Citation:
Jethmalani Yogita and Green M. Erin *, Using Yeast to Define the Regulatory Role of Protein Lysine Methylation, Current Protein & Peptide Science 2020; 21(7) . https://dx.doi.org/10.2174/1389203720666191023150727

DOI:
https://dx.doi.org/10.2174/1389203720666191023150727

HHS Public Access
Author manuscript
*Curr Protein Pept Sci*. Author manuscript; available in PMC 2021 January 01.

Published in final edited form as:
*Curr Protein Pept Sci*. 2020 ; 21(7): 690–698. doi:10.2174/1389203720666191023150727.

# Using yeast to define the regulatory role of protein lysine methylation

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## Abstract

The post-translational modification (PTM) of proteins are crucial for cells to survive under diverse environmental conditions and to respond to stimuli. PTMs are known to govern a broad array of cellular processes including signal transduction and chromatin regulation. The PTM lysine methylation has been extensively studied within the context of chromatin and the epigenetic regulation of the genome. However, it has also emerged as a critical regulator of non-histone proteins important for signal transduction pathways. While the number of known non-histone protein methylation events is increasing, the molecular functions of many of these modifications are not yet known. Proteomic studies of the model system *Saccharomyces cerevisiae* suggest lysine methylation may regulate a diversity of pathways including transcription, RNA processing, translation, and signal transduction cascades. However, there has still been relatively little investigation of lysine methylation as a broad cellular regulator beyond chromatin and transcription. Here, we outline our current state of understanding of non-histone protein methylation in yeast and propose ways in which the yeast system can be leveraged to develop a much more complete picture of molecular mechanisms through which lysine methylation regulates cellular functions.

### Keywords

post-translational modification; methylation; yeast; signaling; transcription; translation

## 1. INTRODUCTION

The ability of cells to respond rapidly to their environment is dependent on an array of signal transduction cascades, which frequently rely on dynamic changes in protein-protein interactions, post-translational modification (PTM) of proteins, and the activation and repression of specialized gene expression programs. In particular, PTMs such as phosphorylation, acetylation, ubiquitylation, and methylation, have all been implicated in nuclear and cytoplasmic signaling events important for regulating cellular responses to stress and environmental changes. Of these, the role for methylation as a key signaling regulator has been recently expanding [36] The amino acids lysine, arginine, glutamate, aspartate, and

CONFLICT OF INTEREST
The authors declare no conflict of interest.

histidine can each be methylated [74], and these marks are dynamically regulated by methyltransferases and demethylases, which add or remove the marks, respectively. In this review, we focus on methylation at lysine residues, as this PTM has been detected on a diversity of protein targets. In particular, lysine methylation is well-characterized as a regulator of gene expression programs and genomic functions through the modification of histone proteins. However, there is also extensive biochemical and proteomic evidence for its presence on numerous other types of proteins, often referred to as non-histone protein methylation. Here, we focus on defining the current understanding of non-histone lysine methylation in the model eukaryote *Saccharomyces cerevisiae* and highlight approaches that can be applied to this easily-manipulated system to more completely elucidate the role of lysine methylation in regulating signaling events throughout the proteome.

## 2. BUDDING YEAST LYSINE METHYLTRANSFERASES

Lysine methyltransferases (KMTs) described to date contain one of two protein domains known to confer catalytic activity on the enzymes: the SET domain (named for Su(var)3-9, enhancer of zeste, and trithorax) and the seven-β-strand domain [3]. The majority of the KMTs with well-characterized activity in *Saccharomyces cerevisiae* are members of the SET domain family, which encompasses 12 proteins in budding yeast that can be loosely divided into two groups based on likely substrate specificity [12]. The first group is comprised of six proteins termed Set1 through Set6, which are known or predicted to target histones for methylation (Figure 1). Of these, Set1, Set2, and Set5 are known to catalyze methylation at H3K4, H3K36 and H4K5, K8, and K12, respectively (Table 1; [5, 19, 63]). Yeast Set1 is also reported to methylate the kinetochore component Dam1 [72], however it is not yet known whether Set2 or Set5 target non-histone proteins. Set5, and another member of this sub-group, Set6, are homologous to the mammalian SMYD proteins [6], which have been reported to have both histone and non-histone targets. While the subcellular localization of Set6 is not yet known, Set5 is found in both the nucleus and cytoplasm [19], suggesting it is also likely to have non-histone substrates.

The other two members of this sub-group, Set3 and Set4, contain divergent SET domains which contain amino acid alterations that are likely to impede catalytic activity [64]. Indeed, to date, methyltransferase activity has not been detected for either protein, although each protein has been reported to interact with chromatin [32, 50, 65]. In addition, Set3 is in a complex with two histone deacetylases, Hos2 and Hst1, as well as other factors [50], and has a well-documented role in the regulation of gene expression [32, 33].

The second sub-group of SET domain proteins appear to be primarily non-histone protein methyltransferases (Figure 1). Four enzymes within this group, named Rkm1 through Rkm4, modify ribosomal proteins (Table 1; [14, 52-54, 69]). In addition, Efm1 is known to target elongation factor eEF1A, responsible for bringing aminoacyl-tRNAs to the ribosome [14] and Ctm1 methylates cytochrome c [51]. Our current understanding of the biological roles for these non-histone SET domain methyltransferases is discussed in further detail below.

The other structural class of KMTs are the seven-β-strand proteins, which contain an alternating seven strand β-sheet with a C-terminal β-hairpin stacked between α-helices

within their catalytic domain [40, 48, 59]. In yeast, the enzymes Dot1, Rkm5, and Efm2 through Efm7 are members of this class (Figure 1; Table 1). Dot1, orthologous to mammalian Dot1L, is the only known histone methyltransferase of the seven-β-strand family in yeast, and is an H3K79 methyltransferase [43, 66]. Rkm5 and the Efm family are reported as non-histone protein methyltransferases, targeting ribosomal proteins and translation elongation factors, respectively [14, 38, 68], suggesting similar substrate specificity to SET domain-containing enzymes. Below, we also discuss the known roles of seven-β-strand KMTs in protein regulation via methylation.

## 3. BIOLOGICAL FUNCTIONS OF NON-HISTONE PROTEIN METHYLTRANSFERASES IN YEAST

### 3.1. Histone and non-histone methylation by Set1

Set1, the catalytic component of the COMPASS complex, is well-characterized as the sole histone H3K4 mono-, di-, and tri-methyltransferase in yeast and is linked to gene activation and repression in different cellular contexts [5, 27]. In addition, yeast cells lacking Set1 show severe mitotic defects and disrupted transcriptional programs throughout the cell cycle [2, 5, 26, 44]. While there appears to be a role for H3K4 methylation in some of these defects [2], Set1 has also been reported to methylate the kinetochore component Dam1 at K233 [72] in a manner dependent on the other members of the COMPASS complex as well as H2B K123 ubiquitylation [34]. Methylation of Dam1 has been shown to impede nearby phosphorylation of the protein by the Aurora kinase Ipl1, which targets Dam1 to regulate kinetochore-microtubule attachments [11], representing a phospho-methyl switch interaction [72]. To date, Dam1 is the only reported non-histone substrate of Set1, but it raises the possibility that Set1 may target other proteins for methylation in yeast, outside of its conserved role as a histone methyltransferase. While there is no direct ortholog of Dam1 in mammals, mammalian Set1 orthologs have been linked to diverse mitotic functions [1, 4] suggesting potential conservation of function within this KMT family. Further studies in yeast and mammalian systems may yet reveal new substrates for Set1/COMPASS and orthologous enzymes.

### 3.2. The Rkm enzymes: The SET domain-containing Rkm1 through Rkm4 and seven-β-strand Rkm5

Post-translational modification of the ribosome has been extensively probed using mass spectrometry. In yeast, at least six of the components of the large ribosomal subunit were identified as targets of methylation, including Rpl1ab, Rpl12ab, Rpl23ab, Rpl3, Rpl42ab, and Rpl43ab, and small subunit components such as Rps18ab are also known to be methylated [35, 52-54, 68, 69]. The SET domain-containing enzyme Rkm1 (ribosomal protein lysine (K) methyltransferase 1) catalyzes di-methylation of Rpl23ab at K105 and K109 [53, 54], as well as mono-methylation of Rps18ab at K48 [14]. Deletion of *RKM1* does not confer any obvious defects in ribosome assembly, stability, or translation [53, 54], however a more detailed analysis of yeast lacking Rkm1 or its specific methylation sites may reveal condition-specific roles for this modification. Structural analysis indicates that the methylated lysines within Rp123ab may interact with the small subunit 18S ribosomal RNA

[61, 62, 69], however further analysis is required to determine how methylation may regulate this interaction. Interestingly, Rkm1 itself is methylated and heavily phosphorylated in cells [71], suggesting the possibility of dynamic regulation of this enzyme.

Another large subunit protein, Rpl12ab, has also been shown to be tri-methylated by the enzyme Rkm2 primarily at K3 [52, 69], although it may have a secondary site at K10 [69]. Rpl12ab is similarly known to interact with the large subunit rRNA and this interaction may be influenced by lysine methylation, however further studies will be required to determine the functional significance of this modification. Similar to genetic experiments with *RKM1*, deletion of *RKM2* does not confer significant translational or ribosome stability defects on yeast [52].

The large subunit protein Rpl42ab is located in the ribosomal tRNA exit site and is in contact with the large subunit rRNA and tRNA [31]. It is mono-methylated by Rkm3 at K40 and Rkm4 at K55 [69]. Based on structural analysis, these mono-methyl marks have been predicted to modulate hydrogen bonding interactions between the protein and either the large subunit rRNA or tRNAs. Functional genetic analysis shows that deletion of *RKM4* confers increased sensitivity to the general translation inhibitor cycloheximide [69], suggesting a role for K55 methylation in protecting cells during translation stress.

Finally, the enzyme Rkm5 is currently the only known seven-β-strand KMT which targets ribosomal proteins in yeast. Rkm5 mono-methylates Rpl1ab at K46 in yeast, and it appears to be a stoichiometric methylation, as there is no un-methylated K46 in cells [68]. Rkm5 appears to be restricted to fungal species, suggesting it may have a specialized role, although its target Rpl1ab is well-conserved and the amino acids adjacent to the modified lysine are also highly conserved in eukaryotes. Further genetic analysis of *RKM5* mutant cells and of the target lysine in Rpl1ab may reveal the functional role for this methylation mark.

### 3.3. The cytochrome c methyltransferase Ctm1

The critical electron carrier cytochrome c (encoded by the *CYC1* gene in yeast) has been reported to contain methylated lysines at specific positions in certain plants and fungi. In budding yeast, the SET domain KMT Ctm1 trimethylates K72 of cytochrome c [51]. Interestingly, Ctm1 is only found in the cytoplasm, and not in mitochondria [51]. Early reports on cytochrome c suggested that cytosolic apocytochrome c is co-translationally methylated [16, 47]. Consistently, Ctm1-mediated methylation of Cyc1 has been proposed to help facilitate its import into the inter-membrane space of mitochrondria [10, 46], and protein-protein interaction studies suggest that methylation of Cyc1 promotes its association with at least two factors important for its function and import into the inter-membrane space, Evr1 and Cyc3 [70]. It is not yet known whether Ctm1 has additional substrates, and while there is not a clear mammalian ortholog for Ctm1 in mammals, extensive PTMs on cytochrome c have been detected and are known to regulate its function [30].

### 3.4. Elongation factor KMTs: Efm1 through Efm7

Protein components of the translation machinery are known to be highly methylated and are frequently detected in methyl-lysine proteomic studies [14, 42, 67, 73]. In particular, elongation factors are abundant targets of methyltransferases in budding yeast [14] including

the conserved proteins eEF1A, eEF2, and the yeast-specific protein EF3. To date, there are seven characterized KMTs known to target elongation factors, which include the SET domain-containing enzyme Efm1 and the seven-β-strand proteins Efm2 through Efm7. eEF1A is a highly conserved elongation factor that has been associated with diverse cellular functions, though one of its primary responsibilities is to bring aminoacyl tRNAs to the A-site of translating ribosomes [58]. Studies of methyltransferase activity in yeast have revealed eEF1A to be a highly-methylated protein targeted by multiple methyltransferases [22, 29]. Initial mass spectrometry analysis of eEF1A methyl marks suggested loss of mono-methylation in *efm1Δ* cells, particularly at K30, which is within a non-conserved region of the protein [14, 38]. However, a later study investigating the stoichiometry of lysine methyl marks showed that *efm1Δ* cells still retain K30me1, and that deletion of Efm4/See1 also reduced eEF1A K30 me1 [24]. The double mutant combination has not been reported to be tested to determine the epistatic relationship between these two enzymes. It is possible that both Efm1 and Efm4/See1 separately target eEF1A K30, or they potentially interact with each other or function together to catalyze this methylation. Although Efm4/See1 is able to directly methylate eEF1A at K316 *in vitro*, it does not methylate K30 *in vitro*, whereas Efm1 is able to methylate K30 and K253 *in vitro* [21, 38]. Further biochemical investigation may reveal whether these proteins are associated with complexes or other co-factors that direct methylation of eEF1A K30 in cells. Additionally, there are relatively few phenotypes associated with loss of Efm1 in cells, so further genetic analysis will help elucidate potential functions of this methyl mark in translation or other eEF1A-dependent pathways.

In addition to K30 methylation, eEF1A is methylated at a number of other sites, including di-methylation at K3, tri-methylation at K79, di-methylation at K316, and mono-methylation at K390 [14, 38]. Methylation at these sites is catalyzed by the seven-β-strand KMTs Efm7, Efm5, Efm4/See1, and Efm6, respectively. As mentioned above, Efm4/See1 is a potential K30 methyltransferase, but also catalyzes eEF1A K316me2 [14, 38]. Unlike Efm1, Efm4/See1 is highly conserved, with its closest human ortholog likely to be METTL10 [38], which has been demonstrated to tri-methylate eEF1A at K318 in mammalian cells, which is equivalent to K316 in yeast [60]. Phenotypic analysis of cells lacking Efm4/See1 show a defect in endocytosis [41] and implicate it in promoting virus replication in yeast [37]. In particular, *efm4Δ* cells show reduced viral replication of multiple types of viruses in yeast and decreased protein half-life of viral proteins [37]. Mutation of multiple methylation sites in eEF1A showed similar phenotypes, although further investigation is required to directly link specific methylation sites to eEF1A function in pathways required for viral replication and protein stability.

Mass spectrometry studies of cell extracts and *in vitro* methylation assays with recombinant proteins have demonstrated a clear dependence of tri-methylation at K79 of eEF1A on the KMT Efm5 [17, 23]. The K79 residue of eEF1A is well-conserved, and methylation is predicted at this site in other eukaryotes [17], although conservation of Efm5 is less penetrant. Orthologs of this enzyme are found in mammals and other vertebrates, although not in all fungal species, suggesting a possible condition-specific or non-essential role for this modification [17]. The human enzyme N6AMT2 has been shown to have similar trimethyltransferase activity on K79 of human eEF1A and is the expected ortholog of yeast Efm5 [23]. Efm6 has also been identified as the mono-methyltransferase targeting K390 of

yeast eEF1A, although it does not have a clear mammalian ortholog and methylation at this site in humans does not appear to be conserved [28]. Finally, Efm7 has been shown to possess both N-terminal methyltransferase activity and to perform methylation on the adjacent lysine at K3 of eEF1A [23], suggesting a dual specificity enzyme. Together, these findings represent the extensive progress in characterizing the enzymes responsible for lysine methylation of this essential translation factor. However, there remain substantial gaps in our understanding of the functional roles of these methylation marks. The molecular and genetic tools of *S. cerevisiae* should provide a valuable avenue for further investigation of the functional consequences of these marks on protein synthesis and other pathways.

In addition to eEF1A, the elongation factor eEF2 is also subject to lysine methylation. Efm2 performs di-methylation at K613 of eEF2 [14] and Efm3 catalyzes eEF2 K509 tri-methylation [18]. K613me2 appears to be a fungal-specific mark and Efm2 is not highly conserved [18], whereas Efm3 is orthologous to the human enzyme FAM86A, which targets an analogous residue (K525) on human eEF2 [15]. Intriguingly, yeast cells lacking either Efm2 or Efm3 do show translation-associated defects. Both *efm2Δ* and *efm3Δ* cells are sensitive to some translation inhibitors [18], [15]. In one report, cells missing Efm2 (but not Efm3) showed a modest increase in stop codon read-through using a reporter assay, and no change in amino acid misincorporation or frameshifting was detected in either mutant compared to wildtype cells [18]. However, a second report demonstrated that Efm3 helps prevent −1 ribosomal frameshifting [15], suggesting that methylation at K509 may be important for translational accuracy. Further biochemical and molecular studies will be required to determine the mechanism by which methylation by either Efm2 or Efm3 affects eEF2 function and the fidelity of protein synthesis.

## 4. ADVANCING OUR UNDERSTANDING OF LYSINE METHYLATION SIGNALING THROUGH PROTEOMIC, BIOCHEMICAL, AND GENETIC ASSAYS IN YEAST

Although significant progress has been made in characterizing the protein methylome in yeast, the extent to which lysine methylation broadly regulates protein function is still not known. Proteomic studies suggest wide-spread lysine methylation on a diversity of protein targets. Table 2 lists methyl-lysine marks which have been identified in yeast by mass spectrometry but have not yet been ascribed to an enzyme. Due to limitations in current approaches and only a small number of conditions tested, this list of methylated proteins is almost certainly incomplete. Additionally, there are candidate KMTs for which no substrates have yet been identified [9, 14, 49, 73] and most KMTs have only been linked to a single substrate (see Table 1), although it is expected that a number of these enzymes will target more than one substrate.

The primary methods that have been applied to identifying lysine methylated proteins in yeast have been mass spectrometry-based approaches that rely on variations of heavy methyl stable isotope labeling of amino acids in cell culture (SILAC) and analyses of whole cell lysates or fractionated lysates [9, 67, 73]. The use of affinity purification with sequence non-specific methyl-lysine reader domains or immunoprecipitation with pan-methyl lysine

antibodies has been used in numerous contexts in mammalian cells [7, 8, 20, 39, 42, 45] however it has not been applied extensively in yeast. These approaches may be particularly useful within the context of gene knockout strains for known or candidate KMTs in order to specifically identify modification sites and their cognate enzymes.

Another strategy for identifying candidate targets of KMTs is based on consensus site identification or motif searching within the proteome. *In vitro* methylation assays have been used on peptide arrays spotted with sequences templated from known substrates for individual enzymes [55-57]. More recently, a sensitive, high-throughput approach has been applied to identifying preferential motifs for a series of human KMTs using lysine-oriented peptide libraries in which amino acid identities are randomized in the +/− 3 positions of the lysine. Recombinant KMTs are used for *in vitro* methylation assays on the peptides, and identified motifs can be used to search the proteome for potential substrates [13]. This method successfully identified motifs for a number of mammalian KMTs and validated potential new substrates using biochemical approaches. Hamey *et al.* [21] used a similar approach named methyltransferase motif analysis by mass spectrometry (MT-MAMS) in which mass spectrometry is used to quantify methylation by recombinant enzymes on pools of peptides of lysine- or arginine-containing peptides. This work identified potential new substrates for both lysine and arginine MTs and specifically tested a small number of yeast enzymes, including Efm1. The use of motif identification to ascertain candidate methylation substrates has potential for uncovering diverse new substrates in yeast, particularly given the small size of the proteome and the seemingly low redundancy between enzymatic activities. The number of candidate substrates with a particular motif are likely to be smaller than in mammalian systems, making a follow-up biochemical screen with recombinant enzymes or an *in vivo* mass spectrometry analysis feasible.

Further application of these proteomic strategies to yeast, as well as improvement of reagents for enriching for methylated proteins in cells, will certainly expand our understanding as to the extent of lysine methylation decorating the proteome and the types of substrates that are frequent targets for these modifications. For example, while histones, ribosomal proteins, and translation elongation factors are clearly abundant targets of many of the KMTs described to date, there are likely less abundant targets of KMTs that still remain to be identified. In addition, the dynamic regulation of non-histone protein methylation in yeast has not been extensively addressed. For example, of the currently known lysine demethylases in yeast, none of them have been linked to demethylation of non-histone substrates. Additionally, the current set of proteomic experiments identifying lysine methyl marks have largely been performed under standard growth conditions in the lab, however there are likely to be dynamic changes in the protein methylome in different environmental conditions. New marks may be identified and alterations to the stoichiometry of known marks may change when cells are subjected to different environmental conditions or stress. This is relatively straightforward to achieve in yeast, and many of the molecular interactions governing stress response pathways are known. Furthermore, collections of mutant strains, including straightforward mutation of specific modification sites in the endogenous gene locus of candidate substrates, can be quickly evaluated for well-characterized phenotypes linked to known signaling pathways. Combined with the lower redundancy in the enzymatic machinery regulating lysine methylation and the smaller proteome, further leveraging the

proteomic, molecular, and genetic tools available in yeast will substantially advance our understanding of the mechanisms through which lysine methylation regulates protein function.

## ACKNOWLEDGEMENTS

Y.J. and E.M.G. conceived of and wrote the manuscript. This work was supported in part by NIH grants R01GM124342 and R21AG064507 to E.M.G.

## REFERENCES

[1]. Ali A; Veeranki SN; Chinchole A; Tyagi S MLL/WDR5 Complex Regulates Kif2A Localization to Ensure Chromosome Congression and Proper Spindle Assembly during Mitosis. Dev Cell, 2017, 41(6), 605–622.e607. [PubMed: 28633016]

[2]. Beilharz TH; Harrison PF; Miles DM; See MM; Le UM; Kalanon M; Curtis MJ; Hasan Q; Saksouk J; Margaritis T; Holstege F; Geli V; Dichtl B Coordination of Cell Cycle Progression and Mitotic Spindle Assembly Involves Histone H3 Lysine 4 Methylation by Set1/COMPASS. Genetics, 2017, 205(1), 185–199. [PubMed: 28049706]

[3]. Black JC; Van Rechem C; Whetstine JR Histone lysine methylation dynamics: establishment, regulation, and biological impact. Mol Cell, 2012, 48(4), 491–507. [PubMed: 23200123]

[4]. Blobel GA; Kadauke S; Wang E; Lau AW; Zuber J; Chou MM; Vakoc CR A reconfigured pattern of MLL occupancy within mitotic chromatin promotes rapid transcriptional reactivation following mitotic exit. Mol Cell, 2009, 36(6), 970–983. [PubMed: 20064463]

[5]. Briggs SD; Bryk M; Strahl BD; Cheung WL; Davie JK; Dent SY; Winston F; Allis CD Histone H3 lysine 4 methylation is mediated by Set1 and required for cell growth and rDNA silencing in Saccharomyces cerevisiae. Genes Dev, 2001, 15(24), 3286–3295. [PubMed: 11751634]

[6]. Calpena E; Palau F; Espinó;s C; Galindo MI Evolutionary History of the Smyd Gene Family in Metazoans: A Framework to Identify the Orthologs of Human Smyd Genes in Drosophila and Other Animal Species. PLoS One, 2015, 10(7), e0134106. [PubMed: 26230726]

[7]. Cao XJ; Arnaudo AM; Garcia BA Large-scale global identification of protein lysine methylation in vivo. Epigenetics, 2013, 8(5), 477–485. [PubMed: 23644510]

[8]. Carlson SM; Moore KE; Green EM; Martín GM; Gozani O Proteome-wide enrichment of proteins modified by lysine methylation. Nat Protoc, 2014, 9(1), 37–50. [PubMed: 24309976]

[9]. Caslavka Zempel KE; Vashisht AA; Barshop WD; Wohlschlegel JA; Clarke SG Determining the Mitochondrial Methyl Proteome in Saccharomyces cerevisiae using Heavy Methyl SILAC. J Proteome Res, 2016, 15(12), 4436–4451. [PubMed: 27696855]

[10]. Cessay KJ; Bergman LW; Tuck MT Further investigations regarding the role of trimethyllysine for cytochrome c uptake into mitochondria. Int J Biochem, 1991, 23(7-8), 761–768. [PubMed: 1650724]

[11]. Cheeseman IM; Anderson S; Jwa M; Green EM; Kang J; Yates JR; Chan CS; Drubin DG; Barnes G Phospho-regulation of kinetochore-microtubule attachments by the Aurora kinase Ipl1p. Cell, 2002, 111(2), 163–172. [PubMed: 12408861]

[12]. Clarke SG Protein methylation at the surface and buried deep: thinking outside the histone box. Trends Biochem Sci, 2013, 38(5), 243–252. [PubMed: 23490039]

[13]. Cornett EM; Dickson BM; Krajewski K; Spellmon N; Umstead A; Vaughan RM; Shaw KM; Versluis PP; Cowles MW; Brunzelle J; Yang Z; Vega IE; Sun ZW; Rothbart SB A functional proteomics platform to reveal the sequence determinants of lysine methyltransferase substrate selectivity. Sci Adv, 2018, 4(11), eaav2623.

[14]. Couttas TA; Raftery MJ; Padula MP; Herbert BR; Wilkins MR Methylation of translation-associated proteins in Saccharomyces cerevisiae: Identification of methylated lysines and their methyltransferases. Proteomics, 2012, 12(7), 960–972. [PubMed: 22522802]

[15]. Davydova E; Ho AY; Malecki J; Moen A; Enserink JM; Jakobsson ME; Loenarz C; Falnes P Identification and characterization of a novel evolutionarily conserved lysine-specific

methyltransferase targeting eukaryotic translation elongation factor 2 (eEF2). J Biol Chem, 2014, 289(44), 30499–30510. [PubMed: 25231979]

[16]. DiMaria P; Polastro E; DeLange RJ; Kim S; Paik WK Studies on cytochrome c methylation in yeast. J Biol Chem, 1979, 254(11), 4645–4652. [PubMed: 220257]

[17]. Dzialo MC; Travaglini KJ; Shen S; Loo JA; Clarke SG A new type of protein lysine methyltransferase trimethylates Lys-79 of elongation factor 1A. Biochem Biophys Res Commun, 2014, 455(3-4), 382–389. [PubMed: 25446118]

[18]. Dzialo MC; Travaglini KJ; Shen S; Roy K; Chanfreau GF; Loo JA; Clarke SG Translational roles of elongation factor 2 protein lysine methylation. J Biol Chem, 2014, 289(44), 30511–30524. [PubMed: 25231983]

[19]. Green EM; Morrison AJ; Gozani O New marks on the block: Set5 methylates H4 lysines 5, 8 and 12. Nucleus, 2012, 3(4), 335–339. [PubMed: 22688645]

[20]. Guo A; Gu H; Zhou J; Mulhern D; Wang Y; Lee KA; Yang V; Aguiar M; Kornhauser J; Jia X; Ren J; Beausoleil SA; Silva JC; Vemulapalli V; Bedford MT; Comb MJ Immunoaffinity enrichment and mass spectrometry analysis of protein methylation. Mol Cell Proteomics, 2014, 13(1), 372–387. [PubMed: 24129315]

[21]. Hamey JJ; Separovich RJ; Wilkins MR MT-MAMS: Protein Methyltransferase Motif Analysis by Mass Spectrometry. J Proteome Res, 2018, 17(10), 3485–3491. [PubMed: 30156103]

[22]. Hamey JJ; Wilkins MR Methylation of Elongation Factor 1A: Where, Who, and Why? Trends Biochem Sci, 2018, 43(3), 211–223. [PubMed: 29398204]

[23]. Hamey JJ; Winter DL; Yagoub D; Overall CM; Hart-Smith G; Wilkins MR Novel N-terminal and Lysine Methyltransferases That Target Translation Elongation Factor 1A in Yeast and Human. Mol Cell Proteomics, 2016, 15(1), 164–176. [PubMed: 26545399]

[24]. Hart-Smith G; Chia SZ; Low JK; McKay MJ; Molloy MP; Wilkins MR Stoichiometry of Saccharomyces cerevisiae lysine methylation: insights into non-histone protein lysine methyltransferase activity. J Proteome Res, 2014, 13(3), 1744–1756. [PubMed: 24517342]

[25]. Huh WK; Falvo JV; Gerke LC; Carroll AS; Howson RW; Weissman JS; O'Shea EK Global analysis of protein localization in budding yeast. Nature, 2003, 425(6959), 686–691. [PubMed: 14562095]

[26]. Jaiswal D; Jezek M; Quijote J; Lum J; Choi G; Kulkarni R; Park D; Green EM Repression of Middle Sporulation Genes in Saccharomyces cerevisiae by the Sum1-Rfm1-Hst1 Comples is Maintained by Set1 and H3K4 Methylation. G3 (Bethesda), 2017, 7(12), 3971–3982. [PubMed: 29066473]

[27]. Jaiswal D; Turniansky R; Green EM Choose Your Own Adventure: The Role of Histone Modifications in Yeast Cell Fate. J Mol Biol, 2017, 429(13), 1946–1957. [PubMed: 27769718]

[28]. Jakobsson ME; Davydova E; Małecki J; Moen A; Falnes P Saccharomyces cerevisiae Eukaryotic Elongation Factor 1A (eEF1A) Is Methylated at Lys-390 by a METTL21-Like Methyltransferase. PLoS One, 2015, 10(6), e0131426. [PubMed: 26115316]

[29]. Jakobsson ME; Małecki J; Falnes P Regulation of eukaryotic elongation factor 1 alpha (eEF1A) by dynamic lysine methylation. RNA Biol, 2018, 15(3), 314–319. [PubMed: 29447067]

[30]. Kalpage HA; Bazylianska V; Recanati MA; Fite A; Liu J; Wan J; Mantena N; Malek MH; Podgorski I; Heath EI; Vaishnav A; Edwards BF; Grossman LI; Sanderson TH; Lee I; Hüttemann M Tissue-specific regulation of cytochrome c by post-translational modifications: respiration, the mitochondrial membrane potential, ROS, and apoptosis. FASEB J, 2019, 33(2), 1540–1553. [PubMed: 30222078]

[31]. Kawai S; Murao S; Mochizuki M; Shibuya I; Yano K; Takagi M Drastic alteration of cycloheximide sensitivity by substitution of one amino acid in the L41 ribosomal protein of yeasts. J Bacteriol, 1992, 174(1), 254–262. [PubMed: 1729213]

[32]. Kim T; Buratowski S Dimethylation of H3K4 by Set1 recruits the Set3 histone deacetylase complex to 5' transcribed regions. Cell, 2009, 137(2), 259–272. [PubMed: 19379692]

[33]. Kim T; Xu Z; Clauder-Münster S; Steinmetz LM; Buratowski S Set3 HDAC mediates effects of overlapping noncoding transcription on gene induction kinetics. Cell, 2012, 150(6), 1158–1169. [PubMed: 22959268]

[34]. Latham JA; Chosed RJ; Wang S; Dent SY Chromatin signaling to kinetochores: transregulation of Dam1 methylation by histone H2B ubiquitination. Cell, 2011, 146(5), 709–719. [PubMed: 21884933]

[35]. Lee SW; Berger SJ; Martinović S; Pasa-Tolić L; Anderson GA; Shen Y; Zhao R; Smith RD Direct mass spectrometric analysis of intact proteins of the yeast large ribosomal subunit using capillary LC/FTICR. Proc Natl Acad Sci U S A, 2002, 99(9), 5942–5947. [PubMed: 11983894]

[36]. Levy D Lysine methylation signaling of non-histone proteins in the nucleus. Cell Mol Life Sci, 2019, 76(15), 2873–2883. [PubMed: 31123776]

[37]. Li Z; Gonzalez PA; Sasvari Z; Kinzy TG; Nagy PD Methylation of translation elongation factor 1A by the METTL10-like See1 methyltransferase facilitates tombusvirus replication in yeast and plants. Virology, 2014, 448, 43–54. [PubMed: 24314635]

[38]. Lipson RS; Webb KJ; Clarke SG Two novel methyltransferases acting upon eukaryotic elongation factor 1A in Saccharomyces cerevisiae. Arch Biochem Biophys, 2010, 500(2), 137–143. [PubMed: 20510667]

[39]. Liu H; Galka M; Mori E; Liu X; Lin YF; Wei R; Pittock P; Voss C; Dhami G; Li X; Miyaji M; Lajoie G; Chen B; Li SS A method for systematic mapping of protein lysine methylation identifies functions for HP1β in DNA damage response. Mol Cell, 2013, 50(5), 723–735. [PubMed: 23707759]

[40]. Martin JL; McMillan FM SAM (dependent) I AM: the S-adenosylmethionine-dependent methyltransferase fold. Curr Opin Struct Biol, 2002, 12(6), 783–793. [PubMed: 12504684]

[41]. Martín-Granados C; Riechers SP; Stahl U; Lang C Absence of See1p, a widely conserved Saccharomyces cerevisiae protein, confers both deficient heterologous protein production and endocytosis. Yeast, 2008, 25(12), 871–877. [PubMed: 19160456]

[42]. Moore KE; Carlson SM; Camp ND; Cheung P; James RG; Chua KF; Wolf-Yadlin A; Gozani O A general molecular affinity strategy for global detection and proteomic analysis of lysine methylation. Mol Cell, 2013, 50(3), 444–456. [PubMed: 23583077]

[43]. Ng HH; Feng Q; Wang H; Erdjument-Bromage H; Tempst P; Zhang Y; Struhl K Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association. Genes Dev, 2002, 16(12), 1518–1527. [PubMed: 12080090]

[44]. Nislow C; Ray E; Pillus L SET1, a yeast member of the trithorax family, functions in transcriptional silencing and diverse cellular processes. Mol Biol Cell, 1997, 8(12), 2421–2436. [PubMed: 9398665]

[45]. Ong SE; Mittler G; Mann M Identifying and quantifying in vivo methylation sites by heavy methyl SILAC. Nat Methods, 2004, 1(2), 119–126. [PubMed: 15782174]

[46]. Paik WK; Park KS; Frost BF; Kim S Effect of enzymatic methylation on the import of in vitro synthesized apocytochrome C into mitochondria. Adv Exp Med Biol, 1988, 231, 317–325. [PubMed: 2843008]

[47]. Park KS; Frost B; Tuck M; Ho LL; Kim S; Paik WK Enzymatic methylation of in vitro synthesized apocytochrome c enhances its transport into mitochondria. J Biol Chem, 1987, 262(30), 14702–14708. [PubMed: 2822698]

[48]. Petrossian T; Clarke S Bioinformatic Identification of Novel Methyltransferases. Epigenomics, 2009, 1(1), 163–175. [PubMed: 20582239]

[49]. Petrossian TC; Clarke SG Multiple Motif Scanning to identify methyltransferases from the yeast proteome. Mol Cell Proteomics, 2009, 8(7), 1516–1526. [PubMed: 19351663]

[50]. Pijnappel WW; Schaft D; Roguev A; Shevchenko A; Tekotte H; Wilm M; Rigaut G; Séraphin B; Aasland R; Stewart AF The S. cerevisiae SET3 complex includes two histone deacetylases, Hos2 and Hst1, and is a meiotic-specific repressor of the sporulation gene program. Genes Dev, 2001, 15(22), 2991–3004. [PubMed: 11711434]

[51]. Polevoda B; Martzen MR; Das B; Phizicky EM; Sherman F Cytochrome c methyltransferase, Ctm1p, of yeast. J Biol Chem, 2000, 275(27), 20508–20513. [PubMed: 10791961]

[52]. Porras-Yakushi TR; Whitelegge JP; Clarke S A novel SET domain methyltransferase in yeast: Rkm2-dependent trimethylation of ribosomal protein L12ab at lysine 10. J Biol Chem, 2006, 281(47), 35835–35845. [PubMed: 17005568]

[53]. Porras-Yakushi TR; Whitelegge JP; Clarke S Yeast ribosomal/cytochrome c SET domain methyltransferase subfamily: identification of Rpl23ab methylation sites and recognition motifs. J Biol Chem, 2007, 282(17), 12368–12376. [PubMed: 17327221]

[54]. Porras-Yakushi TR; Whitelegge JP; Miranda TB; Clarke S A novel SET domain methyltransferase modifies ribosomal protein Rpl23ab in yeast. J Biol Chem, 2005, 280(41), 34590–34598. [PubMed: 16096273]

[55]. Rathert P; Dhayalan A; Ma H; Jeltsch A Specificity of protein lysine methyltransferases and methods for detection of lysine methylation of non-histone proteins. Mol Biosyst, 2008, 4(12), 1186–1190. [PubMed: 19396382]

[56]. Rathert P; Dhayalan A; Murakami M; Zhang X; Tamas R; Jurkowska R; Komatsu Y; Shinkai Y; Cheng X; Jeltsch A Protein lysine methyltransferase G9a acts on non-histone targets. Nat Chem Biol, 2008, 4(6), 344–346. [PubMed: 18438403]

[57]. Rathert P; Zhang X; Freund C; Cheng X; Jeltsch A Analysis of the substrate specificity of the Dim-5 histone lysine methyltransferase using peptide arrays. Chem Biol, 2008, 15(1), 5–11. [PubMed: 18215768]

[58]. Sasikumar AN; Perez WB; Kinzy TG The many roles of the eukaryotic elongation factor 1 complex. Wiley Interdiscip Rev RNA, 2012, 3(4), 543–555. [PubMed: 22555874]

[59]. Schubert HL; Blumenthal RM; Cheng X Many paths to methyltransfer: a chronicle of convergence. Trends Biochem Sci, 2003, 28(6), 329–335. [PubMed: 12826405]

[60]. Shimazu T; Barjau J; Sohtome Y; Sodeoka M; Shinkai Y Selenium-based S-adenosylmethionine analog reveals the mammalian seven-beta-strand methyltransferase METTL10 to be an EF1A1 lysine methyltransferase. PLoS One, 2014, 9(8), e105394. [PubMed: 25144183]

[61]. Spahn CM; Beckmann R; Eswar N; Penczek PA; Sali A; Blobel G; Frank J Structure of the 80S ribosome from Saccharomyces cerevisiae--tRNA-ribosome and subunit-subunit interactions. Cell, 2001, 107(3), 373–386. [PubMed: 11701127]

[62]. Spahn CM; Gomez-Lorenzo MG; Grassucci RA; Jørgensen R; Andersen GR; Beckmann R; Penczek PA; Ballesta JP; Frank J Domain movements of elongation factor eEF2 and the eukaryotic 80S ribosome facilitate tRNA translocation. EMBO J, 2004, 23(5), 1008–1019. [PubMed: 14976550]

[63]. Strahl BD; Grant PA; Briggs SD; Sun ZW; Bone JR; Caldwell JA; Mollah S; Cook RG; Shabanowitz J; Hunt DF; Allis CD Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression. Mol Cell Biol, 2002, 22(5), 1298–1306. [PubMed: 11839797]

[64]. Tran K; Green EM SET domains and stress: uncovering new functions for yeast Set4. Curr Genet, 2018.

[65]. Tran K; Jethmalani Y; Jaiswal D; Green EM Set4 is a chromatin-associated protein, promotes survival during oxidative stress, and regulates stress response genes in yeast. J Biol Chem, 2018, 293(37), 14429–14443. [PubMed: 30082318]

[66]. van Leeuwen F; Gafken PR; Gottschling DE Dot1p modulates silencing in yeast by methylation of the nucleosome core. Cell, 2002, 109(6), 745–756. [PubMed: 12086673]

[67]. Wang K; Zhou YJ; Liu H; Cheng K; Mao J; Wang F; Liu W; Ye M; Zhao ZK; Zou H Proteomic analysis of protein methylation in the yeast Saccharomyces cerevisiae. J Proteomics, 2015, 114, 226–233. [PubMed: 25109467]

[68]. Webb KJ; Al-Hadid Q; Zurita-Lopez CI; Young BD; Lipson RS; Clarke SG The ribosomal l1 protuberance in yeast is methylated on a lysine residue catalyzed by a seven-beta-strand methyltransferase. J Biol Chem, 2011, 286(21), 18405–18413. [PubMed: 21460220]

[69]. Webb KJ; Laganowsky A; Whitelegge JP; Clarke SG Identification of two SET domain proteins required for methylation of lysine residues in yeast ribosomal protein Rpl42ab. J Biol Chem, 2008, 283(51), 35561–35568. [PubMed: 18957409]

[70]. Winter DL; Abeygunawardena D; Hart-Smith G; Erce MA; Wilkins MR Lysine methylation modulates the protein-protein interactions of yeast cytochrome C Cyc1p. Proteomics, 2015, 15(13), 2166–2176. [PubMed: 25755154]

[71]. Winter DL; Hart-Smith G; Wilkins MR Characterization of Protein Methyltransferases Rkm1, Rkm4, Efm4, Efm7, Set5 and Hmt1 Reveals Extensive Post-Translational Modification. J Mol Biol, 2018, 430(1), 102–118. [PubMed: 29183786]

[72]. Zhang K; Lin W; Latham JA; Riefler GM; Schumacher JM; Chan C; Tatchell K; Hawke DH; Kobayashi R; Dent SY The Set1 methyltransferase opposes Ipl1 aurora kinase functions in chromosome segregation. Cell, 2005, 122(5), 723–734. [PubMed: 16143104]

[73]. Zhang M; Xu JY; Hu H; Ye BC; Tan M Systematic Proteomic Analysis of Protein Methylation in Prokaryotes and Eukaryotes Revealed Distinct Substrate Specificity. Proteomics, 2018, 18(1).

[74]. Zhang X; Huang Y; Shi X Emerging roles of lysine methylation on non-histone proteins. Cell Mol Life Sci, 2015, 72(22), 4257–4272. [PubMed: 26227335]

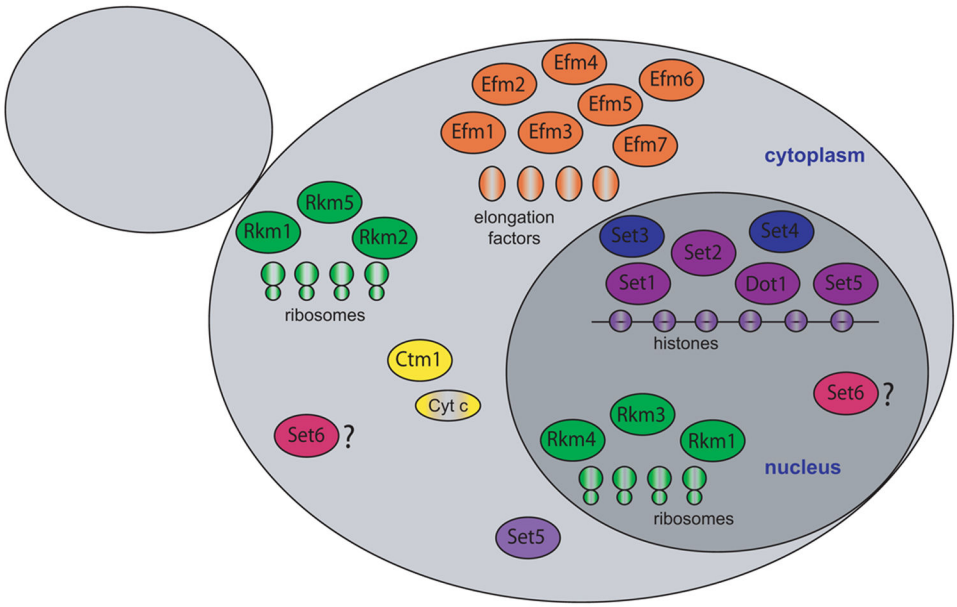


**Figure 1.**

The subcellular localization and known substrates of SET domain and seven-β-strand lysine methyltransferases in yeast. The known substrates are highlighted in the same color as the enzymes. Enzymes without defined KMT activity (Set3, Set4) are shown in dark purple. The localization of Set6 is not known and indicated with question marks. See Table 1 for references.

**Table 1:**

A summary of known and putative yeast lysine methyltransferases (KMTs), their substrates, methylated residues, and localization. Protein localization has been determined through direct experimentation as described in the referenced citations, through high-throughput studies of subcellular localization in yeast [25], or extrapolated based on substrate identification (Rkm2 and Rkm5 only).

| Yeast Protein | KMT Superfamily | Known substrates | Methylated Lysines | Methyl state | Localization | Reference |
|---|---|---|---|---|---|---|
| **Set1** | SET | H3 | K4 | me3 | Nucleus | [5], [72] |
| | | Dam1 | K233 | | | |
| **Set2** | SET | H3 | K36 | me3 | Nucleus | [63] |
| **Set3** | SET | | No KMT activity | | Nucleus | [50] |
| **Set4** | SET | | No KMT activity | | Nucleus | [65] |
| **Set5** | SET | H4 | K5,K8,K12 | me1 | Nucleus/ Cytoplasm | [19] |
| **Set6** | SET | Unknown | - | - | Unknown | - |
| **Rkm1** | SET | Rpl23ab<br>Rps18ab | K105, K109<br>K48 | me2<br>me1 | Nucleus/ Cytoplasm | [54], [53]<br>[14] |
| **Rkm2** | SET | Rpl12ab | K10 | me3 | Cytoplasm(?) | [52] |
| **Rkm3** | SET | Rpl42ab | K40 | me1 | Nucleus | [69] |
| **Rkm4** | SET | Rpl42ab | K55 | me1 | Nucleus | [69] |
| **Ctm1** | SET | Cyc1 | K72 | me3 | Cytoplasm | [51] |
| **Efm1** | SET | eEF1A | K30, K253 | me1 | Cytoplasm | [14], [67] |
| **Efm2** | Seven-β-strand | eEF2 | K613 | me2 | Cytoplasm | [14] |
| **Efm3** | Seven-β-strand | eEF2 | K509 | me3 | Cytoplasm | [18] |
| **Efm4/See1** | Seven-β-strand | eEF1A | K316 | me2 | Cytoplasm | [14], [38] |
| **Efm5** | Seven-β-strand | eEF1A | K79 | me3 | Cytoplasm | [17], [23] |
| **Efm6** | Seven-β-strand | eEF1A | K390 | me1 | Cytoplasm | [28] |
| **Efm7** | Seven-β-strand | eEF1A | K3 | me2 | Cytoplasm | [23] |
| **Rkm5** | Seven-β-strand | Rpl1ab | K46 | me1 | Cytoplasm(?) | [68] |
| **Dot1** | Seven-β-strand | H3 | K79 | me3 | Nucleus | [43], [66] |

**Table 2:**

Lysine-methylated proteins identified in yeast with still undetermined KMTs

| Reference | Methyl-lysine detection method | Proteins and methyl marks identified |
|---|---|---|
| **Couttas et al.** [14] | Protein enrichment, pan-methyl lysine antibody detection, MS/MS | eEF2K15me3; EF3K187me3, K196me3,K789me3; Rkm1K528me1; Rps 18K48me1; Rpl11K75me3; Rpl18K50me3; Rpl42K40me1 |
| **Carlson et al.** [8] | Methyl-lysine affinity pull-down (MBT domain) and MS/MS | Ssa4; Npl3; Rps9 |
| **Zhang et al.** [73] | Heavy-methyl SILAC | Sin3K1328; Jip4K862 |
| **Caslavka Zempel et al.** [9] | Subcellular fractionation and heavy-methyl SILAC | Ssa2/4K421/422me1; Rpl23K106me3, K110me1, me2, K120me2; eEF1A K35me1; Pst2 K144me1; Ecm10K558me1; Mnp1K56me2; Rps2K49me1; Lat1K340me2; Mrpl40K186me1; Ald5K308me3; Hsp60K490me3; Ald4K345me3; Aco1K360me2 |