

## Wobble uridine modifications—a reason to live, a reason to die?!

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### ABSTRACT

Wobble uridines (U<sub>34</sub>) are generally modified in all species. U<sub>34</sub> modifications can be essential in metazoans but are not required for viability in fungi. In this review, we provide an overview on the types of modifications and how they affect the physico-chemical properties of wobble uridines. We describe the molecular machinery required to introduce these modifications into tRNA posttranscriptionally and discuss how posttranslational regulation may affect the activity of the modifying enzymes. We highlight the activity of anticodon specific RNases that target U<sub>34</sub> containing tRNA. Finally, we discuss how defects in wobble uridine modifications lead to phenotypes in different species. Importantly, this review will mainly focus on the cytoplasmic tRNAs of eukaryotes. A recent review has extensively covered their bacterial and mitochondrial counterparts.<sup>1</sup>

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### Wobble uridine modifications are essential in evolution

Among the plethora of chemical posttranscriptional modifications that are found on tRNA, those of wobble uridine (U<sub>34</sub>) are peculiar, because U<sub>34</sub> is almost invariably modified in any organism.<sup>2,3</sup> This phenomenon implies a strong evolutionary pressure to maintain wobble uridines modified and is further affirmed by the analysis of minimal genomes. In *Mollicutes* species that have drastically reduced their genome size during evolution, uridine thiolation (s<sup>2</sup>U) is part of an essential core module of translation.<sup>4</sup> Furthermore, recent attempts of synthetic biologists to generate a minimal genome based on *Mycoplasma mycoides* found modification systems of U<sub>34</sub> to be required for rapid growth under laboratory conditions.<sup>5</sup> Surprisingly however, the absence of U<sub>34</sub> modification does not cause lethality in *Caenorhabditis elegans* and most yeasts but is essential in some strain backgrounds of *Saccharomyces cerevisiae* and in mice.<sup>6–12</sup> Furthermore, several human diseases are linked to defects in U<sub>34</sub> modifying enzymes.<sup>13–15</sup> This apparent discrepancy between essentiality during evolution and variable effects in different organisms makes wobble uridines even more worth exploring.





We know a lot about U<sub>34</sub> modifications from the research of many laboratories working mainly on baker's yeast. Theoretically, 16 anticodons carry a uridine at their wobble position. UAA and UGA, 2 codons that would require U<sub>34</sub>-containing tRNAs for decoding are nonsense codons and therefore recognized by the Eukaryotic Release Factor 1 and 3 (eRF1 and eRF3) GTPase complex.<sup>16,17</sup> However, the modification machinery is of such broad specificity that U<sub>34</sub> in suppressor tRNA<sup>Ser</sup><sub>UUA</sub> is modified.<sup>6</sup> tRNA<sup>Arg</sup><sub>UCG</sub> does not exist in yeast and

tRNA<sup>Leu</sup><sub>UAG</sub> is unmodified.<sup>18</sup> tRNA<sup>Ile</sup><sub>UAU</sub> carries a pseudouridine (Ψ<sub>34</sub>).<sup>19</sup> The remaining 11 tRNA species are decorated by 4 types of modifications: First, 5-carbamoylmethyluridine (ncm<sup>5</sup>U<sub>34</sub>), the most abundant modification, is present on tRNA<sup>Val</sup><sub>UAC</sub>, tRNA<sup>Ser</sup><sub>UGA</sub>, tRNA<sup>Pro</sup><sub>UGG</sub>, tRNA<sup>Thr</sup><sub>UGU</sub> and tRNA<sup>Ala</sup><sub>UGC</sub>. Second, tRNA<sup>Leu</sup><sub>UAA</sub> is further 2-O-methylated to 5-carbamoyl-methyl-2-O-methyluridine (ncm<sup>5</sup>U<sub>34</sub>m). Third, 5-methoxycarbonylmethyluridine (mcm<sup>5</sup>U<sub>34</sub>) is found on tRNA<sup>Arg</sup><sub>UCU</sub> and tRNA<sup>Gly</sup><sub>UCC</sub>. Finally, 3 tRNAs are further decorated by a 2-thio group to form 5-methoxycarbonylmethyl-2-thiouridine (mcm<sup>5</sup>s<sup>2</sup>U<sub>34</sub>): tRNA<sup>Gln</sup><sub>UUG</sub>, tRNA<sup>Lys</sup><sub>UUU</sub> and tRNA<sup>Glu</sup><sub>UUC</sub> (Fig. 1).<sup>20,21</sup>

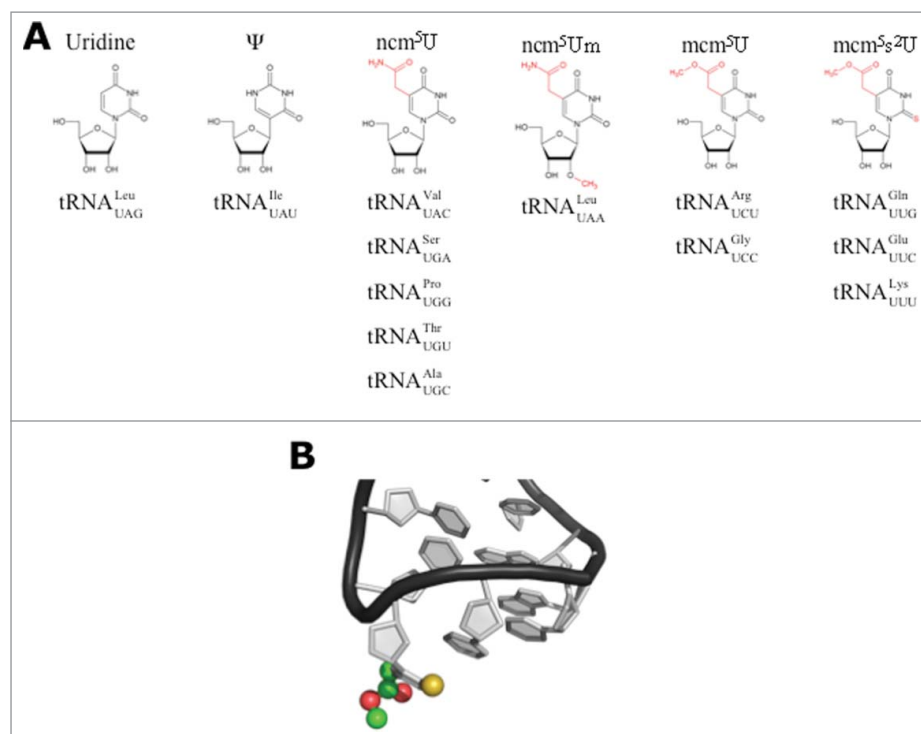
### The physicochemical properties of wobble uridine modifications

The multitude of modifications speaks for particular requirements in the U<sub>34</sub> position. But what are the effects of these modifications on the chemical and structural properties of uridine? In his wobble hypothesis, Francis Crick proposed that U<sub>34</sub> recognizes A and G in the third position of the codon.<sup>22</sup> However, he could not account for the extent of modified nucleotides in tRNA as these modifications were not known. Later adaptations of the wobble hypothesis have attempted to integrate how modifications of U<sub>34</sub> affect codon recognition.<sup>20,23–26</sup>

Uridines are structurally flexible and form only weak stacking interactions with neighboring nucleosides. This is because uridines—even when decorated by 5'-modifications—adopt a C(2') endo conformation, which is relatively flexible.<sup>27,28</sup> However, the presence of 2-thio modifications leads to the adaptation of a C(3') endo, gauche plus [C(4')-C(5')], anti

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**Figure 1.** Modifications of wobble uridines ( $U_{34}$ ). (A) Uridine derivatives that can be found at the wobble position ( $U_{34}$ ) in anticodons of cytoplasmic tRNAs from eukaryotes. Modifications to uridine are indicated in red. (structures taken from Modomics<sup>21</sup>). Abbreviations:  $ncm^5U$ : 5-carbamoylmethyluridine;  $ncm^5Um$ : 5-carbamoylmethyl-2'-O-methyluridine;  $mcm^5U$ : 5-methoxycarbonyl-methyluridine;  $mcm^5s^2U$ : 5-methoxycarbonylmethyl-2-thiouridine. (B) Detail of the structure of the anticodon stem loop (ASL) of human  $tRNA_{UUU}^{Lys}$ .  $mcm^5s^2U$  is highlighted by spheres. Sulfur (yellow), carbon (green), oxygen (red). Note that the nucleobases are arranged in a stacking confirmation (The structure is based on PDB 1FIR).

structure.<sup>27-29</sup> This conformation is hydrophobic, more restricted and appears to be best suited for anticodon base-stacking, therefore stabilizing the anticodon stem loop (ASL) and favoring the interaction with A in the codon.<sup>27,28</sup> The conformational stability of  $s^2U_{34}$  is similar to  $U_{34}m$ , which also occurs mainly in its C(3') endo form.<sup>30,31</sup> While,  $s^2U_{34}$  enhances the stability of the stacking of the triplet significantly over unmodified  $U_{34}$ ,<sup>32,33</sup> the stabilizing effect of the C(3') endo conformation appears to be more important for the codon-anticodon interaction than an improvement of base stacking.<sup>29,34</sup> Furthermore, 2-thio modifications do not only restrict and stabilize the conformation of the nucleoside that carries  $s^2$  but also of a 3'-adjacent unmodified uridine.<sup>35,36</sup> Importantly, a stabilization of the interactions between 2-thiolated anticodons with their codon partners has been observed in binding models and in the context of the ribosome.<sup>36-38</sup> While the role of  $s^2U_{34}$  modification is to restrict codon conformation, the role of  $xm^5$  modifications is to open up the rigidity to stabilize  $U \bullet G$  wobble pairing.<sup>39</sup> In general, the ribosomal grip constrains the positioning of the mRNA more than that of the ASL. Therefore,  $G_{34} \bullet U_3$  wobble pairs can form in unmodified anticodons, while  $U_{34} \bullet G_3$  pairs are much less stable, if they form at all.<sup>26,40-42</sup>

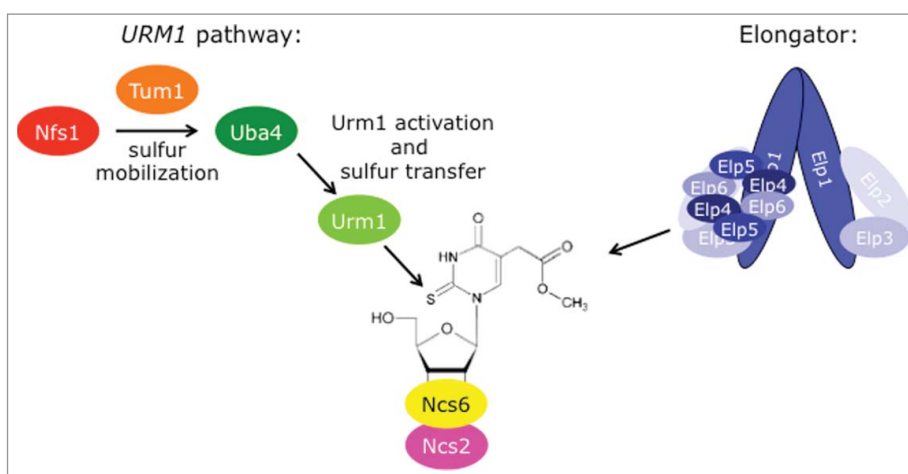
Structural studies found that near-cognate tRNAs do not necessarily take up a wobble confirmation but that non-standard base pairing can retain Watson-Crick geometry.<sup>43</sup> This structural arrangement is supported by the presence of  $U_{34}$  modifications. Indeed,  $mcm^5s^2U_{34}$  modifications change the physicochemical behavior of the base. While  $s^2$  of  $mcm^5s^2U_{34}$  is required for proper positioning of the nucleoside, the main function of the  $mcm^5$  modification is to modify the electron

structure of the ring and to shift the keto-enol equilibrium toward enol thus enabling hydrogen bonding between  $U_{34} \bullet G$  pairs.<sup>44</sup> Therefore, while  $s^2U$  rigidifies the ASL structure thereby favoring an interaction with A-ending codons, the  $xm^5$  modifications relax the conformation and enable pairing with G-ending codons. However, when analyzing codon-translation speed by ribosome profiling the absence of both types of modifications seems to affect translation speed similarly.<sup>45</sup>

Nevertheless, also the context of the anticodon matters. For instance,  $N^6$ -threonylcarbamoyladenosine ( $t^6A_{37}$ ) synergizes with  $U_{34}$  modifications to order the ASL such that it will bind to the correct mRNA codons. Only when  $U_{34}$  and  $A_{37}$  are fully modified, will the ASL of  $tRNA_{UUU}^{Lys}$  bind to AAA and AAG programmed ribosomes<sup>41</sup> and fulfill the function proposed by Crick.<sup>22</sup> Therefore,  $U_{34}$  modifications contribute to the pre-structuring of the anticodon loop to achieve optimal translation.<sup>42</sup>

### Wobble uridine modifications require multiple pathways

Two pathways are responsible for placing the two main classes of modifications on wobble uridine: The *URM1* pathway is required for 2-thiolation and the Elongator complex needed for generating  $xm^5U_{34}$  modifications (Fig. 2). Pseudouridine Synthase (Pus1) introduces pseudouridine at  $U_{34}$  in  $tRNA_{UAU}^{Ile}$ .<sup>46,47</sup> Finally, during the 2'-O-methylation of  $tRNA_{UAA}^{Leu}$   $tRNA$  Methyltransferase (Trm7) uses S-adenosylmethionine (SAM) as a methyl donor together with its cofactor Regulator of Ty1 Transposition 10 (Rtt10).<sup>48,49</sup> However, the absence of 2'-O-



**Figure 2.** Model of the *URM1* pathway and the Elongator complex. Schematic representation of the 2 pathways cooperating in  $mcm^5s^2U_{34}$  formation. In the *URM1* pathway (left), sulfur is mobilized by Nfs1 with the help of Tum1. Uba4 activates Urm1, leading to a thiocarboxylate at Urm1's C-terminus, which acts as a sulfur carrier. Finally, the Ncs2•Ncs6 complex binds to and activates tRNA in the thiolation reaction and transfers the sulfur from Urm1 to uridine. The Elongator complex consists of twice Eip1-Eip6. Eip1 dimerizes via its C-terminus and acts as a platform for Eip2 and Eip3 binding in a wing-like structure. A ring of Eip4-Eip6 binds to one of the wings (Handedness is only partially represented in this model).<sup>102,103</sup>

methylation in tRNA<sup>Leu</sup><sub>UAA</sub> does not significantly contribute to phenotypes observed in *trm7Δ* yeast since overexpression of tRNA<sup>Phe</sup><sub>GAA</sub> is sufficient for rescue experiments, suggesting that the physiological role of this modification in  $U_{34}$  is minor.<sup>49</sup> Interestingly,  $xm^5U_{34}$  formation is independent of the presence of  $s^2U_{34}$  while the reverse is not true. In *S. cerevisiae*  $s^2U_{34}$  levels are reduced in the absence of *mcm*<sup>5,11,50</sup> In humans, mice and *S. pombe* *mcm*<sup>5</sup> is even strictly required for  $s^2U_{34}$  formation.<sup>51,52</sup>

### The *URM1* pathway

Ubiquitin Related Modifier 1 (Urm1) is an ubiquitin like protein (Ubl) first described as a protein modifier in a process called urmylation.<sup>53-55</sup> It was identified through its sequence homology to bacterial sulfur-carrier proteins<sup>53</sup> and is considered to be a molecular fossil because it is the Ubl that most closely resembles the ancestors of this class of protein modifiers.<sup>56,57</sup> Importantly, Urm1 is the only Ubl described as a protein modifier and at the same time to act in sulfur transfer, placing it at the evolutionary intersection of both pathways.<sup>50,58-67</sup> Like every Ubl, Urm1 is activated by an E1-like enzyme Ubiquitin Activating 4 (Uba4).<sup>53</sup> While the N-terminus of Uba4 contains an E1 domain, its C-terminal harbors a rhodanese homology domain (RHD).<sup>68,69</sup> The RHD has been suggested to act as an E2 domain for protein conjugation of Urm1.<sup>70</sup> However, there is no experimental support for this mechanism and the functionality of the domain rather points toward sulfur transfer. Two additional core members of the pathway are Needs Cla4 to Survive 2 and 6 (Ncs2 and Ncs6),<sup>55,71</sup> which form a complex in *S. cerevisiae*, *C. elegans* and *S. pombe*.<sup>8,11</sup> Difficulty in reconstituting the complex *in vitro* has prevented its detailed analysis. It is clear that Ncs6, an iron-sulfur (Fe/S) cluster containing protein,<sup>72</sup> and its worm homolog TUT-1 can bind to tRNA *in vitro*<sup>11</sup> and *in vivo*.<sup>8</sup> The protein carries 2 predicted zinc-finger domains and a P-loop.<sup>11,73,74</sup> Interestingly, the number of tRNA species that bind to Ncs6 *in vitro* exceeds the number of *mcm*<sup>5</sup> $s^2U_{34}$  targets.<sup>11</sup>

Thus, Ncs2 may provide specificity to the complex. Alternatively, Ncs2 may stabilize or activate Ncs6. NCS2 is the closest homolog of NCS6 in the yeast genome. However, the critical residues required for enzymatic function are mutated. The observation that only one catalytic subunit is required in *S. cerevisiae* suggests a similar mechanism in enzymes that form a homo-dimer such as TtuA.<sup>75</sup> While *URM1*, *UBA4*, *NCS2* and *NCS6* are required for 2-thiolation, ThioUridine Modification 1 (*TUM1*), a gene coding for a protein with 2 RHDs, is not essential for thiolation. However, in the absence of Tum1, levels of  $s^2U_{34}$  are significantly reduced and the ratio between modified and unmodified tRNA changes.<sup>11,58,63,67</sup>

In addition to this core set of proteins, there is another group that is essential for thiolation by more generally affecting sulfur pathways. NiFS like 1 (Nfs1), a cysteine desulfurase that converts cysteine into alanine using pyridoxal phosphate as a cofactor acts upstream of several cellular sulfur pathways.<sup>76</sup> During this reaction, Nfs1 feeds sulfane sulfur to its acceptor protein. Uba4 uses the sulfur and transfers it into the downstream cascade for 2-thiolation.<sup>62</sup> The exact mechanism of Tum1 in this reaction is unclear. Tum1 receives sulfur from Nfs1 and appears to stimulate its activity.<sup>63</sup> This suggests that it acts as an enhancer of the transfer reaction or as a sulfur relay. Since Tum1 is not essential for 2-thiolation, the transfer step via Tum1 can be bypassed. Therefore, Tum1 may affect 2-thiolation through an indirect mechanism by rerouting sulfur pathways. Finally, proteins required for iron-sulfur (Fe/S) cluster biogenesis and assembly are essential for 2-thiolation. These are: Cytosolic Iron-sulfur protein Assembly 1 (Cia1), Nucleotide Binding Protein 35 (Nbp35) and Cytosolic Fe/S cluster Deficient 1 (Cfd1), which are all components of the CIA complex.<sup>77,78</sup> The CIA complex works in conjunction with ISCU homologs 1 and 2 (Isu1 and Isu2), 2 proteins that reside in the mitochondrial matrix and are required for Fe/S cluster generation.<sup>79</sup> Additional proteins required for Fe/S cluster formation are, therefore, likely to affect 2-thiolation but have not been tested specifically. Whether Fe/S cluster formation affects  $s^2U_{34}$  formation directly or indirectly via the formation of *mcm*<sup>5</sup> $U_{34}$

will need to be determined.<sup>80</sup> Recently, a Fe/S cluster was identified in Ncs6 and its archaeal homolog TtuC.<sup>72</sup> However, its requirement for  $s^2U_{34}$  formation remains to be demonstrated.

### The Elongator complex

The Elongator complex is at the heart of  $mcm^5$  and  $ncm^5$  side chain formation at  $U_{34}$ . The complex has been reported to act in numerous cellular processes including transcription, DNA damage response, exocytosis, telomere gene silencing, DNA demethylation and wobble uridine modification.<sup>6,81-88</sup> Importantly, all known phenotypes of Elongator-minus yeast, except for the defect in  $U_{34}$  modification itself, can be rescued by over-expression of tRNA that are normally  $mcm^5s^2U_{34}$  modified.<sup>12,45,51,88-95</sup> Nevertheless, the question of whether different functions exist in other species is very persistent, in part due to the fact that tRNA overexpression experiments in metazoans are more difficult to perform than in yeast.

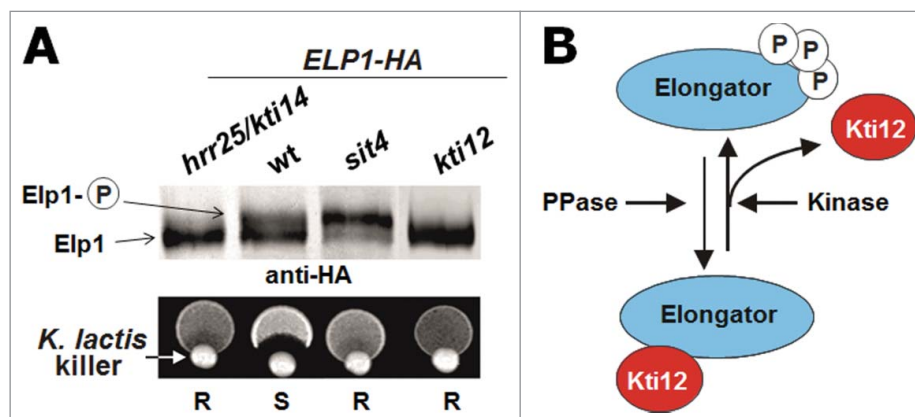
The Elongator complex consists of 6 subunits: Elp1-Elp6.<sup>81,96,97</sup> Each is present twice in the holo complex, which can be divided in two sub-complexes: Elp1-Elp3 and Elp4-Elp6. The latter subunits, between 30–50 kDa in size, have very similar RecA folds and assemble into a heterohexameric ring structure that resembles RecA-like ATPase complexes.<sup>98</sup> However, the subunits lack the P-loop motif, which is characteristic for ATPases.<sup>98</sup> Importantly, the Elp4-Elp6 complex binds tRNA in an ATP dependent manner, where high levels of ATP decrease the affinity of tRNA for the complex. The Elp1-Elp3 subcomplex contains Elp3, which is the catalytic subunit of the complex.<sup>96,99</sup> Elp3 carries an N-terminal radical SAM binding domain and a C-terminal histone acetyltransferase (HAT) domain.<sup>96,100</sup> Interestingly, in the archaeon *Methanocaldococcus infernus* a homolog of Elp3 is sufficient for  $U_{34}$  modification, while all other Elongator subunit genes are absent from its genome.<sup>101</sup> Elp1 and Elp2 are both WD40 domain containing proteins. Two subunits of Elp1 dimerize via their C-terminal domains, while one subunit of Elp2 and Elp3 associates with each Elp1 subunit at either side.<sup>102,103</sup> This generates a wing-

like structure. Surprisingly, in the holo complex, a ring of Elp4-Elp6 is associated with the front of the left wing (Fig. 2) resulting in an asymmetric assembly, which is in contrast to previous models.<sup>98,102-105</sup> The role of the asymmetry still needs to be determined as well as the position of the tRNA in the complex.

In addition to the Elongator complex, *Kluyveromyces lactis* Toxin Insensitive 11–14 (Kti11-Kti14), Suppressor of Initiation of Transcription 4 (Sit4), Sit4 associated protein 185 and 190 (Sap185 and Sap190), tRNA Methyltransferase 9 and 112 (Trm9 and Trm112) are required for  $mcm^5U_{34}$  and  $ncm^5U_{34}$  formation. Elp1-Elp6, Kti11, Kti12, Kti14, Sit4, Sap185 and Sap190 are essential for the formation of  $cm^5U_{34}$ , which is believed to be the precursor of  $mcm^5$  and  $ncm^5$ .<sup>5,6,58,106</sup> The lack of Kti13 leads to reduced levels of  $xm^5$  formation.<sup>6,58</sup> Finally, the Trm9•Trm112 complex uses SAM to synthesize  $mcm^5U_{34}$ .<sup>107</sup> Whether  $cm^5U_{34}$  or an intermediate precursor serves as the direct methylase substrate is not clear, yet. The observation that both *trm9* and *trm112* mutants accumulate  $ncm^5U_{34}$  and  $ncm^5s^2U_{34}$ , however, suggests the existence of an enzyme required for formation of  $ncm^5U_{34}$  (and  $ncm^5s^2U_{34}$ ) from  $cm^5U_{34}$ .<sup>107,108</sup> The identity of this activity is still not known, leading to contradicting ideas of how to rationalize these later steps of the  $U_{34}$  modification pathway.<sup>106-110</sup>

### Elongator regulation by phosphorylation

Strikingly, tRNAs that carry Elongator-dependent  $mcm^5s^2U_{34}$  can be cleaved by zymocin between anticodons position 34 and 35.<sup>111-113</sup> Zymocin is a trimeric ( $\alpha\beta\gamma$ ) tRNase toxin complex produced from *K. lactis* that kills yeasts including *S. cerevisiae*.<sup>114-116</sup> In line with this lethal mode of tRNase action, genetic studies have shown that mutations in Elongator genes trigger zymocin resistance and additional Elongator related factors (Kti11-Kti14, Sit4, Sap185 and Sap190) were genetically identified on the basis of zymocin survivor screens.<sup>58,112,117-136</sup> Rather than affecting the assembly or the integrity of the Elongator complex, these proteins appear to be regulatory.



**Figure 3.** Phosphomodification of Elongator subunit Elp1. (A) Elp1 electrophoretic mobility shifts based on anti-HA Western blots are diagnostic for Elongator de-/phosphorylation.<sup>130,134</sup> In the *kti12* and the kinase-dead *hrr25/kti14* mutants, hypophosphorylated forms of Elp1-HA accumulate while *sit4* phosphatase mutants induce Elp1-HA hyperphosphorylation. Wild-type (*wt*) cells maintain both isoforms of Elp1-HA, which mediate sensitivity (S) to growth inhibition by the tRNase toxin zymocin (killer assay; lower panel; for details see text). Zymocin resistance (R) associates with Elp1 phosphorylation defects in *kti12*, *hrr25/kti14* and *sit4* mutants. (B) Elongator phosphorylation model. Kti12 interacts with Elongator (and kinase Hrr25) thereby potentially activating Elp1 phosphorylation. In support of this, Elp1 is found to be hypophosphorylated in *kti12* and *hrr25/kti14* cells (see A). PPase: protein phosphatase (Sit4); Kinase: Hrr25/Kti14.

Consistent with this, a casein kinase 1 (CK1) isozyme (Kti14, also called: Hrr25), type-2A protein phosphatases (Sit4•Sap185; Sit4•Sap190) and an Elongator interactor (Kti12)<sup>124,127,129</sup> were all shown to affect the phosphorylation state of Elp1, which through dimerization assembles holo-Elongator.<sup>98,102,103,137</sup> Elp1 is present as a hypophosphorylated isoform in *kti12* and *hrr25* mutants and is hyperphosphorylated in *sit4* mutants, while wild-type cells maintain both forms (Fig. 3).<sup>130,131,134</sup> This suggests that Elongator function may be phosphoregulated, which is in line with reports that tRNA modifications including  $mcm^5s^2U_{34}$  can change in response to chemical stress and cell cycle progression.<sup>106,138-140</sup>

Subsequently, phosphorylation sites on Elp1 and other Elongator subunits were identified using mass spectrometry.<sup>141,142</sup> Among those mapped on Elp1, 2 (Ser-1198, Ser-1202) appear to be directly phosphorylated by Hrr25 confirming the genetic data that the CK1 isozyme has Elongator kinase activity. The analysis of phosphosite mutations revealed that Elp1 phosphorylation largely plays a positive role for Elongator activity.<sup>142</sup> Accordingly, profiling modified  $U_{34}$  nucleosides in tRNAs from these phosphosite mutants by LC-MS/MS showed loss of  $mcm^5U_{34}$  and  $mcm^5U_{34}$  formation.<sup>142</sup> The finding that normal phospholevels at Ser-1209 were detectable in a kinase-dead *hrr25* mutant<sup>134</sup> implies that (at least) one additional Elongator kinase ought to exist.<sup>142</sup> Furthermore, altered interaction between Elongator, Hrr25 and Kti12 was seen in several Elp1 phosphosite mutants, in line with data showing that *hrr25* mutants unable to phosphorylate Elp1 affect Elongator association with Kti12.<sup>134,142</sup> The data, therefore, suggest that normal Elongator interaction with Kti12 and proper tRNA modification are facilitated by phosphorylation of Elp1.

### Regulation of $s^2U_{34}$ formation

Whether the *URM1* pathway is regulated is not clear. Most reports describing changes in  $mcm^5s^2U_{34}$  levels in response to chemical stress do not separate the contribution of  $mcm^5$  and  $s^2$  formation.<sup>138,143,144</sup> However, the analysis of 2-thiolation has shown that  $s^2U_{34}$  levels are decreased in response to high temperatures or in growth media lacking a sulfur source.<sup>140,145-148</sup> This is not a consequence of de-thiolation but depends on active transcription of tRNA by RNA polymerase III, since the use of inhibitors or temperature sensitive alleles of RNA polymerase III prevents the decrease in  $s^2U_{34}$ .<sup>140,147</sup> Interestingly, the decrease in  $s^2U_{34}$  is reversible when yeast is shifted back to ambient temperature, which seems like a prerequisite for active regulation. However, the kinetics for up- and downregulation of 2-thiolated tRNA are in the range of several hours.<sup>140,147</sup> This is in contrast to the idea that modification-specific changes to translation could provide a fast switch under stress conditions. The environmental stress response that elicits an extensive transcriptional change in response to various stress conditions peaks after 30 min.<sup>149</sup> A translational rewiring by reducing 2-thiolation would therefore accompany or even follow the transcriptional response to high temperatures rather than being an active driver of such a cellular transition.<sup>140</sup>

But what could be mechanisms for regulation? Different high-throughput analyses have identified phosphosites in

Tum1, Ncs2 and Ncs6.<sup>150,151</sup> However, to date none of these sites has been shown to affect  $s^2U_{34}$  formation *in vivo*. Interestingly, Uba4 and Ncs6 were identified as targets for protein urmylation<sup>65,152</sup> and similarly ATP3BP, the human Ncs6 homolog.<sup>60</sup> However, these studies were performed using increased levels of Urm1 and oxidizing reagents. To show the *in vivo* relevance of urmylation it will be crucial to perform similar experiments under physiological conditions.

### The role of phosphorylation for $U_{34}$ regulation

Although the precise role for Elp1 phosphorylation is unclear, two options can be envisaged. On the one hand, phosphorylation could act as an 'on/off' switch for Elongator's  $U_{34}$  modifying activity, for example, in response to cellular stress. If translation of some mRNAs were dependent on  $U_{34}$  modification and hence tunable by Elongator,<sup>12,90,91,153,154</sup> this raises the possibility that Elongator is part of a translational control-mechanism that functions through its role as a  $U_{34}$  modifier. Such role is consistent with loss-of-function phenotypes associated with Elp1 phosphosite substitutions, kinase-dead *hrr25* mutations and inhibition by ATP analogs of an analog-sensitive Hrr25-I82G kinase variant.<sup>134,142</sup> Although Hrr25 operates in many cellular functions,<sup>155</sup> which complicates the analysis of Elp1 phosphorylation signals, its kinase activity is required for full functionality of ribosomes and  $U_{34}$  containing tRNAs.<sup>134,142,156-158</sup> This is congruent with a role of the kinase in the regulation of a cell's capacity for proper mRNA translation and protein synthesis. Finally, *hrr25* and Elongator mutants are sensitive to DNA damaging mutagens.<sup>87,159</sup> Since efficient translation of the *RNR1* message coding for RiboNucleotide Reductase subunit 1, involved in the DNA damage response (DDR) requires  $U_{34}$ -modified tRNAs,<sup>160</sup> Elp1 phosphorylation may link up to the known role the Hrr25 kinase plays in expression of other DDR genes, i.e. *RNR2* and *RNR3*.<sup>159</sup>

On the other hand, Elongator might require dynamic sequential phosphorylation and dephosphorylation cycles of Elp1 to carry out its tRNA modification reaction.<sup>101,161</sup> It was shown that phosphatase mutants like *sit4* or *sap185sap190* trapped Elp1 in a slower-migrating, hyperphosphorylated form whereas *hrr25* kinase and *kti12* mutants led to the presence of a fast-migrating, hypophosphorylated Elp1 isoform (Fig. 3). Both types of mutations cause loss-of-function phenotypes, suggesting that the functionality of Elongator requires sequential de-/phosphorylation of Elp1. Thus, dynamic de-/phosphorylation may impact on the catalytic activity of Elp3, its localization or even its ability to interact with accessory factors or substrate tRNAs. Importantly, the C-terminus of Elp1, which is phosphorylated, is required for dimerization and is adjacent to a basic region that is crucial for Elongator activity and tRNA binding.<sup>102,103,142,162</sup> Thus, Elp1 phosphorylation may affect holo-Elongator dimerization as well as interaction with partner proteins or its tRNA substrates.

The dynamic model is further supported by the observation that the right balance between hypo- and hyperphosphorylated Elp1 isoforms appears critical for Elongator activity (Fig. 3). This may explain why the presence of exclusively one of the two isoforms results in Elongator loss of function (Fig. 3) and antagonistic de-/phosphorylation of Elp1 by phosphatase/

kinase activities may indeed control its activity.<sup>121,129-131,134</sup> Although this contradicts the idea that Elp1 phosphorylation acts as an 'on/off' switch, data showing that loss of U<sub>34</sub> modifications is similar in *elp1*, *hrr25*, *kti12*, *sit4* and *sap185sap190* mutants with opposite Elp1 phosphorylation states (Fig. 3) agree with the dynamic phosphorylation model.<sup>58,131</sup> However, this would predict that mimicking constitutive phosphorylation on at least some of the Elp1 phosphosites may inhibit Elongator function. Surprisingly, all of the phosphomimetic *ELP1* alleles tested in zymocin assays conferred growth arrest by the tRNase indicating proper Elongator functioning in the U<sub>34</sub> modification pathway.<sup>142</sup> However, whether these mutations are not fully phosphomimetic and thus allowed for residual Elongator activity, has not been analyzed. Thus, although removal of phosphorylation sites provides evidence that Elp1 phosphorylation acts positively on Elongator function, a requirement for dynamic de-/phosphorylation still needs to be shown. Furthermore, it is possible that inhibitory Elp1 phosphosites exist, which were not identified by Abdel-Fattah and colleagues.<sup>142</sup> For example, although Elp1 phosphorylation at Thr-1212 was not detected by MS/MS, a substitution of this residue caused zymocin resistance suggesting that it represents a phosphosite *in vivo*.<sup>142</sup> To conclude, while there is clearly more to learn about Elongator phosphorylation, experimental evidence demonstrates that the kinase Hrr25 affects Elongator's tRNA modification function by phosphorylating (potentially reversible) phosphoacceptor sites in the Elp1 subunit.

### Elongator regulation through Kti11, Kti12 and Kti13 proteins

Although the precise role of Kti12 is unclear, the yeast protein, its plant ortholog (DRL1/ELO4) and PSTK, a tRNA binding kinase, carry N-terminal P-loop motifs typical of nucleotide binding proteins.<sup>118,163-166</sup> Consistent with a functional role for this domain, a P-loop truncation of Kti12 triggers defects typical of Elongator mutants.<sup>124</sup> Importantly, Kti12 supports Elp1 phosphorylation and interacts with the Hrr25 kinase in an Elongator-dependent fashion.<sup>121,124,125,167</sup> *kti12* knockouts abolish Hrr25 interaction with Elongator, cause loss of U<sub>34</sub> modification and trigger the formation of hypophosphorylated Elp1 isoforms (Fig. 3) similar to a *hrr25* kinase mutant.<sup>58,127,134</sup> This led to the proposal that Kti12, through recruitment of Hrr25 to Elongator, positively acts on Elongator's U<sub>34</sub> modification function.<sup>134</sup> Intriguingly, *KTI12* overexpression triggers the accumulation of hyperphosphorylated Elp1 isoforms and suppresses zymocin sensitivity, which is typical of *sit4* phosphatase mutants (Fig. 3).<sup>118,130,133</sup> This effect, however, is likely not caused by altered Elongator interactions in contrast to Elp1 phosphorylation defects, which enhance Kti12 association.<sup>134,142</sup> Furthermore, zymocin suppression through excess Kti12 can be rescued by overexpression of *SIT4*.<sup>130</sup> These genetic interactions suggest a negative role of Kti12 for Sit4 phosphatase function. In support of this idea, multi-copy *KTI12* was found to suppress the rapamycin-resistance of a mutant lacking Resistant to Rapamycin Deletion 1 (Rrd1), a Sit4 activator protein and TOR pathway component, which has not been reported to directly relate to Elongator function.<sup>92</sup> It will be interesting to study whether this additional role for

Kti12 can be separated from its ability to regulate Elongator phosphorylation.

Two additional Elongator regulatory factors are Kti11/Dph3 and Kti13/Ats1. Kti11 is a metal binder and electron transfer protein that copurifies with Fe/S cluster containing complexes including Elongator and Dph1•Dph2.<sup>126,168,169</sup> Moreover, it forms a dimer with Kti13 shown to promote U<sub>34</sub> modification by Elongator and diphthamide synthesis by Dph1•Dph2.<sup>132,136,170,171</sup> Diphthamide is an exotic modification of translation Elongation Factor 2 (EF2),<sup>142,172</sup> which catalyzes ribosomal translocation during translation elongation and is, therefore, essential for protein synthesis and cell viability. Diphthamide-modified EF2 can be inhibited by cytotoxic ADP ribosylases<sup>173</sup> including diphtheria toxin (DT).<sup>172,174</sup> As a consequence, *KTI11* mutations confer resistance against DT and the zymocin tRNase.<sup>123,132,172</sup> Thus, Kti11 and Kti13 appear to partake in U<sub>34</sub> anticodon modifications by Elongator and diphthamide synthesis on EF2<sup>174-176</sup> presumably by providing electrons<sup>170,171</sup> to the Fe/S clusters in Elp3<sup>100</sup> and Dph1•Dph2.<sup>177</sup> With the recent identification of a Kti11 reductase in yeast (Cbr1) that affects Elongator activity,<sup>178</sup> it will be important to clarify the precise roles of Kti11 and Kti13 and study their potential to modulate the electron flow required for both the tRNA and the EF2 modification pathways.

### U<sub>34</sub> modification dependent tRNase ribotoxins

Zymocin inhibits yeast growth through a complex mode of action that involves chitinolysis of the cell wall, tRNase toxin ( $\gamma$ -toxin) uptake, anticodon cleavage and depletion of tRNAs, eventually resulting in cell death.<sup>111,112,114,115,117</sup> Thus, zymocinicity relies on the inhibition of mRNA translation and protein biosynthesis, reminiscent of bacterial anti-phage tRNase and colicin-type anticodon nucleases.<sup>112,179-181</sup> Importantly, up-regulating tRNA repair by overproducing the 2-component system from T4 phage (Rnl1-Pnkp) or tRNA ligase from plants (AtTRL1) suppresses anticodon cleavage and depletion of tRNA<sup>Glu</sup><sub>UUC</sub>.<sup>116</sup> This suggested that the incision generated at the 3' side of the modified wobble base is compatible with sealing and healing functions of heterologous ligases, and that tRNA repair can be an efficient antidote toward lethal anticodon damage by microbial tRNases.<sup>116</sup> This principle was further supported by findings that the inability of the yeast tRNA ligase Trl1 to repair the anticodon damage can be rectified by shuffling genetically engineered ligase constructs. Interestingly, when the domains from AtTRL1 and Trl1 were swapped, the plant ligase domain plus the yeast healing domain rescued *S. cerevisiae* cells against depletion of tRNA<sup>Glu</sup><sub>UUC</sub>. Thus, differences in the ligase component of the plant versus yeast enzymes likely account for tRNase antidote functions.<sup>116</sup> Intriguingly, PaT, a zymocin-related tRNase ribotoxin complex from *Pichia acaciae*, exploits a second site resulting in nucleotide excision rather than incision.<sup>111,182</sup> Hence, tRNA damage by PaT evades reconstitution of a functional ASL by the repair system, which is why plant AtTRL1 ligase fails to suppress PaT toxicity.<sup>182,183</sup>

Other than suppressing zymocin action through direct repair, tRNA protection can also be provided by higher-than-normal levels of tRNA<sup>Gln</sup><sub>UUG</sub>, tRNA<sup>Glu</sup><sub>UUC</sub> and tRNA<sup>Lys</sup><sub>UUU</sub>, which are

targeted for cleavage by the ribotoxin. In fact, a screen for zymocin insensitive mutants identified several tRNA<sup>Glu</sup><sub>UUC</sub> loci as copy-dependent suppressors of the tRNase ribotoxin.<sup>118</sup> Interestingly, this suppression can be efficiently countered by overexpressing the catalytic subunit of U<sub>34</sub> methylase (Trm9•Trm112), suggesting that it is hypomodified tRNA<sup>Glu</sup><sub>UUC</sub>, which in excess is able to bypass zymocin.<sup>58,113</sup> However, full resistance to zymocin is only conferred by mutations in *ELP*, *KTI* or *URM1* pathway genes that trigger the loss of mcm<sup>5</sup>s<sup>2</sup>U<sub>34</sub> modifications in tRNA.

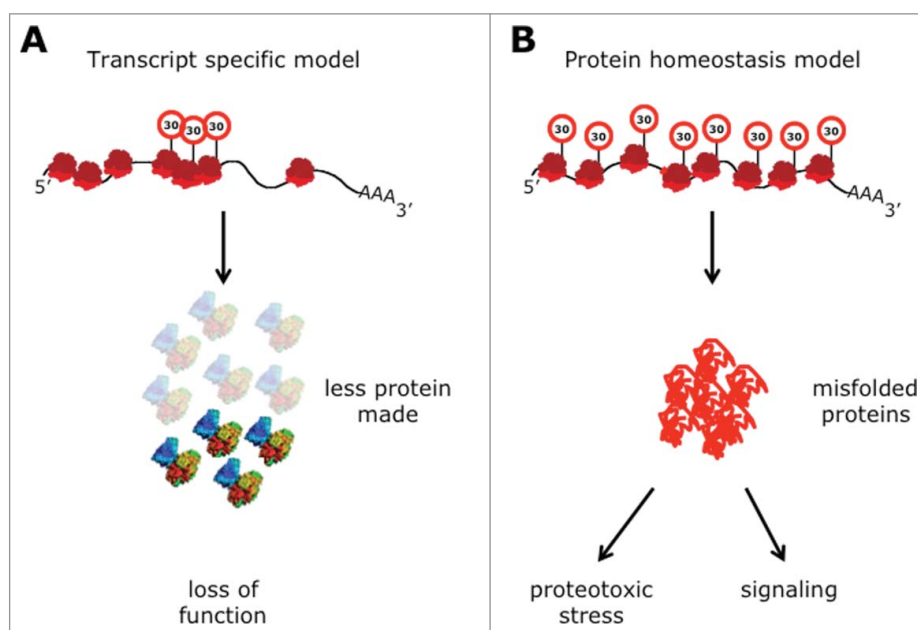
Since the U<sub>34</sub> modification pathways are conserved and elements of the Elongator pathway can be functionally exchanged between yeast and plants,<sup>135,184</sup> it was obvious to apply the yeast tRNase toxins to metazoans. Preliminary findings indicate that tRNase expression not only inhibits yeast growth but also affects the viability of vertebrate cells (RS & SL, data not shown). This is consistent with HeLa cell growth inhibition as well as hypersensitivity reactions in response to transient tRNase induction and tRNA cleavage *in planta*.<sup>185,186</sup> Similarly, bacterial PrrC-type anticodon nucleases were found to be lethal when expressed in yeast.<sup>187</sup> Importantly, using cytotoxic tRNase to study the formation of microbial biofilms suggests that tRNA cleavage may also be used in cell-cell communications.<sup>181,187</sup> Thus, it is tempting to exploit tRNase ribotoxins as anti-proliferative agents for use in biomedical interventions against infections by microbial, fungal or viral pathogens or to prevent undesired growth of tumor cells whose proliferation heavily relies on protein synthesis and therefore, proper tRNA function.<sup>181,188</sup>

### The origin of phenotypes of U<sub>34</sub> defects

Exploiting U<sub>34</sub> modifications to kill competing yeasts is a curious strategy for pathogenicity. But what are the molecular

mechanisms that underlie the pleiotropic phenotypes that we observe in organisms with inappropriate levels of U<sub>34</sub> modification? The absence of wobble uridine modifications is accompanied by increased sensitivity to biotic and abiotic stresses and defects in numerous cellular processes including transcription, DDR, exocytosis, telomere gene silencing and DNA demethylation.<sup>6,81-85,87,88</sup> In prokaryotes, s<sup>2</sup>U<sub>34</sub> deficient tRNAs are poor substrates for aminoacyl-tRNA synthetases.<sup>189</sup> This however, does not appear to be the case in eukaryotes and can, therefore, not explain the observed phenotypes.<sup>20</sup>

Most strikingly in yeast, except for the U<sub>34</sub> modification defects, all known phenotypes can be rescued by overexpression of tRNAs that would normally be decorated by mcm<sup>5</sup>s<sup>2</sup>U<sub>34</sub> (see above). In *S. cerevisiae*, overexpression of tRNA<sup>Gln</sup><sub>UUG</sub> and tRNA<sup>Lys</sup><sub>UUU</sub> efficiently rescues the defects. This is in good agreement with ribosome-profiling experiments, which found that the codons CAA and AAA decoded by tRNA<sup>Gln</sup><sub>UUG</sub> and tRNA<sup>Lys</sup><sub>UUU</sub>, respectively, slow down during translation while a slow down at GAA (decoded by tRNA<sup>Glu</sup><sub>UUC</sub>) was not detected consistently.<sup>45,91</sup> Computational attempts to identify transcripts that are enriched in A-ending codons as well as several screens and proteomics studies have reported targets that are downregulated at the protein level in U<sub>34</sub> modification mutants.<sup>143,146,160,190</sup> Interestingly, it is possible to rescue the levels of some of these target proteins by using engineered gene constructs that have AAA codons exchanged by AAG.<sup>51,191</sup> However, in contrast to tRNA overexpression, none of these synthetic codon rescue experiments has suppressed the underlying phenotype. Failure to do so can have several reasons: First, the phenotypes may be triggered by the loss of function of a group of proteins rather than by the absence of individual proteins (Fig. 4A). Second, instead of a loss-of-function phenotype, the effects may be triggered by a cytotoxic gain-of-function (Fig. 4B). This concept is based on the recent findings that



**Figure 4.** Two models to explain phenotypes of U<sub>34</sub> modification mutants. (A) Specific mRNA enriched in codons that depend on tRNA modifications are translated at lower rates. This leads to reduced levels of the protein encoded by this transcript triggering a loss-of-function phenotype. (B) Ribosomes slow down when translating codons that depend on tRNA modifications. The slowdown perturbs the optimized equilibrium between speed of protein synthesis and protein folding. The increased rate of protein stress leads to a systemic failure in protein homeostasis and the aggregation of endogenous proteins that associates with it in a toxic gain-of-function scenario. This can either affect viability of the cells directly or by changing cellular signaling (Street signs with “30” indicate slow speed of ribosomes).

U<sub>34</sub> modification mutants as well as other dysfunctional tRNA modification pathways are characterized by protein homeostasis defects.<sup>45,139,154</sup> In yeast, these lead to the aggregation of endogenous metastable proteins in the cytoplasm.<sup>45</sup> In mouse brains, the same modification defects induce the unfolded protein response in the endoplasmic reticulum, which triggers differentiation defects in neuronal precursors, leading to microcephaly.<sup>52</sup> Thus, proteotoxic stress provides an alternative mechanism (Fig. 4B) to explain the pleiotropic phenotypes. In this scenario, codon-specific translation defects perturb the equilibrium of mRNA-translation dynamics and peptide-chain folding that has been optimized during evolution to ensure accurate protein synthesis, folding and homeostasis. As a result, proteotoxic stress alone or in combination with the standard model that favors reduction of individual proteins (Fig. 4A), is likely the main trigger for pleiotropic defects by severely rearranging the cellular proteome or by interfering with downstream signaling of the affected cells.

Importantly, the underlying mechanism of U<sub>34</sub> defects has significant consequences for our options to remedy phenotypes, particularly in the context of human disease: The loss-of-function model suggests that the identification of undertranslated mRNAs will lead to treatment options by enhancing the activity of their encoded proteins. In contrast, the protein-homeostasis model suggests that instead of repairing individual proteins, our response rather has to focus on alleviating proteotoxicity or signaling output that is induced downstream of proteotoxic stress.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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## References

- Armengod ME, Meseguer S, Villarroya M, Prado S, Moukadiri I, Ruiz-Partida R, Garzón MJ, Navarro-González C, Martínez-Zamora A. Modification of the wobble uridine in bacterial and mitochondrial tRNAs reading NNA/NNG triplets of 2-codon boxes. *RNA Biol* 2015; 11:1495-507; <https://doi.org/10.4161/15476286.2014.992269>
- Jackman JE, Alfonzo JD. Transfer RNA modifications: nature's combinatorial chemistry playground. *WIREs RNA* 2012; 4:35-48; PMID:23139145; <https://doi.org/10.1002/wrna.1144>
- Helm M, Alfonzo JD. Posttranscriptional RNA Modifications: playing metabolic games in a cell's chemical Legoland. *Chem Biol* 2014; 21:174-85; PMID:24315934; <https://doi.org/10.1016/j.chembiol.2013.10.015>
- Grosjean H, Breton M, Sirand-Pugnet P, Tardy F, Thiaucourt F, Citti C, Barré A, Yoshizawa S, Fourmy D, de Crécy-Lagard V, et al. Predicting the Minimal Translation Apparatus: Lessons from the Reductive Evolution of Mollicutes. *PLoS Genet* 2014; 10:e1004363; PMID:24809820; <https://doi.org/10.1371/journal.pgen.1004363>
- Hutchison CA, Chuang R-Y, Noskov VN, Assad-Garcia N, Deerinck TJ, Ellisman MH, Gill J, Kannan K, Karas BJ, Ma L, et al. Design and synthesis of a minimal bacterial genome. *Science* 2016; 351:aad6253; PMID:27013737; <https://doi.org/10.1126/science.aad6253>
- Huang B, Johansson MJO, Byström AS. An early step in wobble uridine tRNA modification requires the Elongator complex. *RNA* 2005; 11:424-36; PMID:15769872; <https://doi.org/10.1261/rna.7247705>
- Bjork GR, Huang B, Persson OP, Byström AS. A conserved modified wobble nucleoside (mcm<sup>5</sup>s<sup>2</sup>U) in lysyl-tRNA is required for viability in yeast. *RNA* 2007; 13:1245-55; PMID:17592039; <https://doi.org/10.1261/rna.558707>
- Dewez M, Bauer F, Dieu M, Raes M, Vandehaute J, Hermant D. The conserved Wobble uridine tRNA thiolase Ctu1-Ctu2 is required to maintain genome integrity. *Proc Natl Acad Sci USA* 2008; 105:5459-64; PMID:18391219; <https://doi.org/10.1073/pnas.0709404105>
- Chen C, Tuck S, Byström AS. Defects in tRNA modification associated with neurological and developmental dysfunctions in *Caenorhabditis elegans* elongator mutants. *PLoS Genet* 2009; 5:e1000561; PMID:19593383; <https://doi.org/10.1371/journal.pgen.1000561>
- Chen Y-T, Hims MM, Shetty RS, Mull J, Liu L, Leyne M, Slaugenhaupt SA. Loss of mouse Ikbkap, a subunit of elongator, leads to transcriptional deficits and embryonic lethality that can be rescued by human IKBKAP. *Mol Cell Biol* 2009; 29:736-44; PMID:19015235; <https://doi.org/10.1128/MCB.01313-08>
- Leidel S, Pedrioli PGA, Bucher T, Brost RL, Costanzo M, Schmidt A, Aebersold R, Boone C, Hofmann K, Peter M. Ubiquitin-related modifier Urm1 acts as a sulphur carrier in thiolation of eukaryotic transfer RNA. *Nature* 2009; 458:228-32; PMID:19145231; <https://doi.org/10.1038/nature07643>
- Klassen R, Grunewald P, Thüring KL, Eichler C, Helm M, Schaffrath R. Loss of Anticodon Wobble Uridine Modifications Affects tRNA<sup>Lys</sup> Function and Protein Levels in *Saccharomyces cerevisiae*. *PLoS ONE* 2015; 10:e0119261; PMID:25747122; <https://doi.org/10.1371/journal.pone.0119261>
- Torres AG, Battle E, Ribas de Pouplana L. Role of tRNA modifications in human diseases. *Trends Mol Med* 2014; 20:306-14; PMID:24581449; <https://doi.org/10.1016/j.molmed.2014.01.008>
- Sarin LP, Leidel SA. Modify or die? - RNA modification defects in metazoans. *RNA Biol* 2014; 11:1555-67; PMID:25692999; <https://doi.org/10.4161/15476286.2014.992279>
- Kojic M, Wainwright B. The Many Faces of Elongator in Neurodevelopment and Disease. *Front Mol Neurosci* 2016; 9:1-10; PMID:26834556; <https://doi.org/10.3389/fnmol.2016.00115>
- Stansfield I, Jones KM, Kushnirov VV, Dagkesamanskaya AR, Poznyakovskii AI, Paushkin SV, Nierras CR, Cox BS, Ter-Avanesyan MD, Tuite MF. The products of the SUP45 (eRF1) and SUP35 genes interact to mediate translation termination in *Saccharomyces cerevisiae*. *EMBO J* 1995; 14:4365-73; PMID:7556078
- Zhouravleva G, Frolova L, Le Goff X, Le Guellec R, Inge-Vechtomov S, Kisselev L, Philippe M. Termination of translation in eukaryotes is governed by two interacting polypeptide chain release factors, eRF1 and eRF3. *EMBO J* 1995; 14:4065-72; PMID:7664746



18. Randerath E, Gupta RC, Chia LL, Chang SH, Randerath K. Yeast tRNA Leu UAG. Purification, properties and determination of the nucleotide sequence by radioactive derivative methods. *Eur J Biochem* 1979; 93:79-94; PMID: 374075; <https://doi.org/10.1111/j.1432-1033.1979.tb12797.x>
19. Szweykowska-Kulinska Z, Senger B, Keith G, Fasiolo F, Grosjean H. Intron-dependent formation of pseudouridines in the anticodon of *Saccharomyces cerevisiae* minor tRNA<sup>Leu</sup>. *EMBO J* 1994; 13:4636-44; PMID:7925304
20. Johansson MJO, Esberg A, Huang B, Bjork GR, Bystrom AS. Eukaryotic wobble uridine modifications promote a functionally redundant decoding system. *Mol Cell Biol* 2008; 28:3301-12; PMID:18332122; <https://doi.org/10.1128/MCB.01542-07>
21. Dunin-Horkawicz S, Czerwoniec A, Gajda MJ, Feder M, Grosjean H, Bujnicki JM. MODOMICS: a database of RNA modification pathways. *Nucleic Acids Res* 2006; 34:D145-9; PMID:16381833; <https://doi.org/10.1093/nar/gkj084>
22. Crick FH. Codon-anticodon pairing: the wobble hypothesis. *J Mol Biol* 1966; 19:548-55; PMID:5969078; [https://doi.org/10.1016/S0022-2836\(66\)80022-0](https://doi.org/10.1016/S0022-2836(66)80022-0)
23. Agris PF. Wobble position modified nucleosides evolved to select transfer RNA codon recognition: a modified-wobble hypothesis. *Biochimie* 1991; 73:1345-9; PMID:1799628; [https://doi.org/10.1016/0300-9084\(91\)90163-U](https://doi.org/10.1016/0300-9084(91)90163-U)
24. Yokoyama S, Nishimura S. Modified Nucleosides and Codon Recognition. In: tRNA: Structure, Biosynthesis, and Function, Soll D and RajBhandary UL (eds); American Society for Microbiology, Washington 1995; 207-223; <https://doi.org/10.1128/9781555818333.ch12>
25. Agris PF. Decoding the genome: a modified view. *Nucleic Acids Res* 2004; 32:223-38; PMID:14715921; <https://doi.org/10.1093/nar/gkh185>
26. Murphy FV, Ramakrishnan V, Malkiewicz A, Agris PF. The role of modifications in codon discrimination by tRNA<sup>Lys</sup>UUU. *Nat Struct Mol Biol* 2004; 11:1186-91; PMID:15558052; <https://doi.org/10.1038/nsmb861>
27. Sierzputowska H, Sochacka E, Malkiewicz A, Kuo KC, Gehrke CW, Agris PF. Chemistry and Structure of Modified Uridines in the Anticodon, Wobble Position of Transfer-RNA Are Determined by Thiolation. *J Am Chem Soc* 1987; 109:7171-7; <https://doi.org/10.1021/ja00257a044>
28. Agris PF, Sierzputowska H, Smith W, Malkiewicz A, Sochacka E, Nawrot B. Thiolation of Uridine Carbon-2 Restricts the Motional Dynamics of the Transfer-RNA Wobble Position Nucleoside. *J Am Chem Soc* 1992; 114:2652-6; <https://doi.org/10.1021/ja00033a044>
29. Yokoyama S, Yamaizumi Z, Nishimura S, Miyazawa T. 1H NMR studies on the conformational characteristics of 2-thiopyrimidine nucleotides found in transfer RNAs. *Nucleic Acids Res* 1979; 6:2611-26; PMID:379825; <https://doi.org/10.1093/nar/6.7.2611>
30. Kawai G, Yamamoto Y, Kamimura T, Masegi T, Sekine M, Hata T, Iimori T, Watanabe T, Miyazawa T, Yokoyama S. Conformational rigidity of specific pyrimidine residues in tRNA arises from posttranscriptional modifications that enhance steric interaction between the base and the 2'-hydroxyl group. *Biochemistry* 1992; 31:1040-6; PMID:1310418; <https://doi.org/10.1021/bi00119a012>
31. Yamamoto Y, Yokoyama S, Miyazawa T, Watanabe K, Higuchi S. NMR analyses on the molecular mechanism of the conformational rigidity of 2-thioribothymidine, a modified nucleoside in extreme thermophile tRNAs. *FEBS Lett* 1983; 157:95-9; PMID:6190675; [https://doi.org/10.1016/0014-5793\(83\)81123-5](https://doi.org/10.1016/0014-5793(83)81123-5)
32. Testa SM, Disney MD, Turner DH, Kierzek R. Thermodynamics of RNA-RNA duplexes with 2- or 4-thiouridines: implications for antisense design and targeting a group I intron. *Biochemistry* 1999; 38:16655-62; PMID:10600128; <https://doi.org/10.1021/bi991187d>
33. Larsen AT, Fahrenbach AC, Sheng J, Pian J, Szostak JW. Thermodynamic insights into 2-thiouridine-enhanced RNA hybridization. *Nucleic Acids Res* 2015; 43:7675-87; PMID:26240387; <https://doi.org/10.1093/nar/gkv761>
34. Davis DR, Durant PC. Nucleoside modifications affect the structure and stability of the anticodon of tRNA<sup>Lys,3</sup>. *Nucleosides Nucleotides* 1999; 18:1579-81; PMID:10474235; <https://doi.org/10.1080/07328319908044790>
35. Smith WS, Sierzputowska-Gracz H, Sochacka E, Malkiewicz A, Agris PF. Chemistry and Structure of Modified Uridine Dinucleosides are Determined by Thiolation. *J Am Chem Soc* 1992; 114:7989-97; <https://doi.org/10.1021/ja00047a005>
36. Kumar RK, Davis DR. Synthesis and studies on the effect of 2-thiouridine and 4-thiouridine on sugar conformation and RNA duplex stability. *Nucleic Acids Res* 1997; 25:1272-80; PMID:9092639; <https://doi.org/10.1093/nar/25.6.1272>
37. Houssier C, Degée P, Nicoghosian K, Grosjean H. Effect of uridine dethiolation in the anticodon triplet of tRNA(Glu) on its association with tRNA(Phe). *J Biomol Struct Dyn* 1988; 5:1259-66; PMID:2482764; <https://doi.org/10.1080/07391102.1988.10506468>
38. Ashraf SS, Sochacka E, Cain R, Guenther R, Malkiewicz A, Agris PF. Single atom modification (O->S) of tRNA confers ribosome binding. *RNA* 1999; 5:188-94; PMID:10024171; <https://doi.org/10.1017/S1355838299981529>
39. Kurata S, Weixlbaumer A, Ohtsuki T, Shimazaki T, Wada T, Kirino Y, Takai K, Watanabe K, Ramakrishnan V, Suzuki T. Modified Uridines with C5-methylene Substituents at the First Position of the tRNA Anticodon Stabilize U:G Wobble Pairing during Decoding. *J Biol Chem* 2008; 283:18801-11; PMID:18456657; <https://doi.org/10.1074/jbc.M800233200>
40. Ogle JM, Murphy FV, Tarry MJ, Ramakrishnan V. Selection of tRNA by the ribosome requires a transition from an open to a closed form. *Cell* 2002; 111:721-32; PMID:12464183; [https://doi.org/10.1016/S0092-8674\(02\)01086-3](https://doi.org/10.1016/S0092-8674(02)01086-3)
41. Yarian C, Marszalek M, Sochacka E, Malkiewicz A, Guenther R, Misiewicz A, Agris PF. Modified nucleoside dependent Watson-Crick and wobble codon binding by tRNA<sup>Lys</sup>UUU species. *Biochemistry* 2000; 39:13390-5; PMID:11063576; <https://doi.org/10.1021/bi001302g>
42. Grosjean H, Westhof E. An integrated, structure- and energy-based view of the genetic code. *Nucleic Acids Res* 2016; 44:8020-40; PMID:27448410; <https://doi.org/10.1093/nar/gkw608>
43. Demeshkina N, Jenner L, Westhof E, Yusupov M, Yusupova G. A new understanding of the decoding principle on the ribosome. *Nature* 2012; 484:256-9; PMID:22437501; <https://doi.org/10.1038/nature10913>
44. Vendeix FAP, Murphy FV, Cantara WA, Leszczynska G, Gustilo EM, Sproat B, Malkiewicz A, Agris PF. Human tRNA<sup>Lys</sup>UUU is pre-structured by natural modifications for cognate and wobble codon binding through keto-enol tautomerism. *J Mol Biol* 2012; 416:467-85; PMID:22227389; <https://doi.org/10.1016/j.jmb.2011.12.048>
45. Nedialkova DD, Leidel SA. Optimization of Codon Translation Rates via tRNA Modifications Maintains Proteome Integrity. *Cell* 2015; 161:1606-18; PMID:26052047; <https://doi.org/10.1016/j.cell.2015.05.022>
46. Simos G, Tekotte H, Grosjean H, Segref A, Sharma K, Tollervey D, Hurt EC. Nuclear pore proteins are involved in the biogenesis of functional tRNA. *EMBO J* 1996; 15:2270-84; PMID:8641292
47. Motorin Y, Keith G, Simon C, Foiret D, Simos G, Hurt E, Grosjean H. The yeast tRNA:pseudouridine synthase Pus1p displays a multi-site substrate specificity. *RNA* 1998; 4:856-69; PMID:9671058; <https://doi.org/10.1017/S1355838298980396>
48. Pintard L, Lecoite F, Bujnicki JM, Bonnerot C, Grosjean H, Lapeyre B. Trm7p catalyses the formation of two 2'-O-methylriboses in yeast tRNA anticodon loop. *EMBO J* 2002; 21:1811-20; PMID:11927565; <https://doi.org/10.1093/emboj/21.7.1811>
49. Guy MP, Podyma BM, Preston MA, Shaheen HH, Krivos KL, Limbach PA, Hopper AK, Phizicky EM. Yeast Trm7 interacts with distinct proteins for critical modifications of the tRNA<sup>Phe</sup> anticodon loop. *RNA* 2012; 18:1921-33; PMID:22912484; <https://doi.org/10.1261/rna.035287.112>
50. Nakai Y, Nakai M, Hayashi H. Thio-modification of yeast cytosolic tRNA requires a ubiquitin-related system that resembles bacterial sulfur transfer systems. *J Biol Chem* 2008; 283:27469-76; PMID:18664566; <https://doi.org/10.1074/jbc.M804043200>

51. Fernández-Vázquez J, Vargas-Pérez I, Sansó M, Buhne K, Carmona M, Paulo E, Hermand D, Rodríguez-Gabriel M, Ayté J, Leidel S, et al. Modification of tRNA<sup>Lys</sup>UUU by elongator is essential for efficient translation of stress mRNAs. *PLoS Genet* 2013; 9:e1003647; PMID:23874237; <https://doi.org/10.1371/journal.pgen.1003647>
52. Laguesse S, Creppe C, Nedialkova DD, Prévot P-P, Borgs L, Huysseune S, Franco B, Duysens G, Krusy N, Lee G, et al. A Dynamic Unfolded Protein Response Contributes to the Control of Cortical Neurogenesis. *Dev Cell* 2015; 35:553-67; PMID:26651292; <https://doi.org/10.1016/j.devcel.2015.11.005>
53. Furukawa K, Mizushima N, Noda T, Ohsumi Y. A protein conjugation system in yeast with homology to biosynthetic enzyme reaction of prokaryotes. *J Biol Chem* 2000; 275:7462-5; PMID:10713047; <https://doi.org/10.1074/jbc.275.11.7462>
54. Goehring AS, Rivers DM, Sprague GF. Attachment of the ubiquitin-related protein Urm1p to the antioxidant protein Ahp1p. *Eukaryotic Cell* 2003; 2:930-6; PMID:14555475; <https://doi.org/10.1128/EC.2.5.930-936.2003>
55. Goehring AS, Rivers DM, Sprague GF. Urm1ylation: a ubiquitin-like pathway that functions during invasive growth and budding in yeast. *Mol Biol Cell* 2003; 14:4329-41; PMID:14551258; <https://doi.org/10.1091/mbc.E03-02-0079>
56. Singh S, Tonelli M, Tyler RC, Bahrami A, Lee MS, Markley JL. Three-dimensional structure of the AAH26994.1 protein from *Mus musculus*, a putative eukaryotic Urm1. *Protein Sci* 2005; 14:2095-102; PMID:16046629; <https://doi.org/10.1110/ps.051577605>
57. Xu J, Zhang J, Wang L, Zhou J, Huang H, Wu J, Zhong Y, Shi Y. Solution structure of Urm1 and its implications for the origin of protein modifiers. *Proc Natl Acad Sci USA* 2006; 103:11625-30; PMID:16864801; <https://doi.org/10.1073/pnas.0604876103>
58. Huang B, Lu J, Byström AS. A genome-wide screen identifies genes required for formation of the wobble nucleoside 5-methoxycarbonylmethyl-2-thiouridine in *Saccharomyces cerevisiae*. *RNA* 2008; 14:2183-94; PMID:18755837; <https://doi.org/10.1261/rna.1184108>
59. Pedrioli PGA, Leidel S, Hofmann K. Urm1 at the crossroad of modifications. "Protein Modifications: Beyond the Usual Suspects" Review Series. *EMBO Rep* 2008; 9:1196-202; PMID:19047990; <https://doi.org/10.1038/embor.2008.209>
60. Schlieker CD, Van der Veen AG, Damon JR, Spooner E, Ploegh HL. A functional proteomics approach links the ubiquitin-related modifier Urm1 to a tRNA modification pathway. *Proc Natl Acad Sci USA* 2008; 105:18255-60; PMID: 19017811; <https://doi.org/10.1073/pnas.0808756105>
61. Schmitz J, Chowdhury MM, Hänzelmann P, Nimtz M, Lee E-Y, Schindelin H, Leimkuhler S. The sulfurtransferase activity of Uba4 presents a link between ubiquitin-like protein conjugation and activation of sulfur carrier proteins. *Biochemistry* 2008; 47:6479-89; PMID:18491921; <https://doi.org/10.1021/bi800477u>
62. Nakai Y, Umeda N, Suzuki T, Nakai M, Hayashi H, Watanabe K, Kagamiyama H. Yeast Nfs1p is involved in thio-modification of both mitochondrial and cytoplasmic tRNAs. *J Biol Chem* 2004; 279:12363-8; PMID:14722066; <https://doi.org/10.1074/jbc.M312448200>
63. Noma A, Sakaguchi Y, Suzuki T. Mechanistic characterization of the sulfur-relay system for eukaryotic 2-thiouridine biogenesis at tRNA wobble positions. *Nucleic Acids Res* 2009; 37:1335-52; PMID:19151091; <https://doi.org/10.1093/nar/gkn1023>
64. Petroski MD, Salvesen GS, Wolf DA. Urm1 couples sulfur transfer to ubiquitin-like protein function in oxidative stress. *Proc Natl Acad Sci USA* 2011; 108:1749-50; PMID:21245332; <https://doi.org/10.1073/pnas.1019043108>
65. Van der Veen AG, Schorpp K, Schlieker C, Buti L, Damon JR, Spooner E, Ploegh HL, Jentsch S. Role of the ubiquitin-like protein Urm1 as a noncanonical lysine-directed protein modifier. *Proc Natl Acad Sci USA* 2011; 108:1763-70; PMID:21209336; <https://doi.org/10.1073/pnas.1014402108>
66. Van der Veen AG, Ploegh HL. Ubiquitin-Like Proteins. *Annu Rev Biochem* 2012; 81:323-57; PMID:22404627; <https://doi.org/10.1146/annurev-biochem-093010-153308>
67. Jüdes A, Bruch A, Klassen R, Helm M, Schaffrath R. Sulfur transfer and activation by ubiquitin-like modifier system Uba4•Urm1 link protein urmylation and tRNA thiolation in yeast. *Microb Cell* 2016; 423-433; <https://doi.org/10.15698/mic2016.11.539>
68. Hofmann K, Bucher P, Kajava AV. A model of Cdc25 phosphatase catalytic domain and Cdk-interaction surface based on the presence of a rhodanese homology domain. *J Mol Biol* 1998; 282:195-208; PMID:9733650
69. Bordo D, Bork P. The rhodanese/Cdc25 phosphatase superfamily. Sequence-structure-function relations. *EMBO Rep* 2002; 3:741-6; PMID:12151332; <https://doi.org/10.1093/embo-reports/kvf150>
70. Hochstrasser M. Evolution and function of ubiquitin-like protein-conjugation systems. *Nat Cell Biol* 2000; 2:E153-7; PMID:10934491; <https://doi.org/10.1038/35019643>
71. Goehring AS, Mitchell DA, Tong AHY, Keniry ME, Boone C, Sprague GF. Synthetic lethal analysis implicates Ste20p, a p21-activated protein kinase, in polarisome activation. *Mol Biol Cell* 2003; 14:1501-16; PMID:12686605
72. Liu Y, Vinyard DJ, Reesbeck ME, Suzuki T, Manakongtreecheep K, Holland PL, Brudvig GW, Soll D. A [3Fe-4S] cluster is required for tRNA thiolation in archaea and eukaryotes. *Proc Natl Acad Sci USA* 2016; published online 24.10.2016; PMID:27791189; <https://doi.org/10.1073/pnas.1615732113>
73. Kambampati R, Lauhon CT. MnmA and IscS are required for in vitro 2-thiouridine biosynthesis in *Escherichia coli*. *Biochemistry* 2003; 42:1109-17; PMID:12549933; <https://doi.org/10.1021/bi026536+>
74. Mueller EG, Palenchar PM. Using genomic information to investigate the function of ThiI, an enzyme shared between thiamin and 4-thiouridine biosynthesis. *Protein Sci* 1999; 8:2424-7; PMID:10595545; <https://doi.org/10.1110/ps.8.11.2424>
75. Nakagawa H, Kuratani M, Goto-Ito S, Ito T, Katsura K, Terada T, Shirouzu M, Sekine S-I, Shigi N, Yokoyama S. Crystallographic and mutational studies on the tRNA thiouridine synthetase TtuA. *Proteins* 2013; 81:1232-44; PMID:23444054; <https://doi.org/10.1002/prot.24273>
76. Kolman C, Soll D. SPL1-1, a *Saccharomyces cerevisiae* mutation affecting tRNA splicing. *J Bacteriol* 1993; 175:1433-42; PMID:8444805; <https://doi.org/10.1128/jb.175.5.1433-1442.1993>
77. Nakai Y, Nakai M, Lill R, Suzuki T, Hayashi H. Thio modification of yeast cytosolic tRNA is an iron-sulfur protein-dependent pathway. *Mol Cell Biol* 2007; 27:2841-7; PMID:17283054; <https://doi.org/10.1128/MCB.01321-06>
78. Paul VD, Lill R. Biogenesis of cytosolic and nuclear iron-sulfur proteins and their role in genome stability. *Biochim Biophys Acta* 2015; 1853:1528-39; PMID:25583461; <https://doi.org/10.1016/j.bbamcr.2014.12.018>
79. Gerber J, Neumann K, Prohl C, Mühlenhoff U, Lill R. The yeast scaffold proteins Isu1p and Isu2p are required inside mitochondria for maturation of cytosolic Fe/S proteins. *Mol Cell Biol* 2004; 24:4848-57; PMID:15143178; <https://doi.org/10.1128/MCB.24.11.4848-4857.2004>
80. Inigo S, Durand AN, Ritter A, Le Gall S, Termathe M, Klassen R, Tohge T, De Coninck B, Van Leene J, De Clercq R, et al. Glutaredoxin GRXS17 Associates with the Cytosolic Iron-Sulfur Cluster Assembly Pathway. *Plant Physiol* 2016; 172:858-73; PMID:27503603; <https://doi.org/10.1104/pp.16.00261>
81. Otero G, Fellows J, Li Y, de Bizemont T, Dirac AM, Gustafsson CM, Erdjument-Bromage H, Tempst P, Svejstrup JQ. Elongator, a multi-subunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation. *Mol Cell* 1999; 3:109-18; PMID:10024884; [https://doi.org/10.1016/S1097-2765\(00\)80179-3](https://doi.org/10.1016/S1097-2765(00)80179-3)
82. Winkler GS, Petrakis TG, Ethelberg S, Tokunaga M, Erdjument-Bromage H, Tempst P, Svejstrup JQ. RNA polymerase II elongator holoenzyme is composed of two discrete subcomplexes. *J Biol Chem* 2001; 276:32743-9; PMID:11435442; <https://doi.org/10.1074/jbc.M105303200>
83. Winkler GS, Kristjuhan A, Erdjument-Bromage H, Tempst P, Svejstrup JQ. Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels *in vivo*. *Proc Natl*

- Acad Sci USA 2002; 99:3517-22; PMID:11904415; <https://doi.org/10.1073/pnas.022042899>
84. Li Y, Takagi Y, Jiang Y, Tokunaga M, Erdjument-Bromage H, Tempst P, Kornberg RD. A multiprotein complex that interacts with RNA polymerase II elongator. *J Biol Chem* 2001; 276:29628-31; PMID:11390369; <https://doi.org/10.1074/jbc.C100274200>
  85. Rahl PB, Chen CZ, Collins RN. Elp1p, the yeast homolog of the FD disease syndrome protein, negatively regulates exocytosis independently of transcriptional elongation. *Mol Cell* 2005; 17:841-53; PMID:15780940; <https://doi.org/10.1016/j.molcel.2005.02.018>
  86. Svejstrup JQ. Elongator complex: how many roles does it play? *Curr Opin Cell Biol* 2007; 19:331-6; PMID:17466506; <https://doi.org/10.1016/j.ceb.2007.04.005>
  87. Li Q, Fazly AM, Zhou H, Huang S, Zhang Z, Stillman B. The elongator complex interacts with PCNA and modulates transcriptional silencing and sensitivity to DNA damage agents. *PLoS Genet* 2009; 5:e1000684; PMID:19834596; <https://doi.org/10.1371/journal.pgen.1000684>
  88. Chen C, Huang B, Eliasson M, Rydén P, Byström AS. Elongator complex influences telomeric gene silencing and DNA damage response by its role in wobble uridine tRNA modification. *PLoS Genet* 2011; 7:e1002258; PMID:21912530; <https://doi.org/10.1371/journal.pgen.1002258>
  89. Esberg A, Huang B, Johansson MJO, Byström AS. Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. *Mol Cell* 2006; 24:139-48; PMID:17018299; <https://doi.org/10.1016/j.molcel.2006.07.031>
  90. Bauer F, Hermand D. A coordinated codon-dependent regulation of translation by Elongator. *Cell Cycle* 2012; 11; 4524-9; PMID:23165209; <https://doi.org/10.4161/cc.22689>
  91. Zinshteyn B, Gilbert WV. Loss of a conserved tRNA anticodon modification perturbs cellular signaling. *PLoS Genet* 2013; 9:e1003675; PMID:23935536; <https://doi.org/10.1371/journal.pgen.1003675>
  92. Scheidt V, Jüdes A, Bär C, Klassen R. Loss of wobble uridine modification in tRNA anticodons interferes with TOR pathway signaling. *Microb Cell* 2014; 1:416-426; <https://doi.org/10.15698/mic2014.12.179>
  93. Tigano M, Ruotolo R, Dallabona C, Fontanesi F, Barrientos A, Donnini C, Ottonello S. Elongator-dependent modification of cytoplasmic tRNA<sup>Lys</sup>UUU is required for mitochondrial function under stress conditions. *Nucleic Acids Res* 2015; 43:8368-80; PMID:26240381; <https://doi.org/10.1093/nar/gkv765>
  94. Tükenmez H, Xu H, Esberg A, Byström AS. The role of wobble uridine modifications in +1 translational frameshifting in eukaryotes. *Nucleic Acids Res* 2015; 43:9489-99; PMID:26283182; <https://doi.org/10.1093/nar/gkv832>
  95. Klassen R, Bruch A, Schaffrath R. Independent suppression of ribosomal +1 frameshifts by different tRNA anticodon loop modifications. *RNA Biol* 2016; published online 12.12.2016; PMID:27937809; <https://doi.org/10.1080/15476286.2016.1267098>
  96. Wittschieben BO, Otero G, de Bizemont T, Fellows J, Erdjument-Bromage H, Ohba R, Li Y, Allis CD, Tempst P, Svejstrup JQ. A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. *Mol Cell* 1999; 4:123-8; PMID:10445034; [https://doi.org/10.1016/S1097-2765\(00\)80194-X](https://doi.org/10.1016/S1097-2765(00)80194-X)
  97. Krogan NJ, Greenblatt JF. Characterization of a six-subunit holo-elongator complex required for the regulated expression of a group of genes in *Saccharomyces cerevisiae*. *Mol Cell Biol* 2001; 21:8203-12; PMID:11689709; <https://doi.org/10.1128/MCB.21.23.8203-8212.2001>
  98. Glatt S, Létoquart J, Faux C, Taylor NMI, Séraphin B, Müller CW. The Elongator subcomplex Elp456 is a hexameric RecA-like ATPase. *Nat Struct Mol Biol* 2012; 19:314-20; PMID:22343726; <https://doi.org/10.1038/nsmb.2234>
  99. Wittschieben BO, Fellows J, Du W, Stillman DJ, Svejstrup JQ. Overlapping roles for the histone acetyltransferase activities of SAGA and elongator in vivo. *EMBO J* 2000; 19:3060-8; PMID:10856249; <https://doi.org/10.1093/emboj/19.12.3060>
  100. Paraskevopoulou C, Fairhurst SA, Lowe DJ, Brick P, Onesti S. The Elongator subunit Elp3 contains a Fe<sub>4</sub>S<sub>4</sub> cluster and binds S-adenosylmethionine. *Mol Microbiol* 2006; 59:795-806; PMID:16420352; <https://doi.org/10.1111/j.1365-2958.2005.04989.x>
  101. Selvadurai K, Wang P, Seimetz J, Huang RH. Archaeal Elp3 catalyzes tRNA wobble uridine modification at C5 via a radical mechanism. *Nat Chem Biol* 2014; 10:810-2; PMID:25151136; <https://doi.org/10.1038/nchembio.1610>
  102. Setiাপutra DT, Cheng DT, Lu S, Hansen JM, Dalwadi U, Lam CH, To JL, Dong M-Q, Yip CK. Molecular architecture of the yeast Elongator complex reveals an unexpected asymmetric subunit arrangement. *EMBO Rep* 2016; Published online 21.11.2016; PMID:27872205; <https://doi.org/10.15252/embr.201642548>
  103. Dauden MI, Kosinski J, Kolaj-Robin O, Desfosses A, Ori A, Faux C, Hoffmann NA, Onuma OF, Breunig KD, Beck M, et al. Architecture of the yeast Elongator complex. *EMBO Rep* 2016; 18: 264-279; PMID:27974378; <https://doi.org/10.15252/embr.201643353>
  104. Glatt S, Séraphin B, Müller CW. Elongator: transcriptional or translational regulator? *Transcription* 2012; 3:273-6; PMID:22889844; <https://doi.org/10.4161/trns.21525>
  105. Glatt S, Müller CW. Structural insights into Elongator function. *Curr Opin Struct Biol* 2013; 23:235-42; PMID:23510783; <https://doi.org/10.1016/j.sbi.2013.02.009>
  106. Gu C, Begley TJ, Dedon PC. tRNA modifications regulate translation during cellular stress. *FEBS Lett* 2014; 588:4287-96; PMID:25304425; <https://doi.org/10.1016/j.febslet.2014.09.038>
  107. Mazauric M-H, Dirick L, Purushothaman SK, Bjork GR, Lapeyre B. Trm112p is a 15-kDa zinc finger protein essential for the activity of two tRNA and one protein methyltransferases in yeast. *J Biol Chem* 2010; 285:18505-15; PMID:20400505; <https://doi.org/10.1074/jbc.M110.113100>
  108. Chen C, Huang B, Anderson JT, Byström AS. Unexpected accumulation of mcm<sup>5</sup>U and mcm<sup>5</sup>s<sup>2</sup>U in a *trm9* mutant suggests an additional step in the synthesis of mcm<sup>5</sup>U and mcm<sup>5</sup>s<sup>2</sup>U. *PLoS ONE* 2011; 6: e20783; PMID:21687733; <https://doi.org/10.1371/journal.pone.0020783>
  109. Kalhor HR, Clarke S. Novel methyltransferase for modified uridine residues at the wobble position of tRNA. *Mol Cell Biol* 2003; 23:9283-92; PMID:14645538; <https://doi.org/10.1128/MCB.23.24.9283-9292.2003>
  110. Deng W, Babu IR, Su D, Yin S, Begley TJ, Dedon PC. Trm9-Catalyzed tRNA Modifications Regulate Global Protein Expression by Codon-Biased Translation. *PLoS Genet* 2015; 11: e1005706; PMID:26670883; <https://doi.org/10.1371/journal.pgen.1005706>
  111. Lu J, Huang B, Esberg A, Johansson MJO, Byström AS. The *Kluyveromyces lactis*  $\gamma$ -toxin targets tRNA anticodons. *RNA* 2005; 11:1648-54; PMID:16244131; <https://doi.org/10.1261/rna.2172105>
  112. Jablonowski D, Schaffrath R. Zymocin, a composite chitinase and tRNase killer toxin from yeast. *Biochem Soc Trans* 2007; 35:1533-7; PMID:18031261; <https://doi.org/10.1042/BST0351533>
  113. Jablonowski D, Zink S, Mehlgarten C, Daum G, Schaffrath R. tRNA-Glu wobble uridine methylation by Trm9 identifies Elongator's key role for zymocin-induced cell death in yeast. *Mol Microbiol* 2006; 59:677-88; PMID:16390459; <https://doi.org/10.1111/j.1365-2958.2005.04972.x>
  114. Meinhardt PDF, Schaffrath DR. Extranuclear Inheritance: Cytoplasmic linear double-stranded DNA killer elements of the dairy yeast *Kluyveromyces lactis*. In: *Progress in Botany*, Esser K, Lüttge U, Kadereit JW, Beyschlag W (eds); Springer Verlag, Berlin Heidelberg New York; 62:51-70
  115. Lu J, Esberg A, Huang B, Byström AS. *Kluyveromyces lactis*  $\gamma$ -toxin, a ribonuclease that recognizes the anticodon stem loop of tRNA. *Nucleic Acids Res* 2008; 36:1072-80; PMID:18096622; <https://doi.org/10.1093/nar/gkm1121>
  116. Nandakumar J, Schwer B, Schaffrath R, Shuman S. RNA repair: an antidote to cytotoxic eukaryal RNA damage. *Mol Cell* 2008; 31:278-86; PMID:18657509; <https://doi.org/10.1016/j.molcel.2008.05.019>
  117. Schaffrath R, Breunig KD. Genetics and molecular physiology of the yeast *Kluyveromyces lactis*. *Fungal Genet Biol* 2000; 30:173-90; PMID:11035939; <https://doi.org/10.1006/fgbi.2000.1221>

118. Butler AR, White JH, Folawiyo Y, Edlin A, Gardiner D, Stark MJ. Two *Saccharomyces cerevisiae* genes which control sensitivity to G1 arrest induced by *Kluyveromyces lactis* toxin. *Mol Cell Biol* 1994; 14:6306-16; PMID:8065362; <https://doi.org/10.1128/MCB.14.9.6306>
119. Kishida M, Tokunaga M, Katayose Y, Yajima H, Kawamura-Watabe A, Hishinuma F. Isolation and genetic characterization of pGKL killer-insensitive mutants (iki) from *Saccharomyces cerevisiae*. *Biosci Biotechnol Biochem* 1996; 60:798-801; PMID:8704309; <https://doi.org/10.1271/bbb.60.798>
120. Yajima H, Tokunaga M, Nakayama-Murayama A, Hishinuma F. Characterization of IKI1 and IKI3 genes conferring pGKL killer sensitivity on *Saccharomyces cerevisiae*. *Biosci Biotechnol Biochem* 1997; 61:704-9; PMID:9145530; <https://doi.org/10.1271/bbb.61.704>
121. Frohloff F, Fichtner L, Jablonowski D, Breunig KD, Schaffrath R. *Saccharomyces cerevisiae* Elongator mutations confer resistance to the *Kluyveromyces lactis* zymocin. *EMBO J* 2001; 20:1993-2003; PMID:11296232; <https://doi.org/10.1093/emboj/20.8.1993>
122. Frohloff F, Jablonowski D, Fichtner L, Schaffrath R. Subunit communications crucial for the functional integrity of the yeast RNA polymerase II elongator ( $\gamma$ -toxin target (TOT)) complex. *J Biol Chem* 2003; 278:956-61; PMID:12424236; <https://doi.org/10.1074/jbc.M210060200>
123. Fichtner L, Schaffrath R. KTI11 and KTI13, *Saccharomyces cerevisiae* genes controlling sensitivity to G1 arrest induced by *Kluyveromyces lactis* zymocin. *Mol Microbiol* 2002; 44:865-75; PMID:11994165; <https://doi.org/10.1046/j.1365-2958.2002.02928.x>
124. Fichtner L, Frohloff F, Bürkner K, Larsen M, Breunig KD, Schaffrath R. Molecular analysis of KTI12/TOT4, a *Saccharomyces cerevisiae* gene required for *Kluyveromyces lactis* zymocin action. *Mol Microbiol* 2002; 43:783-91; PMID:11929532; <https://doi.org/10.1046/j.1365-2958.2002.02794.x>
125. Fichtner L, Frohloff F, Jablonowski D, Stark MJR, Schaffrath R. Protein interactions within *Saccharomyces cerevisiae* Elongator, a complex essential for *Kluyveromyces lactis* zymocin action. *Mol Microbiol* 2002; 45:817-26; PMID:12139626; <https://doi.org/10.1046/j.1365-2958.2002.03055.x>
126. Fichtner L, Jablonowski D, Schierhorn A, Kitamoto HK, Stark MJR, Schaffrath R. Elongator's toxin-target (TOT) function is nuclear localization sequence dependent and suppressed by post-translational modification. *Mol Microbiol* 2003; 49:1297-307; PMID:12940988; <https://doi.org/10.1046/j.1365-2958.2003.03632.x>
127. Mehlgarten C, Schaffrath R. Mutant casein kinase I (Hrr25p/Kti14p) abrogates the G1 cell cycle arrest induced by *Kluyveromyces lactis* zymocin in budding yeast. *Mol Genet Genomics* 2003; 269:188-96; PMID:12756531; <https://doi.org/10.1007/s00438-003-0807-5>
128. Jablonowski D, Frohloff F, Fichtner L, Stark MJ, Schaffrath R. *Kluyveromyces lactis* zymocin mode of action is linked to RNA polymerase II function via Elongator. *Mol Microbiol* 2001; 42:1095-105; PMID:11737649; <https://doi.org/10.1046/j.1365-2958.2001.02705.x>
129. Jablonowski D, Butler AR, Fichtner L, Gardiner D, Schaffrath R, Stark MJ. Sit4p protein phosphatase is required for sensitivity of *Saccharomyces cerevisiae* to *Kluyveromyces lactis* zymocin. *Genetics* 2001; 159:1479-89; PMID:11779790
130. Jablonowski D, Fichtner L, Stark MJR, Schaffrath R. The yeast elongator histone acetylase requires Sit4-dependent dephosphorylation for toxin-target capacity. *Mol Biol Cell* 2004; 15:1459-69; PMID:14718557; <https://doi.org/10.1091/mbc.E03-10-0750>
131. Jablonowski D, Täubert J-E, Bär C, Stark MJR, Schaffrath R. Distinct subsets of Sit4 holophosphatases are required for inhibition of *Saccharomyces cerevisiae* growth by rapamycin and zymocin. *Eukaryotic Cell* 2009; 8:1637-47; PMID:19749176; <https://doi.org/10.1128/EC.00205-09>
132. Bär C, Zabel R, Liu S, Stark MJR, Schaffrath R. A versatile partner of eukaryotic protein complexes that is involved in multiple biological processes: Kti11/Dph3. *Mol Microbiol* 2008; 69:1221-33; PMID:18627462; <https://doi.org/10.1111/j.1365-2958.2008.06350.x>
133. Mehlgarten C, Zink S, Rutter J, Schaffrath R. Dosage suppression of the *Kluyveromyces lactis* zymocin by *Saccharomyces cerevisiae* ISR1 and UGP1. *FEMS Yeast Res* 2007; 7:722-30; PMID:17367514; <https://doi.org/10.1111/j.1567-1364.2007.00216.x>
134. Mehlgarten C, Jablonowski D, Breunig KD, Stark MJR, Schaffrath R. Elongator function depends on antagonistic regulation by casein kinase Hrr25 and protein phosphatase Sit4. *Mol Microbiol* 2009; 73:869-81; PMID:19656297; <https://doi.org/10.1111/j.1365-2958.2009.06811.x>
135. Mehlgarten C, Jablonowski D, Wrackmeyer U, Tschitschmann S, Sondermann D, Jäger G, Gong Z, Byström AS, Schaffrath R, Breunig KD. Elongator function in tRNA wobble uridine modification is conserved between yeast and plants. *Mol Microbiol* 2010; 76:1082-94; PMID:20398216; <https://doi.org/10.1111/j.1365-2958.2010.07163.x>
136. Zabel R, Bär C, Mehlgarten C, Schaffrath R. Yeast alpha-tubulin suppressor Ats1/Kti13 relates to the Elongator complex and interacts with Elongator partner protein Kti11. *Mol Microbiol* 2008; 69:175-87; PMID:18466297; <https://doi.org/10.1111/j.1365-2958.2008.06273.x>
137. Xu H, Lin Z, Li F, Diaio W, Dong C, Zhou H, Xie X, Wang Z, Shen Y, Long J. Dimerization of elongator protein 1 is essential for Elongator complex assembly. *Proc Natl Acad Sci USA* 2015; 112:10697-702; PMID:26261306; <https://doi.org/10.1073/pnas.1502597112>
138. Chan CTY, Dyavaiah M, Demott MS, Taghizadeh K, Dedon PC, Begley TJ. A Quantitative Systems Approach Reveals Dynamic Control of tRNA Modifications during Cellular Stress. *PLoS Genet* 2010; 6:e1001247; PMID:21187895; <https://doi.org/10.1371/journal.pgen.1001247>
139. Patil A, Chan CTY, Dyavaiah M, Rooney JP, Dedon PC, Begley TJ. Translational infidelity-induced protein stress results from a deficiency in Trm9-catalyzed tRNA modifications. *RNA Biol* 2012; 9:990-1001; PMID:22832247; <https://doi.org/10.4161/rna.20531>
140. Alings F, Sarin LP, Fufezan C, Drexler HCA, Leidel SA. An evolutionary approach uncovers a diverse response of tRNA 2-thiolation to elevated temperatures in yeast. *RNA* 2015; 21:202-12; PMID:25505025; <https://doi.org/10.1261/rna.048199.114>
141. Souldard A, Cremonesi A, Moes S, Schütz F, Jenö P, Hall MN. The rapamycin-sensitive phosphoproteome reveals that TOR controls protein kinase A toward some but not all substrates. *Mol Biol Cell* 2010; 21:3475-86; PMID:20702584; <https://doi.org/10.1091/mbc.E10-03-0182>
142. Abdel-Fattah W, Jablonowski D, Di Santo R, Thüring KL, Scheidt V, Hammermeister A, Have Ten S, Helm M, Schaffrath R, Stark MJR. Phosphorylation of E1p1 by Hrr25 is required for elongator-dependent tRNA modification in yeast. *PLoS Genet* 2015; 11:e1004931; PMID:2569479; <https://doi.org/10.1371/journal.pgen.1004931>
143. Chan CTY, Pang YLJ, Deng W, Babu IR, Dyavaiah M, Begley TJ, Dedon PC. Reprogramming of tRNA modifications controls the oxidative stress response by codon-biased translation of proteins. *Nat Commun* 2012; 3:937; PMID:22760636; <https://doi.org/10.1038/ncomms1938>
144. Chan CTY, Deng W, Li F, Demott MS, Babu IR, Begley TJ, Dedon PC. Highly Predictive Reprogramming of tRNA Modifications Is Linked to Selective Expression of Codon-Biased Genes. *Chem Res Toxicol* 2015; 28:978-88; PMID:25772370; <https://doi.org/10.1021/acs.chemrestox.5b00004>
145. Han L, Kon Y, Phizicky EM. Functional importance of  $\Psi_{38}$  and  $\Psi_{39}$  in distinct tRNAs, amplified for tRNA<sup>Gln(UUG)</sup> by unexpected temperature sensitivity of the s<sup>2</sup>U modification in yeast. *RNA* 2015; 21:188-201; PMID:25505024; <https://doi.org/10.1261/rna.048173.114>
146. Tyagi K, Pedrioli PGA. Protein degradation and dynamic tRNA thiolation fine-tune translation at elevated temperatures. *Nucleic Acids Res* 2015; 43:4701-12; PMID:25870413; <https://doi.org/10.1093/nar/gkv322>
147. Damon JR, Pincus D, Ploegh HL. tRNA thiolation links translation to stress responses in *Saccharomyces cerevisiae*. *Mol Biol Cell* 2015; 26:270-82; PMID:25392298; <https://doi.org/10.1091/mbc.E14-06-1145>
148. Laxman S, Sutter BM, Wu X, Kumar S, Guo X, Trudgian DC, Mirzaei H, Tu BP. Sulfur Amino Acids Regulate Translational Capacity

- and Metabolic Homeostasis through Modulation of tRNA Thiola-  
tion. *Cell* 2013; 154:416-29; PMID:23870129; <https://doi.org/10.1016/j.cell.2013.06.043>
149. Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO. Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* 2000; 11:4241-57; PMID:11102521; <https://doi.org/10.1091/mbc.11.12.4241>
  150. Bodenmiller B, Campbell D, Gerrits B, Lam H, Jovanovic M, Picotti P, Schlapbach R, Aebersold R. PhosphoPep—a database of protein phosphorylation sites in model organisms. *Nat Biotechnol* 2008; 26:1339-40; PMID:19060867; <https://doi.org/10.1038/nbt1208-1339>
  151. Stark C, Su T-C, Breitkreutz A, Lourenco P, Dahabieh M, Breitkreutz B-J, Tyers M, Sadowski I. PhosphoGRID: a database of experimentally verified in vivo protein phosphorylation sites from the budding yeast *Saccharomyces cerevisiae*. *Database (Oxford)* 2010; 2010: bap026; PMID:20428315; <https://doi.org/10.1093/database/bap026>
  152. Jüdes A, Ebert F, Bär C, Thüning KL, Harrer A, Klassen R, Helm M, Stark MJR, Schaffrath R. Urymylation and tRNA thiolation functions of ubiquitin-like Uba4-Urm1 systems are conserved from yeast to man. *FEBS Lett* 2015; 589:904-9; PMID:25747390; <https://doi.org/10.1016/j.febslet.2015.02.024>
  153. Dedon PC, Begley TJ. A System of RNA Modifications and Biased Codon Use Controls Cellular Stress Response at the Level of Translation. *Chem Res Toxicol* 2014; 27:330-7; PMID:24422464; <https://doi.org/10.1021/tx400438d>
  154. Klassen R, Ciftci A, Funk J, Bruch A, Butter F, Schaffrath R. tRNA anticodon loop modifications ensure protein homeostasis and cell morphogenesis in yeast. *Nucleic Acids Res* 2016; PMID:27496282; <https://doi.org/10.1093/nar/gkw705>
  155. Cheong JK, Virshup DM. Casein kinase 1: Complexity in the family. *Int J Biochem Cell Biol* 2011; 43:465-9; PMID:21145983; <https://doi.org/10.1016/j.biocel.2010.12.004>
  156. Schäfer T, Maco B, Petfalski E, Tollervey D, Böttcher B, Aebi U, Hurt E. Hrr25-dependent phosphorylation state regulates organization of the pre-40S subunit. *Nature* 2006; 441:651-5; PMID:16738661; <https://doi.org/10.1038/nature04840>
  157. Ray P, Basu U, Ray A, Majumdar R, Deng H, Maitra U. The *Saccharomyces cerevisiae* 60 S ribosome biogenesis factor Tif6p is regulated by Hrr25p-mediated phosphorylation. *J Biol Chem* 2008; 283:9681-91; PMID:18256024; <https://doi.org/10.1074/jbc.M710294200>
  158. Ghalei H, Schaub FX, Doherty JR, Noguchi Y, Roush WR, Cleveland JL, Stroupe ME, Karbstein K. Hrr25/CK1 $\delta$ -directed release of Ltv1 from pre-40S ribosomes is necessary for ribosome assembly and cell growth. *J Cell Biol* 2015; 208:745-59; PMID:25778921; <https://doi.org/10.1083/jcb.201409056>
  159. Ho Y, Mason S, Kobayashi R, Hoekstra M, Andrews B. Role of the casein kinase I isoform, Hrr25, and the cell cycle-regulatory transcription factor, SBF, in the transcriptional response to DNA damage in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 1997; 94:581-6; PMID:9012827; <https://doi.org/10.1073/pnas.94.2.581>
  160. Begley U, Dyavaiah M, Patil A, Rooney JP, DiRenzo D, Young CM, Conklin DS, Zitomer RS, Begley TJ. Trm9-catalyzed tRNA modifications link translation to the DNA damage response. *Mol Cell* 2007; 28:860-70; PMID:18082610; <https://doi.org/10.1016/j.molcel.2007.09.021>
  161. Glatt S, Zabel R, Kolaj-Robin O, Onuma OF, Baudin F, Graziadei A, Taverniti V, Lin T-Y, Baymann F, Séraphin B, et al. Structural basis for tRNA modification by Elp3 from *Dehalococcoides mccartyi*. *Nat Struct Mol Biol* 2016; 23:794-802; PMID:27455459; <https://doi.org/10.1038/nsmb.3265>
  162. Di Santo R, Bandau S, Stark MJR. A conserved and essential basic region mediates tRNA binding to the Elp1 subunit of the *Saccharomyces cerevisiae* Elongator complex. *Mol Microbiol* 2014; 92:1227-42; PMID:24750273; <https://doi.org/10.1111/mmi.12624>
  163. Leipe DD, Wolf YI, Koonin EV, Aravind L. Classification and evolution of P-loop GTPases and related ATPases. *J Mol Biol* 2002; 317:41-72; PMID:11916378; <https://doi.org/10.1006/jmbi.2001.5378>
  164. Nelissen H, Clarke JH, De Block M, De Block S, Vanderhaeghen R, Zielinski RE, Dyer T, Lust S, Inzé D, Van Lijsebettens M. DRL1, a homolog of the yeast TOT4/KTI12 protein, has a function in meristem activity and organ growth in plants. *Plant Cell* 2003; 15:639-54; PMID:12615938; <https://doi.org/10.1105/tpc.007062>
  165. Carlson BA, Xu X-M, Kryukov GV, Rao M, Berry MJ, Gladyshev VN, Hatfield DL. Identification and characterization of phosphoseryl-tRNA<sup>[Ser]<sup>Sec</sup></sup> kinase. *Proc Natl Acad Sci USA* 2004; 101:12848-53; PMID:15317934; <https://doi.org/10.1073/pnas.0402636101>
  166. Jun SE, Cho K-H, Hwang J-Y, Abdel-Fattah W, Hammermeister A, Schaffrath R, Bowman JL, Kim G-T. Comparative analysis of the conserved functions of Arabidopsis DRL1 and yeast KTI12. *Mol Cells* 2015; 38:243-50; PMID:25518926; <https://doi.org/10.14348/molcells.2015.2297>
  167. Petrakis TG. Physical and Functional Interaction between Elongator and the Chromatin-associated Kti12 Protein. *J Biol Chem* 2005; 280:19454-60; PMID:15772087; <https://doi.org/10.1074/jbc.M413373200>
  168. Proudfoot M, Sanders SA, Singer A, Zhang R, Brown G, Binkowski A, Xu L, Lukin JA, Murzin AG, Joachimiak A, et al. Biochemical and structural characterization of a novel family of cystathionine beta-synthase domain proteins fused to a Zn ribbon-like domain. *J Mol Biol* 2008; 375:301-15; PMID:18021800; <https://doi.org/10.1016/j.jmb.2007.10.060>
  169. Dong M, Su X, Dzikovski B, Dando EE, Zhu X, Du J, Freed JH, Lin H. Dph3 is an electron donor for Dph1-Dph2 in the first step of eukaryotic diphthamide biosynthesis. *J Am Chem Soc* 2014; 136:1754-7; PMID:24422557; <https://doi.org/10.1021/ja4118957>
  170. Glatt S, Zabel R, Vonkova I, Kumar A, Netz DJ, Pierik AJ, Rybin V, Lill R, Gavin A-C, Balbach J, et al. Structure of the Kti11/Kti13 heterodimer and its double role in modifications of tRNA and eukaryotic elongation factor 2. *Structure* 2015; 23:149-60; PMID:25543256; <https://doi.org/10.1016/j.str.2014.11.008>
  171. Kolaj-Robin O, McEwen AG, Cavarelli J, Séraphin B. Structure of the Elongator cofactor complex Kti11/Kti13 provides insight into the role of Kti13 in Elongator-dependent tRNA modification. *FEBS J* 2015; 282:819-33; PMID:25604895; <https://doi.org/10.1111/febs.13199>
  172. Liu S, Milne GT, Kuremsky JG, Fink GR, Leppla SH. Identification of the proteins required for biosynthesis of diphthamide, the target of bacterial ADP-ribosylating toxins on translation elongation factor 2. *Mol Cell Biol* 2004; 24:9487-97; PMID:15485916; <https://doi.org/10.1128/MCB.24.21.9487-9497.2004>
  173. Deng Q, Barbieri JT. Molecular mechanisms of the cytotoxicity of ADP-ribosylating toxins. *Annu Rev Microbiol* 2008; 62:271-88; PMID:18785839; <https://doi.org/10.1146/annurev.micro.62.081307.162848>
  174. Schaffrath R, Abdel-Fattah W, Klassen R, Stark MJR. The diphthamide modification pathway from *Saccharomyces cerevisiae* - revisited. *Mol Microbiol* 2014; 94:1213-26; PMID:25352115; <https://doi.org/10.1111/mmi.12845>
  175. Schaffrath R, Stark MJR. Decoding the biosynthesis and function of diphthamide, an enigmatic modification of translation elongation factor 2 (EF2). *Microb Cell* 2014; 1:203-5; <https://doi.org/10.15698/mic2014.06.151>
  176. Su X, Lin Z, Lin H. The biosynthesis and biological function of diphthamide. *Crit Rev Biochem Mol Biol* 2013; 48:515-21; PMID:23971743; <https://doi.org/10.3109/10409238.2013.831023>
  177. Zhu X, Dzikovski B, Su X, Torelli AT, Zhang Y, Ealick SE, Freed JH, Lin H. Mechanistic understanding of Pyrococcus horikoshii Dph2, a [4Fe-4S] enzyme required for diphthamide biosynthesis. *Mol Biosyst* 2011; 7:74-81; PMID:20931132; <https://doi.org/10.1039/C0MB00076K>
  178. Lin Z, Dong M, Zhang Y, Lee EA, Lin H. Cbr1 is a Dph3 reductase required for the tRNA wobble uridine modification. *Nat Chem Biol* 2016; 12:995-7; PMID:27694803; <https://doi.org/10.1038/nchembio.2190>
  179. Kaufmann G. Anticodon nucleases. *Trends Biochem Sci* 2000; 25:70-4; PMID:10664586; [https://doi.org/10.1016/S0968-0004\(99\)01525-X](https://doi.org/10.1016/S0968-0004(99)01525-X)

180. Satwika D, Klassen R, Meinhardt F. Anticodon nuclease encoding virus-like elements in yeast. *Appl Microbiol Biotechnol* 2012; 96:345-56; PMID:22899498; <https://doi.org/10.1007/s00253-012-4349-9>
181. Ogawa T. tRNA-targeting ribonucleases: molecular mechanisms and insights into their physiological roles. *Biosci Biotechnol Biochem* 2016; 80:1037-45; PMID:26967967; <https://doi.org/10.1080/09168451.2016.1148579>
182. Meineke B, Kast A, Schwer B, Meinhardt F, Shuman S, Klassen R. A fungal anticodon nuclease ribotoxin exploits a secondary cleavage site to evade tRNA repair. *RNA* 2012; 18:1716-24; PMID:22836353; <https://doi.org/10.1261/rna.034132.112>
183. Klassen R, Paluszynski JP, Wemhoff S, Pfeiffer A, Fricke J, Meinhardt F. The primary target of the killer toxin from *Pichia acaciae* is tRNA<sup>Gln</sup>. *Mol Microbiol* 2008; 69:681-97; PMID:18532979; <https://doi.org/10.1111/j.1365-2958.2008.06319.x>
184. Chen Z, Zhang H, Jablonowski D, Zhou X, Ren X, Hong X, Schaffrath R, Zhu J-K, Gong Z. Mutations in ABO1/ELO2, a subunit of holo-Elongator, increase abscisic acid sensitivity and drought tolerance in *Arabidopsis thaliana*. *Mol Cell Biol* 2006; 26:6902-12; PMID:16943431; <https://doi.org/10.1128/MCB.00433-06>
185. Kheir E, Bär C, Jablonowski D. Cell growth control by tRNase ribotoxins from bacteria and yeast. In: Méndez-Vilas A, editor. *Science and Technology against Microbial Pathogens: Research, Development and Evaluation*. London (United Kingdom): World Scientific Publishing Co. Pte. Ltd; 2011:398-402.
186. Leitner J, Retzer K, Malenica N, Bartkeviciute R, Lucyshyn D, Jager G, Korbei B, Byström AS, Luschnig C. Meta-regulation of *Arabidopsis* auxin responses depends on tRNA maturation. *Cell Rep* 2015; 11:516-26; PMID:25892242; <https://doi.org/10.1016/j.celrep.2015.03.054>
187. Meineke B, Schwer B, Schaffrath R, Shuman S. Determinants of eukaryal cell killing by the bacterial ribotoxin PrrC. *Nucleic Acids Res* 2011; 39:687-700; PMID:20855293; <https://doi.org/10.1093/nar/gkq831>
188. Uthman S, Kheir E, Bär C, Jablonowski D. Growth inhibition strategies based on antimicrobial microbes/toxins. In: Méndez-Vilas A, editor. *Science against Microbial Pathogens: Communicating Current Research and Technological Advances*. Vol. 2. Badajoz (Spain): FORMATEX; 2011:1321-29.
189. Sylvers LA, Rogers KC, Shimizu M, Ohtsuka E, Soll D. A 2-thiouridine derivative in tRNA<sup>Glu</sup> is a positive determinant for aminoacylation by *Escherichia coli* glutamyl-tRNA synthetase. *Biochemistry* 1993; 32:3836-41; PMID:8385989; <https://doi.org/10.1021/bi00066a002>
190. Rezgui VAN, Tyagi K, Ranjan N, Konevega AL, Mittelstaet J, Rodnina MV, Peter M, Pedrioli PGA. tRNA tK<sup>UUU</sup>, tQ<sup>UUG</sup>, and tE<sup>UUC</sup> wobble position modifications fine-tune protein translation by promoting ribosome A-site binding. *Proc Natl Acad Sci USA* 2013; 110:12289-94; PMID:23836657; <https://doi.org/10.1073/pnas.1300781110>
191. Bauer F, Matsuyama A, Candiracci J, Dieu M, Scheliga J, Wolf DA, Yoshida M, Hermand D. Translational Control of Cell Division by Elongator. *CellReports* 2012; 1:424-33; PMID:22768388; <https://doi.org/10.1016/j.celrep.2012.04.001>