

Influence of Wobble Uridine Modifications on Eukaryotic Translation

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Cover Image: 3D structure of a tRNA molecule (see Figure 3B for details).

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*Success is not final, failure is not fatal:
it is the courage to continue that counts.*

Winston Churchill

Table of Contents

Papers in This Thesis	VI
Abbreviations	VII
Abstract	VIII
Introduction	1
1. The Translation Process and Translational Control	1
1.1. Translation Initiation.....	1
1.2. Translation Elongation.....	3
1.3. Translation Termination and Ribosome Recycling.....	5
1.4. Translation Errors and Frameshifting.....	5
2. Transfer RNA	8
2.1. tRNA Modifications in <i>Saccharomyces cerevisiae</i>	9
2.2. Formation of tRNA Wobble Uridine Modifications in <i>S. cerevisiae</i>	11
3. The Role of Wobble Uridine Modifications in Yeast	13
4. Elongator Complex	15
4.1. The Elongator Complex in <i>S. cerevisiae</i>	16
4.2. Regulation of Elongator Complex Activity in <i>S. cerevisiae</i>	18
4.3. Elongator Complexes in Multicellular Organisms.....	20
4.4. The Elongator Complex and Diseases.....	22
Results and Discussion	23
Conclusions	31
Acknowledgements	32
References	34
Papers (I-IV)	55

Papers in This Thesis:

This thesis is based on the following papers and manuscripts, which are referred to in the text by their roman numerals. Paper I. and IV. are reproduced with permission from the publishers.

- I. The role of wobble uridine modifications in +1 translational frameshifting in eukaryotes.
Tükenmez H.[†], Xu H.[†], Esberg A., Byström A.S.
Nucleic Acids Research, (2015), doi: 10.1093/nar/gkv832.
[†]These authors contribute equally.
- II. Elongator complex enhances Rnr1p levels in response to DNA damage by influencing Ixr1p expression.
Tükenmez H., Karlsborn T., Mahmud A.K.M.F., Chen C., Xu F., Byström A.S.
(Manuscript)
- III. Loss of ncm⁵ and mcm⁵ wobble uridine side chains results in an altered metabolic profile.
Karlsborn T., Mahmud A.K.M.F.[†], **Tükenmez H.**[†], Byström A.S.
(Manuscript)
[†]These authors contribute equally.
- IV. Familial dysautonomia (FD) patients have reduced levels of the modified wobble nucleoside mcm⁵s²U in tRNA.
Karlsborn T., **Tükenmez H.**, Chen C., Byström A.S.
Biochemical and Biophysical Research Communications, (2014), doi: 10.1016/j.bbrc.2014.10.116.

The following papers are not included in this thesis;

Karlsborn T., **Tükenmez H.**, Mahmud A.K.M.F., Xu F., Xu H., Byström A.S.
Elongator, a conserved complex required for wobble uridine modifications in eukaryotes. RNA Biol. 2014;11(12):1519-28. doi: 10.4161/15476286.2014.992276.

Tükenmez H., Magnussen H.M., Kovermann M., Byström A.S., Wolf-Watz M.
Linkage between fitness of yeast cells and adenylate kinase catalysis. (Accepted in PLoS One)

Abbreviations

eIFs	: Eukaryotic initiation factors
4E-BP	: eIF4E binding protein
PABP	: Poly(A)-binding protein
uORF	: Upstream open reading frames
eEFs	: Eukaryotic elongation factors
PTC	: Peptidyl transferase center
PKA	: cAMP-dependent protein kinase
eRFs	: Eukaryotic release factors
I	: Inosine
Cm	: 2'-O-methylcytidine
ncm⁵U	: 5-carbamoylmethyluridine
ncm⁵Um	: 5-carbamoylmethyl-2'-O-methyluridine
mcm⁵U	: 5-methoxycarbonylmethyluridine
mcm⁵s²U	: 5-methoxycarbonylmethyl-2-thiouridine
m¹G	: 1-methylguanosine
t⁶A	: N6-threonylcarbamoyladenine
i⁶A	: N6-isopentenyladenine
Ψ	: Pseudouridine
m⁵C	: 5-methylcytidine
m⁷G	: 7-methylguanosine
Um	: 2'-O-methyluridine
ac⁴C	: N4-acetylcytidine
HisRS	: Histidyl-tRNA synthetase
RTD	: Rapid tRNA decay
cm⁵U	: 5-carboxymethyluridine
AdoMet	: S-adenosylmethionine
xm⁵U	: 5-methyluridine derivatives
mnm⁵s²U	: 5-methylaminomethyl-2-thiouridine
HAT	: Histone acetyltransferase
Pol II	: RNA polymerase II
Acetyl-CoA	: Acetyl coenzyme A
PCNA	: Proliferating cell nuclear antigen
GEF-like	: Guanine exchange factor-like
JNK	: c-Jun N-terminal kinase
FD	: Familial dysautonomia
ALS	: Amyotrophic lateral sclerosis
RE	: Rolandic epilepsy
ID	: Intellectual disability

Abstract

Elongator is a conserved six subunit protein (Elp1p-Elp6p) complex that is required for the formation of mcm^5 and mcm^5 side chains at wobble uridines in transfer RNAs (tRNAs). Moreover, loss-of-function mutations in any gene encoding an Elongator subunit results in translational defects and a multitude of phenotypic effects. This thesis is based on investigations of effects of wobble uridine modifications on translation.

In *Saccharomyces cerevisiae*, $mcm^5U_{34^-}$, $mcm^5U_{34^-}$ and $mcm^5s^2U_{34^-}$ -modified wobble nucleosides in tRNAs are important for proper codon-anticodon interactions. My colleagues and I (hereafter we) showed that mcm^5 and s^2 groups at wobble uridine in tRNAs are vital for maintaining the reading frame during translation, as absence of these modifications increases the frequency of +1 frameshifting. We also showed that +1 frameshifting events at lysine AAA codons in Elongator mutants are due to slow entry of the hypomodified tRNA $_{s^2UUU}^{Lys}$ to the ribosomal A-site.

Ixr1p is a protein that plays a key role in increasing production of deoxynucleotides (dNTPs) in responses to DNA damage, via induction of Ribonucleotide reductase 1 (Rnr1p), in *S. cerevisiae*. We showed that expression of Ixr1p is reduced in *elp3Δ* mutants due to a post-transcriptional defect, which results in lower levels of Rnr1p in responses to DNA damage. Collectively, these results suggest that high sensitivity of Elongator mutants to DNA damaging agents might be partially due to reductions in Ixr1p expression and hence Rnr1p levels.

Elongator mutant phenotypes are linked to several cellular processes. To probe the mechanisms involved we investigated the metabolic perturbations associated with absence of a functional *ELP3* gene in *S. cerevisiae*. We found that its absence results in widespread metabolic perturbations under both optimal (30°C) and semi-permissive (34°C) growth conditions. We also found that changes in levels of certain metabolites (but not others) were ameliorated by elevated levels of hypomodified tRNAs, suggesting that amelioration of perturbations of these metabolites might be sufficient for suppression of the Elongator mutant phenotypes.

A mutation in the *IKBKAP* (*hELP1*) gene results in lower levels of the full-length hELP1 protein, which causes a neurodegenerative disease in humans called familial dysautonomia (FD). We showed that the levels of mcm^5s^2U -modified wobble nucleoside in tRNAs are lower in both brain tissues and fibroblast cell lines derived from FD patients than in corresponding materials derived from healthy individuals. This suggests that FD may result from inefficient translation due to partial loss of mcm^5s^2U -modified nucleosides in tRNAs.

Introduction

1. The Translation Process and Translational Control

Gene expression is a fundamental process that converts the genetic information contained in genes into functional RNA or protein products. This process occurs in several steps: transcription, RNA splicing, post-transcriptional RNA modifications, translation and post-translational protein modifications. In the translation step, genetic information contained in mRNA sequences is converted into amino acid chains, which eventually form proteins. In eukaryotes, translation proceeds through four major phases: initiation, elongation, termination and ribosome recycling (**Figure 1**) (**Rodnina and Wintermeyer, 2009**).

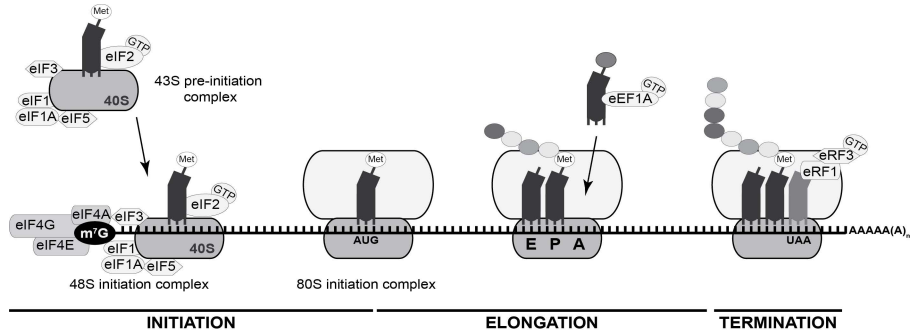


Figure 1. Factors involved in the initiation, elongation and termination phases of translation in eukaryotes.

1.1. Translation Initiation

The first step of translation initiation is formation of a ternary complex containing eukaryotic initiation factor 2 (eIF2), GTP and initiator methionine tRNA ($\text{tRNA}_i^{\text{Met}}$) (**Figure 1**) (**Rodnina and Wintermeyer, 2009**). This ternary complex subsequently binds to a 40S ribosomal subunit with the help of other eukaryotic initiation factors (eIF1, eIF1A, eIF3 and eIF5) to form a 43S pre-initiation complex (**Hinnebusch, 2011; Jackson et al., 2010; Sonenberg and Hinnebusch, 2009**). The eIF4F complex (formed by eIF4E, eIF4A and eIF4G) is a cap-binding protein complex, which is responsible for recruiting initiation factors to the 5' end of mRNA and unwinding secondary structures of the mRNA by its ATP-dependent helicase activity. With assistance of an eIF4F complex, the 43S pre-initiation

complex is recruited to the 5' end of the m⁷G-capped mRNA and starts scanning downstream of the mRNA to find a complementary codon to tRNA_i^{Met}. Recognition of the first AUG start codon by the tRNA_i^{Met} in the 43S pre-initiation complex results in formation of a stable 48S initiation complex (**Sonenberg and Hinnebusch, 2009**). However, several kinds of errors may occur, such as “leaky scanning”, resulting in skipping of the first AUG start codon and initiation of translation at a second AUG codon or a non-AUG codon (**Slusher et al., 1991; Werner et al., 1987; Wolfe et al., 1994**). Upon formation of a 48S initiation complex, eIF5 promotes hydrolysis of eIF2-bound GTP, thereby releasing the tRNA_i^{Met} into the P-site of the ribosome (**Das and Maitra, 2001**). This event is followed by dissociation of the eukaryotic initiation factors from the 48S initiation complex and eIF5B-assisted recruitment of a 60S ribosomal subunit (**Pestova et al., 2000**). Formation of an 80S initiation complex concludes translation initiation, and the ribosome enters the elongation stage of translation.

Numerous factors may affect translation initiation, including functional inhibition of the eIF4F cap-binding protein complex (**Jackson et al., 2010; Raught and Gingras, 2007**). For instance, during nutrient starvation eIF4E-binding protein (4E-BP) becomes hypo-phosphorylated and tightly binds to eIF4E. This interaction blocks the binding site of the eIF4G on eIF4E, thereby inhibiting formation of the eIF4F complex and hence recruitment of the 43S pre-initiation complex to mRNA. Secondary structures in 5' UTRs of mRNAs also inhibit translation initiation, and must be unwound by the eIF4F complex for efficient scanning of the mRNA to locate the start codon (**Jackson et al., 2010; Raught and Gingras, 2007**). Moreover, it has been shown that additional helicases such as Ded1p (in yeast) are needed along with the eIF4F complex to improve scanning through secondary structures (**Berthelot et al., 2004; Chuang et al., 1997; Sen et al., 2015**). Furthermore, eIF4G can interact with the poly(A)-binding proteins (PABPs) located at the 3' end of mRNAs, resulting in formation of a closed loop, which facilitates initiation of the translation of polyadenylated mRNAs (**Costello et al., 2015; Hinton et al., 2007; Kahvejian et al., 2005**).

Another important mechanism involved in translation initiation control is phosphorylation of initiation factors such as the α -subunit of eIF2 (**Dever et al., 2007**). Phosphorylation of eIF2 α at Serine 51 inhibits eIF2B-mediated recycling of eIF2 to the GTP-bound form required for formation of the ternary pre-translation initiation complex, thereby inhibiting the process (**Rowlands et al., 1988**). This phosphorylation event can be activated by various factors (**Ron and Harding, 2007**). For instance, under amino acid

starvation, eIF2 α is phosphorylated by the activated Gen2 protein (**Harding et al., 2000; Wek et al., 1995**). In yeast, eIF2 α phosphorylation inhibits translation initiation globally, but also activates transcription of more than 30 amino acid biosynthetic genes by increasing translation of *GCN4* mRNA (**Rolfes and Hinnebusch, 1993**), which contains four upstream open reading frames (uORFs) located upstream of the main *GCN4* ORF (**Hinnebusch, 1997; Hinnebusch and Natarajan, 2002**). After translation of the first uORF (uORF1), the 60S ribosomal subunit dissociates and the 40S ribosomal subunit resumes scanning and initiates translation at downstream uORFs (uORF2, uORF3, or uORF4) by reacquiring an active ternary complex. Translation of downstream uORFs results in ribosome dissociation, leaving the main *GCN4* ORF untranslated (**Hinnebusch, 1997; Hinnebusch and Natarajan, 2002**). Under amino acid starvation there are limited amounts of the active ternary complex due to eIF2 α phosphorylation. Therefore, some of the 40S ribosomal subunits bypass translation of the downstream uORFs (uORF2-uORF4) due to low abundance of the active ternary complex and initiate translation from the first AUG start codon of *GCN4* ORF (**Hinnebusch, 1997; Hinnebusch and Natarajan, 2002**).

1.2. Translation Elongation

In the translation elongation phase polypeptide chains are formed by cycles of aminoacylated-tRNA (aa-tRNA) delivery to the mRNA and peptide bond formation (**Figure 1**) (**Rodnina and Wintermeyer, 2009**). It begins with recruitment of a ternary complex consisting of aa-tRNA, GTP and eukaryotic elongation factor 1A (eEF1A) to the A-site of an 80S ribosome. A proper interaction between the anticodon of the aa-tRNA in the ternary complex and the codon of the mRNA at the ribosomal A-site triggers conformational changes in the 40S ribosomal subunit. This results in GTP-hydrolysis of the ternary complex by eEF1A, allowing the aa-tRNA to enter the ribosomal A-site. The inactive eEF1A-GDP is then recycled to eEF1A-GTP by eEF1B, enabling formation of a new ternary complex with another aa-tRNA. The large (60S) ribosomal subunit contains a highly conserved peptidyl transferase center (PTC) which is responsible for peptide bond formation during translation elongation and hydrolysis of the peptidyl-tRNA during translation termination. Once an aa-tRNA is in the A-site, the PTC catalyses formation of a peptide bond between the amino acid of the aa-tRNA in the A-site and the C terminus of the polypeptide chain attached to the tRNA in the P-site. Establishment of the peptide bond involves the acceptor stem of the P-site tRNA shifting to the E-site, resulting in formation

of an 'A/P hybrid state', while the anticodon stems of the tRNAs remain in their original positions. GTP hydrolysis catalysed by eukaryotic elongation factor 2 (eEF2) allows the ribosome to move three nucleotides along the mRNA. Consequently, the deacylated-tRNA in the P-site and the peptidyl-tRNA in the A-site translocates into the E- and P-sites of the ribosome. After this translocation process, the deacylated-tRNA leaves the ribosome from the E-site and the ribosomal A-site becomes available for recruitment of the next ternary complex. This elongation process is repeated until a stop codon is located in the ribosomal A-site.

In yeast, a third eukaryotic elongation factor (eEF3) is also required for translation elongation (**Kapp and Lorsch, 2004; Taylor et al., 2007**). This is a ribosome-dependent ATPase that binds to the ribosome close to the E-site (**Andersen et al., 2006**). It putatively assists in release of the deacylated tRNA from the ribosomal E-site and hence stimulates binding of the ternary complex to the A-site (**Andersen et al., 2006; Kurata et al., 2013; Triana-Alonso et al., 1995**).

Eukaryotic elongation factors undergo posttranscriptional modifications that influence the translation elongation process. For instance, phosphorylations of both eEF1A and eEF1B by a number of protein kinases putatively increase translation rates by altering their catalytic properties (**Chang and Traugh, 1998; Eckhardt et al., 2007; Fan et al., 2010; Janssen et al., 1991; Lamberti et al., 2007; Mulner-Lorillon et al., 1994; Peters et al., 1995; Sivan et al., 2011; Venema et al., 1991**). In mammals, eEF2 can be phosphorylated at threonine 56 by an eEF2 kinase (eEF2K) (**Nairn and Palfrey, 1987; Ryazanov et al., 1988a; Ryazanov et al., 1988b**). This phosphorylation event is regulated by activation of the eEF2K, mediated via either autophosphorylation triggered by a Ca²⁺-calmodulin interaction or multiple phosphorylation events catalysed by cAMP-dependent protein kinase (PKA) (**Diggle et al., 1998; Nairn and Palfrey, 1987; Redpath and Proud, 1993; Ryazanov et al., 1988a; Ryazanov et al., 1988b**). The phosphorylation of eEF2 interferes with its binding to ribosomes and consequently inhibits translocation of ribosomes during translation elongation (**Hizli et al., 2013; Ovchinnikov et al., 1990; Price et al., 1991**). eEF2 is also modified with diphthamide at a histidine residue conserved between archaea and eukaryotes, a modification that makes eEF2 a substrate for ADP ribosylation catalysed by diphtheria toxin produced by *Corynebacterium diphtheria* (**Dunlop and Bodley, 1983**). ADP ribosylation inhibits the activity of eEF2 and thus prevents translation (**Oppenheimer and Bodley, 1981**). Furthermore, absence of the diphthamide modification results in increased rates of frameshifting in yeast cells and severe

developmental defects in mice (**Bar et al., 2008; Liu et al., 2012; Ortiz et al., 2006; Uthman et al., 2013**).

1.3. Translation Termination and Ribosome Recycling

The translation termination phase starts with entry of a stop codon (UAA, UGA, or UAG) into the ribosomal A-site and ends with release of the polypeptide chain and ribosome recycling (**Figure 1**) (**Dever and Green, 2012; Jackson et al., 2012**). In eukaryotes, this process is assisted by two eukaryotic release factors: eRF1 (a release factor that recognizes all three stop codons) and eRF3 (a class II release factor that assists translation termination via GTP hydrolysis). These release factors form an eRF1/eRF3/GTP complex that binds to the ribosomal A-site once a stop codon is present. Upon GTP hydrolysis by eRF3, eRF1 promotes hydrolysis of peptidyl-tRNA and consequently release of the polypeptide chain.

The translation termination process is followed by either recycling of ribosomal subunits or reinitiation of translation. Ribosome recycling starts with ATP hydrolysis catalysed by ABCE1 protein in humans (Rliip in yeast), which promotes ribosome splitting and thus dissociation of the ribosomal subunits, release factors and deacylated-tRNA (**Barthelme et al., 2011; Pisarev et al., 2010**). In addition, it has been suggested that translation initiation factors (eIF1, eIF1A, eIF2D and eIF3) assist detachment of the 40S ribosomal subunit from mRNA and the deacylated-tRNA during ribosome recycling (**Dmitriev et al., 2010; Pisarev et al., 2007; Skabkin et al., 2010**). After release of a polypeptide chain, the ribosome might reinitiate translation allowing the 40S ribosomal subunit to either resume scanning for another ORF located on the same mRNA or re-translate the same ORF with the aid of closed loop formation (**Hinnebusch and Lorsch, 2012; Kozak, 1984**).

1.4. Translation Errors and Frameshifting

Translation is a highly energy-consuming process and mRNAs must be decoded with high efficiency and fidelity. During translation process, errors may occur at low frequencies (**Kurland, 1992**). In most cases, the errors will not interfere with the function or stability of the resulting proteins. For instance, a single amino acid change due to a missense error will not affect a protein, unless it significantly changes the protein's conformation or occurs in a position that is important for the protein's function or stability. However, some translation errors, such as frameshifting errors, are more

detrimental as they change the reading frame and consequently the entire amino acid sequence of the protein downstream of the frameshifting site (**Kurland, 1992; Parker, 1989**).

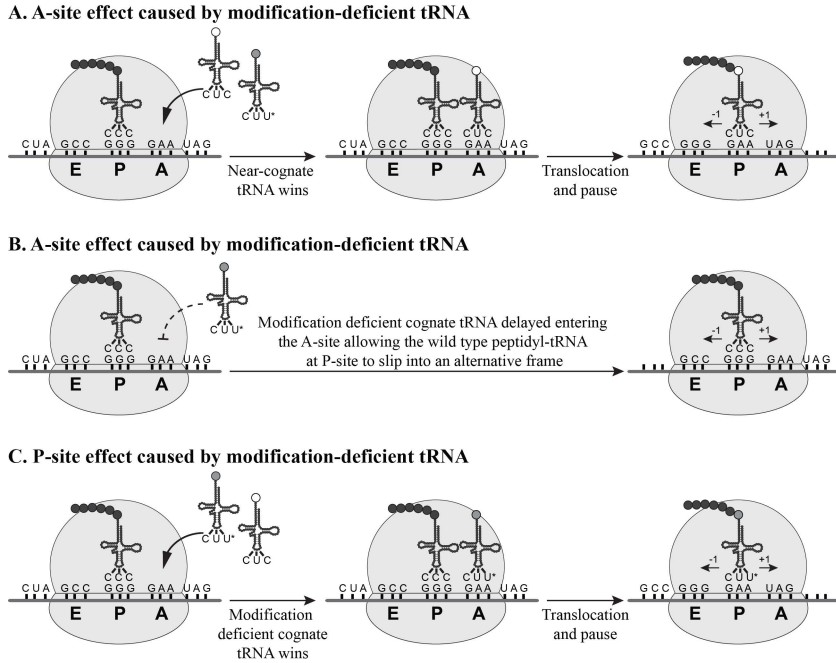


Figure 2. Dual-error frameshifting model. Modification-deficient tRNAs can induce frameshifting by an A- or P- site effect, or combination thereof, as illustrated in A-C, respectively. (A) Lack of tRNA modification reduces the efficiency of the A-site's acceptance of the ternary complex, allowing the site's acceptance of a ternary complex with a near-cognate aminoacyl-tRNA. After translocation to the P-site, the near-cognate peptidyl-tRNA slips into either the -1 or +1 frame, as its fit is not optimal. (B) Lack of tRNA modification reduces the efficiency of the A-site's acceptance of the ternary complex, resulting in a ribosomal pause that allows the peptidyl-tRNA in the P-site to slip into an alternative (-1 or +1) frame. (C) The modification-deficient tRNA is accepted in the A-site and translocated to the P-site where it slips into either the -1 or +1 frame due to a reduced ribosomal grip.

Frameshift errors occur around 10-fold less frequently than missense errors (**Kurland, 1992; Parker, 1989**). However, alterations in the tRNA structure such as lack of a modified nucleoside may affect reading frame maintenance by increasing the frequency of frameshifting (**Atkins and Björk, 2009; Björk and Hagervall, 2014**). For instance, the modified wobble nucleoside 5-methylaminomethyl-2-thiouridine (mnm^{5s2}U₃₄) present in a subset of bacterial tRNA isoacceptors is important for proper reading frame maintenance, as its deficiency increases frameshifting frequencies (**Brierley et al., 1997; Licznar et al., 2003; Maynard et al., 2012; Urbonavicius et al., 2001; Urbonavicius et al., 2003**). Similarly, in yeast lack of either cyclic N6-threonylcarbamoyladenine (ct⁶A) at position 37 or pseudouridine (Ψ) at positions 38 and 39 in tRNA increases the frequency of +1 frameshifting (**El Yacoubi et al., 2011; Lecoite et al., 2002; Lin et al., 2010; Miyauchi et al., 2013**). Hence, the modification status of tRNA isoacceptors plays an important role in maintenance of the proper reading frame and prevention of frameshifting errors in both bacteria and yeast. Induction of frameshifting errors by tRNA modification deficiency is explained with a well-established peptidyl-tRNA slippage model (**Atkins and Björk, 2009; Björk and Hagervall, 2014; Björk et al., 1999; Farabaugh, 1996a, b; Farabaugh and Björk, 1999; Gallant et al., 2000; Jäger et al., 2013; Näsvalld et al., 2009; Urbonavicius et al., 2001**).

According to this model, modification-deficient aminoacyl-tRNA present in an aa-tRNA·eEF1A·GTP ternary complex induces frameshifting by an A-site effect, P-site effect, or a combination thereof (**Figure 2**). In one type of A-site effect of modification deficiency, lack of tRNA modification causes a defect in the cognate aminoacyl-tRNA selection step, allowing acceptance of a ternary complex with a near-cognate wild type aminoacyl-tRNA in the A-site. After translocation to the P-site, the near-cognate peptidyl-tRNA might slip into either the -1 or +1 frame if the fit of tRNA is not optimal (**Figure 2A**). In another type of A-site effect, the efficiency of acceptance of the ternary complex with a modification-deficient cognate aminoacyl-tRNA in the ribosomal A-site is reduced by a lack of modification, resulting in a ribosomal pause that may cause the peptidyl-tRNA to slip one nucleoside forward or backwards (**Figure 2B**). In a P-site effect, a modification-deficient tRNA is accepted in a ribosomal A-site and translocated to the P-site, where its fit is not optimal, thus the peptidyl-tRNA slips into an alternative (-1 or +1) reading frame due to a reduced ribosomal grip (**Figure 2C**).

2. Transfer RNA

Transfer RNAs (tRNAs) are small RNA molecules involved in decoding mRNAs into protein by carrying amino acids to the ribosomes. tRNAs are often represented 2-dimensionally as cloverleaf structures consisting of four base-paired stems (the acceptor-, D-, anticodon- and T Ψ C-stems), three non-base-paired loops (D-, anticodon- and T Ψ C-loops), a CCA-tail and a variable arm that may differ in size among tRNA species (**Figure 3A**) (**Phizicky and Hopper, 2010**). Interaction between the D- and T Ψ C-loops causes tRNA to fold into an L-shaped tertiary structure with the anticodon-loop and CCA-tail positioned at two distal ends (**Figure 3B**) (**Kim et al., 1974**). With the help of aminoacyl tRNA synthetases, amino acids bind covalently to the CCA-tail of the tRNAs (**Ibba and Soll, 2000**). The tRNAs are generally 75 to 90 nucleotides long and generated by transcription of tRNA genes by RNA polymerase III (Pol III). Once the precursor tRNAs are transcribed, they undergo a series of post-transcriptional processing steps before they participate in translation (**Phizicky and Hopper, 2010**).

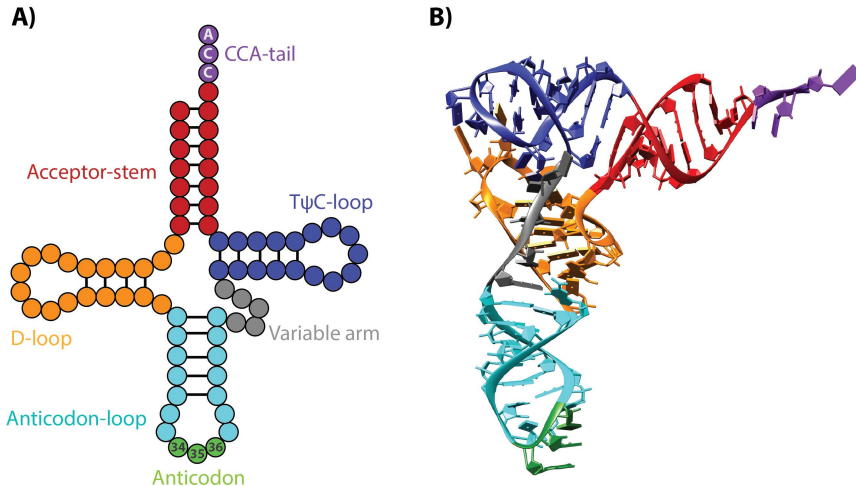


Figure 3. Schematic representations of the secondary (A) and tertiary (B) structures of yeast tRNA^{Phe} showing (in both cases) the acceptor-stem (red), D-loop and D-stem (orange), anticodon-loop (cyan), anticodon (green), variable arm (grey), T Ψ C-loop and T Ψ C-stem (blue) and CCA-tail (purple).

Figure 3B was generated using the UCSF Chimera package (**Pettersen et al., 2004**) from the crystal structure of yeast phenylalanine tRNA (pdb.code: 1EHZ) (**Shi and Moore, 2000**).

2.1. tRNA Modifications in *Saccharomyces cerevisiae*

Transfer RNAs are post-transcriptionally modified at various positions with a wide range of modifications depending on the tRNA species and organism involved. On average, each tRNA is modified at eight positions and more than 100 types of tRNA modifications have been found across the three domains of life (**Machnicka et al., 2013**). Fifty of these tRNA modifications have been found in eukaryotic tRNAs and 25 of them can be found in *Saccharomyces cerevisiae* cytosolic tRNAs, at 36 different positions (**Figure 4A**) (**Johansson and Byström, 2005; Phizicky and Alfonzo, 2010; Phizicky and Hopper, 2010**).

Most of the tRNAs are modified at position 34 (wobble position) and position 37 in the anticodon-loop, and they often affect the tRNAs' decoding ability by altering codon-anticodon interactions (**Agris, 1991; Björk and Hagervall, 2014; Johansson et al., 2008; Lim, 1994**). Therefore, loss of the anticodon-loop modifications often results in severe growth defects, and even cell death in yeast probably due to inefficient decoding. For instance, Tad2p and Tad3p form a heterodimeric enzyme that is responsible for adenine deamination, and hence formation of inosine at wobble position (I_{34}) of seven tRNA species in yeast (**Gerber and Keller, 1999**). Loss-of-function mutations in the *TAD2* or *TAD3* genes result in absence of I_{34} and cell death (**Gerber and Keller, 1999; Tsutsumi et al., 2007**). Furthermore, yeast cells that cannot form $Cm_{32, 34^-}$, $nem^5U_{34^-}$, $nem^5Um_{34^-}$, $mcm^5U_{34^-}$, $mcm^5s^2U_{34^-}$, $m^1G_{37^-}$, $t^6A_{37^-}$, $i^6A_{37^-}$ or $\Psi_{37, 39}$ -modified nucleosides are viable but show translation and growth defects (**Bjork et al., 2001; Dihanich et al., 1987; El Yacoubi et al., 2011; Huang et al., 2005; Johansson et al., 2008; Laten et al., 1978; Lecointe et al., 1998; Pintard et al., 2002**).

The tRNA modifications present outside of the anticodon-loop region have suggested importance for tRNA function and stability (**Phizicky and Alfonzo, 2010; Phizicky and Hopper, 2010**). For example, tRNA_{AAC}^{Val} (lacking both m^7G_{46} and m^5C_{49} nucleosides due to deletion of *TRM8* and *TRM4* genes) is rapidly degraded by the rapid tRNA decay (RTD) pathway (**Alexandrov et al., 2006; Chernyakov et al., 2008**). tRNA_{CGA}^{Ser} and tRNA_{UGA}^{Ser} (lacking Um_{44} and ac^4C_{12} modified nucleosides) in the *tan1Δ trm44Δ* double mutant are also degraded by the RTD pathway

(Chernyakov et al., 2008; Kotelawala et al., 2008), which targets tRNAs according to their structural stability rather than the presence or absence of certain tRNA modifications (Guy et al., 2014).

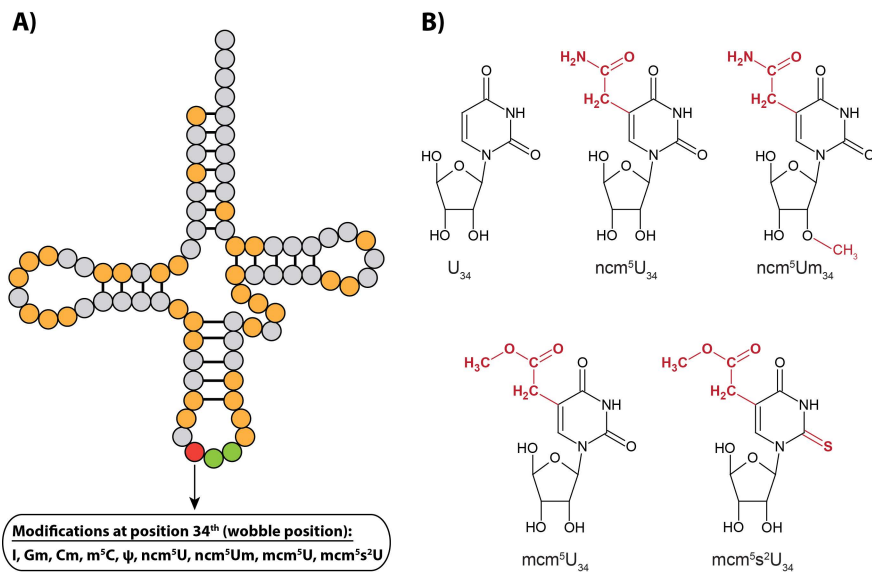


Figure 4. Modifications found in yeast tRNAs. (A) Modified tRNA residues: nucleotides that are modified in some or all tRNA species (orange circles), nucleotides in the anticodon that are modified in some tRNA species (red and green circles), indicated modified nucleosides may be present at the wobble position (red circle). Adapted from (Johansson and Byström, 2005; Phizicky and Hopper, 2010). (B) Chemical structures of unmodified and modified uridines found at the wobble position in yeast tRNA species. The wobble uridine nucleotide can be modified to 5-carbamoylmethyluridine (ncm⁵U), 5-carbamoylmethyl-2'-O-methyluridine (ncm⁵Um), 5-methoxycarbonylmethyl (mcm⁵U) or 5-methoxycarbonylmethyl-2-thiouridine (mcm⁵s²U). The modifications are shown in red. Adapted from (Karlsborn et al., 2014b).

There are other tRNA modifications that contribute specific features to the tRNAs. For instance, tRNA^{His}_{GUG} possesses an extra nucleotide (G₋₁) at the 5'-end, which is added post-transcriptionally by a specific tRNA guanylyl transferase (Cooley et al., 1982) and is crucial for the recognition of

tRNA^{His}_{GUG} by histidyl-tRNA synthetase (HisRS) (**Nameki et al., 1995**). Furthermore, 2'-O-ribosyl phosphate modification at position 64 [Ar(p)₆₄] of tRNA^{Met}_i restricts the interaction between tRNA^{Met}_i and eEF1 α , thereby restricting tRNA^{Met}_i to participation in translation initiation (**Astrom and Bystrom, 1994; Glasser et al., 1991; Shin et al., 2011**). Absence of this modification in the *rit1* Δ mutant results in tRNA^{Met}_i functioning in both initiation and elongation steps of translation (**Astrom and Bystrom, 1994**).

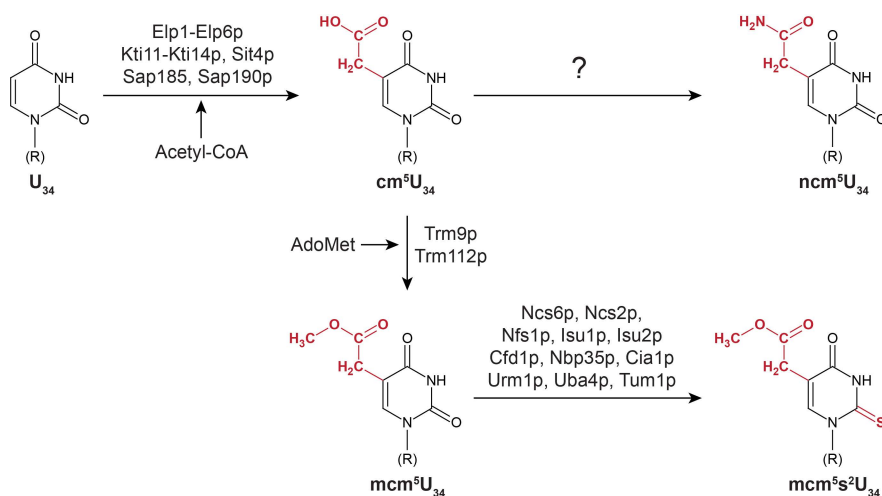


Figure 5. Proteins involved in formation of the 5-methoxycarbonylmethyl (mcm⁵), 5-carbamoylmethyl (ncm⁵) and 2-thio (s²) groups on wobble uridines in *S. cerevisiae*. Acetyl-CoA is used as a donor in formation of the cm⁵ side chain, and AdoMet (S-adenosylmethionine) is used by Trm9p and Trm112p as a methyl donor in formation of the mcm⁵ side chain. The last step in formation of the ncm⁵ remains unknown. The ribose ring of the uridine is shown as (R) and each side group added to uridine is highlighted in red. Adapted from (**Karlsborn et al., 2014b**).

2.2. Formation of tRNA Wobble Uridine Modifications in *S. cerevisiae*

In *S. cerevisiae*, 42 tRNA species are responsible for decoding the 61 sense codons, 13 of them have a uridine at the wobble position (**Hani and**

Feldmann, 1998; Percudani et al., 1997), and only one of these 13 (tRNA^{Leu}_{UAG}) has an unmodified uridine (U₃₄) at the wobble position (**Randerath et al., 1979**). Another (tRNA^{Ile}_{Ψ_{AV}Ψ}) has a pseudouridine (Ψ₃₄) (**Szweykowska-Kulinska et al., 1994**) and the other 11 have one of four related modified uridine nucleosides at the wobble position (**Glasser et al., 1992; Johansson et al., 2008; Keith et al., 1990; Kobayashi et al., 1974; Kuntzel et al., 1975; Lu et al., 2005; Smith et al., 1973; Yamamoto et al., 1985**). These related modified wobble uridine nucleosides are: 5-carbamoylmethyluridine (ncm⁵U₃₄) present in tRNA^{Val}_{ncm⁵UAC}, tRNA^{Ser}_{ncm⁵UGA}, tRNA^{Ala}_{ncm⁵UGC}, tRNA^{Pro}_{ncm⁵UGG} and tRNA^{Thr}_{ncm⁵UGU} (**Johansson et al., 2008; Keith et al., 1990; Yamamoto et al., 1985**); 5-carbamoylmethyl-2'-O-methyluridine (ncm⁵Um₃₄) present in tRNA^{Leu}_{ncm⁵UmAA} (**Glasser et al., 1992**); 5-methoxycarbonylmethyluridine (mcm⁵U₃₄) present in tRNA^{Arg}_{mcm⁵UCU} and tRNA^{Gly}_{mcm⁵UCC} (**Kuntzel et al., 1975; Lu et al., 2005**); and 5-methoxycarbonylmethyl-2-thiouridine (mcm⁵s²U₃₄) present in tRNA^{Gln}_{mcm⁵s²UUG}, tRNA^{Lys}_{mcm⁵s²UUU} and tRNA^{Glu}_{mcm⁵s²UUC} (**Figure 4B**) (**Kobayashi et al., 1974; Lu et al., 2005; Smith et al., 1973**).

In *S. cerevisiae* at least 15 gene products are required to form the mcm⁵ side group and 11 gene products are required to form a 2-thio (s²) group on uridine at the wobble position (**Figure 5**). In order to form the mcm⁵ and ncm⁵ side chains on wobble uridine, Elongator complex (Elp1p-Elp6p), Kti11p-Kti14p, Sit4p, Sap185p and Sap190p are required for the first step: synthesis of an intermediate likely to be 5-carboxymethyluridine (cm⁵U) (**Figure 5**) (**Huang et al., 2005; Huang et al., 2008**). Deletion of any of the *ELP1-ELP6*, *KTI11*, *KTI12*, *KTI14* (*HRR25*), *SIT4* or *SAP185* and *SAP190* genes results in total loss of mcm⁵ and ncm⁵ side chain formation on wobble uridines, whereas deletion of the *KTI13* gene results in reduced levels of these modifications (**Huang et al., 2005; Huang et al., 2008**). The last step in formation of the mcm⁵ side chain requires the Trm9p/Trm112p methyltransferase complex and S-adenosylmethionine (AdoMet) as a methyl donor, but the gene products required for the last step in formation of the ncm⁵ side chain are still unknown (**Figure 5**) (**Chen et al., 2011a; Kalhor and Clarke, 2003; Mazauric et al., 2010**). In addition to the ncm⁵ side chain, the wobble uridine (U₃₄) of tRNA^{Leu}_{ncm⁵UmAA} is also methylated at the 2'-hydroxyl of the ribose (**Figure 4B**) (**Glasser et al., 1992**). Trm7 methyltransferase and AdoMet (as a methyl donor) are required for the 2'-O-methylation both *in vivo* and *in vitro* (**Guy et al., 2012; Pintard et al., 2002**). Formation of the 2-thio (s²) group present in three tRNA species (tRNA^{Gln}_{mcm⁵s²UUG}, tRNA^{Lys}_{mcm⁵s²UUU} and tRNA^{Glu}_{mcm⁵s²UUC}) requires 11 gene products (**Figure 5**) (**Bjork et al., 2007; Dewez et al., 2008; Esberg et al.,**

2006; Huang et al., 2005; Huang et al., 2008; Leidel et al., 2009; Nakai et al., 2008; Nakai et al., 2007; Nakai et al., 2004; Noma et al., 2009; Schlieker et al., 2008). In *S. cerevisiae*, absence of *mcm⁵* side chain results in reduced *s²* formation, whereas in *S. pombe* deletion of the *ELP3/SIN3* gene results in loss of both *mcm⁵* and *s²* groups (**Bauer et al., 2012; Leidel et al., 2009; Nakai et al., 2008; Noma et al., 2009**).

3. The Role of Wobble Uracil Modifications in Yeast

The genetic code consists of 64 codons: 61 sense codons encoding an amino acid and three stop codons. In yeast the 61 sense codons are decoded by 42 tRNA species as certain tRNA species decode more than one sense codon (**Crick, 1966; Hani and Feldmann, 1998; Percudani et al., 1997**). According to Crick's wobble hypothesis, a uracil at position 34 (*U₃₄*) of the anticodon in tRNA can recognize both A and G bases found in the third position of the codon in mRNA (**Crick, 1966**). However, the current, revised version holds that an unmodified *U₃₄* can recognize all four bases (A, G, C and U) (**Agris, 1991; Lim, 1994; Yokoyama and Nishimura, 1995**). Uracils at the wobble position (*U₃₄*) of tRNAs are often modified to 5-methyluracil derivatives (*xm⁵U₃₄*, where x stands for any of several groups and *m⁵* for a methylene carbon directly bound to position 5 of the wobble uracil), in organisms from all three domains of life (**Machnicka et al., 2013; Takai and Yokoyama, 2003**). Additional side groups, such as a 2-thio or a 2'-*O*-methyl, can also be added to *xm⁵U₃₄* to form *xm⁵s²U₃₄* or *xm⁵Um₃₄*. It has been suggested that the modified wobble uracil nucleoside [*xm⁵(s²)U(m)₃₄*] restricts wobble recognition, as part of a mechanism that prevents misreading of U or C (**Yokoyama and Nishimura, 1995; Yokoyama et al., 1985**). Furthermore, the modified wobble uracils are believed to either allow efficient pairing with both A and G or to improve pairing with A while reducing pairing with G (**Johansson et al., 2008; Kruger et al., 1998; Lim, 1994; Murphy et al., 2004; Takai and Yokoyama, 2003; Weissenbach and Dirheimer, 1978; Yarian et al., 2002; Yokoyama and Nishimura, 1995; Yokoyama et al., 1985**).

As the gene products required for the yeast wobble uracil modifications have been identified, effects of the modifications on translation decoding have been investigated using various strategies. Notably, an *in vivo* study of growth properties of yeast mutants lacking certain tRNA genes and genes required for wobble uracil modifications revealed that in most cases *ncm⁵*, *mcm⁵* or *s²* side groups promote reading of both A- and G- ending codons, whereas presence of the esterified methyl

group of mcm⁵ side chains has a modest effect on decoding (**Johansson et al., 2008**). In addition, tRNA_{mcm⁵UGG}^{Pro} can read A-, G-, C- or U- ending codons, regardless of whether or not it has the mcm⁵ side chain at the wobble uridine (**Johansson et al., 2008**).

Effects of wobble uridine modification on translation decoding have also been studied using various reporter systems. For instance, the *SUP4*-encoded ochre suppressor tRNA (which has mcm⁵U₃₄ at the wobble position) can decode cognate UAA stop and (to a lesser extent) near-cognate UAG stop codons (**Huang et al., 2005**). Absence of mcm⁵ modification in *SUP4* suppressor tRNA results in poor decoding of these stop codons (**Johansson et al., 2008**). Another study, involving use of a codon-specific *lacZ* reporter system, found that loss of the esterified methyl group of mcm⁵ side chain in tRNA_{mcm⁵UCU}^{Arg} reduced efficiency of decoding both cognate AGA and near-cognate AGG codons (**Begley et al., 2007**).

Effects of wobble uridine modifications on translation elongation in *S. cerevisiae* have also been investigated recently by ribosome profiling, a technique used to determine genome-wide distributions of ribosomes on mRNA and thus identify codons where elongation of translation is slow. These studies have found that mutants with a defect in mcm⁵s²U₃₄ formation show ribosome accumulation when an AAA, CAA or GAA codon (respectively decoded by tRNA_{mcm⁵s²UUU}^{Lys}, tRNA_{mcm⁵s²UUG}^{Gln} and tRNA_{mcm⁵s²UUC}^{Glu}) is in the ribosomal A-site (**Nedialkova and Leidel, 2015; Zinshteyn and Gilbert, 2013**). Moreover, an *in vitro* study has shown that entry of hypomodified tRNA_{UUU}^{Lys}, isolated from either *elp3Δ* (tRNA_{s²UUU}^{Lys}) or *urm1Δ* (tRNA_{mcm⁵UUU}^{Lys}) mutants, into ribosomal A-site is delayed during elongation of translation (**Rezgui et al., 2013**). These results suggest that the presence of mcm⁵s²U₃₄ plays an important role in ribosomal A-site selection and hence translation elongation.

Ribosome stalling and impairment of ribosomal A-site selection may lead to translation errors such as frameshifting. In bacteria the modified nucleoside mnm⁵s²U₃₄ present in tRNA_{mnm⁵s²UUU}^{Lys}, tRNA_{mnm⁵s²UUG}^{Gln} or tRNA_{mnm⁵s²UUC}^{Glu} is important for proper reading frame maintenance since its absence increases frameshifting events at AAA, CAA and GAA codons (**Bregeon et al., 2001; Brierley et al., 1997; Licznar et al., 2003; Urbonavicius et al., 2001; Urbonavicius et al., 2003**).

Furthermore, occurrence of -1 frameshifting at 4x GAG codon runs is reportedly elevated in *trm9Δ* mutants of *S. cerevisiae*, which cannot form the esterified methyl group of the mcm⁵ side group (**Patil et al., 2012a**).

However, the cited study did not provide extensive information on the role of wobble uridine modifications in reading frame maintenance. Thus, in the study reported in **Paper I** my colleagues and I (hereafter we) further investigated the necessity of ncm^5 , mcm^5 or s^2 side groups at wobble uridines for reading frame maintenance. We found that in most cases absence of the mcm^5 or s^2 side groups results in increased +1 frameshifting at corresponding cognate and near-cognate codons, but absence of the ncm^5 side group or esterified methyl group of mcm^5 has minor effects on frequencies of +1 frameshifting (**Tukenmez et al., 2015**).

Yeast strains with defects in formation of ncm^5 , mcm^5 , and s^2 side groups at wobble uridines show pleiotropic phenotypes (reviewed in (**Karlsborn et al., 2014b**)). Furthermore, all phenotypes tested so far except the defect in tRNA modification can be suppressed by overexpression of hypomodified $\text{tRNA}_{\text{UUU}}^{\text{Lys}}$, $\text{tRNA}_{\text{UUG}}^{\text{Gln}}$ and $\text{tRNA}_{\text{UUC}}^{\text{Glu}}$ in various combinations (**Bauer et al., 2012; Chen et al., 2011a; Dewez et al., 2008; Esberg et al., 2006; Fernandez-Vazquez et al., 2013; Klassen et al., 2015; Nedialkova and Leidel, 2015; Tigano et al., 2015; Tukenmez et al., 2015; Zinshteyn and Gilbert, 2013**). Therefore, it has been suggested that these observed phenotypes are probably caused by inefficient translation of certain mRNAs due to lack of the wobble uridine modifications. According to a proteome-wide study, about 270 proteins are either up- or down-regulated in *S. cerevisiae* strains that cannot form $\text{ncm}^5/\text{mcm}^5$ or s^2 side groups at wobble uridines (**Rezgui et al., 2013**). Moreover, the downregulated proteins are expressed from the genes that are mostly enriched in AAA, CAA and GAA codons, respectively decoded by $\text{tRNA}_{\text{mcm}^5\text{s}^2\text{UUU}}^{\text{Lys}}$, $\text{tRNA}_{\text{mcm}^5\text{s}^2\text{UUG}}^{\text{Gln}}$ and $\text{tRNA}_{\text{mcm}^5\text{s}^2\text{UUC}}^{\text{Glu}}$ (**Rezgui et al., 2013**).

In summary, all these findings suggest that mRNAs enriched in AAA, CAA and GAA codons are most likely to induce translation errors that result in reduced protein expression and hence various pleiotropic phenotypes in strains lacking wobble uridine modifications. Therefore, in the study described in **Paper II** we further investigated the influence of AAA and CAA codon enrichments on protein expression in an *elp3Δ* strain of *S. cerevisiae*.

4. Elongator Complex

The Elongator complex was first identified in *S. cerevisiae* as a histone acetyltransferase (HAT) complex consisting of three subunits (Elp1p, Elp2p and Elp3p), which associates with the hyper-phosphorylated elongation form of RNA polymerase II (Pol II) (**Otero et al., 1999; Wittschieben et al.,**

1999). Three more Elongator subunits (Elp4, Elp5 and Elp6) forming an Elongator sub-complex were subsequently discovered (**Krogan and Greenblatt, 2001; Li et al., 2001; Winkler et al., 2001**). Based on the crystal structure of the Elp456 sub-complex, it was suggested that the Elongator complex contains two copies of the Elp1p-Elp3p core complex and a hexameric ring formed by two copies of the Elp4-Elp6 sub-complex (**Glatt et al., 2012; Lin et al., 2012**). Homologs of the Elp3 protein can be found essentially in all archaea, but not the other Elongator subunits (**Paraskevopoulou et al., 2006; Selvadurai et al., 2014**). Moreover, an *in vitro* study showed that in the presence of acetyl-CoA, an Elp3p homolog from the archaeon *Methanocaldococcus infernus* can modify wobble uridine (U₃₄) in tRNA to cm⁵U₃₄, which is likely the first step in formation of ncm⁵ and mcm⁵ side groups (**Selvadurai et al., 2014**). Orthologues of the Elongator proteins can be found in multicellular eukaryotes and six subunit complexes have been purified from plants and humans (**Chen et al., 2009a; Close et al., 2012; Cohen et al., 2015; Gkampeta et al., 2014; Hawkes et al., 2002; Mei et al., 2014; Nelissen et al., 2010; Nelissen et al., 2005; Okada et al., 2010; Simpson et al., 2009; Xu et al., 2015b; Zhu et al., 2015**). In addition, mutations in Elongator genes in the worm (*Caenorhabditis elegans*), mouse (*Mus musculus*), plant (*Arabidopsis thaliana*) and humans cause defects in formation of wobble uridine modifications as observed in yeast Elongator mutants (**Chen et al., 2009a; Karlsborn et al., 2014a; Lin et al., 2013; Mehlgarten et al., 2010**).

4.1. The Elongator Complex in *S. cerevisiae*

The Elongator complex in *S. cerevisiae* is reportedly involved in several cellular processes, including RNA polymerase II (Pol II) transcription, exocytosis, DNA damage responses, telomeric gene silencing and wobble uridine modifications (**Huang et al., 2005; Li et al., 2001; Otero et al., 1999; Rahl et al., 2005; Winkler et al., 2002; Winkler et al., 2001; Wittschieben et al., 1999**). The complex was initially found to consist of three proteins (Elp1p, Elp2p, and Elp3p) and thought to participate in Pol II transcription due to its association with the hyperphosphorylated elongating form of Pol II (**Otero et al., 1999**). *In vitro* studies suggested that the Elp3 subunit of the Elongator complex has histone acetyltransferase activity that allows it to transfer acetyl groups from acetyl-CoA to histones (**Wittschieben et al., 2000; Wittschieben et al., 1999**). Moreover, an *in vivo* study showed that inactivation of the *ELP3* gene results in decreased histone (H3 and H4) acetylation (**Winkler et al., 2002**). However, the role

of Elongator complex in transcription elongation and histone acetylation was subsequently questioned, as chromatin immuno-precipitation experiments failed to detect the Elongator complex on actively transcribed genes and it was found to be largely localized in the cytosol (**Huh et al., 2003; Pokholok et al., 2002; Rahl et al., 2005**). The cytoplasmic role of the complex was initially suggested to be in regulation of exocytosis, as the Elp1p subunit interacts with Sec2p, a guanine nucleotide exchange factor required for polarized transport of secretory vesicles (**Rahl et al., 2005; Salminen and Novick, 1987; Walch-Solimena et al., 1997**).

It was later discovered that null alleles of any gene encoding an Elongator complex subunit results in loss of the 5-carbamoylmethyluridine (ncm⁵U), 5-carbamoylmethyl-2'-O-methyluridine (ncm⁵Um), 5-methoxycarbonylmethyluridine (mcm⁵U) and 5-methoxycarbonylmethyl-2-thiouridine (mcm⁵s²U) wobble nucleosides in a subset of tRNAs (**Huang et al., 2005**). Therefore, it was suggested that the complex is required for an early step in formation of ncm⁵ and mcm⁵ side groups on wobble uridines (**Huang et al., 2005**). This discovery raised questions regarding the Elongator complex's role in transcription and exocytosis, as the pleiotropic phenotypes observed in Elongator mutants may be caused by defects in translation due to loss of tRNA wobble uridine modifications. Accordingly, it was found that the pleiotropic perturbations observed in Elongator mutants, including the defects in transcription and exocytosis, could be suppressed by elevated levels of hypomodified tRNA^{Lys}_{s²UUU} and tRNA^{Gln}_{s²UUG} lacking mcm⁵ side chains at wobble position (**Esberg et al., 2006**). Another role for the Elongator complex was suggested to be in DNA repair, as Elongator mutants display partial loss of telomeric gene silencing and increased sensitivity to DNA damaging agents, and both of these phenotypes can be suppressed by elevated levels of hypomodified tRNA^{Lys}_{s²UUU}, tRNA^{Gln}_{s²UUG} and tRNA^{Glu}_{s²UUC} (**Chen et al., 2011b; Li et al., 2009**). Overall, all phenotypes tested to date in both *S. cerevisiae* and *S. pombe* Elongator mutants are suppressed by overexpression of these hypomodified tRNAs in various combinations (**Bauer et al., 2012; Chen et al., 2011b; Esberg et al., 2006; Fernandez-Vazquez et al., 2013; Klassen et al., 2015; Nedialkova and Leidel, 2015; Scheidt, 2014; Tigano et al., 2015; Tukenmez et al., 2015; Zinshteyn and Gilbert, 2013**).

In addition, an *ncs2Δ* mutant unable to form the 2-thio (s²) group but not the mcm⁵ group at the wobble position in tRNA^{Lys}_{mcm⁵UUU}, tRNA^{Gln}_{mcm⁵UUG} and tRNA^{Glu}_{mcm⁵UUC} displays identical but weaker phenotypes than Elongator mutants. Similarly, phenotypes observed in the *ncs2Δ* mutant can be suppressed by elevated levels of hypomodified tRNA^{Lys}_{mcm⁵UUU},

tRNA_{mcm⁵UUG}^{Gln} and tRNA_{mcm⁵UUC}^{Glu} in various combinations (**Chen et al., 2011b; Esberg et al., 2006; Leidel et al., 2009; Nedialkova and Leidel, 2015**). These observations indicated that the primary role of the Elongator complex is linked to tRNA wobble uridine modifications and the pleiotropic phenotypes observed in Elongator mutants were considered results of defects in translation caused by hypomodified tRNAs. However, it is still not known if the pleiotropic phenotypes are caused by a global reduction of protein expression or reduced protein expression from a subset of mRNAs. Thus, in the study reported in **Paper III** we investigated the metabolic perturbations in the *elp3Δ* strain and whether elevated levels of hypomodified tRNA_{s²UUU}^{Lys}, tRNA_{s²UUG}^{Gln} and tRNA_{s²UUC}^{Glu} can restore levels of the affected metabolites.

4.2. Regulation of Elongator Complex Activity in *S. cerevisiae*

Another phenotype of *S. cerevisiae* Elongator mutants is resistance to zymocin, a three-subunit anti-yeast toxin produced by certain strains of *Kluyveromyces lactis* (**Butler et al., 1991; Butler et al., 1994; Frohloff et al., 2001; Kishida et al., 1996**). The α- and β- subunits are required for the toxin to attach to the wall of a susceptible cell and transfer the cytotoxic γ-subunit into its cytoplasm (**Butler et al., 1991; Schaffrath and Meinhardt, 2005; Stark and Boyd, 1986**). Two types of *S. cerevisiae* mutants are resistant to this toxin. Type I mutants are resistant to exogenous zymocin due to perturbations that inhibit take-up of the toxin, while type II mutants are resistant to both exogenous zymocin and endogenous expression of the γ-toxin (**Butler et al., 1991; Schaffrath and Meinhardt, 2005**). It was later discovered that the γ-toxin is an endonuclease that cleaves tRNA_{mcm⁵s²UUU}^{Lys}, tRNA_{mcm⁵s²UUG}^{Gln} and tRNA_{mcm⁵s²UUC}^{Glu} and between mcm⁵s²U₃₄ and U₃₅ (**Lu et al., 2005**). Presence of both mcm⁵ and s² side groups at wobble uridines is necessary for these tRNAs to be substrates for the γ-toxin (**Huang et al., 2008; Lu et al., 2008; Lu et al., 2005**). Therefore, Elongator mutants lacking mcm⁵ side chains at wobble uridines are resistant to exogenous zymocin and endogenous expression of γ-toxin. According to genetic screens, strains with mutations in the *TRM9* gene, Elongator subunit genes (*ELP1-ELP6*), killer toxin insensitive genes (*KTI11-KTI14*), the *SIT4* gene or both the *SAP185* and *SAP190* genes are type II resistant mutants that cannot form mcm⁵ and mcm⁵ side chains at wobble uridines (**Butler et al., 1991; Butler et al., 1994; Frohloff et al., 2001; Huang et al., 2005; Huang et al., 2008;**

Jablonowski et al., 2001; Kishida et al., 1996; Schaffrath and Meinhardt, 2005).

Phosphorylation and de-phosphorylation of Elp1p, the largest subunit of the Elongator complex, play crucial roles in activity of the complex (**Jablonowski et al., 2004; Mehlgarten et al., 2009**). De-phosphorylation of Elp1p requires the type 2A phosphatase Sit4p and two Sit4p-associated proteins (Sap185p and Sap190p) (**Jablonowski et al., 2004**), whereas its phosphorylation requires the casein kinase Kti14p (Hrr25p) (**Mehlgarten et al., 2009**). It has been suggested that Sit4p and Kti14p regulate activity of the Elongator complex in an antagonistic manner via their respective Elp1p phosphorylation and de-phosphorylation activities (**Jablonowski et al., 2004; Mehlgarten et al., 2009**). Recently, nine *in vivo* phosphorylation sites of Elp1p were identified and two of these sites (Ser₁₁₉₈ and Ser₁₂₀₂) were identified as direct phosphorylation sites for Kti14p (**Abdel-Fattah et al., 2015**). It was also discovered that binding of Kti14p to the Elongator complex is dependent on Kti12p (**Mehlgarten et al., 2009**). Kti12p interacts with the Elongator complex, but this interaction does not influence the complex's formation or stability (**Fichtner et al., 2002; Petrakis et al., 2005**). Moreover, both deletion and overexpression of the *KTI12* gene result in resistance to zymocin (**Butler et al., 1994**). A deletion of the *KTI12* gene results in loss of mcm⁵U₃₄ and mcm⁵S²U₃₄ nucleosides, suggesting that Kti12p participates in formation of modified wobble uridine nucleosides (**Huang et al., 2008**). However, the function of Kti12p in regulation of the Elongator complex activity remains unclear.

In addition to phosphorylation or de-phosphorylation of the Elp1p, proteolysis of Elp1p is another proposed mechanism for regulation of Elongator complex activity (**Fichtner et al., 2003**). Previous studies revealed that Elp1p exists in two major forms: full-length and an N-terminal truncated form (**Fichtner et al., 2003; Krogan and Greenblatt, 2001**). It was also recently reported that proteolytic cleavage of Elp1p (between residues Lys₂₀₃ and Ala₂₀₄) is catalysed by the vacuolar protease Prb1p (**Xu et al., 2015a**). Deletion of Kti11p was shown to increase levels of the N-terminal truncated form of Elp1p, thus Kti11p was linked with regulation of Elongator complex activity (**Bar et al., 2008; Fichtner et al., 2003**). However, the proteolytic cleavage of Elp1p was subsequently shown to be an artifact occurring during protein extraction (**Xu et al., 2015a**). Besides the formation of mcm⁵ and mcm⁵ side chains at wobble uridines, Kti11p (Dph3p) is involved in biosynthesis of the diphthamide modification on eukaryotic elongation factor 2 (eEF2) (**Liu and Leppla, 2003; Liu et al., 2004**), in which it acts as an electron donor for iron-sulphur (Fe-S) clusters in Dph1p and Dph2p (**Dong et al., 2014**).

Therefore, it was proposed that Kti11p also acts as an electron donor for the Fe-S cluster in Elp3p in regulation of Elongator complex activity (**Dong et al., 2014**). It was also discovered that Kti11p forms a stable complex with Kti13p, a guanine exchange factor-like (GEF-like) protein (**Kolaj-Robin et al., 2015; Zabel et al., 2008**), and proposed that formation of the heterodimeric Kti11p-Kti13p complex plays an important role in regulation of the electron transfer capacity of Kti11p (**Kolaj-Robin et al., 2015**).

4.3. Elongator Complexes in Multicellular Organisms

Orthologues of the Elongator complex subunits can be found in multicellular organisms (**Chen et al., 2009a; Close et al., 2012; Hawkes et al., 2002; Nelissen et al., 2010; Nelissen et al., 2005; Okada et al., 2010; Simpson et al., 2009; Winkler et al., 2001**). As in yeast, mutations in genes encoding subunits of the complex in the worm *C. elegans*, plant *A. thaliana*, mouse *M. musculus* and humans result in loss of wobble uridine modifications (**Chen et al., 2009a; Karlsborn et al., 2014a; Lin et al., 2013; Mehlgarten et al., 2010**). In *C. elegans*, ELPC-1 (a homolog of yeast Elp1p) and ELPC-3 (a homolog of yeast Elp3p) subunits of the Elongator complex are strongly expressed in a subset of chemosensory neurons required for salt chemotaxis learning (**Chen et al., 2009a**). Inactivation of the *elpc-1* or *elpc-3* gene causes posttranscriptional reduction of neuropeptides and acetylcholine in the synaptic cleft, resulting in both neural function and developmental defects (**Chen et al., 2009a**). In addition, ELPC-3 protein has been suggested to acetylate lysine 40 (K40) of α -tubulin (**Solinger 2010**). However, the *C. elegans elpc-3* mutant does not reportedly display a defect in K40 acetylation of α -tubulin (**Chen et al., 2009a**). Furthermore, MEC-17 (ATAT1) and ATAT2 proteins were later discovered to be the sole α -tubulin acetylases in *C. elegans*, as double *mec-17 atat2* mutants lack K40 acetylation of α -tubulin (**Akella et al., 2010; Shida et al., 2010**). In the fruit fly *Drosophila melanogaster*, the Elp3 protein was proposed to play an active role in larval- and neuro-development (**Singh et al., 2010; Walker et al., 2011**). It was suggested that Elp3p is required for acetylation of Bruchpilot, a protein required for neuronal differentiation, thus mutations in the *ELP3* gene could result in neurodevelopmental defects in *D. melanogaster* (**Miskiewicz et al., 2011**). In the plant *A. thaliana*, mutations in the *ELO2*, *ELO3* or *ELO1* genes (respectively encoding homologs of the yeast Elongator subunits Elp1p, Elp3p and Elp4p) cause defects in cell proliferation resulting in narrow leaves and reduced root growth (**Nelissen et al., 2005**). It was also proposed that the Elongator complex plays an important role in regulating

plant's responses to abiotic stresses (**Nelissen et al., 2005; Zhou et al., 2009**). In mice, conditional inactivation of the *Ikbkap* gene, encoding a homolog of the yeast Elp1p, results in meiotic defects during spermatogenesis (**Lin et al., 2013**). Moreover, homozygous *Ikbkap*-knockout (*ikbkap*^{-/-}) mice die during embryonic development (**Chen et al., 2009b**). It was also discovered that knockdown of homologs of the yeast Elongator subunits Elp1p, Elp3p or Elp4p in mice zygotes impairs paternal DNA demethylation during embryogenesis (**Okada et al., 2010**). Similarly to the *C. elegans* ELPC-3 protein, the Elp3p homolog in mice has been implicated in K40 acetylation of α -tubulin (**Creppe et al., 2009**). However, the Atat1 protein was later identified as the major α -tubulin acetylase in mice (**Kim et al., 2013**). Recent ribosome profiling studies have revealed that in both mice and *C. elegans* loss of wobble uridine modifications leads to increased ribosomal pausing at their cognate codons (**Laguesse et al., 2015; Nedialkova and Leidel, 2015**). Based on this, it was suggested that alteration of codon translation speed in mice triggers endoplasmic reticulum stress and the unfolded protein response resulting in intermediate progenitors and hence microcephaly (**Laguesse et al., 2015**).

In mammalian cells, IKAP (hELP1) was first identified as a scaffold protein involved in pro-inflammatory cytokine signalling, as it supports assembly of active NIK-IKK kinase complexes in the I κ B pathway (**Cohen et al., 1998**). However, it was later discovered that IKAP does not associate with IKKs and does not participate in cytokine-induced NF- κ B activation (**Krappmann et al., 2000**). IKAP was also suggested to be involved in regulation of mammalian stress responses, as it interacts with the c-Jun N-terminal kinase (JNK) and possibly regulates activation of JNK (**Holmberg et al., 2002**). In addition, IKAP-depleted cells display defects in cell motility due to reduced expression of actin cytoskeleton modulators such as gelsolin, caveolin-1 and paxilin (**Close et al., 2006**). The reduced expression of these proteins was linked to inefficient histone acetylation upon loss of IKAP (**Close et al., 2006**). However, the indications that IKAP-depleted cells have reduced levels of proteins involved in cell motility were subsequently questioned due to failure to repeat these results and the observation that IKAP-depleted cells display defects in actin cytoskeleton organization due to mislocalization of filamin A (**Johansen et al., 2008**). Later studies also suggested that the cell motility defects observed in IKAP-depleted cells are linked to destabilization of microtubules caused by reduction in JNK protein expression and increased expression of the microtubule destabilizing protein SCG10 (**Abashidze et al., 2014; Cheishvili et al., 2011**). Further suggestions are that DERP6/hELP5 and C3ORF75/ELP6 proteins play important roles in the migration, invasion and tumorigenicity of melanoma cells (**Close et al., 2012**). All these

observations indicate that the Elongator complex has a conserved role in formation of wobble uridine modifications in multicellular organisms, and phenotypes associated with perturbation of the complex observed in worms, mice and humans might be linked to loss of these modifications.

4.4. The Elongator Complex and Human Diseases

Several diseases have been linked to mutations in genes encoding human Elongator subunits. For instance, familial dysautonomia (FD) is a neurodegenerative disease caused by a recessive mutation in the *IKBKAP* gene encoding IKAP/hELP1 protein (**Anderson et al., 2001; Slaugenhaupt et al., 2001**). This mutation is located in intron 20 of the *IKBKAP* gene, and causes an *IKBKAP* mRNA splicing defect resulting in a C-terminal truncation of IKAP protein (**Dong et al., 2002; Slaugenhaupt et al., 2001**). This splicing defect has suggested tissue-specificity, as FD patients have reduced levels of full-length IKAP in brain tissues, but IKAP levels are not affected in lymphoblasts (**Cuajungco et al., 2003; Slaugenhaupt et al., 2001**). Furthermore, findings reported in **Paper IV** indicate that the reduced IKAP protein levels in fibroblast cell lines and brain tissues derived from FD patients lead to lower levels of the modified wobble uridine nucleosides (mcm⁵s²U) in tRNAs (**Karlsborn et al., 2014a**).

Variants of the other Elongator subunits are also related to several diseases. For instance, allelic variants of the human *ELP3* gene have been associated with amyotrophic lateral sclerosis (ALS), a progressive motor neuron disease (**Simpson et al., 2009**). It has also been suggested that variants of the human *ELP4* (*hELP4*) gene are linked to Rolandic epilepsy (RE), a neurodevelopmental disorder affecting 0.2% of the population (**Strug et al., 2009**). However, the proposed link between the *hELP4* gene and RE disorder was subsequently questioned, as a recent study detected no enrichment of mutant forms of the *hELP4* gene in affected patients (**Reinthaler et al., 2014**). Moreover, missense variants of the human *ELP2* (*hELP2*) gene have been linked to intellectual disability (ID), as they have been discovered in genomic DNA from patients with severe ID (**Cohen et al., 2015**). Despite these findings the connections between the Elongator complex and human diseases is still unclear. However, the conserved role of the Elongator complex in formation of wobble uridine modifications indicates that the diseases are likely caused by translation defects associated with loss of these modifications.

Results and Discussion

This section briefly summarizes the rationale, methods, findings and implications of the studies reported in the four appended papers (I-IV).

Paper I: The role of wobble uridine modifications in +1 translational frameshifting in eukaryotes

Translation, where genetic information in mRNA species is converted into corresponding proteins, is the most energy-consuming process in cells. Although the translation machinery decodes mRNAs with high efficiency and fidelity, errors inevitably occur at low frequencies (**Kurland, 1992**). Translational errors such as frameshift errors are detrimental to the function or stability of the generated proteins as they completely change the reading frame downstream of the frameshifting site, resulting in changes in amino acid sequences and/or truncated proteins (**Kurland, 1992; Parker, 1989**). Frameshift errors are not as frequent as other common translation errors, such as missense errors, but there are many examples of alterations in tRNA structure increasing frameshifting frequencies (**Atkins and Björk, 2009; Björk and Hagervall, 2014; Kurland, 1992; Parker, 1989**). In bacteria, the modified wobble nucleoside 5-methylaminomethyl-2-thiouridine ($mnm^{5s^2}U_{34}$) present in a subset of bacterial tRNA isoacceptors is important for proper reading frame maintenance, as its absence increases frameshifting frequencies (**Brierley et al., 1997; Licznar et al., 2003; Maynard et al., 2012; Urbonavicius et al., 2001; Urbonavicius et al., 2003**). Similarly, a previous study showed that the esterified methyl group of 5-methoxycarbonylmethyluridine (mcm^5U_{34}) is important for preventing -1 frameshifting in yeast, but not where the frameshift error occurs (**Patil et al., 2012a**). Therefore, it was important to investigate whether or not the presence of nem^5 , mcm^5 or s^2 groups at wobble uridines in yeast tRNA isoacceptors is crucial for reading frame maintenance.

To analyse the role of wobble uridine modifications in reading frame maintenance, we used yeast mutants that cannot form the s^2 group (*tuc1Δ*), nem^5 or mcm^5 side chains (*elp3Δ*) or the esterified methyl group of the mcm^5 side chain (*trm9Δ*). A *Renilla (R-luc)/Firefly (F-luc)* bicistronic reporter system was used to determine levels of +1 frameshifting in both wild type and mutant strains. The reporter system contains a single frameshifting window consisting of a slippery codon (at which the peptidyl-tRNA will slip) and a test codon (assayed for ribosomal A-site selection) followed by an in-frame stop codon. This frameshifting window is inserted between the *Renilla*

and *Firefly* genes in such way that the *Firefly* luciferase is only expressed when +1 frameshifting occurs within this window. The level of +1 frameshifting was then determined by dividing the ratio of *F-luc/R-luc* generated from the frameshifting test construct by the ratio of *F-luc/R-luc* generated from the in-frame control. We discovered that in most cases lack of the mcm⁵ and/or s² groups in tRNA isoacceptors results in higher levels of +1 frameshifting with codons decoded by these tRNAs, suggesting that presence of these modifications is vital for reading frame maintenance. We also found little difference in +1 frameshifting rates in the presence and absence of the ncm⁵ group or the esterified methyl group in the mcm⁵ side chain, thus they seem to play minor roles in this type of frameshifting.

Frameshifting errors caused by tRNA modification deficiency are well-explained with a peptidyl-tRNA slippage model, according to which peptidyl-tRNA slippage may be induced by either an A-site effect, P-site effect, or a combination thereof (**Atkins and Björk, 2009; Björk and Hagervall, 2014; Björk et al., 1999; Farabaugh, 1996a, b; Farabaugh and Björk, 1999; Gallant et al., 2000; Jäger et al., 2013; Näsvalld et al., 2009; Urbonavicius et al., 2001**). The *Renilla/Firefly* bicistronic reporter system was designed to detect +1 frameshifting events, but not to distinguish between an A- or a P-site effect caused by modification deficiency. Thus, we decided to use an altered version of the *HIS4A::Ty1::lacZ* reporter system to investigate whether or not the +1 frameshifting event induced by lack of mcm⁵ groups is due to an A-site effect. In yeast, expression of the *TyB* gene of the Ty retrotransposon requires a programmed +1 frameshifting event. Only a seven nucleotide sequence (CUU-AGG-C) is required for the +1 frameshifting event, so only two tRNA species (tRNA_{UAG}^{Leu} and tRNA_{CCU}^{Arg}) participate in it (**Belcourt and Farabaugh, 1990**). The low availability of tRNA_{CCU}^{Arg} results in low rates of ribosomal A-site selection, causing a pause and therefore slippage of tRNA_{UAG}^{Leu} at the CUU codon (P-site) into the +1 frame (UU-A) (**Belcourt and Farabaugh, 1990**). The *HIS4A::Ty1::lacZ* reporter system consists of the first 100 nucleotides of the *HIS4* gene followed by seven nucleotides (CUU-AGG-C) of the *TyB* gene and the *LacZ* gene in a +1 frame. These seven nucleotides in the *TyB* gene are required for a +1 frameshifting event, and thus expression of the *LacZ* gene. We changed the arginine AGG codon decoded by tRNA_{CCU}^{Arg} in the CUU-AGG-C sequence into a lysine AAA codon decoded by tRNA_{mcm⁵s²UUU}^{Lys} to determine whether or not the +1 frameshifting induced by lack of the mcm⁵ group is caused by slow ribosomal A-site selection. We found that expression of the *LacZ* gene is around ten-fold higher in the *elp3Δ* mutant lacking the mcm⁵ group in tRNA_{s²UUU}^{Lys} than in the

wild type strain. Furthermore, overexpression of the hypomodified tRNA^{Lys}_{s²UUU} in the *elp3Δ* mutant reduced the *LacZ* expression level to around three-fold higher than in the wild type strain. Thus, elevated levels of hypomodified tRNA^{Lys}_{s²UUU} in the *elp3Δ* mutant seem to improve its selection in the ribosomal A-site, as it reduces levels of +1 frameshifting. All these findings indicate that the induced +1 frameshifting observed in the CUU-AAA-C frameshifting window is caused by slippage of the peptidyl-tRNA^{Leu}_{UAG} due to slow entry of the hypomodified tRNA^{Lys}_{s²UUU} into the ribosomal A-site, therefore this frameshifting event is consistent with the A-site effect caused by modification deficiency model.

Mutants unable to form *mcm*⁵, *mcm*⁵ or *s*² groups at wobble uridines display pleiotropic phenotypes which can be suppressed by overexpression of hypomodified tRNAs (Bauer et al., 2012; Chen et al., 2011b; Esberg et al., 2006; Fernandez-Vazquez et al., 2013; Klassen et al., 2015; Nedialkova and Leidel, 2015; Tigano et al., 2015; Zinshteyn and Gilbert, 2013). This suggests that the observed phenotypes are caused by inefficient translation due to poor decoding of codons read by hypomodified tRNAs. Our data show that absence of *mcm*⁵ or *s*² groups at wobble uridines increases levels of +1 frameshifting errors, which might be partially responsible for the mentioned mutant phenotypes.

Paper II: Elongator complex enhances Rnr1p levels in response to DNA damage by influencing Ixr1p expression

As already mentioned, the Elongator complex is a six subunit protein (Elp1p-Elp6p) complex required for an early step in formation of the *mcm*⁵ and *mcm*⁵ groups at wobble uridines in a subset of tRNA isoacceptors (Huang et al., 2005). Loss-of-function mutations in any gene encoding an Elongator complex subunit result in loss of these wobble uridine modifications and a plethora of phenotypes, including high sensitivity to DNA damaging agents such as methyl methanesulfonate (MMS) (reviewed in (Karlsborn et al., 2014b)). All tested mutant phenotypes except the tRNA modification defects can be suppressed by overexpression of hypomodified tRNAs, suggesting that the observed mutant phenotypes are due to inefficient translation of mRNAs (Bauer et al., 2012; Chen et al., 2011b; Esberg et al., 2006; Fernandez-Vazquez et al., 2013; Klassen et al., 2015; Nedialkova and Leidel, 2015; Tigano et al., 2015; Zinshteyn and Gilbert, 2013). Previous studies also implicated that absence of the *mcm*⁵*s*²U₃₄ nucleoside causes inefficient translation of mRNAs enriched in

lysine AAA (read by tRNA^{Lys}_{mcm⁵s²UUU}) and/or glutamine CAA (read by tRNA^{Gln}_{mcm⁵s²UUG}) codons (**Bauer et al., 2012; Fernandez-Vazquez et al., 2013; Rezgui et al., 2013**).

In the study reported in Paper II we performed a genome wide identification of genes rich in CAA (glutamine) codons and this analysis led to identification of the *IXR1* gene. We showed that Ixr1p levels are around 50% lower in the *elp3Δ* mutant than in the wild type strain, but they can be partially restored by overexpression of hypomodified tRNA^{Lys}_{s²UUU}, tRNA^{Gln}_{s²UUG} and tRNA^{Glu}_{s²UUC} without altering *IXR1* mRNA levels. These observations indicate that reduced Ixr1p expression in the *elp3Δ* mutant is due to a post-transcriptional defect. Ixr1p plays an important role in responses to DNA damage, as it is required for increases in dNTP production via induction of Ribonucleotide reductase 1 (Rnr1p) (**Tsaponina et al., 2011**). Therefore, we investigated whether or not Rnr1p induction is influenced by reduced Ixr1p expression in the *elp3Δ* mutant, and found that treatment with the alkylating agent methyl methanesulfonate (MMS) increases Rnr1p levels 1.7- and 1.3-fold in the wild type and *elp3Δ* strains, respectively. In addition, we discovered that reduced Rnr1p levels can be partially suppressed by elevated levels of the aforementioned hypomodified tRNAs in the *elp3Δ* mutant, but not the *ixr1Δ elp3Δ* double mutant. Overall, these results indicate that reduced expression of Ixr1p in the *elp3Δ* mutant is the main cause of lower Rnr1p levels with or without MMS treatment. Hence, the high sensitivity to DNA damaging agents observed in Elongator mutants might be partially due to reductions in Ixr1p expression and hence Rnr1p levels in responses to DNA damage.

It has been previously reported that absence of the *TRM9* gene, required for formation of the esterified methyl group of the mcm⁵ side chain (**Kalhor and Clarke, 2003**), results in lower Rnr1p levels under normal growth conditions as well as in responses to DNA damage (**Begley et al., 2007**). However, we observed no difference in either Ixr1p or Rnr1p levels between the *trm9Δ* and wild type strains, with or without MMS treatment. The earlier publications also suggested that the reduced Rnr1p levels in the *trm9Δ* mutant may be due to inefficient translation of the *RNR1* mRNA, as changing the codons read by tRNAs with the Trm9p-dependent modification in the *RNR1* gene reportedly restored Rnr1p expression in the *trm9Δ* mutant (**Patil et al., 2012a; Patil et al., 2012b**). However, some of the codon changes introduced in the *RNR1* gene in the cited studies were doubtful, and no correlations between mRNA and protein levels were described, so it is difficult to draw meaningful conclusions from these previous experiments.

Earlier studies also showed that impaired expression of a gene in the mutants lacking wobble uridine modifications can be ameliorated by changing the codons read by tRNAs dependent on wobble uridine modifications to corresponding codons read by tRNAs lacking these modifications (**Bauer et al., 2012; Fernandez-Vazquez et al., 2013**). As expression of the glutamine CAA codon-rich *IXR1* gene is partially restored by elevated levels of tRNA^{Lys}_{s²UUU} and tRNA^{Gln}_{s²UUG} in the *elp3Δ* mutant, we decided to mutate all lysine AAA and glutamine CAA codons read by these tRNAs to corresponding G-ending near-cognate AAG and CAG codons read by tRNA^{Lys}_{CUU} and tRNA^{Gln}_{CUG} to improve its expression in the *elp3Δ* mutant. The codon alterations in the *IXR1* gene ameliorated the low Ixr1p levels in the *elp3Δ* mutant, but did not fully restore them to levels in the wild type strain. Therefore, we changed all codons (172 in total) in the *IXR1* gene that are read by tRNAs possessing the Elongator complex-dependent wobble uridine modifications in a further attempt to fully restore Ixr1p expression in the *elp3Δ* mutant. However, the improvement in Ixr1p levels this induced was similar to the improvement observed when only lysine and glutamine codons were changed. Moreover, elevated levels of hypomodified tRNA^{Lys}_{s²UUU}, tRNA^{Gln}_{s²UUG} and tRNA^{Glu}_{s²UUC} were still capable of increasing Ixr1p expression from the optimized versions of the *IXR1* gene, although no codons in the optimized *IXR1* genes require these tRNAs during translation. Overall, full-restoration of reduced Ixr1p levels in the *elp3Δ* mutant requires both codon alterations in the *IXR1* gene and overexpression of the mentioned hypomodified tRNAs. Thus, these results suggest that the *elp3Δ* mutant has secondary defect(s) in other post-transcriptional processes that contributes to the reduced Ixr1p expression.

It was previously shown that the reduced protein expression from lysine AAA-enriched *CDR2* and *ATF1* genes in *S. pombe* mutants lacking wobble uridine modifications can be suppressed by either elevating levels of hypomodified tRNAs or changing lysine AAA codons to AAG codons (**Bauer et al., 2012; Fernandez-Vazquez et al., 2013**). However, only the codon changes in the *ATF1* gene were verified to improve translation of the *ATF1* mRNA without altering the mRNA levels (**Fernandez-Vazquez et al., 2013**). Our results indicate that the reductions in expression of proteins in Elongator mutants may be synergistically caused by inefficient decoding of mRNAs rich in codons read by tRNAs with Elongator complex-dependent wobble uridine modifications and diverse secondary defects, such as translational initiation or post-translational modifications. Thus, the events that lead to reduced protein expression in Elongator mutants seem to be very complex.

Paper III: Loss of *nmc*⁵ and *mcm*⁵ wobble uridine side chains results in an altered metabolic profile

As previously mentioned, the Elongator complex is a highly-conserved six subunit protein (Elp1p-Elp6p) complex that is involved in an early step in the formation of the *nmc*⁵ and *mcm*⁵ groups at wobble uridines in a subset of tRNA isoacceptors **(Huang et al., 2005)**. In *S. cerevisiae*, Elongator mutants display a multitude of phenotypes linked to several cellular processes (reviewed in **(Karlsborn et al., 2014b)**). All phenotypes reportedly observed in Elongator mutants, except the tRNA modification defects, can be suppressed by elevated levels of hypomodified tRNAs, suggesting that the phenotypes are mainly caused by defects in translation **(Bauer et al., 2012; Chen et al., 2011b; Esberg et al., 2006; Fernandez-Vazquez et al., 2013; Klassen et al., 2015; Nediakova and Leidel, 2015; Tigano et al., 2015; Zinshteyn and Gilbert, 2013)**. Previous studies proposed that absence of the *mcm*⁵ group at wobble uridines causes inefficient translation of mRNAs rich in lysine AAA and/or glutamine CAA codons, respectively read by hypomodified tRNA^{Lys}_{S²UUU} and tRNA^{Gln}_{S²UUG} **(Bauer et al., 2012; Fernandez-Vazquez et al., 2013; Rezgui et al., 2013)**. Moreover, ribosome profiling studies revealed that in Elongator mutants ribosomal pausing occurs more frequently at lysine AAA and glutamine CAA codons **(Nediakova and Leidel, 2015; Zinshteyn and Gilbert, 2013)**. However, whether the Elongator mutant phenotypes are caused by global alterations in protein expression or reduced protein expression from a subset of mRNAs, was still not clear. Therefore, we decided to investigate the metabolic alterations that occur in the absence of wobble uridine modifications.

Metabolite levels in an *elp3Δ* strain harbouring either an empty low-copy *LEU2* vector (*elp3Δ*-l.c.-empty) or the same vector containing a wild-type *ELP3* gene (*elp3Δ*-l.c.-*ELP3*) were determined by non-targeted gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF-MS). As the Elongator mutants are temperature sensitive, we decided to monitor the metabolic alterations both at the optimal growth temperature (30°C) and a semi-permissive growth temperature (34°C). Absence of the *ELP3* gene resulted in changes in levels of 36% of all detected metabolites in strains grown at 30°C, and the changes were most pronounced for glutamine, beta-alanine, ornithine and lysine. In strains grown at 34°C, loss of the *ELP3* gene resulted in changes in levels of 46% of all detected metabolites and the changes were most pronounced for lysine, ornithine, tyrosine and lactic acid. Thus, growth at 34°C resulted in more metabolic alterations than growth at 30°C in the *elp3Δ* (*elp3Δ*-l.c.-empty) strain, relative to the *elp3Δ* strain

complemented with the wild-type *ELP3* gene (*elp3Δ-l.c.-ELP3*). It is possible that some metabolic alterations in the *elp3Δ* strain are transient, and can only be observed under certain stress conditions such as elevated growth temperatures. It is also possible that the elevated temperature induces translational defects in the *elp3Δ* strain, and consequently more metabolic alterations.

We next investigated whether or not the metabolic alterations observed in the *elp3Δ* strain can be suppressed by overexpression of hypomodified tRNA^{Lys}_{s²UUU}, tRNA^{Gln}_{s²UUG} and tRNA^{Glu}_{s²UUC}. We found that only certain metabolic alterations were partially suppressed, regardless of whether the strains were grown at 30°C or 34°C. The only metabolites with alterations in levels that were partially suppressed at both growth temperatures were ornithine and lysine, although we detected weak suppression of changes in several other metabolites. As overexpression of the mentioned tRNAs fully or partially suppresses Elongator mutant phenotypes (Bauer et al., 2012; Chen et al., 2011b; Esberg et al., 2006; Fernandez-Vazquez et al., 2013; Nedialkova and Leidel, 2015; Tigano et al., 2015), it was surprising that overexpression of these tRNAs did not result in similar suppression of changes in metabolite levels. It is possible that partial suppression of these metabolites is sufficient to improve the *elp3Δ* strain's metabolic homeostasis enough to suppress the Elongator mutant phenotypes. However, as the *ncm⁵* or *mcm⁵* side chain is present at wobble uridines in 11 tRNA species, suppression of the metabolic alterations in the *elp3Δ* strain might require simultaneous overexpression of all these hypomodified tRNAs.

Paper IV: Familial dysautonomia (FD) patients have reduced levels of the modified wobble nucleoside *mcm⁵s²U* in tRNA

FD is a neurodegenerative disease caused by a mutation in the donor splicing site of intron 20 of the *IKBKAP* (*hELP1*) gene (Anderson et al., 2001; Slaughaupt et al., 2001). This mutation causes a splicing defect of the *hELP1* mRNA, and consequently results in a C-terminal truncation of hELP1 protein (Dong et al., 2002; Slaughaupt et al., 2001). This splicing defect was suggested to be tissue-specific, as FD patients have reduced levels of the full-length hELP1 protein in brain tissues, but not lymphoblasts (Cuajungco et al., 2003; Slaughaupt et al., 2001). The hELP1 protein is part of the six-subunit Elongator protein complex, and orthologues of these subunits can be found in all eukaryotes (Chen et al., 2009a;

Close et al., 2012; Hawkes et al., 2002; Nelissen et al., 2010; Nelissen et al., 2005; Okada et al., 2010; Otero et al., 1999; Simpson et al., 2009; Winkler et al., 2001). As already noted, in yeast, the Elongator complex is required for formation of mcm⁵U, mcm⁵U and mcm⁵s²U nucleosides at the wobble position in tRNAs, and absence of these modified nucleosides results in translational defects and pleiotropic phenotypes (**Esberg et al., 2006; Huang et al., 2005**). In accordance with observed phenomena in yeast, loss-of-function mutations in any gene encoding subunits of the Elongator complex in the worm *C. elegans*, the plant *A. thaliana* and the mouse *M. musculus* results in diverse phenotypes including loss of wobble uridine modifications (**Chen et al., 2009a; Lin et al., 2013; Mehlgarten et al., 2010; Nelissen et al., 2005; Okada et al., 2010**).

Despite the evidence regarding the Elongator complex's functions in multicellular organisms, the possibility that FD patients might have defects in formation of modified wobble uridines due to reduced levels of full-length hELP1 protein had not been previously addressed. Therefore, we isolated total tRNA samples from brain tissue and fibroblast cell lines derived from FD patients and healthy individuals, and determined levels of the mcm⁵s²U-modified nucleoside by high-performance liquid chromatography (HPLC) in them. We found that levels of mcm⁵s²U modified nucleoside in total tRNA were 29-35% lower in brain tissue derived from FD patients than in corresponding tissue derived from healthy individuals. Similarly, fibroblast cell lines derived from FD patients had 36% lower levels of mcm⁵s²U modified nucleoside in total tRNA than cell lines derived from healthy individuals. We also confirmed the reduction in full-length hELP1 protein levels in fibroblast cell lines derived from FD patients with western blotting, in accordance with previous studies (**Anderson et al., 2001; Cuajungco et al., 2003; Dong et al., 2002; Slaugenhaupt et al., 2001**). The reductions in mcm⁵s²U levels observed in both brain tissue and fibroblast cell lines are similar to the reductions observed in yeast *kti13Δ* mutants (**Huang et al., 2008**). In yeast, absence of the *KTI13* gene results in similar but weaker phenotypes than those of Elongator mutants, suggesting that partial loss of modified nucleosides is sufficient to cause translation defects and hence multiple phenotypes (**Esberg et al., 2006; Zabel et al., 2008**). Moreover, earlier studies showed that translational defects caused by loss of wobble uridine modifications result in reduced neuronal function in both nematodes and mice (**Chen et al., 2009a; Laguesse et al., 2015; Nedialkova and Leidel, 2015**). Therefore, partial loss of the mcm⁵s²U nucleoside in tRNA may be the cause of inefficient translation in brain tissues of FD patients.

Conclusions

1. Presence of mcm^5 and s^2 groups at wobble uridines play an important role in maintaining the reading frame during translation as absence of these modifications results in elevated levels of +1 frameshifting errors. The slow entry of the hypomodified $tRNA_{s^2UUU}^{Lys}$ into ribosomal A-site results in peptidyl-tRNA slippage, which can be suppressed by elevated levels of $tRNA_{s^2UUU}^{Lys}$.
2. Absence of the ncm^5 and mcm^5 groups at wobble uridines in *elp3Δ* mutants results in reduced expression of Ixr1p and consequently lower Rnr1p levels in response to DNA damage. Reduction of the Ixr1p levels in these mutants is due to synergistic effects caused by inefficient decoding of codons read by tRNAs with Elongator-dependent wobble uridine modifications and secondary defects in other post-transcriptional processes.
3. Loss of the ncm^5 and mcm^5 groups at wobble uridines in the *elp3Δ* mutant causes widespread metabolic alterations. Elevated levels of hypomodified $tRNA_{s^2UUU}^{Lys}$, $tRNA_{s^2UUG}^{Gln}$ and $tRNA_{s^2UUC}^{Glu}$ does not suppress all metabolic alterations observed in the *elp3Δ* mutant.
4. Levels of $mcm^5s^2U_{34}$ -modified nucleoside in tRNAs are lower in both brain tissues and fibroblast cell lines derived from Familial dysautonomia patients than in corresponding materials derived from healthy individuals.

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References

- Abashidze, A., Gold, V., Anavi, Y., Greenspan, H., and Weil, M. (2014). Involvement of IKAP in peripheral target innervation and in specific JNK and NGF signaling in developing PNS neurons. *PLoS One* 9, e113428.
- Abdel-Fattah, W., Jablonowski, D., Di Santo, R., Thuring, K.L., Scheidt, V., Hammermeister, A., Ten Have, S., Helm, M., Schaffrath, R., and Stark, M.J. (2015). Phosphorylation of Elp1 by Hrr25 is required for elongator-dependent tRNA modification in yeast. *PLoS Genet* 11, e1004931.
- Agris, P.F. (1991). Wobble position modified nucleosides evolved to select transfer RNA codon recognition: a modified-wobble hypothesis. *Biochimie* 73, 1345-1349.
- Akella, J.S., Wloga, D., Kim, J., Starostina, N.G., Lyons-Abbott, S., Morrisette, N.S., Dougan, S.T., Kipreos, E.T., and Gaertig, J. (2010). MEC-17 is an alpha-tubulin acetyltransferase. *Nature* 467, 218-222.
- Alexandrov, A., Chernyakov, I., Gu, W., Hiley, S.L., Hughes, T.R., Grayhack, E.J., and Phizicky, E.M. (2006). Rapid tRNA decay can result from lack of nonessential modifications. *Molecular cell* 21, 87-96.
- Andersen, C.B., Becker, T., Blau, M., Anand, M., Halic, M., Balar, B., Mielke, T., Boesen, T., Pedersen, J.S., Spahn, C.M., *et al.* (2006). Structure of eEF3 and the mechanism of transfer RNA release from the E-site. *Nature* 443, 663-668.
- Anderson, S.L., Coli, R., Daly, I.W., Kichula, E.A., Rork, M.J., Volpi, S.A., Ekstein, J., and Rubin, B.Y. (2001). Familial dysautonomia is caused by mutations of the IKAP gene. *Am J Hum Genet* 68, 753-758.
- Astrom, S.U., and Bystrom, A.S. (1994). Rit1, a tRNA backbone-modifying enzyme that mediates initiator and elongator tRNA discrimination. *Cell* 79, 535-546.
- Atkins, J.F., and Björk, G.R. (2009). A gripping tale of ribosomal frameshifting: extragenic suppressors of frameshift mutations spotlight P-site realignment. *Microbiol Mol Biol Rev* 73, 178-210.
- Bar, C., Zabel, R., Liu, S., Stark, M.J., and Schaffrath, R. (2008). A versatile partner of eukaryotic protein complexes that is involved in multiple biological processes: Kti11/Dph3. *Mol Microbiol* 69, 1221-1233.
- Barthelme, D., Dinkelaker, S., Albers, S.V., Londei, P., Ermler, U., and Tampe, R. (2011). Ribosome recycling depends on a mechanistic link between the FeS cluster domain and a conformational switch of the twin-ATPase ABCE1. *Proc Natl Acad Sci U S A* 108, 3228-3233.

Bauer, F., Matsuyama, A., Candiracci, J., Dieu, M., Scheliga, J., Wolf, D.A., Yoshida, M., and Hermand, D. (2012). Translational control of cell division by Elongator. *Cell Rep* 1, 424-433.

Begley, U., Dyavaiah, M., Patil, A., Rooney, J.P., DiRenzo, D., Young, C.M., Conklin, D.S., Zitomer, R.S., and Begley, T.J. (2007). Trm9-catalyzed tRNA modifications link translation to the DNA damage response. *Molecular cell* 28, 860-870.

Belcourt, M.F., and Farabaugh, P.J. (1990). Ribosomal frameshifting in the yeast retrotransposon Ty: tRNAs induce slippage on a 7 nucleotide minimal site. *Cell* 62, 339-352.

Berthelot, K., Muldoon, M., Rajkowitsch, L., Hughes, J., and McCarthy, J.E. (2004). Dynamics and processivity of 40S ribosome scanning on mRNA in yeast. *Mol Microbiol* 51, 987-1001.

Björk, G., and Hagervall, T. (2014). Transfer RNA Modification: Presence, Synthesis, and Function. *EcoSal Plus*.

Björk, G.R., Durand, J.M., Hagervall, T.G., Leipuvienė, R., Lundgren, H.K., Nilsson, K., Chen, P., Qian, Q., and Urbonavičius, J. (1999). Transfer RNA modification: influence on translational frameshifting and metabolism. *FEBS Lett* 452, 47-51.

Bjork, G.R., Huang, B., Persson, O.P., and Bystrom, A.S. (2007). A conserved modified wobble nucleoside (mcm5s2U) in lysyl-tRNA is required for viability in yeast. *RNA* 13, 1245-1255.

Bjork, G.R., Jacobsson, K., Nilsson, K., Johansson, M.J., Bystrom, A.S., and Persson, O.P. (2001). A primordial tRNA modification required for the evolution of life? *The EMBO journal* 20, 231-239.

Bregeon, D., Colot, V., Radman, M., and Taddei, F. (2001). Translational misreading: a tRNA modification counteracts a +2 ribosomal frameshift. *Genes Dev* 15, 2295-2306.

Brierley, I., Meredith, M.R., Bloys, A.J., and Hagervall, T.G. (1997). Expression of a coronavirus ribosomal frameshift signal in *Escherichia coli*: influence of tRNA anticodon modification on frameshifting. *J Mol Biol* 270, 360-373.

Butler, A.R., Porter, M., and Stark, M.J. (1991). Intracellular expression of *Kluyveromyces lactis* toxin gamma subunit mimics treatment with exogenous toxin and distinguishes two classes of toxin-resistant mutant. *Yeast* 7, 617-625.

Butler, A.R., White, J.H., Folawiyo, Y., Edlin, A., Gardiner, D., and Stark, M.J. (1994). Two *Saccharomyces cerevisiae* genes which control sensitivity to G1 arrest induced by *Kluyveromyces lactis* toxin. *Molecular and cellular biology* 14, 6306-6316.

Chang, Y.W., and Traugh, J.A. (1998). Insulin stimulation of phosphorylation of elongation factor 1 (eEF-1) enhances elongation activity. *European journal of biochemistry / FEBS* 251, 201-207.

Cheishvili, D., Maayan, C., Cohen-Kupiec, R., Lefler, S., Weil, M., Ast, G., and Razin, A. (2011). IKAP/Elp1 involvement in cytoskeleton regulation and implication for familial dysautonomia. *Human molecular genetics* 20, 1585-1594.

Chen, C., Huang, B., Anderson, J.T., and Bystrom, A.S. (2011a). Unexpected accumulation of mcm(5)U and mcm(5)S(2) (U) in a trm9 mutant suggests an additional step in the synthesis of mcm(5)U and mcm(5)S(2)U. *PLoS One* 6, e20783.

Chen, C., Huang, B., Eliasson, M., Ryden, P., and Bystrom, A.S. (2011b). Elongator complex influences telomeric gene silencing and DNA damage response by its role in wobble uridine tRNA modification. *PLoS Genet* 7, e1002258.

Chen, C., Tuck, S., and Bystrom, A.S. (2009a). Defects in tRNA modification associated with neurological and developmental dysfunctions in *Caenorhabditis elegans* elongator mutants. *PLoS Genet* 5, e1000561.

Chen, Y.T., Hims, M.M., Shetty, R.S., Mull, J., Liu, L., Leyne, M., and Slaughaupt, S.A. (2009b). Loss of mouse Ikbkap, a subunit of elongator, leads to transcriptional deficits and embryonic lethality that can be rescued by human IKBKAP. *Molecular and cellular biology* 29, 736-744.

Chernyakov, I., Whipple, J.M., Kotelawala, L., Grayhack, E.J., and Phizicky, E.M. (2008). Degradation of several hypomodified mature tRNA species in *Saccharomyces cerevisiae* is mediated by Met22 and the 5'-3' exonucleases Rat1 and Xrn1. *Genes Dev* 22, 1369-1380.

Chuang, R.Y., Weaver, P.L., Liu, Z., and Chang, T.H. (1997). Requirement of the DEAD-Box protein ded1p for messenger RNA translation. *Science* 275, 1468-1471.

Close, P., Gillard, M., Ladang, A., Jiang, Z., Papuga, J., Hawkes, N., Nguyen, L., Chapelle, J.P., Bouillenne, F., Svejstrup, J., *et al.* (2012). DERP6 (ELP5) and C3ORF75 (ELP6) regulate tumorigenicity and migration of melanoma cells as subunits of Elongator. *J Biol Chem* 287, 32535-32545.

Close, P., Hawkes, N., Cornez, I., Creppe, C., Lambert, C.A., Register, B., Siebenlist, U., Merville, M.P., Slaughaupt, S.A., Bours, V., *et al.* (2006). Transcription impairment and cell migration defects in elongator-depleted cells: implication for familial dysautonomia. *Molecular cell* 22, 521-531.

Cohen, J.S., Srivastava, S., Farwell, K.D., Lu, H.M., Zeng, W., Lu, H., Chao, E.C., and Fatemi, A. (2015). ELP2 is a novel gene implicated in neurodevelopmental disabilities. *American journal of medical genetics. Part A* 167, 1391-1395.

Cohen, L., Henzel, W.J., and Baeuerle, P.A. (1998). IKAP is a scaffold protein of the IkappaB kinase complex. *Nature* 395, 292-296.

Cooley, L., Appel, B., and Soll, D. (1982). Post-transcriptional nucleotide addition is responsible for the formation of the 5' terminus of histidine tRNA. *Proc Natl Acad Sci U S A* 79, 6475-6479.

Costello, J., Castelli, L.M., Rowe, W., Kershaw, C.J., Talavera, D., Mohammad-Qureshi, S.S., Sims, P.F., Grant, C.M., Pavitt, G.D., Hubbard, S.J., *et al.* (2015). Global mRNA selection mechanisms for translation initiation. *Genome Biol* 16, 10.

Creppe, C., Malinouskaya, L., Volvert, M.L., Gillard, M., Close, P., Malaise, O., Laguesse, S., Cornez, I., Rahmouni, S., Ormenese, S., *et al.* (2009). Elongator controls the migration and differentiation of cortical neurons through acetylation of alpha-tubulin. *Cell* 136, 551-564.

Crick, F.H. (1966). Codon--anticodon pairing: the wobble hypothesis. *J Mol Biol* 19, 548-555.

Cuajungco, M.P., Leyne, M., Mull, J., Gill, S.P., Lu, W., Zagzag, D., Axelrod, F.B., Maayan, C., Gusella, J.F., and Slaugenhaupt, S.A. (2003). Tissue-specific reduction in splicing efficiency of IKBKAP due to the major mutation associated with familial dysautonomia. *Am J Hum Genet* 72, 749-758.

Das, S., and Maitra, U. (2001). Functional significance and mechanism of eIF5-promoted GTP hydrolysis in eukaryotic translation initiation. *Prog Nucleic Acid Res Mol Biol* 70, 207-231.

Dever, T.E., Dar, A.C., and Sicheri, F. (2007). 12 The eIF2 α Kinases.

Dever, T.E., and Green, R. (2012). The elongation, termination, and recycling phases of translation in eukaryotes. *Cold Spring Harbor perspectives in biology* 4, a013706.

Dewez, M., Bauer, F., Dieu, M., Raes, M., Vandenhaute, J., and Hermand, D. (2008). The conserved Wobble uridine tRNA thiolase Ctu1-Ctu2 is required to maintain genome integrity. *Proc Natl Acad Sci U S A* 105, 5459-5464.

Diggle, T.A., Redpath, N.T., Heesom, K.J., and Denton, R.M. (1998). Regulation of protein-synthesis elongation-factor-2 kinase by cAMP in adipocytes. *Biochem J* 336 (Pt 3), 525-529.

Dihanich, M.E., Najarian, D., Clark, R., Gillman, E.C., Martin, N.C., and Hopper, A.K. (1987). Isolation and characterization of MOD5, a gene required for isopentenylation of cytoplasmic and mitochondrial tRNAs of *Saccharomyces cerevisiae*. *Molecular and cellular biology* 7, 177-184.

Dmitriev, S.E., Terenin, I.M., Andreev, D.E., Ivanov, P.A., Dunaevsky, J.E., Merrick, W.C., and Shatsky, I.N. (2010). GTP-independent tRNA delivery to the ribosomal P-site by a novel eukaryotic translation factor. *J Biol Chem* 285, 26779-26787.

Dong, J., Edelman, L., Bajwa, A.M., Kornreich, R., and Desnick, R.J. (2002). Familial dysautonomia: detection of the IKBKAP IVS20(+6T --> C) and R696P mutations and frequencies among Ashkenazi Jews. *American journal of medical genetics* 110, 253-257.

Dong, M., Su, X., Dzikovski, B., Dando, E.E., Zhu, X., Du, J., Freed, J.H., and Lin, H. (2014). Dph3 is an electron donor for Dph1-Dph2 in the first step of eukaryotic diphthamide biosynthesis. *Journal of the American Chemical Society* 136, 1754-1757.

Dunlop, P.C., and Bodley, J.W. (1983). Biosynthetic labeling of diphthamide in *Saccharomyces cerevisiae*. *J Biol Chem* 258, 4754-4758.

Eckhardt, K., Troger, J., Reissmann, J., Katschinski, D.M., Wagner, K.F., Stengel, P., Paasch, U., Hunziker, P., Bortner, E., Barth, S., *et al.* (2007). Male germ cell expression of the PAS domain kinase PASKIN and its novel target eukaryotic translation elongation factor eEF1A1. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* 20, 227-240.

El Yacoubi, B., Hatin, I., Deutsch, C., Kahveci, T., Rousset, J.P., Iwata-Reuyl, D., Murzin, A.G., and de Crécy-Lagard, V. (2011). A role for the universal Kae1/Qri7/YgjD (COG0533) family in tRNA modification. *The EMBO journal* 30, 882-893.

Esberg, A., Huang, B., Johansson, M.J., and Bystrom, A.S. (2006). Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. *Molecular cell* 24, 139-148.

Fan, Y., Schlierf, M., Gaspar, A.C., Dreux, C., Kpebe, A., Chaney, L., Mathieu, A., Hitte, C., Gremy, O., Sarot, E., *et al.* (2010). *Drosophila* translational elongation factor-1gamma is modified in response to DOA kinase activity and is essential for cellular viability. *Genetics* 184, 141-154.

Farabaugh, P.J. (1996a). Programmed translational frameshifting. *Microbiological reviews* 60, 103-134.

Farabaugh, P.J. (1996b). Programmed translational frameshifting. *Annual review of genetics* 30, 507-528.

Farabaugh, P.J., and Björk, G.R. (1999). How translational accuracy influences reading frame maintenance. *The EMBO journal* 18, 1427-1434.

Fernandez-Vazquez, J., Vargas-Perez, I., Sanso, M., Buhne, K., Carmona, M., Paulo, E., Hermand, D., Rodriguez-Gabriel, M., Ayte, J., Leidel, S., *et al.* (2013). Modification of tRNA(Lys) UUU by elongator is essential for efficient translation of stress mRNAs. *PLoS Genet* 9, e1003647.

Fichtner, L., Frohloff, F., Burkner, K., Larsen, M., Breunig, K.D., and Schaffrath, R. (2002). Molecular analysis of KTI12/TOT4, a *Saccharomyces cerevisiae* gene required for *Kluyveromyces lactis* zymocin action. *Mol Microbiol* 43, 783-791.

- Fichtner, L., Jablonowski, D., Schierhorn, A., Kitamoto, H.K., Stark, M.J., and Schaffrath, R. (2003). Elongator's toxin-target (TOT) function is nuclear localization sequence dependent and suppressed by post-translational modification. *Mol Microbiol* 49, 1297-1307.
- Frohloff, F., Fichtner, L., Jablonowski, D., Breunig, K.D., and Schaffrath, R. (2001). *Saccharomyces cerevisiae* Elongator mutations confer resistance to the *Kluyveromyces lactis* zymocin. *The EMBO journal* 20, 1993-2003.
- Gallant, J., Lindsley, D., and Masucci, J. (2000). The unbearable lightness of peptidyl-tRNA. *Ribosome: Structure, Function, Antibiotics, and Cellular Interactions*, 385-396.
- Gerber, A.P., and Keller, W. (1999). An adenosine deaminase that generates inosine at the wobble position of tRNAs. *Science* 286, 1146-1149.
- Gkampeta, A., Fidani, L., Clarimon, J., Kalinderi, K., Katopodi, T., Zafeiriou, D., and Pavlou, E. (2014). Association of brain-derived neurotrophic factor (BDNF) and elongator protein complex 4 (ELP4) polymorphisms with benign epilepsy with centrotemporal spikes in a Greek population. *Epilepsy research* 108, 1734-1739.
- Glasser, A.L., Desgres, J., Heitzler, J., Gehrke, C.W., and Keith, G. (1991). O-ribosyl-phosphate purine as a constant modified nucleotide located at position 64 in cytoplasmic initiator tRNAs(Met) of yeasts. *Nucleic Acids Res* 19, 5199-5203.
- Glasser, A.L., el Adlouni, C., Keith, G., Sochacka, E., Malkiewicz, A., Santos, M., Tuite, M.F., and Desgres, J. (1992). Presence and coding properties of 2'-O-methyl-5-carbamoylmethyluridine (ncm5Um) in the wobble position of the anticodon of tRNA(Leu) (U*AA) from brewer's yeast. *FEBS Lett* 314, 381-385.
- Glatt, S., Letoquart, J., Faux, C., Taylor, N.M., Seraphin, B., and Muller, C.W. (2012). The Elongator subcomplex Elp456 is a hexameric RecA-like ATPase. *Nat Struct Mol Biol* 19, 314-320.
- Guy, M.P., Podyma, B.M., Preston, M.A., Shaheen, H.H., Krivos, K.L., Limbach, P.A., Hopper, A.K., and Phizicky, E.M. (2012). Yeast Trm7 interacts with distinct proteins for critical modifications of the tRNA^{Phe} anticodon loop. *RNA* 18, 1921-1933.
- Guy, M.P., Young, D.L., Payea, M.J., Zhang, X., Kon, Y., Dean, K.M., Grayhack, E.J., Mathews, D.H., Fields, S., and Phizicky, E.M. (2014). Identification of the determinants of tRNA function and susceptibility to rapid tRNA decay by high-throughput in vivo analysis. *Genes Dev* 28, 1721-1732.
- Hani, J., and Feldmann, H. (1998). tRNA genes and retroelements in the yeast genome. *Nucleic Acids Res* 26, 689-696.

Harding, H.P., Novoa, I., Zhang, Y., Zeng, H., Wek, R., Schapira, M., and Ron, D. (2000). Regulated translation initiation controls stress-induced gene expression in mammalian cells. *Molecular cell* 6, 1099-1108.

Hawkes, N.A., Otero, G., Winkler, G.S., Marshall, N., Dahmus, M.E., Krappmann, D., Scheidereit, C., Thomas, C.L., Schiavo, G., Erdjument-Bromage, H., *et al.* (2002). Purification and characterization of the human elongator complex. *J Biol Chem* 277, 3047-3052.

Hinnebusch, A.G. (1997). Translational regulation of yeast GCN4. A window on factors that control initiator-trna binding to the ribosome. *J Biol Chem* 272, 21661-21664.

Hinnebusch, A.G. (2011). Molecular mechanism of scanning and start codon selection in eukaryotes. *Microbiol Mol Biol Rev* 75, 434-467, first page of table of contents.

Hinnebusch, A.G., and Lorsch, J.R. (2012). The mechanism of eukaryotic translation initiation: new insights and challenges. *Cold Spring Harbor perspectives in biology* 4.

Hinnebusch, A.G., and Natarajan, K. (2002). Gcn4p, a master regulator of gene expression, is controlled at multiple levels by diverse signals of starvation and stress. *Eukaryot Cell* 1, 22-32.

Hinton, T.M., Coldwell, M.J., Carpenter, G.A., Morley, S.J., and Pain, V.M. (2007). Functional analysis of individual binding activities of the scaffold protein eIF4G. *J Biol Chem* 282, 1695-1708.

Hizli, A.A., Chi, Y., Swanger, J., Carter, J.H., Liao, Y., Welcker, M., Ryazanov, A.G., and Clurman, B.E. (2013). Phosphorylation of eukaryotic elongation factor 2 (eEF2) by cyclin A-cyclin-dependent kinase 2 regulates its inhibition by eEF2 kinase. *Molecular and cellular biology* 33, 596-604.

Holmberg, C., Katz, S., Lerdrup, M., Herdegen, T., Jaattela, M., Aronheim, A., and Kallunki, T. (2002). A novel specific role for I kappa B kinase complex-associated protein in cytosolic stress signaling. *J Biol Chem* 277, 31918-31928.

Huang, B., Johansson, M.J., and Bystrom, A.S. (2005). An early step in wobble uridine tRNA modification requires the Elongator complex. *RNA* 11, 424-436.

Huang, B., Lu, J., and Bystrom, A.S. (2008). A genome-wide screen identifies genes required for formation of the wobble nucleoside 5-methoxycarbonylmethyl-2-thiouridine in *Saccharomyces cerevisiae*. *RNA* 14, 2183-2194.

Huh, W.K., Falvo, J.V., Gerke, L.C., Carroll, A.S., Howson, R.W., Weissman, J.S., and O'Shea, E.K. (2003). Global analysis of protein localization in budding yeast. *Nature* 425, 686-691.

Ibba, M., and Soll, D. (2000). Aminoacyl-tRNA synthesis. *Annu Rev Biochem* 69, 617-650.

Jablonowski, D., Butler, A.R., Fichtner, L., Gardiner, D., Schaffrath, R., and Stark, M.J. (2001). Sit4p protein phosphatase is required for sensitivity of *Saccharomyces cerevisiae* to *Kluyveromyces lactis* zymocin. *Genetics* 159, 1479-1489.

Jablonowski, D., Fichtner, L., Stark, M.J., and Schaffrath, R. (2004). The yeast elongator histone acetylase requires Sit4-dependent dephosphorylation for toxin-target capacity. *Molecular biology of the cell* 15, 1459-1469.

Jackson, R.J., Hellen, C.U., and Pestova, T.V. (2010). The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol* 11, 113-127.

Jackson, R.J., Hellen, C.U., and Pestova, T.V. (2012). Termination and post-termination events in eukaryotic translation. *Advances in protein chemistry and structural biology* 86, 45-93.

Jäger, G., Nilsson, K., and Björk, G.R. (2013). The phenotype of many independently isolated +1 frameshift suppressor mutants supports a pivotal role of the P-site in reading frame maintenance. *PLoS One* 8, e60246.

Janssen, G.M., Morales, J., Schipper, A., Labbe, J.C., Mulner-Lorillon, O., Belle, R., and Moller, W. (1991). A major substrate of maturation promoting factor identified as elongation factor 1 beta gamma delta in *Xenopus laevis*. *J Biol Chem* 266, 14885-14888.

Johansen, L.D., Naumanen, T., Knudsen, A., Westerlund, N., Gromova, I., Junttila, M., Nielsen, C., Bottzauw, T., Tolkovsky, A., Westermarck, J., *et al.* (2008). IKAP localizes to membrane ruffles with filamin A and regulates actin cytoskeleton organization and cell migration. *Journal of cell science* 121, 854-864.

Johansson, M.J., Esberg, A., Huang, B., Bjork, G.R., and Bystrom, A.S. (2008). Eukaryotic wobble uridine modifications promote a functionally redundant decoding system. *Molecular and cellular biology* 28, 3301-3312.

Johansson, M.J.O., and Byström, A.S. (2005). Transfer RNA modifications and modifying enzymes in *Saccharomyces cerevisiae*. In *Fine-Tuning of RNA Functions by Modification and Editing*, H. Grosjean, ed. (Berlin, Heidelberg: Springer Berlin Heidelberg), pp. 87-120.

Kahvejian, A., Svitkin, Y.V., Sukarieh, R., M'Boutchou, M.N., and Sonenberg, N. (2005). Mammalian poly(A)-binding protein is a eukaryotic translation initiation factor, which acts via multiple mechanisms. *Genes Dev* 19, 104-113.

Kalhor, H.R., and Clarke, S. (2003). Novel methyltransferase for modified uridine residues at the wobble position of tRNA. *Molecular and cellular biology* 23, 9283-9292.

- Kapp, L.D., and Lorsch, J.R. (2004). The molecular mechanics of eukaryotic translation. *Annu Rev Biochem* 73, 657-704.
- Karlsborn, T., Tukenmez, H., Chen, C., and Bystrom, A.S. (2014a). Familial dysautonomia (FD) patients have reduced levels of the modified wobble nucleoside mcmsU in tRNA. *Biochem Biophys Res Commun* 454, 441-445.
- Karlsborn, T., Tukenmez, H., Mahmud, A.K., Xu, F., Xu, H., and Bystrom, A.S. (2014b). Elongator, a conserved complex required for wobble uridine modifications in eukaryotes. *RNA Biol* 11, 1519-1528.
- Keith, G., Desgres, J., Pochart, P., Heyman, T., Kuo, K.C., and Gehrke, C.W. (1990). Eukaryotic tRNAs(Pro): primary structure of the anticodon loop; presence of 5-carbamoylmethyluridine or inosine as the first nucleoside of the anticodon. *Biochimica et biophysica acta* 1049, 255-260.
- Kim, G.W., Li, L., Gorbani, M., You, L., and Yang, X.J. (2013). Mice lacking alpha-tubulin acetyltransferase 1 are viable but display alpha-tubulin acetylation deficiency and dentate gyrus distortion. *J Biol Chem* 288, 20334-20350.
- Kim, S.H., Suddath, F.L., Quigley, G.J., McPherson, A., Sussman, J.L., Wang, A.H., Seeman, N.C., and Rich, A. (1974). Three-dimensional tertiary structure of yeast phenylalanine transfer RNA. *Science* 185, 435-440.
- Kishida, M., Tokunaga, M., Katayose, Y., Yajima, H., Kawamura-Watabe, A., and Hishinuma, F. (1996). Isolation and genetic characterization of pGKL killer-insensitive mutants (iki) from *Saccharomyces cerevisiae*. *Biosci Biotechnol Biochem* 60, 798-801.
- Klassen, R., Grunewald, P., Thuring, K.L., Eichler, C., Helm, M., and Schaffrath, R. (2015). Loss of anticodon wobble uridine modifications affects tRNA(Lys) function and protein levels in *Saccharomyces cerevisiae*. *PLoS One* 10, e0119261.
- Kobayashi, T., Irie, T., Yoshida, M., Takeishi, K., and Ukita, T. (1974). The primary structure of yeast glutamic acid tRNA specific to the GAA codon. *Biochimica et biophysica acta* 366, 168-181.
- Kolaj-Robin, O., McEwen, A.G., Cavarelli, J., and Seraphin, B. (2015). Structure of the Elongator cofactor complex Kti11/Kti13 provides insight into the role of Kti13 in Elongator-dependent tRNA modification. *The FEBS journal* 282, 819-833.
- Kotelawala, L., Grayhack, E.J., and Phizicky, E.M. (2008). Identification of yeast tRNA Um(44) 2'-O-methyltransferase (Trm44) and demonstration of a Trm44 role in sustaining levels of specific tRNA(Ser) species. *RNA* 14, 158-169.
- Kozak, M. (1984). Selection of initiation sites by eucaryotic ribosomes: effect of inserting AUG triplets upstream from the coding sequence for preproinsulin. *Nucleic Acids Res* 12, 3873-3893.

Krappmann, D., Hatada, E.N., Tegethoff, S., Li, J., Klippel, A., Giese, K., Baeuerle, P.A., and Scheidereit, C. (2000). The I kappa B kinase (IKK) complex is tripartite and contains IKK gamma but not IKAP as a regular component. *J Biol Chem* 275, 29779-29787.

Krogan, N.J., and Greenblatt, J.F. (2001). Characterization of a six-subunit holo-elongator complex required for the regulated expression of a group of genes in *Saccharomyces cerevisiae*. *Molecular and cellular biology* 21, 8203-8212.

Kruger, M.K., Pedersen, S., Hagervall, T.G., and Sorensen, M.A. (1998). The modification of the wobble base of tRNAGlu modulates the translation rate of glutamic acid codons in vivo. *J Mol Biol* 284, 621-631.

Kuntzel, B., Weissenbach, J., Wolff, R.E., Tumaitis-Kennedy, T.D., Lane, B.G., and Dirheimer, G. (1975). Presence of the methylester of 5-carboxymethyl uridine in the wobble position of the anticodon of tRNAIII Arg from brewer's yeast. *Biochimie* 57, 61-70.

Kurata, S., Shen, B., Liu, J.O., Takeuchi, N., Kaji, A., and Kaji, H. (2013). Possible steps of complete disassembly of post-termination complex by yeast eEF3 deduced from inhibition by translocation inhibitors. *Nucleic Acids Res* 41, 264-276.

Kurland, C.G. (1992). Translational accuracy and the fitness of bacteria. *Annual review of genetics* 26, 29-50.

Laguesse, S., Creppe, C., Nedialkova, D.D., Prevot, P.P., Borgs, L., Huyseune, S., Franco, B., Duysens, G., Krusy, N., Lee, G., *et al.* (2015). A Dynamic Unfolded Protein Response Contributes to the Control of Cortical Neurogenesis. *Developmental cell* 35, 553-567.

Lamberti, A., Longo, O., Marra, M., Tagliaferri, P., Bismuto, E., Fiengo, A., Viscomi, C., Budillon, A., Rapp, U.R., Wang, E., *et al.* (2007). C-Raf antagonizes apoptosis induced by IFN-alpha in human lung cancer cells by phosphorylation and increase of the intracellular content of elongation factor 1A. *Cell death and differentiation* 14, 952-962.

Laten, H., Gorman, J., and Bock, R.M. (1978). Isopentenyladenosine deficient tRNA from an antisuppressor mutant of *Saccharomyces cerevisiae*. *Nucleic Acids Res* 5, 4329-4342.

Lecoite, F., Namy, O., Hatin, I., Simos, G., Rousset, J.P., and Grosjean, H. (2002). Lack of pseudouridine 38/39 in the anticodon arm of yeast cytoplasmic tRNA decreases in vivo recoding efficiency. *J Biol Chem* 277, 30445-30453.

Lecoite, F., Simos, G., Sauer, A., Hurt, E.C., Motorin, Y., and Grosjean, H. (1998). Characterization of yeast protein Deg1 as pseudouridine synthase (Pus3) catalyzing the formation of psi 38 and psi 39 in tRNA anticodon loop. *J Biol Chem* 273, 1316-1323.

- Leidel, S., Pedrioli, P.G., Bucher, T., Brost, R., Costanzo, M., Schmidt, A., Aebersold, R., Boone, C., Hofmann, K., and Peter, M. (2009). Ubiquitin-related modifier Urm1 acts as a sulphur carrier in thiolation of eukaryotic transfer RNA. *Nature* 458, 228-232.
- Li, Q., Fazly, A.M., Zhou, H., Huang, S., Zhang, Z., and Stillman, B. (2009). The elongator complex interacts with PCNA and modulates transcriptional silencing and sensitivity to DNA damage agents. *PLoS Genet* 5, e1000684.
- Li, Y., Takagi, Y., Jiang, Y., Tokunaga, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R.D. (2001). A multiprotein complex that interacts with RNA polymerase II elongator. *J Biol Chem* 276, 29628-29631.
- Licznar, P., Mejlhede, N., Prere, M.F., Wills, N., Gesteland, R.F., Atkins, J.F., and Fayet, O. (2003). Programmed translational -1 frameshifting on hexanucleotide motifs and the wobble properties of tRNAs. *The EMBO journal* 22, 4770-4778.
- Lim, V.I. (1994). Analysis of action of wobble nucleoside modifications on codon-anticodon pairing within the ribosome. *J Mol Biol* 240, 8-19.
- Lin, C.A., Ellis, S.R., and True, H.L. (2010). The Sua5 protein is essential for normal translational regulation in yeast. *Molecular and cellular biology* 30, 354-363.
- Lin, F.J., Shen, L., Jang, C.W., Falnes, P.O., and Zhang, Y. (2013). Ikbkap/Elp1 deficiency causes male infertility by disrupting meiotic progression. *PLoS Genet* 9, e1003516.
- Lin, Z., Zhao, W., Diao, W., Xie, X., Wang, Z., Zhang, J., Shen, Y., and Long, J. (2012). Crystal structure of elongator subcomplex Elp4-6. *J Biol Chem* 287, 21501-21508.
- Liu, S., Bachran, C., Gupta, P., Miller-Randolph, S., Wang, H., Crown, D., Zhang, Y., Wein, A.N., Singh, R., Fattah, R., *et al.* (2012). Diphthamide modification on eukaryotic elongation factor 2 is needed to assure fidelity of mRNA translation and mouse development. *Proc Natl Acad Sci U S A* 109, 13817-13822.
- Liu, S., and Leppla, S.H. (2003). Retroviral insertional mutagenesis identifies a small protein required for synthesis of diphthamide, the target of bacterial ADP-ribosylating toxins. *Molecular cell* 12, 603-613.
- Liu, S., Milne, G.T., Kuremsky, J.G., Fink, G.R., and Leppla, S.H. (2004). Identification of the proteins required for biosynthesis of diphthamide, the target of bacterial ADP-ribosylating toxins on translation elongation factor 2. *Molecular and cellular biology* 24, 9487-9497.
- Lu, J., Esberg, A., Huang, B., and Bystrom, A.S. (2008). *Kluyveromyces lactis* gamma-toxin, a ribonuclease that recognizes the anticodon stem loop of tRNA. *Nucleic Acids Res* 36, 1072-1080.

- Lu, J., Huang, B., Esberg, A., Johansson, M.J., and Bystrom, A.S. (2005). The Kluyveromyces lactis gamma-toxin targets tRNA anticodons. *RNA* 11, 1648-1654.
- Machnicka, M.A., Milanowska, K., Osman Oglou, O., Purta, E., Kurkowska, M., Olchowik, A., Januszewski, W., Kalinowski, S., Dunin-Horkawicz, S., Rother, K.M., *et al.* (2013). MODOMICS: a database of RNA modification pathways--2013 update. *Nucleic Acids Res* 41, D262-267.
- Maynard, N.D., Macklin, D.N., Kirkegaard, K., and Covert, M.W. (2012). Competing pathways control host resistance to virus via tRNA modification and programmed ribosomal frameshifting. *Molecular systems biology* 8, 567.
- Mazauric, M.H., Dirick, L., Purushothaman, S.K., Bjork, G.R., and Lapeyre, B. (2010). Trm112p is a 15-kDa zinc finger protein essential for the activity of two tRNA and one protein methyltransferases in yeast. *J Biol Chem* 285, 18505-18515.
- Mehlgarten, C., Jablonowski, D., Breunig, K.D., Stark, M.J., and Schaffrath, R. (2009). Elongator function depends on antagonistic regulation by casein kinase Hrr25 and protein phosphatase Sit4. *Mol Microbiol* 73, 869-881.
- Mehlgarten, C., Jablonowski, D., Wrackmeyer, U., Tschitschmann, S., Sondermann, D., Jager, G., Gong, Z., Bystrom, A.S., Schaffrath, R., and Breunig, K.D. (2010). Elongator function in tRNA wobble uridine modification is conserved between yeast and plants. *Mol Microbiol* 76, 1082-1094.
- Mei, Y., Yao, F., Wu, Y., Chu, B., Cheng, C., Liu, Y., Li, X., Zou, X., and Hou, L. (2014). Identification and expression of the elongator protein 2 (Ajelp2) gene, a novel regeneration-related gene from the sea cucumber *Apostichopus japonicus*. *Molecular biology reports* 41, 4985-4996.
- Miskiewicz, K., Jose, L.E., Bento-Abreu, A., Fislage, M., Taes, I., Kasproicz, J., Swerts, J., Sigrist, S., Versees, W., Robberecht, W., *et al.* (2011). ELP3 controls active zone morphology by acetylating the ELKS family member Bruchpilot. *Neuron* 72, 776-788.
- Miyauchi, K., Kimura, S., and Suzuki, T. (2013). A cyclic form of N6-threonylcarbamoyladenine as a widely distributed tRNA hypermodification. *Nature chemical biology* 9, 105-111.
- Mulner-Lorillon, O., Minella, O., Cormier, P., Capony, J.P., Cavadore, J.C., Morales, J., Poulhe, R., and Belle, R. (1994). Elongation factor EF-1 delta, a new target for maturation-promoting factor in *Xenopus* oocytes. *J Biol Chem* 269, 20201-20207.
- Murphy, F.V.t., Ramakrishnan, V., Malkiewicz, A., and Agris, P.F. (2004). The role of modifications in codon discrimination by tRNA(Lys)UUU. *Nat Struct Mol Biol* 11, 1186-1191.

Nairn, A.C., and Palfrey, H.C. (1987). Identification of the major Mr 100,000 substrate for calmodulin-dependent protein kinase III in mammalian cells as elongation factor-2. *J Biol Chem* 262, 17299-17303.

Nakai, Y., Nakai, M., and Hayashi, H. (2008). Thio-modification of yeast cytosolic tRNA requires a ubiquitin-related system that resembles bacterial sulfur transfer systems. *J Biol Chem* 283, 27469-27476.

Nakai, Y., Nakai, M., Lill, R., Suzuki, T., and Hayashi, H. (2007). Thio modification of yeast cytosolic tRNA is an iron-sulfur protein-dependent pathway. *Molecular and cellular biology* 27, 2841-2847.

Nakai, Y., Umeda, N., Suzuki, T., Nakai, M., Hayashi, H., Watanabe, K., and Kagamiyama, H. (2004). Yeast Nfs1p is involved in thio-modification of both mitochondrial and cytoplasmic tRNAs. *J Biol Chem* 279, 12363-12368.

Nameki, N., Asahara, H., Shimizu, M., Okada, N., and Himeno, H. (1995). Identity elements of *Saccharomyces cerevisiae* tRNA(His). *Nucleic Acids Res* 23, 389-394.

Näsvall, S.J., Nilsson, K., and Björk, G.R. (2009). The ribosomal grip of the peptidyl-tRNA is critical for reading frame maintenance. *J Mol Biol* 385, 350-367.

Nedialkova, D.D., and Leidel, S.A. (2015). Optimization of Codon Translation Rates via tRNA Modifications Maintains Proteome Integrity. *Cell* 161, 1606-1618.

Nelissen, H., De Groeve, S., Fleury, D., Neyt, P., Bruno, L., Bitonti, M.B., Vandenbussche, F., Van der Straeten, D., Yamaguchi, T., Tsukaya, H., *et al.* (2010). Plant Elongator regulates auxin-related genes during RNA polymerase II transcription elongation. *Proc Natl Acad Sci U S A* 107, 1678-1683.

Nelissen, H., Fleury, D., Bruno, L., Robles, P., De Veylder, L., Traas, J., Micol, J.L., Van Montagu, M., Inze, D., and Van Lijsebettens, M. (2005). The elongata mutants identify a functional Elongator complex in plants with a role in cell proliferation during organ growth. *Proc Natl Acad Sci U S A* 102, 7754-7759.

Noma, A., Sakaguchi, Y., and Suzuki, T. (2009). Mechanistic characterization of the sulfur-relay system for eukaryotic 2-thiouridine biogenesis at tRNA wobble positions. *Nucleic Acids Res* 37, 1335-1352.

Okada, Y., Yamagata, K., Hong, K., Wakayama, T., and Zhang, Y. (2010). A role for the elongator complex in zygotic paternal genome demethylation. *Nature* 463, 554-558.

Oppenheimer, N.J., and Bodley, J.W. (1981). Diphtheria toxin. Site and configuration of ADP-ribosylation of diphthamide in elongation factor 2. *J Biol Chem* 256, 8579-8581.

- Ortiz, P.A., Ulloque, R., Kihara, G.K., Zheng, H., and Kinzy, T.G. (2006). Translation elongation factor 2 anticodon mimicry domain mutants affect fidelity and diphtheria toxin resistance. *J Biol Chem* *281*, 32639-32648.
- Otero, G., Fellows, J., Li, Y., de Bizemont, T., Dirac, A.M., Gustafsson, C.M., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. (1999). Elongator, a multisubunit component of a novel RNA polymerase II holoenzyme for transcriptional elongation. *Molecular cell* *3*, 109-118.
- Ovchinnikov, L.P., Motuz, L.P., Natapov, P.G., Averbuch, L.J., Wettenhall, R.E., Szyszka, R., Kramer, G., and Hardesty, B. (1990). Three phosphorylation sites in elongation factor 2. *FEBS Lett* *275*, 209-212.
- Paraskevopoulou, C., Fairhurst, S.A., Lowe, D.J., Brick, P., and Onesti, S. (2006). The Elongator subunit Elp3 contains a Fe4S4 cluster and binds S-adenosylmethionine. *Mol Microbiol* *59*, 795-806.
- Parker, J. (1989). Errors and alternatives in reading the universal genetic code. *Microbiological reviews* *53*, 273-298.
- Patil, A., Chan, C.T., Dyavaiah, M., Rooney, J.P., Dedon, P.C., and Begley, T.J. (2012a). Translational infidelity-induced protein stress results from a deficiency in Trm9-catalyzed tRNA modifications. *RNA Biol* *9*, 990-1001.
- Patil, A., Dyavaiah, M., Joseph, F., Rooney, J.P., Chan, C.T., Dedon, P.C., and Begley, T.J. (2012b). Increased tRNA modification and gene-specific codon usage regulate cell cycle progression during the DNA damage response. *Cell cycle* *11*, 3656-3665.
- Percudani, R., Pavesi, A., and Ottonello, S. (1997). Transfer RNA gene redundancy and translational selection in *Saccharomyces cerevisiae*. *J Mol Biol* *268*, 322-330.
- Pestova, T.V., Lomakin, I.B., Lee, J.H., Choi, S.K., Dever, T.E., and Hellen, C.U. (2000). The joining of ribosomal subunits in eukaryotes requires eIF5B. *Nature* *403*, 332-335.
- Peters, H.I., Chang, Y.W., and Traugh, J.A. (1995). Phosphorylation of elongation factor 1 (EF-1) by protein kinase C stimulates GDP/GTP-exchange activity. *European journal of biochemistry / FEBS* *234*, 550-556.
- Petrakis, T.G., Sogaard, T.M., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. (2005). Physical and functional interaction between Elongator and the chromatin-associated Kti12 protein. *J Biol Chem* *280*, 19454-19460.
- Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *J Comput Chem* *25*, 1605-1612.
- Phizicky, E.M., and Alfonzo, J.D. (2010). Do all modifications benefit all tRNAs? *FEBS Lett* *584*, 265-271.

Phizicky, E.M., and Hopper, A.K. (2010). tRNA biology charges to the front. *Genes Dev* 24, 1832-1860.

Pintard, L., Lecointe, F., Bujnicki, J.M., Bonnerot, C., Grosjean, H., and Lapeyre, B. (2002). Trm7p catalyses the formation of two 2'-O-methylriboses in yeast tRNA anticodon loop. *The EMBO journal* 21, 1811-1820.

Pisarev, A.V., Hellen, C.U., and Pestova, T.V. (2007). Recycling of eukaryotic posttermination ribosomal complexes. *Cell* 131, 286-299.

Pisarev, A.V., Skabkin, M.A., Pisareva, V.P., Skabkina, O.V., Rakotondrafara, A.M., Hentze, M.W., Hellen, C.U., and Pestova, T.V. (2010). The role of ABCE1 in eukaryotic posttermination ribosomal recycling. *Molecular cell* 37, 196-210.

Pokholok, D.K., Hannett, N.M., and Young, R.A. (2002). Exchange of RNA polymerase II initiation and elongation factors during gene expression in vivo. *Molecular cell* 9, 799-809.

Price, N.T., Redpath, N.T., Severinov, K.V., Campbell, D.G., Russell, J.M., and Proud, C.G. (1991). Identification of the phosphorylation sites in elongation factor-2 from rabbit reticulocytes. *FEBS Lett* 282, 253-258.

Rahl, P.B., Chen, C.Z., and Collins, R.N. (2005). Elp1p, the yeast homolog of the FD disease syndrome protein, negatively regulates exocytosis independently of transcriptional elongation. *Molecular cell* 17, 841-853.

Randerath, E., Gupta, R.C., Chia, L.L., Chang, S.H., and Randerath, K. (1979). Yeast tRNA Leu UAG. Purification, properties and determination of the nucleotide sequence by radioactive derivative methods. *European journal of biochemistry / FEBS* 93, 79-94.

Raught, B., and Gingras, A.-C. (2007). 14 Signaling to Translation Initiation.

Redpath, N.T., and Proud, C.G. (1993). Cyclic AMP-dependent protein kinase phosphorylates rabbit reticulocyte elongation factor-2 kinase and induces calcium-independent activity. *Biochem J* 293 (Pt 1), 31-34.

Reinthal, E.M., Lal, D., Jurkowski, W., Feucht, M., Steinbock, H., Gruber-Sedlmayr, U., Ronen, G.M., Geldner, J., Haberlandt, E., Neophytou, B., *et al.* (2014). Analysis of ELP4, SRPX2, and interacting genes in typical and atypical rolandic epilepsy. *Epilepsia* 55, e89-93.

Rezgui, V.A., Tyagi, K., Ranjan, N., Konevega, A.L., Mittelstaet, J., Rodnina, M.V., Peter, M., and Pedrioli, P.G. (2013). tRNA tKUUU, tQUUG, and tEUUC wobble position modifications fine-tune protein translation by promoting ribosome A-site binding. *Proc Natl Acad Sci U S A* 110, 12289-12294.

Rodnina, M.V., and Wintermeyer, W. (2009). Recent mechanistic insights into eukaryotic ribosomes. *Curr Opin Cell Biol* 21, 435-443.

Rolfes, R.J., and Hinnebusch, A.G. (1993). Translation of the yeast transcriptional activator GCN4 is stimulated by purine limitation: implications for activation of the protein kinase GCN2. *Molecular and cellular biology* *13*, 5099-5111.

Ron, D., and Harding, H.P. (2007). 13 eIF2 α Phosphorylation in Cellular Stress Responses and Disease.

Rowlands, A.G., Panniers, R., and Henshaw, E.C. (1988). The catalytic mechanism of guanine nucleotide exchange factor action and competitive inhibition by phosphorylated eukaryotic initiation factor 2. *J Biol Chem* *263*, 5526-5533.

Ryazanov, A.G., Natapov, P.G., Shestakova, E.A., Severin, F.F., and Spirin, A.S. (1988a). Phosphorylation of the elongation factor 2: the fifth Ca²⁺/calmodulin-dependent system of protein phosphorylation. *Biochimie* *70*, 619-626.

Ryazanov, A.G., Shestakova, E.A., and Natapov, P.G. (1988b). Phosphorylation of elongation factor 2 by EF-2 kinase affects rate of translation. *Nature* *334*, 170-173.

Salminen, A., and Novick, P.J. (1987). A ras-like protein is required for a post-Golgi event in yeast secretion. *Cell* *49*, 527-538.

Schaffrath, R., and Meinhardt, F. (2005). Kluyveromyces lactis zymocin and other plasmid-encoded yeast killer toxins. In *Microbial Protein Toxins*, M.J. Schmitt, and R. Schaffrath, eds. (Berlin, Heidelberg: Springer Berlin Heidelberg), pp. 133-155.

Scheidt, V., Jüdes, A., Bär, C., Klassen, R., and Raffael Schaffrath (2014). Loss of wobble uridine modification in tRNA anticodons interferes with TOR pathway signaling. *Microbial Cell* *1*, 416 - 424.

Schlieker, C.D., Van der Veen, A.G., Damon, J.R., Spooner, E., and Ploegh, H.L. (2008). A functional proteomics approach links the ubiquitin-related modifier Urm1 to a tRNA modification pathway. *Proc Natl Acad Sci U S A* *105*, 18255-18260.

Selvadurai, K., Wang, P., Seimetz, J., and Huang, R.H. (2014). Archaeal Elp3 catalyzes tRNA wobble uridine modification at C5 via a radical mechanism. *Nature chemical biology* *10*, 810-812.

Sen, N.D., Zhou, F., Ingolia, N.T., and Hinnebusch, A.G. (2015). Genome-wide analysis of translational efficiency reveals distinct but overlapping functions of yeast DEAD-box RNA helicases Ded1 and eIF4A. *Genome Res* *25*, 1196-1205.

Shi, H., and Moore, P.B. (2000). The crystal structure of yeast phenylalanine tRNA at 1.93 Å resolution: a classic structure revisited. *RNA* *6*, 1091-1105.

- Shida, T., Cueva, J.G., Xu, Z., Goodman, M.B., and Nachury, M.V. (2010). The major alpha-tubulin K40 acetyltransferase alphaTAT1 promotes rapid ciliogenesis and efficient mechanosensation. *Proc Natl Acad Sci U S A* *107*, 21517-21522.
- Shin, B.S., Kim, J.R., Walker, S.E., Dong, J., Lorsch, J.R., and Dever, T.E. (2011). Initiation factor eIF2gamma promotes eIF2-GTP-Met-tRNAⁱ(Met) ternary complex binding to the 40S ribosome. *Nat Struct Mol Biol* *18*, 1227-1234.
- Simpson, C.L., Lemmens, R., Miskiewicz, K., Broom, W.J., Hansen, V.K., van Vught, P.W., Landers, J.E., Sapp, P., Van Den Bosch, L., Knight, J., *et al.* (2009). Variants of the elongator protein 3 (ELP3) gene are associated with motor neuron degeneration. *Human molecular genetics* *18*, 472-481.
- Singh, N., Lorbeck, M.T., Zervos, A., Zimmerman, J., and Elefant, F. (2010). The histone acetyltransferase Elp3 plays in active role in the control of synaptic bouton expansion and sleep in *Drosophila*. *Journal of neurochemistry* *115*, 493-504.
- Sivan, G., Aviner, R., and Elroy-Stein, O. (2011). Mitotic modulation of translation elongation factor 1 leads to hindered tRNA delivery to ribosomes. *J Biol Chem* *286*, 27927-27935.
- Skabkin, M.A., Skabkina, O.V., Dhote, V., Komar, A.A., Hellen, C.U., and Pestova, T.V. (2010). Activities of Ligatin and MCT-1/DENR in eukaryotic translation initiation and ribosomal recycling. *Genes Dev* *24*, 1787-1801.
- Slaugenhaupt, S.A., Blumenfeld, A., Gill, S.P., Leyne, M., Mull, J., Cuajungco, M.P., Liebert, C.B., Chadwick, B., Idelson, M., Reznik, L., *et al.* (2001). Tissue-specific expression of a splicing mutation in the IKBKAP gene causes familial dysautonomia. *Am J Hum Genet* *68*, 598-605.
- Slusher, L.B., Gillman, E.C., Martin, N.C., and Hopper, A.K. (1991). mRNA leader length and initiation codon context determine alternative AUG selection for the yeast gene MOD5. *Proc Natl Acad Sci U S A* *88*, 9789-9793.
- Smith, C.J., Teh, H.S., Ley, A.N., and D'Obrenan, P. (1973). The nucleotide sequences and coding properties of the major and minor lysine transfer ribonucleic acids from the haploid yeast *Saccharomyces cerevisiae* S288C. *J Biol Chem* *248*, 4475-4485.
- Sonenberg, N., and Hinnebusch, A.G. (2009). Regulation of translation initiation in eukaryotes: mechanisms and biological targets. *Cell* *136*, 731-745.
- Stark, M.J., and Boyd, A. (1986). The killer toxin of *Kluyveromyces lactis*: characterization of the toxin subunits and identification of the genes which encode them. *The EMBO journal* *5*, 1995-2002.
- Strug, L.J., Clarke, T., Chiang, T., Chien, M., Baskurt, Z., Li, W., Dorfman, R., Bali, B., Wirrell, E., Kugler, S.L., *et al.* (2009). Centrotemporal sharp wave

EEG trait in rolandic epilepsy maps to Elongator Protein Complex 4 (ELP4). *European journal of human genetics : EJHG* 17, 1171-1181.

Szweykowska-Kulinska, Z., Senger, B., Keith, G., Fasiolo, F., and Grosjean, H. (1994). Intron-dependent formation of pseudouridines in the anticodon of *Saccharomyces cerevisiae* minor tRNA(Ile). *The EMBO journal* 13, 4636-4644.

Takai, K., and Yokoyama, S. (2003). Roles of 5-substituents of tRNA wobble uridines in the recognition of purine-ending codons. *Nucleic Acids Res* 31, 6383-6391.

Taylor, D.J., Frank, J., and Kinzy, T.G. (2007). 3 Structure and Function of the Eukaryotic Ribosome and Elongation Factors.

Tigano, M., Ruotolo, R., Dallabona, C., Fontanesi, F., Barrientos, A., Donnini, C., and Ottonello, S. (2015). Elongator-dependent modification of cytoplasmic tRNA^{Lys}UUU is required for mitochondrial function under stress conditions. *Nucleic Acids Res* 43, 8368-8380.

Triana-Alonso, F.J., Chakraborty, K., and Nierhaus, K.H. (1995). The elongation factor 3 unique in higher fungi and essential for protein biosynthesis is an E site factor. *J Biol Chem* 270, 20473-20478.

Tsaponina, O., Barsoum, E., Astrom, S.U., and Chabes, A. (2011). Ixr1 is required for the expression of the ribonucleotide reductase Rnr1 and maintenance of dNTP pools. *PLoS Genet* 7, e1002061.

Tsutsumi, S., Sugiura, R., Ma, Y., Tokuoka, H., Ohta, K., Ohte, R., Noma, A., Suzuki, T., and Kuno, T. (2007). Wobble inosine tRNA modification is essential to cell cycle progression in G(1)/S and G(2)/M transitions in fission yeast. *J Biol Chem* 282, 33459-33465.

Tukenmez, H., Xu, H., Esberg, A., and Bystrom, A.S. (2015). The role of wobble uridine modifications in +1 translational frameshifting in eukaryotes. *Nucleic Acids Res* 43, 9489-9499.

Urbonavicius, J., Qian, Q., Durand, J.M., Hagervall, T.G., and Bjork, G.R. (2001). Improvement of reading frame maintenance is a common function for several tRNA modifications. *The EMBO journal* 20, 4863-4873.

Urbonavicius, J., Stahl, G., Durand, J.M., Ben Salem, S.N., Qian, Q., Farabaugh, P.J., and Bjork, G.R. (2003). Transfer RNA modifications that alter +1 frameshifting in general fail to affect -1 frameshifting. *RNA* 9, 760-768.

Uthman, S., Bar, C., Scheidt, V., Liu, S., ten Have, S., Giorgini, F., Stark, M.J., and Schaffrath, R. (2013). The amidation step of diphthamide biosynthesis in yeast requires DPH6, a gene identified through mining the DPH1-DPH5 interaction network. *PLoS Genet* 9, e1003334.

- Venema, R.C., Peters, H.I., and Traugh, J.A. (1991). Phosphorylation of valyl-tRNA synthetase and elongation factor 1 in response to phorbol esters is associated with stimulation of both activities. *J Biol Chem* 266, 11993-11998.
- Walch-Solimena, C., Collins, R.N., and Novick, P.J. (1997). Sec2p mediates nucleotide exchange on Sec4p and is involved in polarized delivery of post-Golgi vesicles. *The Journal of cell biology* 137, 1495-1509.
- Walker, J., Kwon, S.Y., Badenhorst, P., East, P., McNeill, H., and Svejstrup, J.Q. (2011). Role of elongator subunit Elp3 in *Drosophila melanogaster* larval development and immunity. *Genetics* 187, 1067-1075.
- Weissenbach, J., and Dirheimer, G. (1978). Pairing properties of the methylester of 5-carboxymethyl uridine in the wobble position of yeast tRNA³Arg. *Biochimica et biophysica acta* 518, 530-534.
- Wek, S.A., Zhu, S., and Wek, R.C. (1995). The histidyl-tRNA synthetase-related sequence in the eIF-2 alpha protein kinase GCN2 interacts with tRNA and is required for activation in response to starvation for different amino acids. *Molecular and cellular biology* 15, 4497-4506.
- Werner, M., Feller, A., Messenguy, F., and Pierard, A. (1987). The leader peptide of yeast gene CPA1 is essential for the translational repression of its expression. *Cell* 49, 805-813.
- Winkler, G.S., Kristjuhan, A., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. (2002). Elongator is a histone H3 and H4 acetyltransferase important for normal histone acetylation levels in vivo. *Proc Natl Acad Sci U S A* 99, 3517-3522.
- Winkler, G.S., Petrakis, T.G., Ethelberg, S., Tokunaga, M., Erdjument-Bromage, H., Tempst, P., and Svejstrup, J.Q. (2001). RNA polymerase II elongator holoenzyme is composed of two discrete subcomplexes. *J Biol Chem* 276, 32743-32749.
- Wittschieben, B.O., Fellows, J., Du, W., Stillman, D.J., and Svejstrup, J.Q. (2000). Overlapping roles for the histone acetyltransferase activities of SAGA and elongator in vivo. *The EMBO journal* 19, 3060-3068.
- Wittschieben, B.O., Otero, G., de Bizemont, T., Fellows, J., Erdjument-Bromage, H., Ohba, R., Li, Y., Allis, C.D., Tempst, P., and Svejstrup, J.Q. (1999). A novel histone acetyltransferase is an integral subunit of elongating RNA polymerase II holoenzyme. *Molecular cell* 4, 123-128.
- Wolfe, C.L., Lou, Y.C., Hopper, A.K., and Martin, N.C. (1994). Interplay of heterogeneous transcriptional start sites and translational selection of AUGs dictate the production of mitochondrial and cytosolic/nuclear tRNA nucleotidyltransferase from the same gene in yeast. *J Biol Chem* 269, 13361-13366.

- Xu, H., Bygdell, J., Wingsle, G., and Bystrom, A.S. (2015a). Yeast Elongator protein Ebp1 does not undergo proteolytic processing in exponentially growing cells. *Microbiologyopen* 4, 867-878.
- Xu, H., Lin, Z., Li, F., Diao, W., Dong, C., Zhou, H., Xie, X., Wang, Z., Shen, Y., and Long, J. (2015b). Dimerization of elongator protein 1 is essential for Elongator complex assembly. *Proc Natl Acad Sci U S A* 112, 10697-10702.
- Yamamoto, N., Yamaizumi, Z., Yokoyama, S., Miyazawa, T., and Nishimura, S. (1985). Modified nucleoside, 5-carbamoylmethyluridine, located in the first position of the anticodon of yeast valine tRNA. *Journal of biochemistry* 97, 361-364.
- Yarian, C., Townsend, H., Czestkowski, W., Sochacka, E., Malkiewicz, A.J., Guenther, R., Miskiewicz, A., and Agris, P.F. (2002). Accurate translation of the genetic code depends on tRNA modified nucleosides. *J Biol Chem* 277, 16391-16395.
- Yokoyama, S., and Nishimura, S. (1995). Modified Nucleosides and Codon Recognition[†]. In *tRNA* (American Society of Microbiology).
- Yokoyama, S., Watanabe, T., Murao, K., Ishikura, H., Yamaizumi, Z., Nishimura, S., and Miyazawa, T. (1985). Molecular mechanism of codon recognition by tRNA species with modified uridine in the first position of the anticodon. *Proc Natl Acad Sci U S A* 82, 4905-4909.
- Zabel, R., Bar, C., Mehlgarten, C., and Schaffrath, R. (2008). Yeast alpha-tubulin suppressor Ats1/Kti13 relates to the Elongator complex and interacts with Elongator partner protein Kti11. *Mol Microbiol* 69, 175-187.
- Zhou, X., Hua, D., Chen, Z., Zhou, Z., and Gong, Z. (2009). Elongator mediates ABA responses, oxidative stress resistance and anthocyanin biosynthesis in Arabidopsis. *The Plant journal : for cell and molecular biology* 60, 79-90.
- Zhu, M., Li, Y., Chen, G., Ren, L., Xie, Q., Zhao, Z., and Hu, Z. (2015). Silencing SLEP2L, a tomato Elongator complex protein 2-like gene, inhibits leaf growth, accelerates leaf, sepal senescence, and produces dark-green fruit. *Scientific reports* 5, 7693.
- Zinshteyn, B., and Gilbert, W.V. (2013). Loss of a conserved tRNA anticodon modification perturbs cellular signaling. *PLoS Genet* 9, e1003675.

