,*
eIF5B*	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-2*Hs SUMO-1*	55,59,61 (A)
eIF3A	Hs Ubiquitin*Mm Ubiquitin*Rn Ubiquitin	Hs SUMO-2*Hs SUMO-1	36,55,59,61 (B)*
eIF3B	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-2	35,55,59
eIF3C	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO 1/2	35,59,81
eIF3D	Hs Ubiquitin*Mm Ubiquitin	Rn SUMO-3	35,58,59
eIF3E	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-1/2	35,59,81
eIF3F	Hs Ubiquitin*Mm Ubiquitin		59,74©
eIF3G	Hs Ubiquitin*Mm Ubiquitin		35,36,59
eIF3H	Hs Ubiquitin*Mm Ubiquitin*Rn Ubiquitin		35,36,59 (D)*
eIF3I	Hs Ubiquitin	Sc SUMO*Hs SUMO-1/2	35,36,59,60,82-84
eIF3J	Hs Ubiquitin*Mm Ubiquitin		36,59
eIF3K	Hs Ubiquitin*Mm Ubiquitin		35,36*
eIF3L	Hs Ubiquitin*Mm Ubiquitin		35,59
eIF3M	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-1	35,59,83
eIF3X		Hs SUMO-2	55
eIF4A1	Hs Ubiquitin*Mm Ubiquitin*Rn Ubiquitin	Dm SUMO*Rn SUMO-3*Hs SUMO-1/2*At SUMO	35,36,55-62 (D)
eIF4A2	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-1	35,59,61
eIF4E	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-1	36,46,59
eIF4GI	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-1/2	36,57,59,61
eIF4GII	Hs Ubiquitin		35
eIF4GIII	Hs Ubiquitin*Mm Ubiquitin		35,36
eIF5A	Hs Ubiquitin*Mm Ubiquitin*Rn Ubiquitin	Hs SUMO-1/2	35,59,83 (D)
PABP1	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-2*Sc SUMO	35,55,57,59,85
PABP4	Hs Ubiquitin*Mm Ubiquitin	Hs SUMO-2	35,55,59

NOTES Hs: human, Rn: rat, Mm: mouse, Sc: S. cerevisiae, At: Arabidospsis. (A) (2010) CST Curation Set: 9913; Year: 2010; SILAC: N; Biosample/Treatment: AMO-1(cell line)/Velcade; Disease: -; Specificity of Antibody Used to Purify Peptides prior to MS2: anti-UbK Antibody Used to Purify Peptides prior to MS2: Ubiquitin (D4A7A10) XP(R) Rabbit mAb Cat#: 3925, PTMScan(R) Ubiquitin Branch Motif (K-e-GG) Immunoaffinity Beads Cat#: 1990. (B) (2008) CST Curation Set: 3970; Year: 2008; SILAC: N; Biosample/Treatment: brain(tissue)/untreated; Disease: -; Specificity of Antibody Used to Purify Peptides prior to MS2: anti-UbK. (C) (2009) CST Curation Set: 8668; Year: 2009; SILAC: N; Biosample/Treatment: RPMI-8266(cell line)/Velcade; Disease: -; Specificity of Antibody Used to Purify Peptides prior to MS2: anti-UbK Antibody Used to Purify Peptides prior to MS2: Ubiquitin (D4A7A10) XP(R) Rabbit mAb Cat#: 3925, PTMScan(R) Ubiquitin Branch Motif (K-e-GG)

More detailed studies on the role of modification of a number of the individual proteins by ubiquitin, SUMO and in one case, ISG15 have also been reported. We summarize here what is known about the roles of these post-translational modifications and how they might affect translation rates in mammalian cells.

eIF4E

Regulation of eIF4E levels is important for normal cell growth, as disruption of its expression or its over-production leads to aberrant cell growth or oncogenesis.[38] Additionally, eIF4E protein levels increase during differentiation e.g.,[39] eIF4E is both mono- and poly-ubiquitylated[40,41] and this has been demonstrated to occur mainly on K159.[40] This modification is enhanced by the E3 ubiquitin ligase, Chip (carboxy terminus of Hsp-70 interacting protein) which is known to have a role in regulating protein quality control.[42] A mutant form of eIF4E that is unable to interact with eIF4G or 4E-BP1 is more highly ubiquitylated than wild type eIF4E. This results in increased degradation by the proteasome of the mutant form, consistent with a role for ubiquitylation of eIF4E in a quality control process, removing inactive forms of the protein from the cell.[40] A role for ubiquitylation in quality control is supported by a number of observations. First, that binding of eIF4E to 4E-BP1 (eIF4E binding protein that is also regulated by ubiquitylation—see below) suppresses ubiquitylation and degradation and that only non-ubiquitylated eIF4E binds eIF4G. Second, overexpression of 4E-BP1 prevents ubiquitin-mediated degradation of eIF4E. Third, heat shock (45°C 10 min, conditions that would result in a degree of protein misfolding) also induces ubiquitylation of eIF4E, as does exposure to another form of stress, cadmium chloride.[41]

While poly-ubiquitylation clearly has a role in targeted destruction of eIF4E, little work has been performed to determine whether there is a different role for mono-ubiquitylation in regulating levels or subcellular localization of eIF4E. In contrast, the biological significance of eIF4E phosphorylation and its effect on translation have been studied over many years; however, the role of phosphorylation in modulating the activity of the protein is still not completely understood, although enhanced levels of eIF4E phosphorylation are associated with a number of human tumors.[43,44] Biophysical studies have suggested that phosphorylation of eIF4E decreases its affinity for the mRNA cap of mRNA, possibly allowing rapid recycling of eIF4E between competing mRNAs.[45] However, it has also been suggested that phosphorylation of S209 causes a retractable salt bridge to form with K159 (the ubiquitylation site) which leads to increased binding of capped mRNA.[40] Mutation of K159 to alanine but not arginine, reduces association with cap analogs, indicating that a positive charge is required at this position. Despite the fact that the K159R mutant cannot be ubiquitylated, it has been proposed that mono-ubiquitylation may stabilize the distance between S209 and K159, or that ubiquitin itself may form part of the bridge between S209 and K159.[40]

eIF4E is also modified by SUMO,[32,46] in a process that is promoted by HDAC2 (histone deacetylase 2).[46] Sumoylation occurs on several lysine residues, namely K36, 49, 162, 206 and 212. Interestingly, unlike what has been observed with a number of other proteins, such as IkBa and PCNA,[34,47] sumoylation and ubiquitylation of eIF4E do not occur on the same lysine residues. Sumoylation of eIF4E is dependent on phosphorylation, but the reverse is not true: inability to sumoylate eIF4E does not affect its ability to be phosphorylated.[32] Sumoylation results in the induction of translation of a subset of mRNAs required for cell proliferation and apoptosis. A mutant form of eIF4E that cannot be sumoylated is still able to bind m^7GTP, indicating that capbinding is unaffected. However, compared with wild type protein, the mutant form binds significantly better to 4E-BP1 than it does to eIF4G, and is unable to form stable eIF4F complexes. It has been suggested that sumoylation induces a conformational change in eIF4E producing a change in interaction surfaces resulting in release from 4E-BP1 and promoting interaction with eIF4G. The inability of the mutant protein to be sumoylated results in an increase in the amount of eIF4E interacting with 4E-BP1.[32] While overexpression of wild type eIF4E in NIH-3T3 cells results in increased expression of eIF4Eregulated genes, this is not observed when unsumoylatable eIF4E is overexpressed.[32] At this time is unclear whether sumoylation of eIF4E has any effect of global rates of translation or rates of export of specific mRNAs from the nucleus.

4EHP

4EHP, also known as eIF4E2, binds to the m^7GTP cap in a manner similar to that of eIF4E. However, unlike eIF4E, it does not bind eIF4G and therefore does not allow ribosome recruitment. It thus competes with eIF4E for the mRNA and prevents translation.[48] It is targeted for ubiquitylation[49] and interestingly, also for modification with another Ubl, ISG15.[50] Curiously, the E3 ligase HHARI, which has recently been shown to be a marker of cellular proliferation,[51] stimulates both ubiquitylation and ISGylation of 4EHP.[49,50] Proteomic studies have identified K239 as a ubiquitylation site, but this has not been verified in a detailed study. In contrast, ISGylation, which occurs on K134 and K222, has been analyzed in some detail.[50] Binding studies indicate that ISGylated 4EHP has a higher affinity for m7GTP than the unmodified form. It has been proposed that this modification is used by cells to inhibit translation of specific mRNAs in innate immune responses. Interestingly, despite its similarity to 4EHP, eIF4E is not ISGylated.

4E-BP Family

The eIF4E binding proteins (4E-BPs) are key regulators of protein synthesis.[1-3] As their name suggests, they function by interacting with eIF4E. This inhibits eIF4E function by preventing it from interacting with eIF4G to form the mature eIF4F complex. The 4E-BP proteins are phosphorylated following activation of mTORC1, in response to changes in growth conditions, and interaction of eIF4E with 4E-BP1 and -2 occurs with the hypophosphorylated form.[1-5] A key factor in the regulation of translation initiation is that the relative levels of eIF4E and 4E-BP1 and -2 are highly controlled.[52] The hypophosphorylated form, but not the hyperphosphorylated form, of 4E-BP1 is unstable if not bound to eIF4E. Under these conditions, 4E-BP1 is ubiquitylated and targeted for proteasome-mediated proteolysis.[52,53] The role of ubiquitylation was identified following some rather unexpected results obtained when knockdown of eIF4E using shRNA was demonstrated to have no

effect on protein synthesis.[52] This was subsequently shown to be due to concomitant degradation of 4E-BP1, which resulted in the release of eIF4E molecules to compensate for the loss brought about by the reduced expression. K57, a lysine residue conserved between all 3 4E-BPs, was identified by the Sonenberg lab as the ubiquitylation site in 4E-BP1,[52] and a screen of an siRNA library identified the KLHL25-CUL3 as the E3 ubiquitin ligase responsible for 4EBP1 degradation. Knockdown of KLHL25 resulted in a decrease in translation, consistent with it having a role in controlling levels of 4E-BP1.[52]

Proteasome activity (presumed to be a result of poly-ubiquitylation) has also been demonstrated to be required for the formation of a truncated form of 4E-BP1 (tr4E-BP) in murine erythroleukemia (MEL) cells containing activated p53.[54] This truncated form is 3 kDa smaller than full-length protein, is unphosphorylated and relatively stable. It also binds to eIF4E in preference to the full-length protein. It has been proposed that the production of this p53-induced form may be contributing to the ability of p53 to regulate apoptosis and malignancy.

eIF4A

Two isoforms of eIF4A have been identified in proteomic screens as being modified by ubiquitin and SUMO.[35,36,55-62] In contrast to what is observed with some of the other initiation factors, modified peptides from both eIF4A1 and eIF4A2 are highly abundant in the proteomic screens designed to identify ubiquitylation sites, implying that modification is likely to have a key role (s) in the regulation of the function of these 2 proteins. In the ubiquitin screens, most of the modified sites identified in the human eIF4A proteins were also observed in the mouse proteins, suggesting that they are likely to be true 'hits' and not false positives. Interestingly, eIF4A2 (but not eIF4A1) and translational repression have both been shown to be essential for miRNA-mediated gene regulation.[63] However, the post-translational modification of these proteins by ubiquitin or Ubls has not been analyzed in detail and to date there are no reports on whether it affects the activity of the eIF4A protein or miRNA-mediated translational control.

In a role unrelated to its function in translation, ubiquitylation of *Drosophila* eIF4A has been shown to be linked with Decapentaplegic (Dpp) signaling.[64] Additionally, rice DRM2 (required for RNA-directed DNA methylation) interacts with eIF4A via its ubiquitin associated (UBA) domain, (although whether this occurs with a ubiquitylated form has not been analyzed).[65]

eIF4G

There are 3 isoforms of the scaffold protein, eIF4G, eIF4GI-III. As observed with eIF4A, diGly-modified peptides from these proteins are abundant in proteomic screens designed to identify ubiquitylation sites,[35,36,57,59,61] and again most are observed in both the human and mouse proteins. In eIF4GI these sites (6 in total, 4 common to both human and mouse) map to lysine residues occurring between amino acids 593–925 which map close to, or in the region of, the eIF4E and eIF4A/3 binding sites. The abundance of these modified tryptic fragments and their position in the protein suggests that this post-translational modification is likely to be important for regulating the functions of these proteins, possibly by affecting the interaction of eIF4G with other members of the

eIF4F complex. Again, these modifications have not been analyzed in detail and to date there are no reports on whether they affect the activity of eIF4GI. In addition to this modification by ubiquitin, eIF4GI has been shown to be sumoylated in both fission yeast and human cells.[66] Sumoylation of *S. pombe* eIF4G is increased following exposure of cells to 1 M KCl or arsenite, conditions which result in the formation of stress granules. In vitro sumoylation studies have identified 2 sumoylation sites in mammalian eIF4GI, K1368 and K1588, residing in the eIF3/4A binding site and the Mnk-binding domain, respectively. (Mnks (MAP kinase-interacting kinases) are kinases which bind to the C-terminus of eIF4G and phosphorylate eIF4E which is bound to the N-terminus of eIF4G.[67]) These data suggest that sumoylation may be affecting interactions of eIF4GI with associated proteins, e.g., eIF4E, and possibly the assembly of eIF4G into stress granules.

Paip2

Poly(A)-binding protein (PABP) is regulated through the interaction with 2 proteins, Paip1 and Paip2.[5,6] Paip1, which also interacts with eIF3g, is eIF4G-like and is stimulatory for translation, while Paip2 represses PABP function by decreasing the affinity of PABP for polyadenylated mRNA, thus inhibiting translation. Paip1 and Paip2 both have 2 domains, PAM1 and PAM2 which interact with PABP. This interaction occurs through RRM-1 and PABC domains, respectively.[68] Additionally, PAM2 is capable of interacting with EDD (a member of the HECT domain family of E3 ubiquitin ligases) which also contains a PABC domain.[69] In cells where levels of PABP are depleted, Paip2A is ubiquitylated in an EDD-dependent manner prior to proteasome-mediated degradation.[70] Interestingly, the affinity of the PAM2 domain of Paip2 for the PABC domain of PABP is greater than that of the affinity for the PABC of EDD. Thus, it is proposed that interaction of PABP with Paip2 competes with EDD for interaction with PAM2 on Paip2, and that this normally prevents ubiquitylation of Paip2.[70] However, in apparently contradictory work, it has been demonstrated that during human cytomegalovirus infection PABP levels rise concomitantly with the levels of both Paip2 and EDD1. The reason for this is not known, but it has been proposed that it may provide cells with a process to allow rapid changes in protein levels if necessary.[71] Paip2B is also polyubiquitylated, although at a somewhat lower level that Paip2, and is hence more stable.[72]

eIF3

Proteomic studies have identified many of the eIF3 subunits as targets for ubiquitylation and/or sumoylation. However, independent of these studies, eIF3f is the only eIF3 subunit where the function of these modifications has been studied in any detail. eIF3f is a non-core subunit of the eIF3 complex. It can act both as a repressor and as an enhancer of translation (reviewed in[73]). Its role as a translational enhancer came to light in a study on muscle atrophy.[74] Here, eIF3f is ubiquitylated by the MAFbnx/Atrogin1 protein which is a muscle-specific F-box protein ubiquitin E3 ligase.[75] This E3 is upregulated and essential for accelerated muscle protein loss in a number of disorders.[76] Ubiquitylation of eIF3f occurs on multiple (6) lysines in the C-terminus[74] and results in its ubiquitin mediated proteolysis in myotubes undergoing atrophy. Under these conditions both MAFbnx and eIF3f are detected in the nucleus.[75] It has been proposed that this ubiquitylation may be associated with the rapid downregulation of certain proteins during muscle atrophy. eIF3f also interacts with the ubiquitin E3 ligase TRC8 to inhibit protein synthesis. The mechanism by which this occurs is

unknown, but it has been proposed that TRC8 targets an eIF3 subunit for ubiquitylation.[77] Unrelated to its role in translation, eIF3f can act as a deubiquitylating enzyme (DUB). In this capacity it is capable of deubiquitylating, and thus contributing to the activation of, the Notch signaling receptor in *Drosophila*.[78]

Interestingly, recent work has shown that eIF3e is involved in eIF4E phosphorylation; Mnk1 binding to eIF4F is dependent on eIF3e, and eIF3e is sufficient to promote Mnk1-binding to eIF4G.[79] As eIF3e is modified by both ubiquitylation and sumoylation, it would be interesting to know if these modifications of eIF3e also have a role in controlling eIF4E phosphorylation.

Summary

In conclusion, despite the fact that numerous translation initiation factors have been shown to be ubiquitylated and/or sumoylated in proteomic screens, relatively little is known about the effects of the modifications on the functions of individual proteins. In part this is due to the transient nature of these modifications, e.g., in many cases of sumoylation, less than 5% of a particular protein is modified at any one time, and the sumoylated species appear to be very labile in certain organisms due to highly active SUMO-specific proteases. Additionally, since ubiquitylation targets proteins for destruction, analysis of ubiquitylated proteins, other than in the presence of a proteasome inhibitor, is difficult.

The recent use of proteomic screens to identify modified proteins and the modified site(s) suggests that there are many more cases where post-translational modification by ubiquitin or Ubls is likely to affect translation initiation factors. For example, sumoylation of eIF4A1/2 might have a role in regulating both the unwinding of mRNA secondary structure and the ability of eIF4A2 to mediate miRNA-dependent gene expression in mammalian cells. Further work on these modifications is required to fully elucidate their effect on individual proteins and on translational control of gene expression as a whole.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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