# Characterization of Human Lysine-specific Protein Methyltransferases in the METTL21 Subgroup

Thesis for the degree of *Philosophiae Doctor*

By

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# List of papers

**Paper I**

Markus Fusser, Stefan Kernstock, Vinay Kumar Aileni, Wolfgang Egge-Jacobsen, Pål. Ø. Falnes, Arne Klungland. **Lysine Methylation of the Valosin-Containing Protein (VCP) Is Dispensable for Development and Survival of Mice.** *PLoS ONE* (2015) 10(11): e0141472. doi:10.1371/journal. pone.0141472.

**Paper II**

Jędrzej Małecki[1,#], Vinay Kumar Aileni[1,#],Anders Moen[1], Angela Y.Y. Ho[1], Vigdis Sørensen[2], Magnus Jakobsson[1] and Pål Ø. Falnes[1,*]. **The novel lysine specific methyltransferase METTL21B affects mRNA translation through inducible and dynamic methylation of Lys-165 in human eukaryotic elongation factor 1 alpha (eEF1A).** Submitted to *Nucleic Acids Res*. (Under revision).

# Joint first authors

**Paper III**

Vinay Kumar Aileni, Erna Davydova, Anders Moen and Pål Ø. Falnes. **VCP-KMT-mediated lysine methylation of histone H3 peptide sequences grafted onto a VCP-derived scaffold.** (Manuscript)

# Abbreviations

| | |
|---|---|
| 7BS | seven-β-strand |
| Arg (R) | arginine |
| AAA | ATPases associated with diverse cellular activities |
| aa-tRNA | aminoacyl-transfer RNA |
| ADP | adenosine diphosphate |
| ATP | adenosine triphosphate |
| DNA | deoxyribonucleicacid |
| eEF1α | eukaryotic elongation factor 1 alpha |
| eEF1β | eukaryotic elongation factor 1 beta |
| eEF2 | eukaryotic elongation factor 2 |
| Efm | elongation factor methyltransferase |
| ER | endoplasmic reticulum |
| ERAD | ER-associated protein degradation |
| GTP | guanosine triphosphate |
| GDP | guanosine diphosphate |
| H4K4 | histone H3 lysine 4 |
| H4K9 | histone H3 lysine 9 |
| H4K27 | histone H3 lysine 27 |
| H4K36 | histone H3 lysine 36 |
| His (H) | histidine |
| HKMT | histone lysine methyltransferase |
| KMT | lysine methyltransferase |
| KO | knock out |
| LC | liquid chromatography |
| Lys (K) | lysine |

| | |
|---|---|
| MAT | methionine adenosyltransferase |
| mRNA | messenger RNA |
| MS | mass spectrometry |
| MTase | methyltransferase |
| MTF16 | methyltransferase family 16 |
| MW | molecular weight |
| PDB | protein data bank |
| PRMT | protein arginine methyltransferase |
| PTM | post-translational modification |
| RPL | ribosomal protein of the large subunit |
| RNA | ribonucleic acid |
| SAH (AdoHcy) | *S*-adenosyl homocysteine |
| SAM (AdoMet) | *S*-adenosyl methionine |
| SET | Suppressor of variegation, Enhancer of zeste, Trithorax |
| SMYD | SET and MYND-domain containing |
| TAP | tandem affinity purification |
| tRNA | transfer RNA |
| VCP (p97) | valosin-containing protein |
| VCP-KMT | VCP lysine methyltransferase |

# Introduction

## Methyltransferases

Methyltransferases (MTases) are enzymes that catalyze the transfer of a methyl group from a donor molecule to a substrate via nucleophilic substitution (SN) reactions. They play distinct roles in various biological pathways, including epigenetic control, metabolic regulation, protein repair, lipid and small-molecule biosynthesis, by targeting a vast variety of substrates, such as nucleic acids, proteins, lipids, and small molecules (Schubert et al., 2003, Rust et al., 2011). MTases are widespread across different organisms, and recent bioinformatic studies have reported that they account for 1.0% and 1.2% of gene products in humans and yeast, respectively. They show a similar abundance in other organisms, ranging between 0.6 and 1.6%, in *Escherichia coli, Caenorhabditis elegans, Drosophila melanogaster, Arabidopsis thaliana, and Mus musculus* (Katz et al., 2003). The majority of MTases use *S*-denosyl methionine (SAM) as the donor molecule (Cantoni, 1975).

## SAM

SAM is the second-most abundant cellular cofactor after adenosine triphosphate (ATP), and is a sulfonium compound with an adenosyl moiety and a chemically active methyl group (Figure 1) (Fontecave et al., 2004). It is synthesized from an essential amino acid, methionine, and ATP by the enzyme methionine adenosyltransferase (Figure 1) (Mato et al., 2002). SAM is present in all living organisms, and plays a crucial role in cellular biochemistry, primarily via its involvement in the methyl group transfers catalyzed by MTases, but it also serves as a precursor molecule for aminopropylation in the pathway for synthesis of polyamines, and for trans-sulfuration during the synthesis of glutathione (Bottiglieri, 2002). Several studies have shown that aberrant SAM levels are associated with several disorders. For example, abnormal SAM synthesis is associated with chronic liver disease, (in agreement with SAM being primarily synthesized and utilized in the liver). In addition, reduced SAM levels are found in the cerebrospinal fluid of patients with neurological disorders, such as Alzheimer's disease, and SAM can also act as an antidepressant, which might be due to its close metabolic relationship with folate, the deficiency of which can result in depression [reviewed in (Bottiglieri, 2002)].

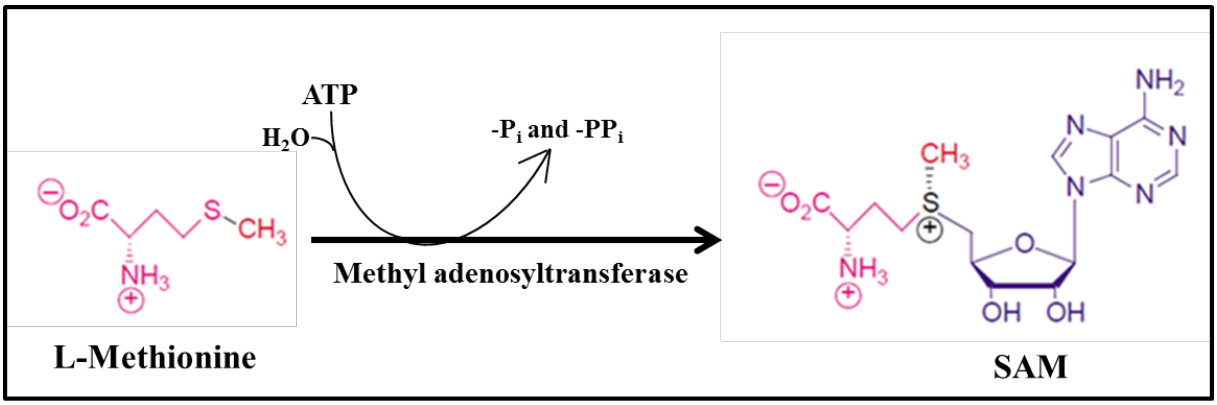


**Figure 1.** Methyl adenosyltransferase catalysed formation of SAM.

# SAM-dependent MTases

SAM-dependent MTases catalyze methylation by binding to the substrate and exposing its nucleophilic entity towards the electrophilic sulfonium group of SAM, and then mediating the transfer of the methyl group to the substrate in an $S_N2$ reaction (Velichkova & Himo, 2005). MTases methylate a wide variety of substrates from simple molecules to complex molecules, and a recent bioinformatics study identified 208 SAM-dependent MTases encoded by the human genome. It was shown that approximately 30% of these MTases were associated with disorders such as cancers and mental disabilities. SAM-dependent MTases belong to several structurally distinct classes, and the distribution of the yeast and human MTases between these classes is shown in Figure 2 (Petrossian & Clarke, 2011).

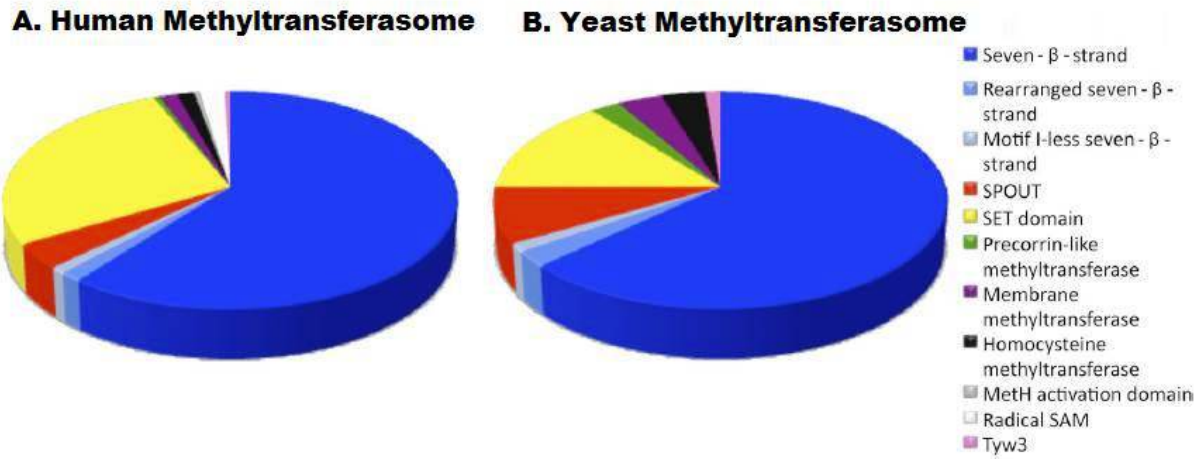


**Figure 2.** Distribution of human (A) and yeast (B) MTases between different MTase classes. Figure from (Petrossian & Clarke, 2011).

## Seven-β-strand MTases

Seven-β-strand (7BS) MTases represent the most common type of MTase, constituting approximately 60-70 % of the human and yeast methyltransferasome, and around 0.6% of the total human proteome (Figure 2). The core 7BS fold includes alternating β-strands (β1–β7) and α-helices (αZ and αA–αE), which together form a Rossmann-fold-like seven-stranded β-sheet, with three helices on each side. Helices Z, A, and B are located on one side of the sheet, with C, D and E located on the other, and all of the β-strands are parallel, with the exception of the seventh strand. β-strands 1, 2, 4, and 5, and α-helix D of the canonical 7BS core fold harbor conserved motifs, denoted as Motif-I, Motif Post-I, Motif-II, and Motif-III (Figure 3). The N-terminally located Motif-I and Motif Post-I constitute the SAM binding sites, and Motif-II and Motif-III constitute the substrate binding sites (Martin & McMillan, 2002, Petrossian & Clarke, 2009). There are some exceptions to this 7BS core fold structure, for example, αC is not always present, and some enzymes have insertions between β5 and αE, and β6 and β7 [reviewed in (Martin & McMillan, 2002)]. In some 7BS MTases, for example protein arginine MTase 3 (PRMT3), the β6 and β7strands are completely absent (Zhang et al., 2000).

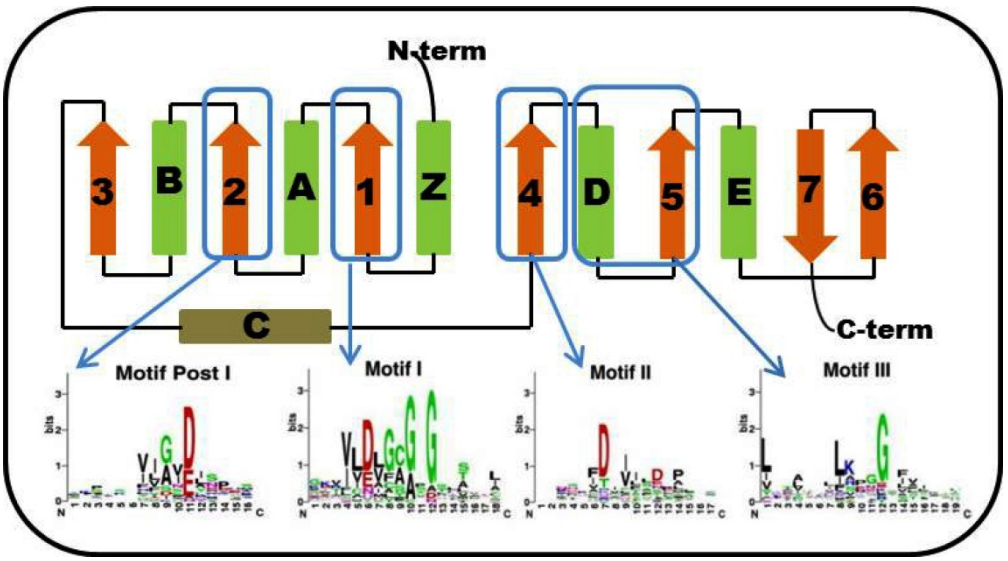


**Figure 3.** A schematic diagram that shows the topology of the seven-β-strand-methyltransferase core fold, including the seven-stranded β-sheet (orange) and helices (green). The position and concensus sequence of the conserved motifs are indicated (Petrossian & Clarke, 2009).

7BS MTases can methylate a wide variety of substrates, such as proteins, DNA, and RNA. For example, METTL21A methylates a lysine residue in members of the heat-shock protein 70 (Hsp70) family (Jakobsson et al., 2013), PRMT5 methylates an arginine residue in the p53 protein (Jansson et al., 2008), DNMT1 methylates DNA (Bestor, 2000), and ALKBH8 methylates tRNA at the wobble position (Songe-Moller et al., 2010).

## SET-domain MTases

The SET is a protein domain named from the first letter of three *Drosophila melanogaster* proteins: The modifier of position effect variegation called suppressor of 3-9 [Su(var)3-9], the polycomb-group chromatin regulator Enhancer of zeste [E(z)], and the trithorax-group chromatin regulator trithorax (trx). SET domain proteins have been found in all studied eukaryotic organisms, and several of them have been identified, including SET7/9, Dim-5, Clr4, and SET8/PreSET7. All of these have a unique fold with a series of β strands, which forms three discrete sheets [reviewed in (Qian & Zhou, 2006)]. SET domain MTases are the second largest group of MTases, constituting 27% of the human and 14% of the yeast methyltransferasome (Figure 2). To date, SET domain MTases have, unlike 7BS MTases, been shown to target only lysine residues. It has been shown that the enzymes of this class primarily methylate lysines on histones, such as histone H3 lysine 4 (H3K4), histone H3 lysine 9 (H3K9), histone H3 lysine 27 (H3K27), and histone H3 lysine 36 (H3K36) (Dillon et al., 2005), and can also methylate several non-histone proteins, such as p53, ERα, TAF7, and TAF10 (Biggar & Li, 2015, Pradhan et al., 2009). Some SET domain proteins can target multiple substrates, for example, SET7/9 can methylate histone H3 at K4, but also ~ 30 other non-histone protein substrates, and the H3K9 MTase G9a has been reported to methylate 17 other non-histone proteins (Biggar & Li, 2015).

## SPOUT and other MTases

SPOUT is another class of MTases, and this class has eight members (Petrossian & Clarke, 2011), which generally have been found to methylate RNA (Tkaczuk et al., 2007, Schapira & Ferreira de Freitas, 2014, Liu et al., 2015). However, one exception is YOR021c (sfm1), which can methylate Arg-146 in the ribosomal subunit protein Rps3, suggesting that the SPOUT MTases may have other non-RNA substrates as well (Young et al., 2012).

In addition to the three major MTases classes mentioned above, there are several other small MTase classes, such as the membrane-bound MTases, which include three human members

with unknown functions, the precorrin-like MTases, which includes only one human member, and the homocysteine MTase class, which has three human members (Petrossian & Clarke, 2011).

# Protein methylation

A relative large proportion of MTases mediate post-translational protein methylation. Lysine methylation was initially identified in the flagella protein of *Salmonella typhimurium* in 1959 [reviewed by (Paik et al., 2007)], and then in 1964 it was found that methylated lysine residues were present in acid-hydrolysates of histones isolated from calf thymus, wheat germ, and various rabbit organs (Murray, 1964). Subsequently, arginine methylated on the nitrogen of its guanidine group (Arg-107) was identified in the myelin basic protein (Brostoff & Eylar, 1971), and it was found that several other residues including histidine, glutamine, glutamate, asparagine, cysteine, and N-terminal and C-terminal residues, are methylated in proteins [reviewed by (Clarke, 2013)]. However, lysine and arginine are the residues most commonly targeted by the MTases.

## Arginine methylation

Arginine is unique among the amino acids, as its guanidine group contains five potential hydrogen bond donors, which are positioned for favourable interactions with biological hydrogen bond acceptors. The two amino groups of guanidine act as methyl group acceptors, and the addition of each methyl group to arginine removes a hydrogen bond donor, which may affect protein-protein interactions. Arginine can exist in three methylated states: ω-NG-monomethylarginine, ω-NG,NG-asymmetrical dimethylarginine, and ω-NG,NG–symmetrical dimethylarginine (Figure 4) [reviewed in (Yang & Bedford, 2013)].

To date, 10 mammalian protein arginine MTases have been identified and denoted as PRMT1–10. All 10 PRMTs belong to the 7BS MTase family, and all of them have been shown to catalyze the transfer of the methyl group from SAM to an arginine residue. There are three types of PRMTs, Type-I (PRMT1, PRMT2, PRMT3, PRMT4, PRMT6 and PRMT8) can form mono-, and asymmetrical dimethyl arginine, type-II (PRMT5 and PRMT7) can form mono-, and symmetrical dimethyl arginine, whereas type-III can form only monomethyl arginine. Notably, PRMT7 can act as both type-II and type-III depending on the nature of the substrate (Yang & Bedford, 2013). The type-I enzyme PRMT1 has very wide substrate specificity, and is responsible for approximately 85% of the total arginine methylation in

cultured RAT1 fibroblasts and in mouse liver (Tang et al., 2000). Other Type-I enzymes, such as PRMT4 and PRMT6, are distinguishable from PRMT1 in their substrate specificity and catalyze the methylation of a few distinct substrates. PRMT4 can associate with steroid receptor coactivators and transcriptional coactivators, and is thus alternatively named coactivator-associated arginine MTase 1 (CARM1). PRMT6 is a nuclear enzyme and its specific substrates include the nuclear scaffold protein HMGA1a/b and DNA polymerase β [reviewed by (Bedford & Clarke, 2009)]. Many of the PRMTs are deregulated in several cancers (Yang & Bedford, 2013), suggesting that PRMT dysregulation is involved in cancer pathology.

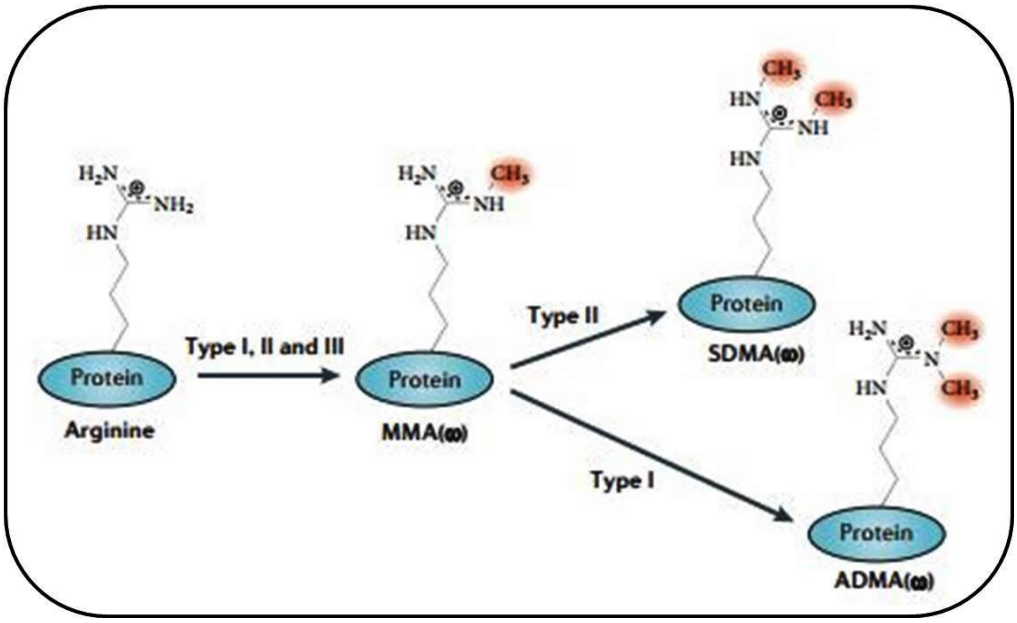


**Figure 4. Types of methylation on arginine residues:** Type-I, Type-II and Type-III PRMTs generate monomethylarginine, Type-I PRMTs generate asymmetrical dimethylarginine, and Type-II PRMTs generate symmetrical dimethylarginine. Figure from (Yang & Bedford, 2013).

## Lysine methylation

Like arginine, lysine is also a basic amino acid, and it has an aliphatic side chain with four carbons and a terminal amino group. This ε-amino group can be enzymatically methylated resulting in mono-, di-, or trimethylated lysine (Figure 5). Methylation increases the bulk and hydrophobicity of the residue without changing its positive charge. The ε-amino group of

lysine can also undergoes several other modifications, and methylation blocks the most common of these, such as acetylation and ubiquitination (Liu et al., 2014). Protein lysine methylation has received substantial attention since the discovery of the first histone specific lysine MTase (KMT); SUV39H1. Thereafter, several other MTases have been discovered, such as G9a, EZH2, SET2, SET7/9, and DOT1. These enzymes were initially identified as histone-specific KMTs, but, for several of them numerous non-histone substrates have also been identified [reviewed in (He et al., 2012)].

Lysine methylation is a dynamic process, as it can be subject to enzymatic demethylation. The first reported lysine demethylase (KDM) was the amine oxidase lysine-specific demethylase 1 (LSD1), which can demethylate only mono- and dimethylated lysines (Shi et al., 2004). Another family of lysine demethylases, containing a JmjC-domain, and capable of demethylating tri-, di-, and monomethylated lysines, was also subsequently identified (Tsukada et al., 2006).

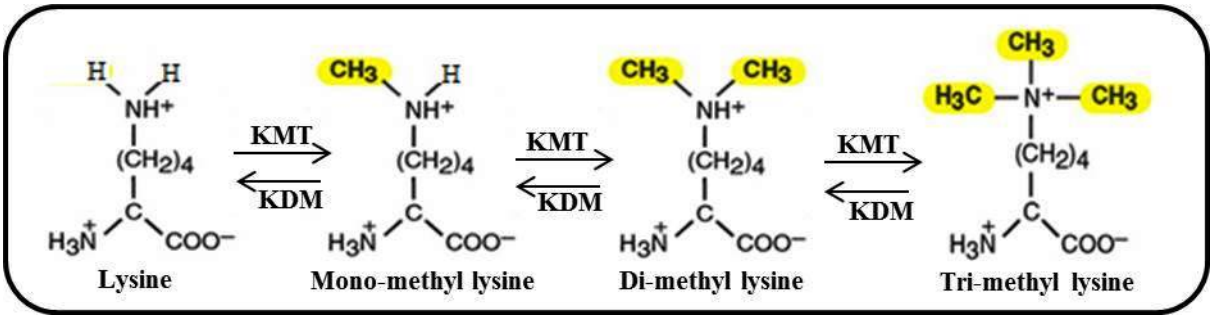


**Figure 5. Lysine methylation:** Chemical structure of un-, mono-, di, and trimethylated lysine. The methylation is catalyzed by lysine methyltransferases (KMT) and demethylation by lysine-specific demethylases (KDMs).

## Lysine methylation of histone proteins

Lysine methylation of histones has been extensively studied and has been proposed to constitute the so-called histone code, together with other post-translational modifications (PTMs) and DNA methylation. Histone lysine methylation primarily occurs on the N-terminus of histone tails. For example, Lys-4, -9, -27, -36, and -79 - on histone H3, and Lys-20 and -59 on histone H4 undergo methylation (Lee et al., 2005). These modified residues on histones can be recognised by various protein domains, referred to as "readers", such as the chromodomain, tudor domain, and WD40 repeat domains, and which again typically are fused

to chromatin modifying domains capable of modulating chromatin structure, gene transcription, and other biological processes (Martin & Zhang, 2005).

Several histone-specific KMTs have been discovered, such as SUV39H1 and G9a, which methylate H3K9 and H3K27, and are responsible for heterochromatin formation, X-chromosome silencing, and transcriptional repression. SMYD1 methylate H3K4, and SMYD2 and ASH1L methylates H3K36, and leading to transcriptional activation. NSD2 methylates the H4K20 residue, which is critical for the accumulation of p53-binding protein1 (53BP1) at DNA damage sites (Pei et al., 2011, Lanouette et al., 2014). To date, all of the characterized human histone KMTs belong to SET domain proteins, with the exception of human DOT1-like (DOT1L) (Feng et al., 2002), which is a member of the 7BS MTase family and methylates H3K79, thereby activating transcription (Nguyen & Zhang, 2011).

## Lysine methylation of non-histone proteins

Although lysine methylation has been extensively studied in histone proteins, various non-histone substrates for protein lysine MTases have also been discovered. Numerous protein lysine MTases (KMTs) are localized in the cytoplasm, and it has been reported that several cytoplasmic proteins are substrates for these enzymes (Hamamoto et al., 2015). The SET domain-containing proteins mostly methylate histones, but a few of these enzymes can methylate a multitude of non-histone proteins also. For example, SET7 methylates histone H3 at Lys4 along with several non-histone proteins, such as p53, DNMT1, and TAF10 (Zhang et al., 2015). In addition to SET domain-containing KMTs, several other MTases that can methylate non-histone proteins have been identified. These are members of the 7BS MTase class, and the first of these non-histone KMTs to be characterized in humans was calmodulin lysine N-methyltransferase, which trimethylates Lys-115 of calmodulin (Magnani et al., 2012). Several other human 7BS KMTs have subsequently been identified, such as METTL21D (VCP-KMT), which can methylate Lys-315 of valosin-containing protein (VCP) (Kernstock et al., 2012, Cloutier et al., 2013), METTL21A (HSPA-KMT), which can methylate Lys-561 in Hsp70 (HSPA1) or Hsc70 (HSPA8) or the corresponding lysine residue in other Hsp70 proteins (Jakobsson et al., 2013), and METTL20 (ETFβ-KMT), which methylates Lys-200 and Lys-203 of ETFβ (Malecki et al., 2015). The research in the current thesis also characterizes a novel MTase that targets a non-histone protein.

Lysine methylation of non-histone proteins generally regulates protein functions, stability, and protein-protein interactions. Both the position and state of methylation of lysine residues

can affect protein functions; for example, SMYD2-mediated monomethylation of p53 at Lys-370 is linked to transcriptionally repressive marks, whereas dimethylation by an unknown enzyme at the same site is linked to activation of p53 (Zhang et al., 2015). SET9-mediated monomethylation of Lys-372 increases the stability of chromatin-bound p53, and is associated with target gene activation, and G9a-mediated dimethylation of the same site is associated with target gene repression (Zhang et al., 2015, Chuikov et al., 2004). It has been reported that Ctm1p-mediated trimethylation of Lys-78 on yeast cytochrome C (Cyc1p) significantly increased its interaction with mitochondrial proteins ERV1p and Cyc3p (Winter et al., 2015). It has also been shown that various other proteins are methylated, such as molecular chaperones (VCP and Hsp70 proteins) (Kernstock et al., 2012), (Jakobsson et al., 2013), ribosomal proteins (Malecki et al., 2016) and translational factors, eEF2 (Davydova et al., 2014), and eukaryotic elongation factor 1 alpha (eEF1α) (Hamey et al., 2016). Also, it was found that mammalian eEF1α was methylated at five lysine residues (Dever et al., 1989), and the human enzymes responsible for two of these five modification events have recently been characterized (Hamey et al., 2016, Shimazu et al., 2014). eEF1α and its PTMs are described in detail below, as the characterization of a novel eEF1α-targeting KMT is the focus of one of the studies presented in this thesis.

# eEF1α (Eukaryotic elongation factor 1 alpha)

Protein translation is a three-step process, consisting of the steps initiation, elongation, and termination. Translational elongation requires several soluble proteins, known as eEFs (eukaryotic elongation factors), one of which is eEF1α, which is activated upon guanosine triphosphate (GTP) binding, and forms a ternary complex with aminoacyl tRNAs. These complexes can decode the genetic information of mRNA and deliver the aminoacyl tRNA to site A of the ribosome. Upon formation of a correct codon-anticodon pair, a conformational change in the ribosome leads to GTP hydrolysis and release of the guanosine diphosphate (GDP) bound eEF1α from the ribosome, after which eEF1α is reactivated to its GTP form by the nucleotide exchange factor eEF1β. Finally, eEF2 catalyzes the translocation of the peptidyl-tRNA mRNA complex from site A to site P of the ribosome and positions the next codon at site A to allow repetition of the process [reviewed in (Abbott & Proud, 2004, Sasikumar et al., 2012)].

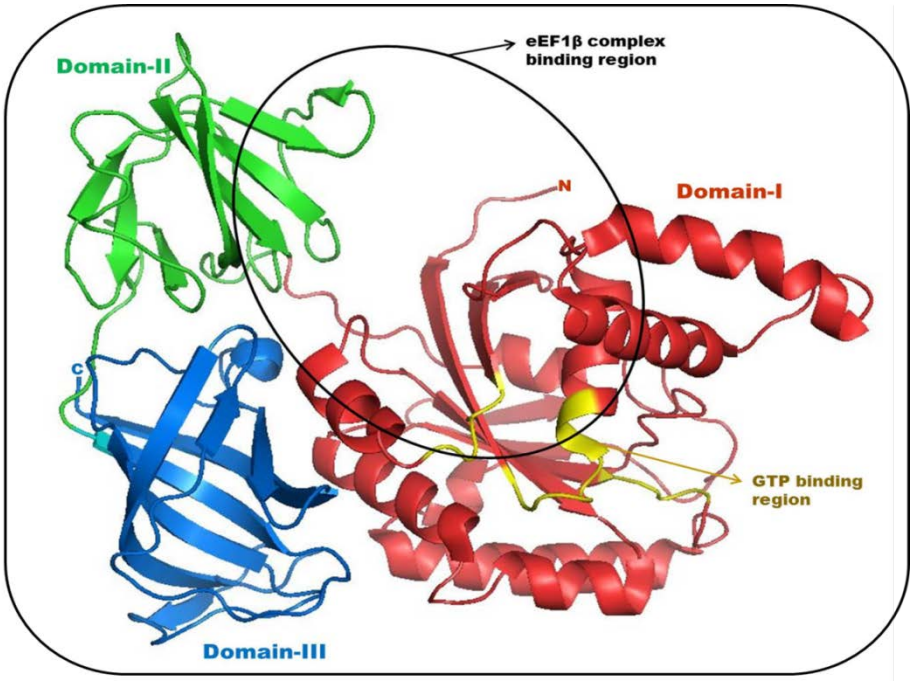


**Figure 6. Crystal structure of eEF1α1:** Domain structures of the yeast eukaryotic elongation factor 1 alpha. Domain-I (red), domain-II (green), domain-III (blue), are indicated. The guanosine triphosphate binding site of domain-I is highlighted in yellow, and the eukaryotic elongation factor 1 beta complex binding regions of domain-I and II are indicated by a black circle [PDB ID: 2B7B (Pittman et al., 2006)].

eEF1α is a highly abundant protein, and constitutes ~3% of total cellular protein. It consists of three structural domains: domain-I (amino acid residues 1–240), domain-II (amino acid residues 241–336), and domain-III (amino acid residues 337–443). Domain-I has a GTP/GDP binding site, domains-I and II have binding sites for the eEF1β complex, which exchanges GDP for GTP, domain-II and domain-III possess an aa-tRNA binding site, and domain-III is an actin bundling domain. It has also been reported that eEF1α localizes in the neuronal spines in association with post-synaptic density (PSD) through domain-III (Figure 6) (Cho et al., 2012, Soares et al., 2009, Li et al., 2013). In higher vertebrates, eEF1α exists as two paralogs, eEF1α1 and eEF1α2, encoded by two different genes with different expression patterns. eEF1α1 is ubiquitously expressed in all tissues throughout development, but is replaced by eEF1α2 in adult muscle and heart. The two eEF1α paralogs have 92% sequence identity, and show similar translational activities, but with different relative affinities towards GTP and GDP (Soares et al., 2009, Svobodova et al., 2015).

Although the canonical role of eEF1α is the delivery of the cognate aa-tRNA to the A-site of the ribosome during the elongation step of protein synthesis, it has also been reported that it is involved in several other cellular functions; a role in the nuclear export of proteins that have the transcription-dependent nuclear export motif, such as von Hippel-Lindau tumor suppressor and poly(A)-binding protein, has been reported (Khacho et al., 2008). eEF1α also plays a role in the degradation of damaged proteins by facilitating their delivery to the proteasome (Chuang et al., 2005). It can also bind to the untranslated regions of viral genomes and polymerases to regulate the viral life cycle; for example, the interaction between eEF1α and reverse transcriptase is crucial for HIV reverse transcription and replication (Li et al., 2015). The specific up-regulation of eEF1α2 has been observed in several cancers, and it activates the phospholipid and Akt signaling pathways that favour cell survival [reviewed in (Abbas et al., 2015)]. eEF1α is also involved in actin bundling, and may have a function in microtubule dynamics (Mateyak & Kinzy, 2010, Novosylna et al., 2016). Conceivably, this multi-functionality of eEF1α could be regulated by the various PTMs that it undergoes.

## PTMs

Eukaryotic EF1α undergoes several common PTMs, such as phosphorylation, acetylation, ubiquitination, and methylation. Due to its abundance and technical advancement in mass spectra, several such PTMs have been identified; over 60 PTMs have been annotated for eEF1α in an online database (Fig.7) (http://www.phosphosite.org/protein Action id =3315), but the majority has not been individually characterized with regard to biological significance. However, several studies have described enzymes catalyzing lysine methylation of eEF1α. Furthermore, other studies have attempted to determine the functional role of ethanolamine phosphoglycerol (EPG) modification of eEF1α in protozoan parasites (Greganova & Butikofer, 2012).

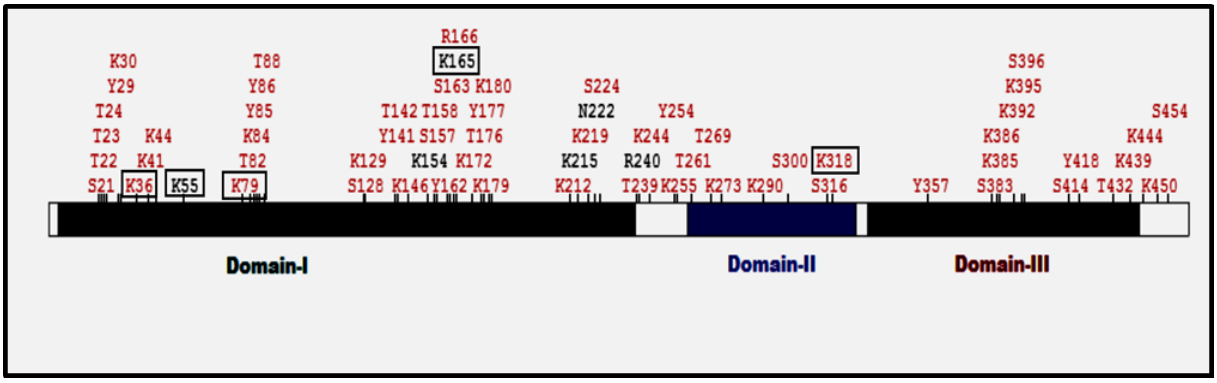


**Figure 7. Post translational modifications of human eEF1α:** Annotated post-translational modification sites on human eukaryotic elongation factor 1 alpha protein. Methylated lysine residues are marked (Adapted from http://www.phosphosite.org/protein Action id =3315).

## Lysine methylation of eEF1α

Several lysine residues on eEF1α undergo methylation, and, more than two decades ago, it was reported that five methylated lysine residues, are present in rabbit eEF1α1, i.e. at positions Lys-36, Lys-55, Lys-79, Lys-165, and Lys-318 (Dever et al., 1989). However, only two of the responsible MTases have been identified to date: METL10, which methylates Lys-318 (Shimazu et al., 2014), and N6AMT2, which methylates Lys-79 (Hamey et al., 2016). Yeast eEF1α has four methylated lysine residues, Lys-30, Lys-79, Lys-316, and Lys-390, of which two are also found in mammals: Lys-79 and Lys-316 corresponding to Lys-318 in mammals (Cavallius et al., 1993). Three of these residues, Lys-79, Lys-316, and Lys-390, are methylated by 7BS MTases - Efm4 elongation factor MTase (EFM) 4, EFM5, and EFM6,

respectively. Lys-30 is methylated by a SET domain MTase (Yhl039w) (Couttas et al., 2012, Lipson et al., 2010). The functional significance of these modifications has not been identified in eukaryotes, but methylation of Ef-Tu, the bacterial orthologue of eEF1α was identified in *E. coli* in response to nutrient deprivation (Kraal et al., 1999). Further, Lys-56 of Ef-Tu was observed to be dimethylated in stationary phase, while it was monomethylated in log phase, suggesting that Ef-Tu methylation might have a regulatory role in the bacterial growth [reviewed in (Polevoda & Sherman, 2007)]. Also, it was reported that the EftM (Ef-Tu modifying enzyme) is responsible for methylation of Ef-Tu at Lys-5 in *Pseudomonas aeruginosa* (Barbier et al., 2013). Further, the functional significance of Ef-Tu methylation was confirmed by subsequent studies showing that EftM deficient *P. aeruginosa* has a strongly reduced respiratory infectivity (Barbier et al., 2013).

## Ethanolamine phosphoglycerol modification of eEF1α

Ethanolamine phosphoglycerol (EPG) is a unique protein modification associated with eEF1α. The sites of EPG attachment have been identified as two glutamate residues, in mammals Glu-301 and Glu-374, in plants Glu-289 and Glu-362, which are located in domain-II and domain-III. Similarly, EPG modified EF1α has been described in a protozoan parasite (*Trypanosoma brucei*), but only with single EPG moiety at the Glu-362 residue, corresponding to the Glu-374 of mammals, a modification that is absent in *S. cerevisiae* and bacteria. The pathway for EPG synthesis and its attachment to eEF1α is elusive, but a recent study showed that phosphatidylethanolamine acts as the ethanolamine donor. On the basis of previous studies (Whiteheart & Hart, 1994, Signorell et al., 2008), a stepwise reaction for EPG attachment on eEF1α was proposed, as shown in Figure 8 [reviewed in (Mittal et al., 2013)]. Although the EPG attachment of eEF1α was first identified over 20 years ago, the biological significance of this modification has not been revealed in mammals and plants. Recently, trypanosomes have been used as a model eukaryote to study the biological significance of EPG attachment on eEF1α by substituting Glu-362 with Asp, and it was observed that the lack of EPG modification on eEF1α did not affect the growth of parasites in culture (Greganova & Butikofer, 2012). However, this modification is preserved during eukaryotic evolution, and is conserved in mammals, plants, and protozoans, which suggests that it plays an essential role in these organisms under certain conditions

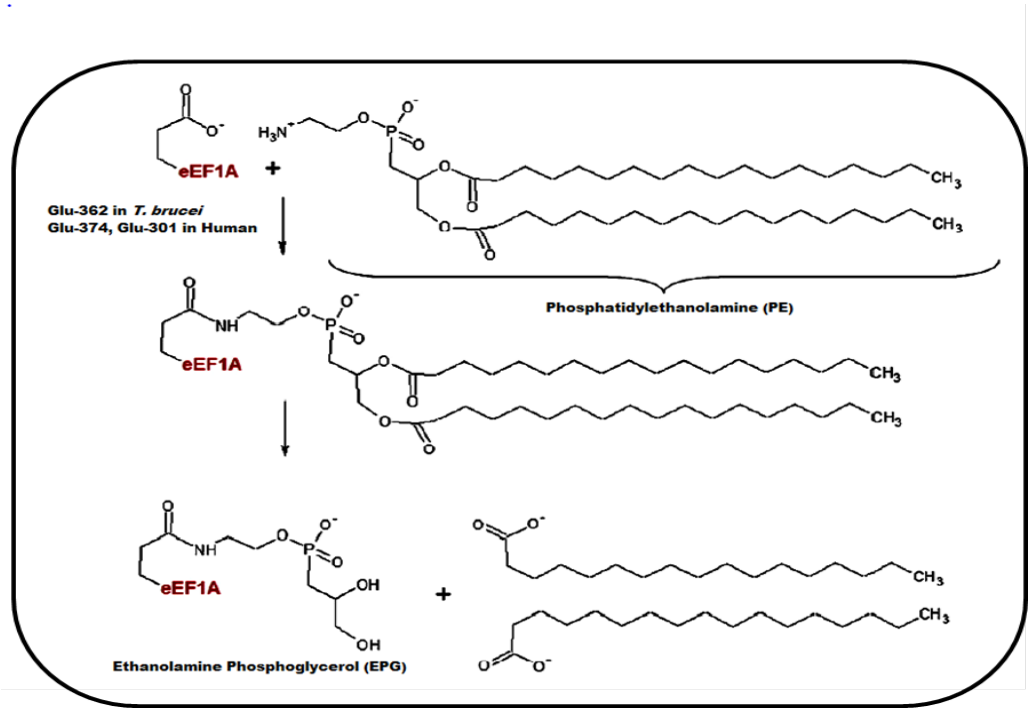


**Figure 8.** Proposed pathway for the ethanolamine phosphoglycerol (EPG) attachment on eEF1α by phosphatidyl ethanolamine (PE) and subsequent deacylation to EPG. Figure from (Mittal et al., 2013).

# VCP (Valosin-containing protein)

VCP/p97/cdc48 belongs to the AAA (ATPases associated with various cellular activities) family of proteins. VCP is abundant, accounting for approximately 1% of total cellular protein, and is highly conserved within eukaryotes and archaebacteria. It is present in all cell types and is localized to the cytoplasm, nucleus, and sub-compartments of the perimembrane (Peters et al., 1990). VCP generally appears as a homo-hexamer, and each monomer consists of an N-terminal domain (residues 1–187 in mammalian VCP), two ATPase domains, referred to as D1 (residues 208–458) and D2 (residues 481–761), a C-terminal tail (residues 762–806), and two linker regions (residues 188–207 and 459–480) connecting the N-terminal and D1 domains, as well as the D1 and D2 domains, respectively (Fig 9A). X-ray crystallography studies have shown that VCP forms two concentric rings; the N-D1 ring has a larger radius than the D2 ring (Fig.9B) [(Zhang et al., 2000), (Xia et al., 2016)]. The two ATPase domains of VCP contribute to its ATPase activity, but D2 accounts for the majority of the ATPase activity and D1 is responsible for heat-induced activity during elevated temperatures or heat-shock conditions (Song et al., 2003).

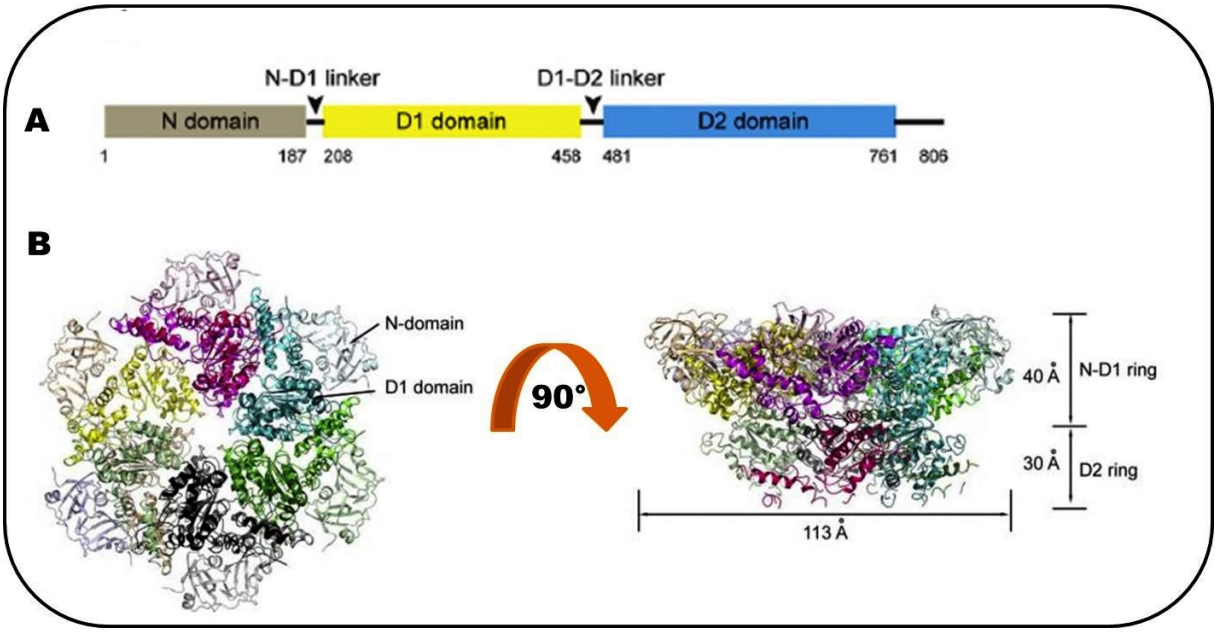


**Figure 9. VCP structure: A.** Schematic representation of the domain organisation of VCP. **B.** Top view (left; down the 6-fold symmetry axis) and side view (right) of the VCP hexameric structure. The six monomers of VCP are shown in different colors. The width and height of the VCP hexamer is indicated. Adopted from (Xia et al., 2016).

It has been shown that VCP is involved in various cellular functions, such as transcriptional regulation, post-mitotic membrane fusion, DNA repair, autophagy, suppression of protein aggregation, and proteolysis (Yamanaka et al., 2012, Meyer & Weihl, 2014). VCP can interact with various cofactors, of which 40 cofactors have been identified in mammals, most of them bind to the N-terminal part of the protein with a small proportion binding to the C-terminal tail (Buchberger et al., 2015). VCP is a crucial player in multiple ubiquitin signaling pathways, protein degradation and protein quality control. For example, in endoplasmic reticulum (ER)-associated protein degradation, misfolded proteins in the ER are immediately reassigned to ubiquitination by the Hrd1 or gp78 E3 ubiquitin ligases, and then VCP recognizes such proteins and segregates them from ER to cytosol by forming a complex with cofactors such as Ufd1 and Npl4. Further, it can unfold and direct the mis-folded proteins to the 26S proteasome complex (Ye et al., 2003, Barthelme & Sauer, 2016, Shmueli et al., 2009). It has also been reported that VCP plays a role in the extraction of ubiquitinated proteins from chromatin (Dantuma et al., 2014).

# PTMs

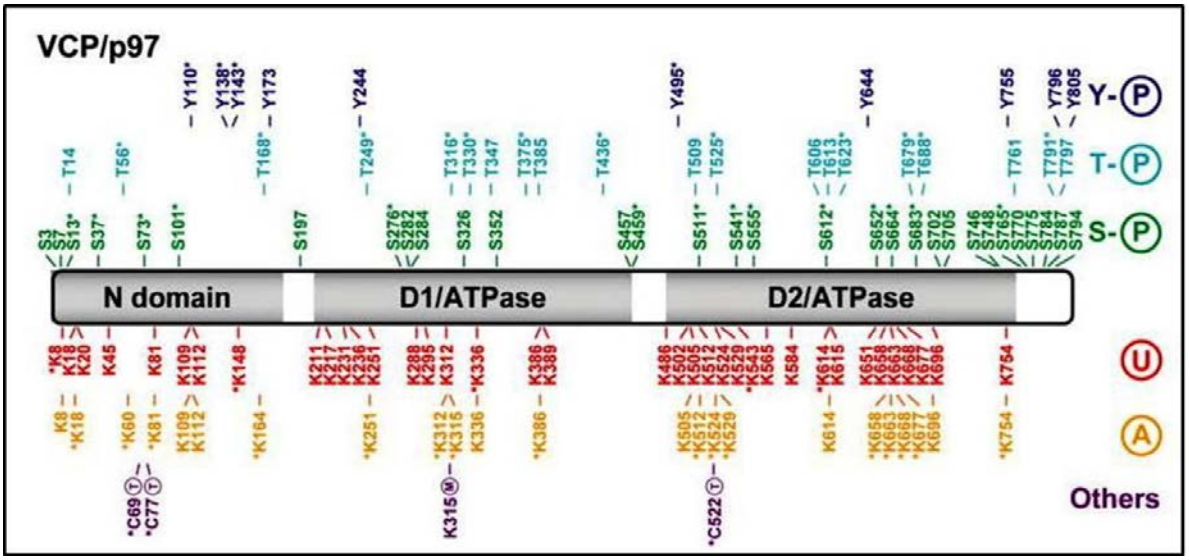


**Figure 10. Post-translational modifications of VCP:** Annotated phosphorylated (P), ubiquitinated (U), acetylated (A) and methylated (M) residues of valosin-containing protein [Figure from (Cloutier & Coulombe, 2013)].

VCP undergoes several post-translational modifications (Fig. 10) (Cloutier & Coulombe, 2013, Mori-Konya et al., 2009), of which, phosphorylation and acetylation are the most-studied. VCP phosphorylation has several functional roles, and has been shown to take place during cold acclimation (Imamura et al., 2003) and sperm capacitation (Ficarro et al., 2003). It was reported that VCP could be phosphorylated at Ser-352, Ser-746 and Ser-748 in response to hypoxia (Klein et al., 2005). Also, phosphorylation of the Tyr-805 in the C-terminus of VCP can block its interaction with proteins that contain a PUB-domain, such as, peptide-N-glycosidase (PNGase) and Ufd3. VCP normally interacts with such proteins by inserting a small motif, which includes Tyr-805 in to the hydrophobic pocket of the PUB-domain, hence, the phosphorylation disrupts the interaction due to its negative charge. Ufd2 and Ufd3 are two VCP cofactors that compete for binding to VCP, and antagonistically regulate the ubiquitiation of substrates. Tyr-805 phosphorylation inhibits the interaction of Ufd3 with VCP, but favours Ufd2 recruitment and thus promotes polyubiquitination instead of deubiquitination (Cloutier & Coulombe, 2013, Zhao et al., 2007). The acetylation of VCP also has some functional roles. Mutational studies of Lys-696 on VCP, to mimic acetylated and deacetylaed states, was shown to increase and decrease ATPase activity, respectively, which indicates that acetylation of Lys-696 could regulate VCP (Mori-Konya et al., 2009). It was recently shown that Lys-315 of VCP is methylated by VCP-KMT (METTL21D), and that it negatively affects the ATPase activity of VCP (Cloutier et al., 2013).

# Aim of the study

The aim of the research presented in this thesis was to investigate uncharacterized members of the METTL21 subgroup within human MTase family 16 (MTF16), and to further assess the biochemical and biological functions of already characterized members. Briefly, identification of the substrate for an uncharacterized member of the MTF16 family (METTL21B), was carried out and evaluated *in vitro* and in cells (Paper II). We have also aimed at revealing the biological significance of METTL21B (eEF1α-KMT3) and METTL21D (VCP-KMT) by using corresponding gene knockout cells and mice (Paper I, II). Finally, this study also encompassed the development of a biotechnological tool by using the VCP-KMT/VCP as a system, enabling the generation of a desired peptide library with inserted lysine methylation (Paper III).

# Summary of papers

## Paper I

**Lysine methylation of the Valosin-containing protein (VCP) is dispensable for development and survival of mice.**

In this paper we have tried to elucidate the biological significance of the VCP-KMT by generating corresponding gene knockout mice. We have observed that VCP-KMT is ubiquitously expressed in all organs of the wild-type mice and confirmed its subcellular localization to the cytoplasm. We have authenticated the absence of the VCP-KMT expression and Lys-315 methylation of VCP in *VCPKMT*$^{-/-}$ mice by various methods. We have investigated the specificity of VCP-KMT using *in vitro* methylation assays by the use of protein extracts from *VCPKMT*$^{-/-}$ mouse organs or human *VCPKMT*-/- cell lines as substrates. Finally, we have concluded that *VCPKMT*$^{-/-}$ mice were viable, fertile and have no significant phenotype compared to the wild-type mice. Hence, we conclude that VCP-KMT mediated methylation of VCP is dispensable for the development and survival of mice under unstressed conditions.

## Paper II

**The novel lysine specific methyltransferase METTL21B mediates inducible and dynamic methylation of Lys-165 in human eukaryotic elongation factor 1 alpha (eEF1A).**

In this study, we have described the functional characterization of the human MTF16 member, METTL21B. We have demonstrated that METTL21B orthologs are limited to vertebrates, and found METTL21B to be localized to the cytoplasm as well as at the centriole. We have determined that METTL21B specifically methylates Lys-165 in the two close paralogs, eEF1α1 and eEF1α2, and that the methylation is GTP- and tRNA-dependent, and does not appear to affect the interactome of eEF1α. Based on these results and, in agreement with current nomenclature, we have suggested that METTL21B is renamed eEF1α-KMT3. Further, we have also reported the dynamic nature of the Lys-165 methylation in eEF1α in Balb/c mouse fibroblast exposed to various types of stress and alterations in growth conditions, and our results also showed elevated methylation of eEF1α at Lys-165 in several cancer-derived cell lines compared to normal rat tissues. In summary, the results presented in this study demonstrated that eEF1α-KMT3 is the enzyme responsible for the Lys-165

methylation of eEF1α, and that the modification is dynamic, inducible and likely to be of regulatory importance.

# Paper III

**VCP-KMT-mediated lysine methylation of histone H3 peptide sequences grafted onto a VCP-derived scaffold.**

This study describes the possibility of using VCP-KMT and VCP as a system to obtain specific lysine methylation of desired peptide sequences grafted onto VCP-derived scaffold. We have determined the D1 domain of the VCP (VCP-D1) as the minimal part of VCP that could be efficiently methylated by VCP-KMT. We have generated VCP-D1/histone H3 chimera proteins and found that they all underwent efficient VCP-KMT mediated methylation *in vitro*. Further, VCP-KMT-mediated methylation was also observed when the chimeric proteins were expressed in the human cells. These results demonstrate that VCP-KMT and VCP-D1 together represent a versatile system for introducing lysine methylation into desired peptide sequences.

# Discussion

## The Human MTF16 family

MTase Family 16 (abbreviated MTF16) is a subclass of the 7BS MTases and includes 10 human members that form a closed group containing a conserved DXXY motif (consensus [D/E] XX [Y/F]) located immediately C-terminal to motif-II [Paper II, Figure 1B; (Kernstock et al., 2012)]. Notably, this motif is highly similar to the DPPY motif (consensus [D/N/S]-P-P-[Y/F/W]) of several DNA methyltransferases (Bheemanaik et al., 2006). Among these 10 members of MTF16, only two, CaM-KMT [calmodulin-lysine M-methyltransferase], which methylates calmodulin (Magnani et al., 2010), and VCP-KMT [valosin-containing protein lysine methyltransferase], which methylates the VCP (Kernstock et al., 2012) were characterised at the time the work encompassed in this thesis was initiated. However, several studies revealing the function of human MTF16 members have been published since then (Jakobsson et al., 2013, Cloutier et al., 2013, Rhein et al., 2014 Malecki et al., 2015, Davydova et al., 2014), but some members still remain to be elucidated.

The characterised members of the human MTF16 family include CaM-KMT, VCP-KMT, HSPA-KMT, eukaryotic elongation factor 2 lysine methyltransferase (eEF2-KMT), ETFβ lysine methyltransferase (ETFβ-KMT), Kin17 lysine methyltransferase (KIN-KMT), and eukaryotic elongation factor 1A lysine methyltransferase 3 (eEF1A-KMT3). All of these MTF16 family members target a single substrate; the sole exception being HSPA lysine methyltransferase (HSPA-KMT), which targets several Hsp70 proteins at the corresponding lysine residue. However, the bacterial ortholog of ETFβ-KMT targets two apparently unrelated substrates, i.e. ETFβ and the ribosomal protein RPL7/L12 (Malecki et al., 2016). All the seven characterized human MTF16 members methylate lysine residues in non-histone proteins. However, the putative yeast ortholog of the yet uncharacterized human MTF16 member METTL18 was reported to methylate a histidine residue in the ribosomal protein RPL3, and was therefore denoted Hpm1 (histone protein methyltransferase 1) (Webb et al., 2010, Al-Hadid et al., 2014). In addition, METTL21C has been shown to undergo auto-methylation at Lys-35, suggesting that also this enzyme is a KMT (Kernstock et al., 2012). Thus, all of the human MTF16 members appear to represent KMTs acting on non-histone proteins, with the possible exceptions of METTL23 which remains uncharacterized and the aforementioned METTL18, which likely is histidine-specific (Table 1).

Almost all of the identified substrates for the human MTF16 family enzymes represent abundant proteins. For example, in a quantitative study of the proteome in a human cell line, VCP, CaM, eEF2, ETFβ, eEF1α, as well as several Hsp70 family members, were listed as highly expressed proteins (Beck et al., 2011). These proteins play crucial roles in various cellular processes, and their methylation, mediated by the respective MTF16 members, will likely affect their functions. Indeed, some previous studies have addressed the significance of these lysine methylations. Efm3/Yjr129c is the yeast ortholog of eEF2-KMT, methylating yeast eEF2 at the lysine residue corresponding to the site targeted by the human enzyme. Knock-out of *efm3* caused hypersensitivity towards the eEF2-specific drug sordarin, and increased frameshifting during protein synthesis, suggesting that eEF2 methylation is required for accurate and robust protein translation in both the yeast and humans (Davydova et al., 2014).

HSPA-KMT–mediated methylation of HSPA8 was shown to reduce its affinity for fibrils formed by α-synuclein, whose aggregation is associated with Parkinson disease, and methylation was shown to abolish the ability of HSPA8 to prevent the formation of such fibrils (Jakobsson et al., 2013). It was also reported that trimethylation of the stress-inducible Hsp70 protein HSPA1 (Lys-561) was predominant in ovarian and breast cancer samples, and the results suggested that Lys-561 methylation status may be used as a diagnostic or prognostic marker (Jakobsson et al., 2015). Previously, Cho et al. reported that SETD1A catalyses the dimethylation of HSPA1 at Lys-561 (Cho et al., 2012), but later work has showed that this is likely incorrect, and that METTL21A is the sole enzyme responsible for methylation of Lys-561 of HSPA1 (Jakobsson et al., 2016). The observed SETD1A dependent modulation of HSPA1-methylation could reflect an indirect effect, caused by regulation of gene expression mediated through SETD1A catalysed histone methylation (Jakobsson et al., 2016). VCP-KMT has also been shown to be involved in the metastasis of cancer (Thiele et al., 2011), and another study reported that *VCPKMT*$^{-/-}$ cells showed reduced migratory/invasive nature compared to corresponding wild-type cells (Kernstock et al., 2012). ETFβ-KMT–mediated methylation of electron transfer flavoprotein (ETFβ) *in vitro* was found to reduce its ability to receive electrons from various dehydrogenases, and furthermore, siRNA-mediated knock-down of ETFβ-KMT enzyme was shown to cause a moderate decrease in the oxygen consumption rate of permeablized cells metabolizing palmitoyl-L-carnitine. These findings support a role of ETFβ-KMT in modulating cellular metabolism (Malecki et al., 2015, Rhein et al., 2014).

**Table 1. The human MTF16 members and their substrates:** Lys, lysine; His, histidine; me1, monomethylation; me2, dimethylation; me3, trimethylation;

*, activity shown for yeast homolog. #, activity shown for bacterial homolog.

| Human MTF16 member | Alternative name | Substrate | Target residue and methylation state | Reference |
|---|---|---|---|---|
| CaM KMT | C2orf34 | CaM | Lys-115me3 | Magnani *et al.*, 2010 |
| METTL18 | C1orf156 | RPL3* | His-243me1 | Webb *et al.*,2010<br>Al-Hadid *et al.*, 2014 |
| METTL21D | VCP-KMT/ C14orf138 | VCP | Lys-315me3 | Kernstock *et al.*, 2012<br>Cloutier *et al.*, 2013 |
| METTL21C | C13orf39 | METTL21C | Lys-35me1 | Krenstock *et al.*, 2012 |
| METTL21A | HSPA-KMT/ FAM119A | HSPA8 and homologs | Lys-561me3 and analogous positions | Jakobsson *et al.*, 2013<br>Cloutier *et al.*, 2013 |
| METTL22 | KIN-KMT/ C16orf68 | KIN | Lys-135me3 | Cloutier *et al.*, 2013 |
| EEF2KMT | FAM86A | eEF2 | Lys-525me3 | Davydova *et al.*, 2014 |
| ETFBKMT | METTL20/ C12orf72 | ETFβ<br><br>RPL7# | Lys-200me1/2/3<br>Lys-203me1/2/3<br>Lys-86 me1 | Malecki *et al.*, 2015<br>Rhein *et al.*, 2014<br>Malecki *et al.*, 2016 |
| METTL21B | eEF1A-KMT3/ FAM119B | eEF1α1 and eEF1α2 | Lys-165me1/2/3 | Paper II |
| METTL23 | C17orf95 | -------- | -------- | -------- |

No substrates have yet been identified for the human MTF16 members, such as METTL23 and METTL21C, but the corresponding genes have been associated with the development of various disorders, primarily involving cognition but also osteoporosis and various types of tumors. Disruption of the *METTL23* gene was shown to lead to mild intellectual disability (Reiff et al., 2014, Bernkopf et al., 2014). Also, METTL23 was shown to interact with GA binding protein transcription factor, alpha subunit (GABPA), a component of a transcription factor complex which regulates expression of several genes (Reiff et al., 2014). It has been shown that overexpressed METTL23 is localized in nucleus and cytoplasm, and alters the expression of GABPA regulated genes such as *THPO* and *ATP5B* (Reiff et al., 2014). Both of these genes have possible roles in cognition and neurodevelopment since they are highly expressed in brain. However, in spite of the observed interaction between GABPA and METTL23, a direct ability of METTL23 to regulate GABPA transcription has not been demonstrated (Reiff et al., 2014). A genome-wide association study has implicated *METTL21C* in the musculoskeletal diseases osteoporosis and sarcopenia. Correspondingly, siRNA-mediated knockdown of METTL21C in mouse cells led to significant reduction in myoblast differentiation through modulation of the NF-kB signaling pathway (Huang et al., 2014). More recently, a study showed that METTL21C levels were altered in the plasma of patients with autism spectrum disorder (Homs et al., 2016), suggesting a possible involvement in such disorders.

# Characterization of METTL21B

A major aim of the present study was to unravel the biochemical function of a previously uncharacterised MTF16 member, METTL21B (eEF1A-KMT3), which was found to methylate Lys-165 of eEF1α (Paper II). The recently determined crystal structure of human METTL21B (Tempel et al., 2014) shows a canonical, seven-stranded β-sheet fold with alternating α-helices; a characteristic feature of the 7BS MTases. A protein sequence alignment of METTL21B orthologs revealed the presence of all of the conserved 7BS hallmark motifs (Motif-I, Post-I and Motif-II), as well as the so-called DXXY-motif [consensus (D/E)-X-X-(Y/F)] shared by MTF16 family members (Paper II, Figure 1B).

To identify the putative orthologs for METTL21B in other organisms, the Basic Local Alignment Search Tool (BLAST) was used to perform a search, which demonstrated that METTL21B is exclusive to vertebrates. However, METTL21B shows a scattered distribution in vertebrates; putative orthologs are found in all mammals, reptiles and amphibians, but not in birds. Also, METTL21B shows a pachy distribution in fishes: for example, zebrafish (*Danio rerio)* does not have METTL21B, but Mexican tetra (*Astyanax mexicanus)* does. Importantly, both of these fishes have the corresponding target lysine residue on eEF1α.

## METTL21B *in vitro* activity

Previously characterized members of the MTF16 family, such as VCP-KMT, HSPA-KMT, eEF2-KMT had been shown to methylate protein substrates (Jakobsson et al., 2013, Kernstock et al., 2012, Davydova et al., 2014). These results suggested that METTL21B may also be a protein methyltransferase. To investigate its activity, we performed an *in vitro* methylation assay with recombinant METTL21B using cellular extract as a source of substrate. In this experiment, a strong methylated band of ~50 kDa, induced by METTL21B in an ATP-dependent manner, appeared (Paper II, Figure. 1C), indicating that METTL21B had protein methyltransferase activity.

To identify the METTL21B substrate, we have methylated cell extracts with recombinant METTL21B and reduced the sample complexity by ion-exchange chromatography, followed by analysis of the corresponding bands by mass spectrometry. Among the identified proteins, the two paralogs of eEF1α (eEF1α1 and eEF1α2) were listed top hits (Paper II, Table 1). As previously reported for rabbit eEF1α (Dever et al., 1989), five methylated lysines have been identified in both of these paralogs: i.e. Lys-36, Lys-55, Lys-79, Lys-165 and Lys-318. Among these, Lys-165 was demonstrated as the target of METTL21B (Paper II, Figure 2B).

To further validate these findings, we cloned and produced wild-type and Lys165Ala mutants of recombinant eEF1α1 and eEF1α2, and incubated them with recombinant METTL21B. We then observed methylation in case of the wild-type proteins, but not of the mutants (Paper II, Figure 4A).

To investigate whether METTL21B is responsible for methylation of eEF1α inside cells, we have purchased *METTL21B* knockout HAP1 cells, and analysed the Lys-165 methylation status of eEF1α1 in wild-type, *METTL21B* knockout (KO) and KO cells complemented with the *METTL21B* gene. The results revealed that Lys-165 was predominantly un-, mono- and dimethylated in the wild-type cells; this finding is similar to what was observed in HeLa and HEK293 cells (Paper II). Lys-165 methylation was completely eliminated in *METTL21B* knockout cells and partially restored in complemented cells (Paper II, Figure 5). Altogether these results confirmed that METTL21B is a highly specific enzyme that targets eEF1α at Lys-165.

METTL21B is the third human enzyme found to methylate eEF1α. The previously identified enzymes were N6AMT2, which methylates Lys-79 and was renamed eEF1A-KMT1 (gene name EEF1AKMT1) (Hamey et al., 2016), and METTL10, which methylates Lys-318 and was suggested renamed eEF1A-KMT2 (Falnes et al., 2016). In accordance with this nomenclature and the specificity of the enzyme substrate, we have suggested METTL21B to be renamed eEF1A-KMT3 (gene name *EEF1AKMT3*).

## METTL21B-mediated methylation is GTP- and tRNA-dependent

Both eEF1α1 and eEF1α2 exist as both GTP- and GDP-bound forms.The GTP-bound form of eEF1α associates with aminoacyl-tRNA and transfers it to the A site of the ribosome during the polypeptide elongation, and through hydrolysis of GTP, eEF1α is converted to the GDP-bound form, and leaves the ribosome to associate with the eEF1β complex, which catalyzes the exchange of GDP for GTP (Voorhees & Ramakrishnan, 2013). Since GTP has a key role in eEF1α function, and since Lys-165 is located within the GTP-binding domain of eEF1α (Paper II, Figure 9), we hypothesised that the observed ATP-dependent activity of the METTL21B in reality reflected of this GTP dependence. Thus, the ATP-dependent activity may be attributable to the presence of nucleotide diphosphate (NDP) kinases in the cellular extract, which may generate GTP from GDP and ATP (Hsu et al., 2015). In order to investigate this, we have methylated the partially purified cellular extract (0.3S fraction) and recombinant eEF1α1 by supplementing the reaction with ATP, guanosine triphosphate (GTP)

and guanosine diphosphate (GDP) (Paper II, Figure 3A-C). The results showed that METTL21B–mediated methylation in cellular extract fractions is supported by both ATP and GTP; however, for recombinant eEF1α1 only GTP supports methylation, indicating that GTP is the true co-factor. In addition, since eEF1α is a tRNA-binding translational factor, we thought that methylation may also be influenced by the presence of tRNA. In agreement with this, our results (Paper II, Figure 3D-F) showed that methylation was completely absent in the RNaseA treated samples, whereas methylation was enhanced by supplementation with tRNA. These results established that METTL21B–mediated methylation is dependent on both GTP and tRNA.

## Biological significance of eEF1α methylation

eEF1α shows high sequence conservation among eukaryotic species, ranging from yeast to humans, and undergoes several post-translational modifications, such as acetylation and methylation. Yeast eEF1α undergoes methylation at the four lysine residues Lys-30, -79, -316, -390, and all the enzymes responsible for these modifications have been identified and denoted Efm1, Efm5, Efm4 and Efm6 respectively (Falnes et al., 2016). In mammals, five lysine residues (Lys-36, -55, -79, -165 and -318) have been shown to be methylated (Dever et al., 1989), of which Lys-79 and Lys-318 were shown to be methylated by eEF1A-KMT1 (N6AMT2) (Hamey et al., 2016) and eEF1A-KMT2 (METTL10) (Shimazu et al., 2014) respectively. To test the possible function of the eEF1α methylation in yeast, Cavallius and colleagues analyzed the eEF1α mutants where all the four methylated lysines had been substituted by arginines, and observed no apparent phenotypic change (Cavallius et al., 1997), indicating that eEF1α methylation is not essential for normal yeast growth. However, mutations in the nucleotide binding region of yeast eEF1α were shown to have functional consequences, such as reductions in the rate of GTP hydrolysis and increased misincorporation of amino acids during translation (Cavallius & Merrick, 1998). Although the eEF1α sequence is highly conserved among eukaryotes, Lys-165, which may interact directly with GTP-binding region, is present in most of the vertebrates, but absent in yeast (Paper II, Figure 9D). METTL21B mediated methylation of Lys-165 in higher eukaryotes may regulate the role of eEF1α via influencing its interaction with GTP/GDP. Notably, the neighboring residue (Arg-166) was already implicated in nucleotide binding, as mutating the corresponding residue (Arg-164) in yeast eEF1α decreased its affinity for GDP (Ozturk & Kinzy, 2008).

We also analyzed *EEF1AKMT3* knockout HAP1 cells by performing ribosome profiling experiments and found that METTL21B mediated methylation of eEF1α affects the gene expression at the level of translation, and that proteins associated with the endoplasmic reticulum (ER) and the unfolded protein response were down-regulated (Paper II, Figure 5D, F). We have also found that Lys-165 methylation of eEF1α in Balb/c cells varied significantly depending on the proliferation state of the cells. Cells with a high degree of confluence had a ~3-fold elevated dimethylation compared to cells proliferating with low density (Paper II, Figure 8A). Similarly, serum starved cells also showed elevated methylation, which was reversed upon restimulated with serum (Paper II, Figure 8A, B). The dynamic nature of METTL21B mediated methylation of eEF1α appears to reflect an active process involving the regulation of *EEF1AKMT3* gene expression, (Paper II, Figure 8C), rather than more generic effect caused by changes in protein turnover or alterations in AdoMet levels due to altered metabolism, since conditions that caused elevated Lys-165 methylation also led to increased *EEF1AKMT3* mRNA expression. The dynamics and regulatory function of lysine methylation on histones have been intensively studied, and, here, several lysine specific demethylases play important roles (Tsukada et al., 2006, Dimitrova et al., 2015). Thus, it will clearly be interesting, in future studies, to investigate whether demethylases are, partly, responsible for the observed dynamics of lysine methylation at Lys-165. However, the apparent reversibility of Lys-165 methylation may also be mediated by a passive mechanism involving down-regulation of METTL21B expression, leading to (relative) hypomethylation of newly synthesized eEF1α.

We have also observed elevated Lys-165 methylation in cancer-derived cells, which, typically, have upregulated stress pathways, when compared with normal tissues (Paper II, Figure 7A, B). Balb/c cells also showed increased levels of Lys-165 methylation upon treatment with drugs that induce ER stress (Paper II, Figure 8D, E). Previously, it was shown that over-expression of eEF1α can prevent apoptosis triggered by ER stressors, and upon withdrawal of growth factors (Talapatra et al., 2002). Similarly, METTL21B mediated methylation of eEF1α was also induced by ER stressors, and thus we speculate that this phenomenon is a part of stress response pathway. eEF1α is considered to form a reversible EF1-heavy complex (eEF1H) with its guanine nucleotide exchange factor eEF1B to mediate the exchange of the aminoacyl-tRNA to ribosome during translation. Previously, it was showed that among the two paralogs of eEF1α, eEF1α1 has more affinity towards the guanine exchange factor complex and eEF1α2 has no or negligible affinity (Mansilla et al., 2002). In

contrast to this, we found that all the components of the eEF1B complex co-purified with similar yields regardless of which of the two paralogues were used as bait in TAP-tagging experiments. eEF1α undergoes methylation at various lysine residues (Dever et al., 1989), which may influence protein-protein interactions, and Lys-165 is one among them. Hence, we have investigated the influence of Lys-165 on the eEF1α interactome by mutating it with alanine, and found that this substitution did not influence the translational related interactome of eEF1α (Paper II, Figure 4B). Besides its canonical role in protein translation, several non-canonical functions were described for eEF1α, and the most intensely studied one among these is the regulation of the cytoskeleton (Sasikumar et al., 2012). eEF1A has been shown to interact with microtubules (Moore et al., 1998), as well as with centrosomal proteins (Fogeron et al., 2013), and we also found that a subpopulation of METTL21B was localized to centrosomes, which represent the major microtubule organizing centers in the cell. Thus, METTL21B-mediated eEF1α methylation may conceivably be specifically involved in eEF1α-mediated regulation of the cytoskeleton. Apart from this, eEF1α was also shown to play an important role in the replication and pathogenesis of RNA viruses, and the P51 subunit of reverse transcriptase and integrase of reverse transcription complex (RTC) of the HIV virus was shown to interact with eEF1α. Furthermore, the viral reverse transcription in cells was sharply down-regulated when eEF1α levels were reduced by treatment with either siRNA (Warren et al., 2012) or didemnin B, which specifically interacts with eEF1α (Li et al., 2015).

# Biological significance of VCP-KMT mediated methylation

VCP-KMT is responsible for the trimethylation of Lys-315 in the VCP monomer (Kernstock et al., 2012, Cloutier et al., 2013). The trimethylated VCP monomers are then assembled into a hexameric complex (Paper I, Figure 1A). VCP-KMT (NVM-1) is upregulated in several metastatic tumors, and has also been implicated in cellular invasion and migration (Thiele et al., 2011). In agreement with this, VCP-KMT knockout cell lines generated in our laboratory showed slow invasion, migration and proliferation compared to wild-type cells (Kernstock et al., 2012). In other studies, it was reported that VCP-KMT–mediated methylation of VCP at Lys-315 is important for its ATPase activity (Cloutier et al., 2013). However, all of these results were exhibited *in vitro* and in cells. Hence, we decided to further elucidate the role of VCP-KMT *in vivo*.

Therefore, we generated constitutive knockout mice by introducing loxP sites flanking exons 1 and 4 of the *VCPKMT* gene followed by continuous breeding with Cre recombinase expressing mice. We investigated and confirmed the lack of *VCPKMT* gene expression in the knockout mice and found its ubiquitous expression in all organs of the wild-type mice (Paper I, Figure 1E). We have also observed that VCP methylation at Lys-315 was completely absent in the knockout mice, and that Lys-315 was completely trimethylated in all the organs of the wild-type mice. This complete trimethylation of VCP may indicate the absence of dynamic regulation (Paper I, Figure 2A, B).

There are several lysine MTases that can target a multitude of substrates (histone and non-histone proteins). For example, SMYD3 can methylate histone H4 and also non-histone proteins such as HSP90 and vascular endothelial growth-factor receptors (VEGFR). The SET-domain containing lysine methyltransferase 7 (SETD7) can methylate multiple proteins like TAF10, P53 and DNMT1 (Biggar & Li, 2015). In contrast to these MTases, VCP-KMT has only one substrate (VCP), indicating that VCP-KMT is highly specific (Paper I, Figure 4A, B). Similarly, all other characterised members of human MTF16 also have a single substrate except HSPA-KMT, which can methylate HSPA1 along with other HSPAs (Jakobsson et al., 2013, Falnes et al., 2016). Previously generated knockout mouse models for KMTs indicate that several methyl modifications on histones, such as those introduced by DOT1L and SMYD2, are essential for embryonic development (Jones et al., 2008, Diehl et al., 2010). Of the other members of human MTF16, CAM-KMT knockout mice exhibit reduced body growth, somatosensory development deficiency and impaired brain function (Haziza et al.,

2015); METTL23 (without a described target) is associated with intellectual disability (Bernkopf et al., 2014). In contrast, VCP-KMT knockout mice do not shown any obvious phenotype. We have tested fertility rate, sex ratio, body growth, and survival of the wild-type and *VCPKMT* knockout mice, and found no significant differences (Paper I, Figure 5).

VCP is an abundant and highly conserved member of the AAA+-ATPases family, and is involved in various cellular functions including transcription regulation and proteolysis (Meyer & Weihl, 2014, Yamanaka et al., 2012). VCP has several interaction partners; most of them associate with the VCP N-terminal domain, and only a few of them bind to the C-terminal domain (Buchberger et al., 2015). VCP mutations have been linked to neurological disorders and also reported to associate with inclusion body myopathy with frontotemporal dementia (IBMPFD) (Watts et al., 2007). The possible effect of Lys-315 trimethylation on ATPase activity of VCP remains unclear, but, Cloutier et al. showed that the truncated version of VCP (VCPΔD2) with D1 domain had diminished ATPase activity when trimethylated by recombinant VCP-KMT (Cloutier et al., 2013). In contrast, the (D2-containing) endogenous VCP from *VCPKMT* knockout and wild-type cells did not show any differences in their ATPase activities (Kernstock et al., 2012). Conceivably, any effect of knock-out on the D1 domain may here be masked by the higher ATPase activity of D2.

Overall, the results in this study (Paper I) show that mice with the complete loss of Lys-315 trimethylation in VCP due to *VCPKMT* knockout were as healthy as wild-type mice, and that VCP-KMT appears to be dispensable with respect to the development and survival of mice under unstressed conditions. As VCP-KMT has been shown to be involved in the development of metastatic cancer (Thiele et al., 2011), it will be of interest to study how these knockout mice respond to tumor transplantation compared to wild-type mice.

# The VCP/VCP-KMT pair as a biotechnological tool

VCP-KMT is a highly specific enzyme and methylates VCP at Lys-315, which is localised in a flexible loop (Paper III, Figure 1B). Other members of the human MTF16 family, such as METTL21A (HSPA-KMT), METTL22 (KIN-KMT) and CaM-KMT, also methylate lysine residues located within the loops in their respective substrates. Previously, it was showed in our laboratory that VCP-KMT mediated methylation of VCP is tolerant to sequence alterations in the proximity of the methylation site (Kernstock et al., 2012). Another human member of MTF16 family, METTL20, methylates lysine residues (Lys-200 and Lys-203) in ETFB, localised in a lysine-rich sequence, $Lys_{(-200)}$-Ala-Lys-Lys-Lys-$Lys_{(-205)}$, found as part of a helix. It was shown that METTL20 is tolerant towards alterations in this motif; the non-methylated lysines in this motif could be simultaneously mutated to arginine without affecting the methylation, and, also the methylation targets Lys-200 and Lys-203 could be individually replaced by arginine without abolishing the methylation at the other site (Malecki et al., 2015). This shows that METTL20 also has some tolerance towards substitutions of residues in the neighbourhood of the methylation site. However, only in the case of CaM-KMT has the effect of systematically mutating residues in the vicinity of the methylation site been investigated, and the results showed that the methylation was abrogated by mutations of residues surrounding the methylation site (Magnani et al., 2012). In the same study, it was shown that the calmodulin variants where amino acid residues in a flexible linker region (residues 68-92), distant from the methylation site Lys-115, were replaced, could still be methylated. Here, methylation was observed after relatively conservative replacements (of polar residues by polar, non-polar residues by non-polar) of the sequence in the linker region (Magnani et al., 2012).

In an approach similar to the one described above (Magnani et al., 2012), we have investigated the tolerance of VCP-KMT towards amino acid alterations in VCP loop where Lys-315 resides. In order to do this, we generated several deletion mutants of the VCP and identified D1 domain as the minimal part that can be expressed, purified and methylated by VCP-KMT with the same efficiency as VCPΔD2 (Paper III, Figure 1 and 2). Based on the D1 domain, we generated histone H3 chimeras, where residues surrounding Lys-315 of D1 were replaced by sequence corresponding to various lysine methylation sites in the N-terminal tail of histone H3 (Paper III, Figure 3B). All these chimeras were expressed, purified and efficiently methylated by VCP-KMT *in vitro*; that is, the level of methylation obtained at the highest enzyme concentrations was similar to that with the wild-type D1 domain (Paper III,

Figure 3C, E). Of note, all of these chimeras (except D1-H3K27, which we were unable to investigate) underwent VCP-KMT mediated methylation inside cells, although with lower efficiency than the wild-type D1-domain (Paper III, Figure 4C). This observation may be partly explained by the chimeras being poorer VCP-KMT substrates, as observed in the *in vitro* experiments; it may also be caused by a higher turnover rate of the chimeras.

The VCP-KMT/D1 system described in this paper can be used as a versatile system to introduce lysine methylation into a desired peptide sequence, and the approach should be readily expandable to the generation of combinatorial libraries of methylated peptides. The VCP-KMT/D1 system has a major advantage in that the varied sequence actually surrounds the methylation site, whereas in a CaM-KMT/CaM system the methylation site is located distant to the sequence being varied (Magnani et al., 2012).

# Protein lysine methyltransferases (KMTs) and cancer

Protein lysine methylation is carried out by various KMTs and many of the respective substrates play important roles in cancer. The roles of histone lysine methyltransferases in cancer development and progression have been studied extensively, and such enzymes have been found to be dysregulated in many cancers. For example, EZH2, is an H3K27 specific methyltransferase found to be over-expressed in various solid tumors, including prostate, breast, colon, lung cancers, and over-expression of EZH2 confers invasiveness to fibroblasts in a manner dependent on its H3K27 methyltransferase activity [reviewed in (Chi et al., 2010)]. It was also reported that suppression of EZH2 by siRNA reduced the tumor size in breast and prostate cancer xenograft models (Gonzalez et al., 2009, Yu et al., 2007). *MLL* is a proto-oncogene, and encodes a methyltransferase which methylates H3K4 (Milne et al., 2002). *MLL* gene rearrangements represent one of the most common abnormalities found in human leukemia, and are present in 80% of infant leukemias and 5-10% of adult acute myeloid leukemias (AML) [reviewed in (Chi et al., 2010)].

Beyond histones, the significance of non-histone protein lysine methylation in human cancer has recently begun to be explored. Accumulated evidence indicates that dysregulation of non-histone protein lysine methylation is involved in the development and progression of cancer. p53 is one of the most important tumor suppressors, and is frequently inactivated during tumorigenesis, and SETD7 mediated methylation of p53 at Lys-372 enhances its stability and transcriptional activity. SMYD2 methylate Lys-370 of p53 and its down regulation by RNA interference enhances p53-mediated apoptosis in cancer cells. SETD8 mediated methylation of Lys-382 suppresses the transcriptional activity of p53 in cancer cells [reviewed in (Hamamoto et al., 2015)]. RB1 is a cell cycle regulator and its methylation at Lys-810 mediated by SMYD2, regulates its interaction with E2F transcription factors, and thus regulates the cell cycle (Cho et al., 2012). Vascular endothelial growth factor receptor 1 (VEGFR1), which is a receptor tyrosine kinase, undergoes SMYD3 mediated methylation at Lys-831, which enhances its kinase activity, leading to increased migration and invasion of cancer cells. Another kinase, MAP3K2, undergoes methylation at Lys-260, which activates RAS-RAF-MEK-ERK signaling, and down-regulation of the responsible methyltransferase, SMYD3, blocks RAS-driven tumorigenesis [reviewed in (Hamamoto et al., 2015)].

Human MTF16 members were shown to methylate a wide variety of proteins, such as translation factors, chaperones and ribosomal proteins (Table 1). VCP-KMT was reported to

be up-regulated in several metastatic tumors (Thiele et al., 2011). It was reported that HSPA-KMT mediated trimethylation of the stress induced Hsp70 protein, HSPA1, at Lys-561 is predominant in various cancers such as ovarian and breast cancer patients. HSPA1 (Lys-561) Methylation was found to be different between breast and ovarian cancers; trimethylation was more prevalent in breast carcinoma while di-, mono- and unmethylated forms were common in ovarian cancers. Moreover, the unmethylated form of Lys-561 found to be associated with poor survival in ovarian cancer (Jakobsson et al., 2015). We also showed that METTL21B mediated methylation of eEF1α was much higher in cancer-derived human cells than in normal rat tissues (Paper II). Hence, it may be of interest to study whether these KMTs are over-expressed in cancer, and if they play a role in cancer development. If so, these enzymes may represent targets for future cancer therapy.

# Conclusion and future perspectives

In conclusion, the studies presented in this thesis have contributed to the characterization and elucidation of the functional role of methyltransferases belonging to the human MTF16 subfamily of seven-β-strand methyltransferases. We showed for the first time that an inducible expression of *METTL21B* occurs in response to alterations in growth conditions of Balb/c cells through serum withdrawal or contact inhibition, and similar effects were observed after treatment of cells with certain ER-stress induced drugs. We have noticed that eEF1α methylation at Lys-165 is high in cancer-derived human cells compared to normal rat organs. Hence, it would be interesting to compare its methylation status between cancer patients and healthy individuals. We have also found that METTL21B is localized in centrioles and since, eEF1α has been shown to involve in cellular cytoskeleton organization, METTL21B may have a crucial role in this process. In another study, we have showed that VCP-KMT is dispensable for the growth and survival of mice but it will be a great interest to study how the *VCPKMT*-/- mice will respond to tumour transplants in comparison to normal mice. Further, we have showed that the VCP-KMT/VCP-D1 enzyme substrate pair may represent a versatile system to generate combinatorial libraries of desired lysine methylated sequences. However, further studies are needed to expand this system to be used as a tool for identifying methyllysine-containing sequences that interact with specific readers of lysine methylation. Also, a few members of the human MTF16 family, as well as several other human 7BS methyltransferases, remain uncharacterized, and the elucidation of these enzymes represent important challenges for future research.

# Bibliography


Abbas, Wasim, Kumar, A. and Herbein, G. 2015. 'The eEF1A Proteins: At the Crossroads of Oncogenesis, Apoptosis, and Viral Infections', *Frontiers in Oncology*, 5, 75.

Abbott, Catherine M., and Christopher G. P. 2004. 'Translation factors: in sickness and in health', *Trends in Biochemical Sciences*, 29, 25-31.

Al-Hadid, Q., Roy, K., Munroe, W., Dzialo, M. C., Chanfreau, G. F. and Clarke, S. G. 2014. 'Histidine methylation of yeast ribosomal protein Rpl3p is required for proper 60S subunit assembly', *Mol Cell Biol*, 34, 2903-16.

Barbier, M., Owings, J. P., Martínez-Ramos, I., Damron, F. H., Gomila, R., Blázquez, J., Goldberg, J. B., Albertí, S. 2013. 'Lysine trimethylation of Ef-Tu mimics platelet-activating factor to initiate Pseudomonas aeruginosa pneumonia'. *mBio* 4(3), e00207-13.

Barthelme, D. and Sauer, R. T. 2016. 'Origin and Functional Evolution of the Cdc48/p97/VCP AAA+ Protein Unfolding and Remodeling Machine', *J Mol Biol*, 428, 1861-9.

Bec, G., Kerjan, P., Zha, X. D. and Waller, J. P. 1989. Valyl-tRNA synthetase from rabbit liver. I. Purification as a heterotypic complex in association with elongation factor 1. *The Journal of biological chemistry*, 264, 21131-21137.

Beck, M., Schmidt, A., Malmstroem, J., Claassen, M., Ori, A., Szymborska, A., Herzog, F., Rinner, O., Ellenberg, J. and Aebersold, R. 2011. 'The quantitative proteome of a human cell line', *Mol Syst Biol*, 7, 549.

Bedford, M. T. 2007. 'Arginine methylation at a glance', *J Cell Sci*, 120, 4243-46.

Bedford, M. T. and Clarke, S. G. 2009. 'Protein Arginine Methylation in Mammals: Who, What, and Why', *Mol Cell*, 33, 1-13.

Bernkopf, M., Webersinke, G., Tongsook, C., Koyani, C. N., Rafiq, M. A., Ayaz, M., Muller, D., Enzinger, C., Aslam, M., Naeem, F., Schmidt, K., Gruber, K., Speicher, M. R., Malle, E., Macheroux, P., Ayub, M., Vincent, J. B., Windpassinger, C. and Duba. H. C. 2014. 'Disruption of the methyltransferase-like 23 gene METTL23 causes mild autosomal recessive intellectual disability', *Hum Mol Genet*, 23, 4015-23.

Bestor, T. H. 2000. 'The DNA methyltransferases of mammals', *Hum Mol Genet*, 9, 2395-402.

Bheemanaik, S., Reddy, Y. V. and Rao, D. N. 2006. 'Structure, function and mechanism of exocyclic DNA methyltransferases', *Biochem J*, 399, 177-90.

Biggar, K. K. and Li, S. S. 2015. 'Non-histone protein methylation as a regulator of cellular signalling and function', *Nat Rev Mol Cell Biol*, 16, 5-17.

Bottiglieri, T. 2002. 'S-Adenosyl-L-methionine (SAMe): from the bench to the bedside--molecular basis of a pleiotrophic molecule', *Am J Clin Nutr*, 76, 1151s-7s.

Brostoff, S. and Eylar, E. H. 1971. 'Localization of methylated arginine in the A1 protein from myelin', *Proc Natl Acad Sci U S A*, 68, 765-9.

Buchberger, A., Schindelin, H. and Hanzelmann, P. 2015. 'Control of p97 function by cofactor binding', *FEBS Lett*, 589, 2578-89.

Cantoni, G. L. 1975. 'Biological methylation: selected aspects', *Annu Rev Biochem*, 44, 435-51.

Carr, S. M., Munro, S., Kessler, B., Oppermann, U. and Thangue, N. B. L. 2011. 'Interplay between lysine methylation and Cdk phosphorylation in growth control by the retinoblastoma protein', *EMBO J*, 30, 317-27.

Cavallius, J., Zoll, W., Chakraburtty, K. and Merrick, W. C. 1993. 'Characterization of yeast EF-1 alpha: non-conservation of post-translational modifications', *Biochim Biophys Acta*, 1163, 75-80.

Chi, P., Allis, C. D. and Wang, G. G. 2010. 'Covalent histone modifications: miswritten, misinterpreted, and miserased in human cancers' *Nat Rev Cancer*, 10(7), 457–469.

Cho, H. S., Shimazu, T., Toyokawa, G., Daigo, Y., Maehara, Y., Hayami, S., Ito, A., Masuda, K., Ikawa, N., Field, H. I., Tsuchiya, E., Ohnuma, S., Ponder, B. A., Yoshida, M., Nakamura, Y. and Hamamoto. R. 2012. 'Enhanced HSP70 lysine methylation promotes proliferation of cancer cells through activation of Aurora kinase B', *Nat Commun*, 3, 1072.

Cho, H., Hayami, S., Toyokawa, G., Maejima, K., Yamane, Y., Suzuki, T., Dohmae, N., Kogure, M., Kang, D., Neal, D. E., Ponder, B. A. J., Yamaue, H., Nakamura, Y. and Hamamoto, R. 2012. 'RB1 Methylation by SMYD2 Enhances Cell Cycle Progression through an Increase of RB1 Phosphorylation', *Neoplasia*, 14, 476–486.

Cho, S. J., Lee, H., Dutta, S., Seog, D. H. and Moon. I. S. 2012. 'Translation elongation factor-1A1 (eEF1A1) localizes to the spine by domain III', *BMB Rep*, 45, 227-32.

Chuang, S. M., Chen, L., Lambertson, D., Anand, M., Kinzy, T. G. and Madura. K. 2005. 'Proteasome-mediated degradation of cotranslationally damaged proteins involves translation elongation factor 1A', *Mol Cell Biol*, 25, 403-13.

Chuikov, S., Kurash, J. K., Wilson, J. R., Xiao, B., Justin, N., Ivanov, G. S., McKinney, K., Tempst, P., Prives, C., Gamblin, S. J., Barlev, N. A. and Reinberg, D. 2004a. 'Regulation of p53 activity through lysine methylation', *Nature*, 432, 353-60.

Chuikov, Sergei, Kurash, J. K., Wilson, J. R., Xiao, B., Justin, N., Ivanov, G. S., McKinney, K., Tempst, P., Prives, C., Gamblin, S. J., Barlev, N. A. and Reinberg, D. 2004b. 'Regulation of p53 activity through lysine methylation', *Nature*, 432, 353-60.

Clarke, S. G. 2013. 'Protein methylation at the surface and buried deep: thinking outside the histone box', *Trends Biochem Sci*, 38, 243-52.

Cloutier, P. and Coulombe, B. 2013. 'Regulation of molecular chaperones through post-translational modifications: decrypting the chaperone code', *Biochim Biophys Acta*, 1829, 443-54.

Cloutier, P., Lavallee-Adam, M., Faubert, D., Blanchette, M. and Coulombe, B. 2013. 'A newly uncovered group of distantly related lysine methyltransferases preferentially interact with molecular chaperones to regulate their activity', *PLoS Genet*, 9, e1003210.

Couttas, T. A., Raftery, M. J., Padula, M. P., Herbert, B. R. and Wilkins, M. R. 2012. 'Methylation of translation-associated proteins in Saccharomyces cerevisiae: Identification of methylated lysines and their methyltransferases', *Proteomics*, 12, 960-72.

Dantuma, N. P., Acs, K. and Luijsterburg, M. S. 2014. 'Should I stay or should I go: VCP/p97-mediated chromatin extraction in the DNA damage response', *Exp Cell Res*, 329, 9-17.

Davydova, E., Ho, A. Y., Malecki, J., Moen, A. Enserink, J. M., Jakobsson, M. E., Loenarz, C. and Falnes, P. O. 2014. 'Identification and characterization of a novel evolutionarily conserved lysine-specific methyltransferase targeting eukaryotic translation elongation factor 2 (eEF2)', *J Biol Chem*, 289, 30499-510.

Dever, T. E., Costello, C. E., Owens, C. L., Rosenberry, T. L. and Merrick, W. C. 1989. 'Location of seven post-translational modifications in rabbit elongation factor 1 alpha including dimethyllysine, trimethyllysine, and glycerylphosphorylethanolamine', *J Biol Chem*, 264, 20518-25.

Diehl, F., Brown, M. A., Amerongen, M. J. M. J., Novoyatleva, T., Wietelmann, A., Harriss, J., Ferrazzi, F., Bottger, V., Harvey, V., Tucker, P. W. and Engel, F. B. 2010. 'Cardiac deletion of Smyd2 is dispensable for mouse heart development', *PLoS One*, 5, e9748.

Dillon, S. C., Zhang, X., Trievel, R. C. and Cheng, X. 2005. 'The SET-domain protein superfamily: protein lysine methyltransferases', *Genome Biol*, 6, 227.

Dimitrova, E., Turberfield, A. H. and Klose, R. J. 2015. 'Histone demethylases in chromatin biology and beyond', *EMBO Rep*, 16, 1620-39.

Falnes, P. O., Jakobsson, M. E., Davydova, E., Ho, A. and Malecki, J. 2016. 'Protein lysine methylation by seven-beta-strand methyltransferases', *Biochem J*, 473, 1995-2009.

Feng, Q., Wang, H., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Struhl, K. and Zhang, Y. 2002. 'Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain', *Curr Biol*, 12, 1052-8.

Ficarro, S., Chertihin, O., Westbrook, V. A., White, F., Jayes, F., Kalab, P., Marto, J. A., Shabanowitz, J., Herr, J. C., Hunt, D. F. and Visconti, P. E. Phosphoproteome analysis of capacitated human sperm. Evidence of tyrosine phosphorylation of a kinase-anchoring protein 3 and valosin-containing protein/p97 during capacitation. J Biol Chem. 2003; 278, 11579–11589. [PubMed: 12509440]

Fogeron, M. L., Müller, H., Schade, S., Dreher, F., Lehmann, V., Kühnel, A., Scholz, A.K., Kashofer, K., Zerck, A., Fauler, B., Lurz, R., Herwig, R., Zatloukal, K., Lehrach, H., Gobom, J., Nordhoff, E. and Lange, B. M. H. (2013) LGALS3BP regulates centriole biogenesis and centrosome hypertrophy in cancer cells. *Nature communications*, 4, 153.

Fontecave, M., Atta, M. and Mulliez, E. 2004. 'S-adenosylmethionine: nothing goes to waste', *Trends Biochem Sci*, 29, 243-9.

Gonzalez, M. E., Li, X., Toy, K., DuPrie, M., Ventura, A. C., Banerjee, M., Ljugman, M., Merajver, S. D. and Kleer, C. G. 2009. 'Down-regulation of Enhancer of Zeste-2 decreases growth of estrogen receptor negative invasive breast carcinoma and requires BRCA1' *Oncogene*, 28(6), 843–853.

Greganova, E. and Butikofer, P. 2012. 'Ethanolamine phosphoglycerol attachment to eEF1A is not essential for normal growth of Trypanosoma brucei', *Sci Rep*, 2, 254.

Halawani, D., LeBlanc, A. C., Rouiller, I., Michnick, S. W., Servant, M. J. and Latterich, M. 2009. 'Hereditary inclusion body myopathy-linked p97/VCP mutations in the NH2 domain and the D1 ring modulate p97/VCP ATPase activity and D2 ring conformation', *Mol Cell Biol*, 29, 4484-94.

Hamamoto, R., Saloura, V. and Nakamura, Y. 2015. 'Critical roles of non-histone protein lysine methylation in human tumorigenesis', *Nat Rev Cancer*, 15, 110-24.

Hamey, J. J., Winter, D. L., Yagoub, D., Overall, C. M., Hart-Smith, G. and Wilkins, M. R. 2016. 'Novel N-terminal and Lysine Methyltransferases That Target Translation Elongation Factor 1A in Yeast and Human', *Mol Cell Proteomics*, 15, 164-76.

Haziza, S., Magnani, R., Lan, D., Keinan, O., Saada, A., Hershkovitz, E., Yanay, N., Cohen, Y., Nevo, Y., Houtz, R. L., Sheffield, V. C., Golan, H. and Parvari, R. 2015. 'Calmodulin Methyltransferase Is Required for Growth, Muscle Strength, Somatosensory Development and Brain Function', *PLoS Genet*, 11, e1005388.

He, Y., Korboukh, I., Jin, J. and Huang, J. 2012. 'Targeting protein lysine methylation and demethylation in cancers', *Acta Biochim Biophys Sin (Shanghai)*, 44, 70-9.

Homs, A., Codina-Sola, M., Rodriguez-Santiago, B., Villanueva, C. M., Monk, D., Cusco, I. and Perez-Jurado, L. A. 2016. 'Genetic and epigenetic methylation defects and implication of the ERMN gene in autism spectrum disorders', *Transl Psychiatry*, 6, e855.

Hsu, Tien, Steeg, P. S., Zollo, M., and Wieland, T. 2015. 'Progress on Nme (NDP kinase/Nm23/Awd) gene family-related functions derived from animal model systems: studies on development, cardiovascular disease, and cancer metastasis exemplified', *Naunyn-Schmiedeberg's Archives of Pharmacology*, 388, 109-17.

Huang, J., Hsu, Y. H., Mo, C., Abreu, E., Kiel, D. P., Bonewald, L. F., Brotto, M. and Karasik, D. 2014. 'METTL21C is a potential pleiotropic gene for osteoporosis and sarcopenia acting through the modulation of the NF-kappaB signaling pathway', *J Bone Miner Res*, 29, 1531-40.

Imamura, S., Ojima, N., Yamashita, M. Cold-inducible expression of the cell division cycle gene CDC48 and its promotion of cell proliferation during cold acclimation in zebrafishcells. FEBS Lett. 2003, 549:14–20. [PubMed: 12914916]

Jakobsson, M. E., Moen, A., Bousset, L., Egge-Jacobsen, W., Kernstock, S., Melki, R. and Falnes, P. O. 2013. 'Identification and characterization of a novel human methyltransferase modulating Hsp70 protein function through lysine methylation', *J Biol Chem*, 288, 27752-63.

Jakobsson, M. E., Moen, A., Davidson, B., and Falnes, P. O. (2015) Hsp70 (HSPA1) Lysine Methylation Status as a Potential Prognostic Factor in Metastatic High-Grade Serous Carcinoma. *PloS one* 10, e0140168

Jakobsson, M. E., Moen, A., and Falnes, P. O. (2016) Correspondence: On the enzymology and significance of HSPA1 lysine methylation. *Nature communications* 7, 11464

Jansson, Martin, Stephen, T,. Durant, Cho, E., Sheahan, S., Edelmann, M., Kessler, B. and Thangue, N. B. L. 2008. 'Arginine methylation regulates the p53 response', *Nat Cell Biol*, 10, 1431-39.

Jones, Brendan, Su, H., Bhat, A., Lei, H., Bajko, J., Hevi, S., Baltus, G. A., Kadam, S., Zhai, H., Valdez, R., Gonzalo, S., Zhang, Y., Li, E. and Chen, T. 2008. 'The Histone H3K79 Methyltransferase Dot1L Is Essential for Mammalian Development and Heterochromatin Structure', *PLoS Genet*, 4, e1000190.

Katz, J. E., Dlakic, M. and Clarke, S. 2003. 'Automated identification of putative methyltransferases from genomic open reading frames', *Mol Cell Proteomics*, 2, 525-40.

Kernstock, S., Davydova, E., Jakobsson, M., Moen, A., Pettersen, S., Maelandsmo, G. M., Egge-Jacobsen, W. and Falnes, P. O. 2012. 'Lysine methylation of VCP by a member of a novel human protein methyltransferase family', *Nat Commun*, 3, 1038.

Khacho, M., Mekhail, K., Pilon-Larose, K., Pause, A., Cote, J. and Lee, S. 2008. 'eEF1A is a novel component of the mammalian nuclear protein export machinery', *Mol Biol Cell*, 19, 5296-308.

Klein, J. B., Barati, M. T., Wu, R., Gozal, D., Sachleben, L. R. Jr., Kausar, H., Trent, J. O., Gozal, E. and Rane, M. J. 2005. 'Akt-mediated Valosin-containing Protein 97 Phosphorylation Regulates Its Association with Ubiquitinated Proteins', *J. Biol. Chem*, 280, 31870-81.

Kraal, B., Lippmann, C. and Kleanthous, C. 1999. 'Translational regulation by modifications of the elongation factor Tu', *Folia Microbiologica*, 44, 131-41.

Lanouette, S., Mongeon, V., Figeys, D. and Couture, J. F. 2014. 'The functional diversity of protein lysine methylation', *Mol Syst Biol*, 10, 724.

Lee, D. Y., Teyssier, C., Strahl, B. D. and Stallcup, M. R. 2005. 'Role of protein methylation in regulation of transcription', *Endocr Rev*, 26, 147-70.

Li, D., Wei, T., Abbott, C. M. and Harrich, D. 2013. 'The unexpected roles of eukaryotic translation elongation factors in RNA virus replication and pathogenesis', *Microbiol Mol Biol Rev*, 77, 253-66.

Li, D., Wei, T., Rawle, D. J., Qin, F., Wang, R., Soares, D. C., Jin, H., Sivakumaran, H., Lin, M. H., Spann, K., Abbott, C. M. and Harrich, D. 2015. 'Specific Interaction between eEF1A and HIV RT Is Critical for HIV-1 Reverse Transcription and a Potential Anti-HIV Target', *PLoS Pathog*, 11, e1005289.

Lipson, R. S., Webb, K. J. and Clarke, S. G. 2010. 'Two novel methyltransferases acting upon eukaryotic elongation factor 1A in Saccharomyces cerevisiae', *Arch Biochem Biophys*, 500, 137-43.

Liu, R. J., Long, T., Zhou, M., Zhou, X. L. and Wang, E. D. 2015. 'tRNA recognition by a bacterial tRNA Xm32 modification enzyme from the SPOUT methyltransferase superfamily', *Nucleic Acids Res*, 43, 7489-503.

Liu, Zexian, Wang, Y., Gao, T., Pan, Z., Cheng, H., Yang, Q., Cheng, Z., Guo, A., Ren, J. and Xue, Y. 2014. 'CPLM: a database of protein lysine modifications', *Nucleic Acids Res*, 42, D531-D36.

Magnani, R., Chaffin, B., Dick, E., Bricken, M. L., Houtz, R. L. and Bradley, L. H. 2012. 'Utilization of a calmodulin lysine methyltransferase co-expression system for the generation of a combinatorial library of post-translationally modified proteins', *Protein Expr Purif*, 86, 83-8.

Magnani, R., Dirk, L. M., Trievel, R. C. and Houtz, R. L. 2010. 'Calmodulin methyltransferase is an evolutionarily conserved enzyme that trimethylates Lys-115 in calmodulin', *Nat Commun*, 1, 43.

Malecki, J., Dahl, H. A., Moen, A., Davydova, E. and Falnes, P. O. 2016. 'The METTL20 Homologue from Agrobacterium tumefaciens Is a Dual Specificity Protein-lysine Methyltransferase That Targets Ribosomal Protein L7/L12 and the beta Subunit of Electron Transfer Flavoprotein (ETFbeta)', *J Biol Chem*, 291, 9581-95.

Malecki, J., Ho, A. Y., Moen, A., Dahl, H. A. and Falnes, P. O. 2015. 'Human METTL20 is a mitochondrial lysine methyltransferase that targets the beta subunit of electron transfer flavoprotein (ETFbeta) and modulates its activity', *J Biol Chem*, 290, 423-34.

Mansilla, F., Friis, I., Jadidi, M., Nielsen, K. M., Clark, B. F. and Knudsen, C. R. (2002) Mapping the human translation elongation factor eEF1H complex using the yeast two-hybrid system. *The Biochemical journal* 365, 669-676

Martin, C. and Zhang, Y. 2005. 'The diverse functions of histone lysine methylation', *Nat Rev Mol Cell Biol*, 6, 838-49.

Martin, J. L. and McMillan, F. M. 2002. 'SAM (dependent) I AM: the S-adenosylmethionine-dependent methyltransferase fold', *Curr Opin Struct Biol*, 12, 783-93.

Mateyak, M. K. and Kinzy, T. G. 2010. 'eEF1A: thinking outside the ribosome', *J Biol Chem*, 285, 21209-13.

Mato, Jose, M., Corrales, F. J., Lu, S. C. and Avila, M. A. 2002. 'S-Adenosylmethionine: a control switch that regulates liver function', *The FASEB Journal*, 16, 15-26.

Meyer, H. and Weihl, C. C. 2014. 'The VCP/p97 system at a glance: connecting cellular function to disease pathogenesis', *J Cell Sci*, 127, 3877-83.

Milne, T. A., Briggs, S. D., Brock, H. W., Martin, M. E., Gibbs, D., Allis, C. D. and Hess, J. L. 2002. 'MLL Targets SET Domain Methyltransferase Activity to *Hox* Gene Promoters', *Mol. Cell*, 10, 1107–1117.

Mittal, Nimisha, Subramanian, G., Bütikofer, P. and Madhubala, M. 2013. 'Unique posttranslational modifications in eukaryotic translation factors and their roles in protozoan parasite viability and pathogenesis', *Molecular and Biochemical Parasitology*, 187, 21-31.

Moore, R. C., Durso, N. A. and Cyr, R. J. (1998) Elongation factor-1α stabilizes microtubules in a calcium/calmodulin-dependent manner. *Cell Motility and the Cytoskeleton*, 41, 168-180

Mori-Konya, C., Kato, N., Maeda, R., Yasuda, K., Higashimae, N., Noguchi, M., Koike, M., Kimura, Y., Ohizumi, H., Hori, S. and Kakizuka, A. 2009. 'p97/valosin-containing protein (VCP) is highly modulated by phosphorylation and acetylation', *Genes Cells*, 14, 483-97.

Murray, K. 1964. 'The occurrence of epsilon-N-methyl lysine in histones', *Biochemistry*, 3, 10-5.

Nguyen, Tram, A. and Zhang, Y. 2011. 'The diverse functions of Dot1 and H3K79 methylation', *Genes Dev*, 25, 1345-58.

Niwa, H., Ewens, C. A., Tsang, C., Yeung, H. O., Zhang, X. and Freemont, P. S. 2012. 'The role of the N-domain in the ATPase activity of the mammalian AAA ATPase p97/VCP', *J Biol Chem*, 287, 8561-70.

Novosylna, O., Doyle, A., Vlasenko, D., Murphy, M., Negrutskii, B. and El'skaya, A. 2016. 'Comparison of the ability of mammalian eEF1A1 and its oncogenic variant eEF1A2 to interact with actin and calmodulin', *Biol Chem*.

Paik, W. K., Paik, D. C. and Kim, S. 2007. 'Historical review: the field of protein methylation', *Trends Biochem Sci*, 32, 146-52.

Pei, Huadong, Zhang, L., Luo, K., Qin, Y., Chesi, M., Fei, F., Bergsagel, P. L., Wang, L., You, Z. and Lou, Z. 2011. 'MMSET regulates histone H4K20 methylation and 53BP1 accumulation at DNA damage sites', *Nature*, 470, 124-28.

Peters, J. M., Walsh, M. J. and Franke, W. W. 1990. 'An abundant and ubiquitous homo-oligomeric ring-shaped ATPase particle related to the putative vesicle fusion proteins Sec18p and NSF', *EMBO J*, 9, 1757-67.

Petrossian, T. C. and Clarke, S. G. 2009. 'Multiple Motif Scanning to identify methyltransferases from the yeast proteome', *Mol Cell Proteomics*, 8, 1516-26.

Petrossian, T. C. and Clarke, S. G. 2011. 'Uncovering the human methyltransferasome', *Mol Cell Proteomics*, 10, M110 000976.

Pittman, Y. R., Valente, L., Jeppesen, M. G., Andersen, G. R., Patel, S. and Kinzy, T. G. 2006. 'Mg2+ and a key lysine modulate exchange activity of eukaryotic translation elongation factor 1B alpha', *J Biol Chem*, 281, 19457-68.

Polevoda, B. and Sherman, F. 2007. 'Methylation of proteins involved in translation', *Mol Microbiol*, 65, 590-606.

Pradhan, S., Chin, H. G., Esteve, P. O. and Jacobsen, S. E. 2009. 'SET7/9 mediated methylation of non-histone proteins in mammalian cells', *Epigenetics*, 4, 383-7.

Qian, C. and Zhou, M. M. 2006. 'SET domain protein lysine methyltransferases: Structure, specificity and catalysis', *Cell Mol Life Sci*, 63, 2755-63.

Reiff, R. E., Ali, B. R., Baron, B., Yu, T. W., Ben-Salem, S., Coulter, M. E., Schubert, C. R., Hill, R. S., Akawi, N. A., Al-Younes, B., Kaya, N., Evrony, G. D., Al-Saffar, M., Felie, J. M., Partlow, J. N., Sunu, C. M., Schembri-Wismayer, P., Alkuraya, F. S., Meyer, B. F., Walsh, C. A., Al-Gazali, L. and Mochida, G. H. 2014. 'METTL23, a transcriptional partner of GABPA, is essential for human cognition', *Hum Mol Genet*, 23, 3456-66.

Rhein, V. F., Carroll, J., He, J., Ding, S., Fearnley, I. M. and Walker, J. E. 2014. 'Human METTL20 methylates lysine residues adjacent to the recognition loop of the electron transfer flavoprotein in mitochondria', *J Biol Chem*, 289, 24640-51.

Rosmarin, A. G., Resendes, K. K., Yang, Z., McMillan, J. N. and Fleming, S. L. 2004. 'GA-binding protein transcription factor: a review of GABP as an integrator of intracellular signaling and protein-protein interactions', *Blood Cells Mol Dis*, 32, 143-54.

Rust, H. L., Zurita-Lopez, C. I., Clarke, S. and Thompson, P. R. 2011. 'Mechanistic studies on transcriptional coactivator protein arginine methyltransferase 1', *Biochemistry*, 50, 3332-45.

Sasikumar, A. N., Perez, W. B. and Kinzy, T. G. 2012. 'The many roles of the eukaryotic elongation factor 1 complex', *Wiley Interdiscip Rev RNA*, 3, 543-55.

Schapira, M. and Ferreira de Freitas, R. 2014. 'Structural biology and chemistry of protein arginine methyltransferases', *Medchemcomm*, 5, 1779-88.

Schubert, H. L., Blumenthal, R. M. and Cheng, X. 2003. 'Many paths to methyltransfer: a chronicle of convergence', *Trends Biochem Sci*, 28, 329-35.

Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A. and Shi, Y. 2004. 'Histone Demethylation Mediated by the Nuclear Amine Oxidase Homolog LSD1' *Cell,* 119, 941-53.

Shimazu, T., Barjau, J., Sohtome, Y., Sodeoka, M. and Shinkai, Y. 2014. 'Selenium-based S-adenosylmethionine analog reveals the mammalian seven-beta-strand methyltransferase METTL10 to be an EF1A1 lysine methyltransferase', *PLoS One*, 9, e105394.

Shmueli, A., Tsai, Y. C., Yang, M., Braun, M. A. and Weissman, A. M. 2009. 'Targeting of gp78 for ubiquitin-mediated proteasomal degradation by Hrd1: cross-talk between E3s in the endoplasmic reticulum', *Biochem Biophys Res Commun*, 390, 758-62.

Signorell, A., Jelk, J., Rauch, M. and Butikofer, P. 2008. 'Phosphatidylethanolamine is the precursor of the ethanolamine phosphoglycerol moiety bound to eukaryotic elongation factor 1A', *J Biol Chem*, 283, 20320-9.

Soares, D. C., Barlow, P. N., Newbery, H. J., Porteous, D. J. and Abbott, C. M. 2009. 'Structural models of human eEF1A1 and eEF1A2 reveal two distinct surface clusters of sequence variation and potential differences in phosphorylation', *PLoS One*, 4, e6315.

Song, Changcheng, Wang, Q. and Li, C. H. 2003. 'ATPase Activity of p97-Valosin-containing Protein (VCP): D2 mediates the major enzyme activity, and D1 contributes to the heat-induced activity', *Journal of Biological Chemistry*, 278, 3648-55.

Songe-Moller, L., Born, E. V. D., Leihne, V., Vagbo, C. B., Kristoffersen, T., Krokan, H. E., Kirpekar, F., Falnes, P. O. and Klungland, A. 2010. 'Mammalian ALKBH8 possesses tRNA methyltransferase activity required for the biogenesis of multiple wobble uridine modifications implicated in translational decoding', *Mol Cell Biol*, 30, 1814-27.

Svobodova, K., Horak, P., Stratil, A., Bartenschlager, H., Poucke, M. V., Chalupova, P., Dvorakova, V., Knorr, C., Stupka, R., Citek, J., Sprysl, M., Palanova, A., Peelman, L. J., Geldermann, H. and Knoll, A. 2015. 'Porcine EEF1A1 and EEF1A2 genes: genomic structure, polymorphism, mapping and expression', *Mol Biol Rep*, 42, 1257-64.

Talapatra, S., Wagner, J. D. and Thompson, C. B. 2002. 'Elongation factor-1 alpha is a selective regulator of growth factor withdrawal and ER stress-induced apoptosis', *Cell Death Differ*, 9, 856-61.

Tang, J., Frankel, A., Cook, R. J., Kim, S., Paik, W. K., Williams, K. R., Clarke, S. and Herschman, H. R. 2000. 'PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells', *J Biol Chem*, 275, 7723-30.

Tempel, W., Hong, B. S., Seitova, A., He, H., Li, Y., Graslund, S., Arrowsmith, C. H., Edwards, A. M., Brown, P. J. and Structural Genomics Consortium. 2014. DOI: 10.2210/pdb4qpn/pdb.

Thiele, W., Novac, N., Mink, S., Schreiber, C., Plaumann, D., Fritzmann, J., Cremers, N., Rothley, M., Schwager, C., Regiert, T., Huber, P. E., Stein, U., Schlag, P., Moll, J.,

Abdollahi, A. and Sleeman, J. P. 2011. 'Discovery of a novel tumour metastasis-promoting gene, NVM-1', *J Pathol*, 225, 96-105.

Tkaczuk, K. L., Dunin-Horkawicz, S., Purta, E. and Bujnicki, J. M. 2007. 'Structural and evolutionary bioinformatics of the SPOUT superfamily of methyltransferases', *BMC Bioinformatics*, 8, 73.

Tsukada, Yu-ichi, Fang, J., Erdjument-Bromage, H., Warren, M. E., Borchers, C. H., Tempst, P. and Zhang, Y. 2006. 'Histone demethylation by a family of JmjC domain-containing proteins', *Nature*, 439, 811-16.

Velichkova, Polina, and Himo, F. 2005. 'Methyl Transfer in Glycine N-Methyltransferase. A Theoretical Study', *The Journal of Physical Chemistry B*, 109, 8216-19.

Voorhees, R. M. and Ramakrishnan, V. (2013) Structural basis of the translational elongation cycle. *Annual review of biochemistry,* 82, 203-236

Warren, K., Wei, T., Li, D., Qin, F., Warrilow, D., Lin, M. H., Sivakumaran, H., Apolloni, A., Abbott, C. M., Jones, A., Anderson, J. L. and Harrich, D. (2012) Eukaryotic elongation factor 1 complex subunits are critical HIV-1 reverse transcription cofactors. *Proceedings of the National Academy of Sciences*, 109, 9587-9592

Watts, G. D., Thomasova, D., Ramdeen, S. K., Fulchiero, E. C., Mehta, S. G., Drachman, D. A., Weihl, C. C., Jamrozik, Z., Kwiecinski, H., Kaminska, A. and Kimonis, V. E. 2007. 'Novel VCP mutations in inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia', *Clin Genet*, 72, 420-6.

Webb, K. J., Zurita-Lopez, C. I., Al-Hadid, Q., Laganowsky, A., Young, B. D., Lipson, R. S., Souda, P., Faull, K. F., Whitelegge, J. P. and Clarke, S. G. 2010. 'A novel 3-methylhistidine modification of yeast ribosomal protein Rpl3 is dependent upon the YIL110W methyltransferase', *J Biol Chem*, 285, 37598-606.

Whiteheart, S. W. and Hart, G. W. 1994. 'Incorporation of [3H]ethanolamine into a single cytosolic protein in a cell free system: ethanolaminylation of EF-1 alpha in vitro', *Arch Biochem Biophys*, 309, 387-91.

Winter, D. L., Abeygunawardena, D., Hart-Smith, G., Erce, M. A. and Wilkins, M. R. 2015. 'Lysine methylation modulates the protein-protein interactions of yeast cytochrome C Cyc1p', *Proteomics*, 15, 2166-76.

Xia, D., Tang, W. K. and Ye, Y. 2016. 'Structure and function of the AAA+ ATPase p97/Cdc48p', *Gene*, 583, 64-77.

Yamanaka, K., Sasagawa, Y. and Ogura, T. 2012a. 'Recent advances in p97/VCP/Cdc48 cellular functions', *Biochim Biophys Acta*, 1823, 130-7.

Yamanaka, Kunitoshi, Sasagawa, Y. and Ogura, T. 2012b. 'Recent advances in p97/VCP/Cdc48 cellular functions', *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1823, 130-37.

Yang, Yanzhong, and Bedford, M. T. 2013. 'Protein arginine methyltransferases and cancer', *Nat Rev Cancer*, 13, 37-50.

Ye, Y., Meyer, H. H. and Rapoport, T. A. 2003. 'Function of the p97-Ufd1-Npl4 complex in retrotranslocation from the ER to the cytosol: dual recognition of nonubiquitinated polypeptide segments and polyubiquitin chains', *J Cell Biol*, 162, 71-84.

Young, B. D., Weiss, D. I., Zurita-Lopez, C. I., Webb, K. J., Clarke, S. G. and McBride, A. E. 2012. 'Identification of methylated proteins in the yeast small ribosomal subunit: a role for SPOUT methyltransferases in protein arginine methylation', *Biochemistry*, 51, 5091-104.

Yu, J., Cao, Q., Mehra, R., Laxman, B., Yu, J., Tomlins, S. A., Creighton, C. A., Dhanasekaran, S. M., Shen, R., Chen, G., Morris, D. S., Marquez, V. E., Shah, R. B., Ghosh, D., Varambally, S. and Chinnaiyan, A. M. 2007. 'Integrative Genomics Analysis Reveals Silencing of b-Adrenergic Signaling by Polycomb in Prostate Cancer' *Cancer Cell* 12, 419–431.

Zhang, X., Huang, Y. and Shi, X. 2015. 'Emerging roles of lysine methylation on non-histone proteins', *Cell Mol Life Sci*, 72, 4257-72.

Zhang, X., Shaw, A., Bates, P. A., Newman, R. H., Gowen, B., Orlova, E., Gorman, M. A., Kondo, H., Dokurno, P., Lally, J., Leonard, G., Meyer, H., Heel, M. V. and Freemont, P. S. 2000. 'Structure of the AAA ATPase p97', *Mol Cell*, 6 1473-84.

Zhang, X., Zhou, L. and Cheng, X. 2000. 'Crystal structure of the conserved core of protein arginine methyltransferase PRMT3', *EMBO J*, 19, 3509-19.

Zhang, X., Wen, H. and Shi, X. (2012) Lysine methylation: beyond histones. *Acta biochimica et biophysica Sinica*, 44, 14-27

Zhao, G., Zhou, X., Wang, L., Li, G., Schindelin, H. and Lennarz, W. J. 2007. 'Studies on peptide:N-glycanase-p97 interaction suggest that p97 phosphorylation modulates endoplasmic reticulum-associated degradation', *Proc Natl Acad Sci U S A*, 104, 8785-90.