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Elongation factor methyltransferase 3 – A novel eukaryotic lysine methyltransferase

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ABSTRACT

Here we describe the discovery of *Saccharomyces cerevisiae* protein YJR129Cp as a new eukaryotic seven-beta-strand lysine methyltransferase. An immunoblotting screen of 21 putative methyltransferases showed a loss in the methylation of elongation factor 2 (EF2) on knockout of YJR129C. Mass spectrometric analysis of EF2 tryptic peptides localised this loss of methylation to lysine 509, in peptide LVEGLKR. *In vitro* methylation, using recombinant methyltransferases and purified EF2, validated YJR129Cp as responsible for methylation of lysine 509 and Efm2p as responsible for methylation at lysine 613. Contextualised on previously described protein structures, both sites of methylation were found at the interaction interface between EF2 and the 40S ribosomal subunit. In line with the recently discovered Efm1 and Efm2 we propose that YJR129C be named elongation factor methyltransferase 3 (Efm3). The human homolog of Efm3 is likely to be the putative methyltransferase FAM86A, according to sequence homology and multiple lines of literature evidence.

1. Introduction

The methylation of proteins is emerging as one of the most widespread protein post-translational modifications [1]. It predominantly occurs on lysine and arginine residues, but has also been documented on glutamine, asparagine, cysteine, histidine and glutamate residues [2]. Lysine methylation is known to affect a wide variety of biological functions by modifying histone proteins, and more recently, non-histone proteins [3,4]. On histones, lysine methylation regulates transcription by facilitating docking of chromatin remodelling proteins and complexes, in order to bring about either transcriptional silencing or activation [3]. More generally, lysine methylation is known to modulate protein–protein interactions and thereby also affects the function of non-histone proteins [4,5].

The first lysine methyltransferases (KMTs) to be discovered were SET domain methyltransferases, however upon the discovery of Dot1 in *Saccharomyces cerevisiae*, which monomethylates histone H3 at lysine 79 [6], it became evident that seven-beta-strand (or Class I) methyltransferases were also able to catalyse lysine methylation. Since then, a number of other seven-beta-strand KMTs have been discovered in both yeast and human. In yeast these are See1, which dimethylates elongation factor 1α at lysine 316, Efm2 which dimethylates elongation factor 2 at lysine 613, and Rkm5 which monomethylates the ribosomal protein Rpl1 at lysine 46 [7–9]. In human these are DOT1L, which monomethylates histone H3 at lysine 79, CaM-KMT, which trimethylates calmodulin (CaM) at lysine 115, VCP-KMT, which trimethylates Valosin-containing protein (VCP) at lysine 315, HSPA-KMT, which trimethylates a number of Hsp70 proteins at a conserved lysine, and METTL22, which trimethylates KIN17 at lysine 135 [10–14]. The catalytic core of seven-beta-strand methyltransferases contains alternating β-strands and α-helices which form a seven-stranded beta sheet surrounded by α-helices [15]. In terms of sequence there are four key motifs that define seven-beta-strand methyltransferases: I, Post-I, II and III [15]. Motifs I and Post-I are involved in binding S-adenosyl L-methionine (AdoMet or SAM), the methyl donor, while Motifs II and III have been proposed to bind the substrate [16].

In recent years, there has been an intense search for new protein methyltransferases. Sequence-based studies, focused on the presence of the seven-beta-strand, SET domain or SPOUT methyltransferase motifs, have predicted the presence of about 30 putative methyltransferases in *S. cerevisiae* [17,18]. The specificity of seven-beta-strand methyltransferases remains challenging to predict, yet these studies have classified putative methyltransferases into those most likely to methylate proteins, as opposed to RNA, fatty acids and lipids, or small molecules. Antibody-based studies of protein methylation patterns, in conjunction with the single gene knockout of methyltransferases, have proven useful for the discovery of putative new enzymes [8,19]. *In vitro* methylation

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assays can then validate these methyltransferases [12–14,19,20]. This direct validation of activity is important as simple knockout analysis of putative methyltransferases may only unveil secondary effects. Ultimately, tandem mass spectrometry is required to map the site specificity of methyltransferases. This allows unequivocal enzyme-substrate relationships to be built, for incorporation into methylproteome networks [5].

Elongation factors 1α and 2 are known to be methylated in eukaryotes [7,8,21]. In particular, we previously reported that *S. cerevisiae* elongation factor 2 (EF2), which catalyses the translocation step of translation elongation, is trimethylated at lysine 509 and dimethylated at lysine 613 [22]. Methylation of lysine 613 was found to be removed in knockout of *EFM2*, but methylation of lysine 509 was not found to change in any knockouts of elongation factor-associated methyltransferases *SEE1*, *EFM1* or *EFM2* [22].

Here we describe the discovery and validation of *S. cerevisiae* protein YJR129Cp as a new eukaryotic seven-beta-strand lysine methyltransferase. Through knockout analysis with antibodies and *in vitro* methylation analyses, we show that the enzyme methylates elongation factor 2 on lysine 509, in the tryptic peptide LVEGLKR. We also confirm that Efm2p is responsible for the dimethylation of EF2 at lysine 613. Interestingly, both sites of methylation are found at EF2 interaction interfaces with the 40S ribosomal subunit. In line with the recently discovered and named Efm1 and Efm2, both which methylate elongation factors, we propose that YJR129C be named elongation factor methyltransferase 3 (Efm3). We finally show that there are multiple lines of literature evidence to suggest that the human homolog of Efm3 is the putative methyltransferase FAM86A.

2. Materials and methods

2.1. Yeast strains, double knockout generation and antibody-based screens

Yeast strains used are shown in Table 1. The double knockout of *EFM2* and YJR129C was generated by deleting *EFM2* from the YJR129C single knockout, by use of a hygromycin B resistance cassette amplified from plasmid pFA6a–hphNT1, according to [23]. Yeast was grown in YEPD at 30 °C to mid-log (~0.8 OD_{600}) before harvest and protein extraction as per [8], except that lysates were not subjected to ultracentrifugation. SDS–PAGE and immunoblotting were performed as per [24] with the following antibodies: anti-trimethyllysine (Immunechem, ICP0601), 1:2000 in 1% BSA overnight; anti-Nε-methyllysine (Immunechem, ICP0501), 1:1000 in 2% BSA overnight; anti-PentaHis HRP-Conjugated antibody (Qiagen, 34460), 1:10,000 in blocking buffer provided overnight.

2.2. Recombinant protein production and in vitro methylation

Efm2p and YJR129Cp were made recombinantly in *Escherichia coli* (Rosetta DE3) by cloning genes into plasmid pET15b (Novagen); expression and lysis was as per [25], with the exception that expression was for 4.5 h at 25 °C. The gene *EFT1* (which codes EF2) was cloned into plasmid pAG426GAL-ccdB, with addition of a C-terminal 6×His-Tag, and transformed into yeast strain Δ*EFM2*ΔYJR129C. EF2 was overexpressed as per [26] and lysis was by bead-beating cell pellets (BioSpec BeadBeater) for 30 s three times in binding/wash buffer 1 (50 mM sodium phosphate buffer, 500 mM NaCl, 40 mM imidazole, 20% (v/v) glycerol, 0.25% (v/v) Triton X-100, 10 mM β-mercaptoethanol, pH 8) with EDTA-free protease inhibitor (Roche). Resultant lysates (from either *E. coli* or *S. cerevisiae*) were clarified at 21,000*g* for 30 min at 4 °C. A 1 mL Ni–NTA Superflow Cartridge (Qiagen) charged with Ni^{2+} was used to purify the 6×His-Tagged proteins as per manufacturer instructions.

For *in vitro* methylation reactions, purified EF2 was incubated with purified Efm2p and/or purified YJR129Cp in the presence of 50 μM AdoMet in *in vitro* methylation buffer (50 mM HEPES–KOH, 20 mM NaCl, 1 mM EDTA, pH 7.4) at 30 °C for 1 h. No enzyme was added for the negative control. Ten microlitres of 6× SDS buffer (350 mM Tris–Cl pH 6.8, 30% (v/v) glycerol, 10% (v/v) SDS, 0.6 M DTT, 0.012% (w/v) bromophenol blue) was added to stop reactions, which were then boiled for 10 min, subjected to SDS–PAGE and immunoblotted with anti-trimethyllysine or anti-PentaHis HRP-Conjugated antibodies. Alternatively, for mass spectrometry, gels were fixed in 25% (v/v) isopropanol/10% (v/v) acetic acid for 10 min, and then stained with Bio-Safe™ Coomassie (BioRad).

2.3. Methylation site analysis by mass spectrometry

Samples for mass spectrometry were prepared according to [27]. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) using collision-induced dissociation was performed on an Orbitrap Velos or Velos Pro as described [27]. Targeted data acquisition was performed with an inclusion list containing the theoretical *m*/*z* values of every potential EF2 doubly- and triply-charged lysine-methylated tryptic peptide (with two missed cleavages), within the range of 350–2000*m*/*z*. Peptides associated with K509 and K613 methylation of EF2, i.e. mono-, di- and tri-methylated forms of LVEGLKR, and mono- and di-methylated forms of DDFKAR, respectively, were identified by comparing observed *m*/*z* values, LC retention times and MS/MS spectra to synthetic $^{13}C(6)$ $^{15}N(4)$ C-terminal arginine labelled versions of these peptides (SpikeTides TQL, JPT, Germany). All peptide identifications were confirmed following previous criteria [22] (for example see Fig. S1). Unmodified forms of LVEGLKR, DDFKAR, and the proteotypic EF2 peptide AGIISAAK, were identified from Mascot sequence database searches (v2.3, Matrix Science) following previous procedures [22]. Relative abundances for methylated and unmethylated peptide ions of interest were monitored using extracted ion chromatograms (XICs) [22]. XICs were obtained using Thermo Xcalibur 2.2 SP1.48; mass ranges were set as the theoretical *m*/*z* for the monoisotopic peak of the peptide ion of interest ±10 ppm. MS data were manually interrogated to confirm that no co-eluting peptide ions contributed to the XICs derived from the peptide ions of interest.

2.4. Analysis and comparison of methyltransferase sequences

YJR129C ortholog search was performed using BLASTp (NCBI) using default parameters against the Swiss-Prot database. Pairwise protein sequence alignment between YJR129C and FAM86A was

Table 1
S. cerevisiae strains used in this study.

Strain	Genotype	Source
Wild type (BY4741)	*MATα his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	EUROSCARF
ΔEFM2	Δ*EFM2*::kanMX4 in BY4741	EUROSCARF
ΔYJR129C	ΔYJR129C::kanMX4 in BY4741	EUROSCARF
Δ*EFM2*ΔYJR129C	Δ*EFM2*::hphNT1, ΔYJR129C::kanNMX4 in BY4741	This study
Δ*EFM2*ΔYJR129C, overexpressing EF2	Above, with the pAG426GAL-*EFT1*-6×His-Tag plasmid	This study

performed using EMBOSS Stretcher (EMBL-EBI) with default parameters.

3. Results

3.1. Deletion of YJR129C results in loss of methylation in EF2

Previously, we showed that the knockout of lysine methyltransferases *EFM1*, *EFM2* and *SEE1* in *S. cerevisiae* did not affect the methylation of lysine 509 in elongation factor 2 (EF2) [22]. To discover the methyltransferase responsible for this methylation, we screened single gene knockouts of 21 putative methyltransferases (Table S1) by immunoblotting whole cell lysates with anti-trimethyllysine and anti-Nε-methyllysine antibodies. The anti-trimethyllysine antibody is known to have specificity for mono-, di- and tri-methylation [28]. Out of all 21 knockouts, ΔYJR129C showed an apparent reduction in methylation on an 89 kDa protein, known to be EF2 (Fig. 1A) [8]. We then generated a double knockout of YJR129C and *EFM2*, and compared its EF2 methylation to that of each individual knockout. Δ*EFM2*ΔYJR129C showed a complete or near complete loss of methylation (Fig. 1A); this was as expected as Efm2p is known to methylate lysine 613 – the other reported site in EF2. Interestingly, EF2 methylation was still weakly detectable with the anti-trimethyllysine antibody in the Δ*EFM2*ΔYJR129C double knockout (Fig. 1A), indicating that there may be other, unidentified methylation sites. Indeed, methylation of lysines 407 and 594 has recently been found on human elongation factor 2 (eEF2) [29,30], which correspond to conserved lysine residues 391 and 578 in yeast EF2.

In order to localise the methylation being lost in the YJR129C knockout, bands corresponding to EF2 from WT, Δ*EFM2*, ΔYJR129C and Δ*EFM2*ΔYJR129C cells were digested by trypsin and analysed by LC–MS/MS. Peptides corresponding to mono-, di- and trimethylation of K509 and mono- and dimethylation of K613 were monitored for changes between strains. In accordance with our previous findings [22], the methylation of K613 was absent in Δ*EFM2*, while the methylation of K509 was not changed (Fig. 1B). Lysine 509 was found to be completely unmethylated, however, in both the knockout of YJR129C and the double knockout of *EFM2* and YJR129C (Fig. 1B). This suggested that YJR129Cp is the methyltransferase responsible for mono-, di- and trimethylation of EF2 at lysine 509.

3.2. YJR129Cp methylates EF2 in vitro at K509

To confirm our previous finding that deletion of *EFM2* corresponds to a loss of methylation at K613 [22] and validate our new finding that deletion of YJR129C corresponds to a loss of methylation at K509, we performed *in vitro* methylation of EF2 by Efm2p and YJR129Cp. EF2 was overexpressed and purified from the Δ*EFM2*ΔYJR129C strain of yeast and incubated with AdoMet and recombinant Efm2p and/or YJR129Cp. Efm2p and YJR129Cp were individually found to methylate EF2 *in vitro*, and additional methylation was observed in the presence of both methyltransferases (Fig. 2A).

EF2 from each *in vitro* methylation reaction was then analysed by LC–MS/MS, and the methylation status of both K509 and K613 were monitored as in the knockout analysis. In the absence of either methyltransferase K509 was entirely unmethylated, however upon incubation with YJR129Cp, a mix of mono-, di- and trimethylated K509 was observed (Fig. 2B). The presence of a mixture of mono-, di, and tri-methylated K509 suggests that YJR129Cp acts via a distributive, rather than processive, mechanism. Similarly, the methylated form of K613 was not observed in the absence of methyltransferases, however upon incubation with Efm2p, K613 was seen to be predominantly dimethylated (Fig. 2B). The methylation status of K509 was not seen to change upon incubation with just Efm2p and the methylation status of K613 was not seen to change upon incubation with just YJR129C, indicating that no functional redundancy exists between the two methyltransferases. Finally, incubation of EF2 with both YJR129Cp and Efm2p resulted in methylation of both K509 and K613. Together these results identify YJR129C as a novel methyltransferase responsible for the mono-, di- and trimethylation of K509 in EF2 and provide *in vitro* confirmation of Efm2p as the methyltransferase responsible for mono- and dimethylation of K613 in EF2. We suggest that YJR129Cp is named elongation factor methyltransferase 3 (Efm3),

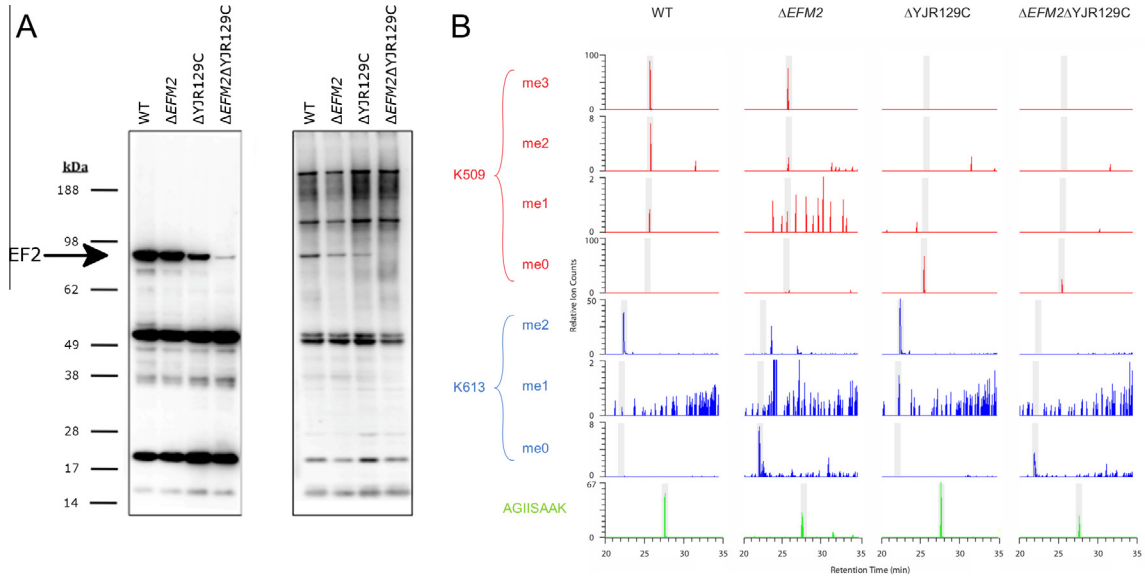

Fig. 1. Deletion of *EFM2* and YJR129C abolishes *in vivo* EF2 methylation on lysines 613 and 509, respectively. (A) Whole cell lysates from wild-type (WT), single gene knockouts of *EFM2*, YJR129C and double knockout (Δ*EFM2*ΔYJR129C) were immunoblotted with anti-trimethyllysine (left) or anti-Nε-methyllysine (right) antibodies. Blots showed that the double knockout resulted in near-complete or complete reduction in methylation of EF2. (B) Bands corresponding to EF2 from A were analysed by LC–MS/MS. The methylation status of K509 (red) and K613 (blue) were analysed by taking mass windows (±10 p.p.m.) corresponding to all relevant methylation states of the tryptic peptides LVEGLKR and DDFKAR, respectively. Elution times of peptides are shaded. The EF2 tryptic peptide AGIISAAK (green) was included for the sake of comparison. K509 was found to be unmethylated in both the single knockout of YJR129C and the double knockout of *EFM2* and YJR129C. K613 was found to be unmethylated in both the single knockout of *EFM2* and the double knockout. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

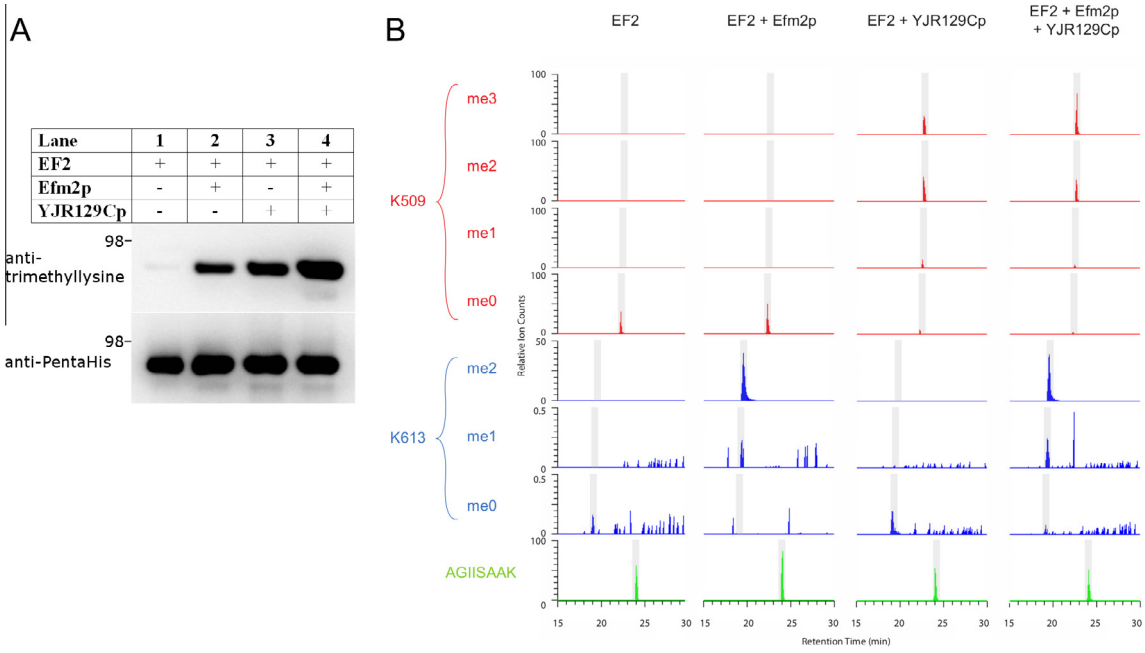

Fig. 2. *In vitro* methylation of EF2 by Efm2p and YJR129Cp at lysines 613 and 509, respectively. (A) EF2 expressed and purified from a background of Δ*EFM2*ΔYJR129C was incubated with no enzyme (lane 1), Efm2p (lane 2), YJR129Cp (lane 3) or both Efm2p and YJR129Cp simultaneously (lane 4), and probed with either anti-trimethyllysine antibody to detect methylation or anti-PentaHis HRP-Conjugated antibody as a loading control. Methylation was observed after treatment with Efm2p or YJR129Cp and additional methylation observed when treated with both enzymes. (B) EF2 from *in vitro* methylation reactions from A were analysed by LC–MS/MS. The methylation status of K509 in LVEGLKR and K613 in DDFKAR were analysed as in Fig. 1B. Elution times of peptides are shaded. K509 was found to be methylated only when YJR129Cp was added, and K613 was found to be methylated only when Efm2p was added. Both modifications were observed after treatment with both enzymes.

in line with the recent discoveries of elongation factor methyltransferases 1 and 2 [7,8].

3.3. Structural significance of EF2 lysine methylation

A structure of *S. cerevisiae* EF2 bound to the whole 80S ribosome, in the presence of the antibiotic sordarin, was reported by Spahn et al. [31]. This was achieved by docking an X-ray structure of EF2·sordarin and atomic models of ribosomal proteins/RNA to an 11.7 Å cryo-EM map of EF2 bound to the whole 80S ribosome. This inferred structure allowed us to explore the possible significance of EF2 methylation. Interestingly, we found both lysines are positioned near the interface of EF2 and the 40S small ribosomal subunit (Fig. 3A). K509 is located proximal to the ribosomal protein Rps23b (Fig. 3B), and may be involved in the interaction between EF2 and the 40S subunit. Indeed it is adjacent to residues N499–P502 of EF2, which sit at the start of an alpha helix predicted to interact with N99 of Rps23b [31]. K509 is further along this same alpha helix, and its ε-nitrogen is estimated to be 5.3 Å from L132 and 6.9 Å from F95 of Rps23b. It is therefore possible that trimethylated K509 may form part of the interaction between EF2 and Rps23b. However, as the model is a mapped cryo-EM and not an X-ray structure, the exact atomic distances between residues may not be accurate and will require confirmation.

K613 is located proximal to helix 33 of the 18S rRNA (Fig. 3C) and in fact was predicted to be one of the residues that interacts with residue 1044 in helix 33 of the 18S rRNA, along with R617 [31]. It is therefore possible that the methylation of K613 has a role in this interaction. Interestingly, the function of methylation in modulating protein–RNA interactions has also been suggested for other translation-associated proteins [2]. In particular, lysine methylation of Rpl42ab at K40 and K55 was suggested to affect its interaction with rRNA [32]. Hence, dimethylation of EF2 at K613 may have a role in modulating its interaction with the 18S rRNA and therefore the ribosome.

3.4. FAM86A is the likely human ortholog of YJR129C

A reciprocal BLASTp search predicted the uncharacterised FAM86A to be the likely human ortholog of YJR129C (expect value = 9×10^{-11}; reciprocal search expect value = 2×10^{-11}). This is in agreement with what was observed by Kernstock et al. when searching for a potential ortholog of the FAM86 proteins [12]. Alignment of YJR129C and FAM86A shows a sequence identity of 22.5% and the presence of distinctive seven-beta-strand motifs I and post-I (Fig. 4). In addition, the DXXY motif described by Kernstock et al. is evident in both proteins [12].

4. Discussion

The *S. cerevisiae* YJR129C gene was predicted to be a protein methyltransferase in two bioinformatic studies of the yeast methyltransferome [17,18]. Additionally, it was clustered with two known protein methyltransferases, YBR271W (*EFM2*) and YIL110W (*HPM1*), in an earlier study [33]. Here we have shown that YJR129C is a novel seven-beta-strand lysine methyltransferase which is responsible for the mono-, di- and tri-methylation of lysine 509 in elongation factor 2. We designate it elongation factor methyltransferase 3 (Efm3) in line with the recent naming of Efm1 and Efm2 [7,8]. Purified Efm3p was found to be active *in vitro*, indicating that it acts either as a monomer or as a homo-multimer. It appears to act through a distributive mechanism, as has been observed for other seven-beta-strand KMTs, such as Dot1 and VCP-KMT [12,34].

The uncharacterised human protein FAM86A belongs to the group of highly similar FAM86 proteins, which also includes FAM86B1, FAM86B2 and FAM86C. In addition to sequence similarity, there is further evidence that FAM86A is likely to be the human ortholog of Efm3. Firstly, YJR129C/Efm3 was the only yeast protein to be grouped with the FAM86 proteins in a bioinformatic characterisation of the yeast and human methyltransferomes [35].

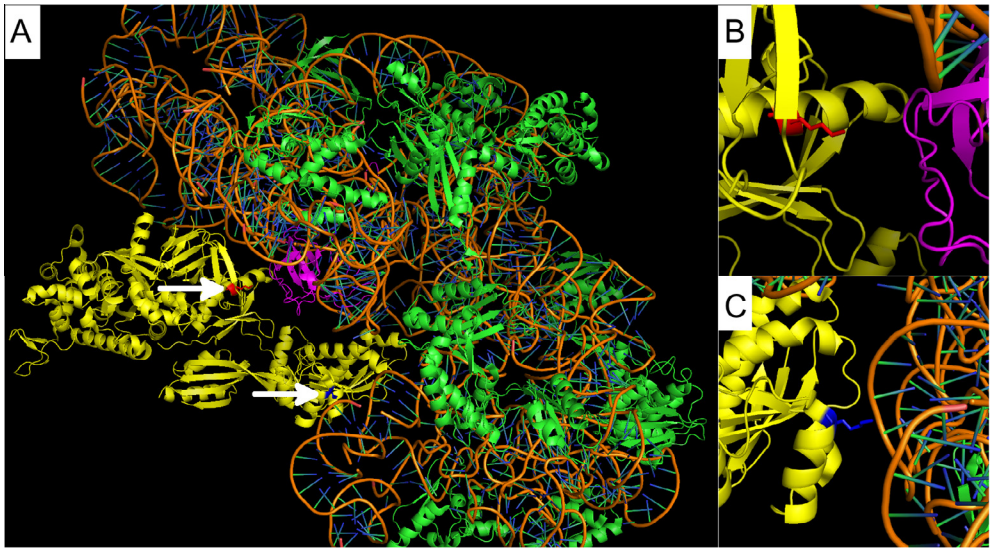

Fig. 3. Lysines 509 and 613 in EF2 are in close proximity to the 40S ribosomal subunit during interaction. EF2 is shown bound to the 40S subunit as determined by Spahn et al. [31] (PDB ID: 1S1H). (A) Overall view of EF2 (yellow) bound to the 40S subunit (proteins in green, rRNA in orange backbone with green/blue bases). White arrows indicate the positions of K509 (red) and K613 (blue). (B) A close-up view of K509 (red) in proximity to Rps23b (magenta). (C) A close-up view of K613 (blue) in proximity to helix 33 of the 18S rRNA. Visualised in PyMOL. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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YJR129C           1 M--NEDLFYDRLHQRCPGKYLLEELETSKSNDVLHAS-RFVCEMELVQKT     47
                    |  .|:...:.|.|....::|......|.....|.|. |...:.||::..
FAM86A            1 MAPEENAGTELLLQSFERRFLAARTLRSFPWQSLEAKLRDSSDSELLRDI     50

YJR129C          48 NAYYCKTIVKMLLDHEWIFAKAFTIVNDGEDEIEIYDYLYEKYIKLLSTG     97
                    .....|..|.:.......:|:.|......:.|....:.|.|.|..|..|.
FAM86A           51 LHKTVKHPVCVKHPPSVKYARCFLSELIKKHEAVHTEPLDELYEALAETL    100

YJR129C          98 KPDPMMKDVVRYRFDEDVKIKIEETPNLISAASTTGFRTWEAALYMGDFL    147
                    .....:....|.......:.:.|:..:||.. |||..||:||||:.::.
FAM86A          101 MAKESTQGHRSYLLPSGGSVTLSESTAIISYG-TTGLVTWDAALYLAEWA    149
                                                  Motif I
YJR129C         148 IHKPLQELAPVQGQDDGKKKLNVLEVGAGTGIVSLVILQKYHEFVNKMYV    197
                    |..|    |....:      .|||:|:|.|:..|.|.:....   :.|:
FAM86A          150 IENP----AVFTNR------TVLELGSGAGLTGLAICKMCRP---RAYI    185
                  Motif Post-I
YJR129C         198 TDGDSNLVETQLKRNFELNNEVRE-------NEPDIKLQRLWWGSDRVPE    240
                    .....:.|.|||:.|..||....|       :.|.:.:.:|.|....|.:
FAM86A          186 FSDCHSRVLEQLRGNVLLNGLSLEADITAKLDSPRVTVAQLDWDVATVHQ    235
                                 DxxY
YJR129C         241 ----DIDLVVGADVTYDPTILPDLCECL---AECLALDRCKLCLLSATIR    283
                        ..|:|:.||||.|.|..:..|...|   |.|....|.....::.|:|
FAM86A          236 LSAFQPDVVIAADVLYCPEAIMSLVGVLRRLAACREHQRAPEVYVAFTVR    285

YJR129C         284 SESTVQLFSQECNKLGLKCTIVTSTEYDANNEIRAMKALQFKPLIAPIRI    333
                    :..|.|||:.|..:.|::.:....|         .|...::..:. :.:
FAM86A          286 NPETCQLFTTELGRAGIRWEVEPRHE---------QKLFPYEEHLE-MAM    325

YJR129C         334 YKITKQ    339
                    ..:|.
FAM86A          326 LNLTL-    330
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Fig. 4. Alignment of yeast YJR129C and human FAM86A. The sequence of YJR129C was aligned with its likely human ortholog, FAM86A, using EMBOSS Stretcher (EMBL-EBI). Distinctive seven-beta-strand motifs I and post-I are shown, along with the DxxY motif described by Kernstock et al. [12]. Vertical lines (|) indicate identical residues; double dots (:) indicate chemically similar residues; single dots (.) indicate dissimilar residues; dashes (-) indicate missing residues.

Secondly, the methylation of K509 in EF2 is conserved in humans. Guo et al. reported dimethylation of K525 in human eEF2 (which corresponds to K509 in *S. cerevisiae*) in a recent screen for protein methylation [1]; this residue may also carry trimethylation. Lastly, eEF2 was found to be an interaction partner of FAM86A in an affinity purification/mass spectrometry experiment [13]. Together, these strongly suggest that FAM86A is the direct human ortholog of YJR129C.

The fact that EF2 is methylated by two different methyltransferases suggests different functional roles for their respective sites. While it is common for histones to have multiple sites of lysine methylation from different methyltransferases, there are few examples of this for non-histone proteins. In *S. cerevisiae*, only EF1α (methylated by See1 and Efm1) and Rpl42ab (methylated by Rkm3 and Rkm4) are known to have multiple lysine methyltransferases; the functional significance of this is currently unknown [7,32]. In human, however, p53 is known to be methylated by four different PKMTs, each having a distinct role in modulating function [4,36]. The methylation of EF2 by both Efm2 and Efm3 therefore suggests distinct roles for the methylation of K613 and K509. It is known that protein methylation can modulate protein–protein and protein–RNA interactions [5,25]. Thus, given

the structural contexts of each lysine at the EF2-ribosome interface, it would be interesting to investigate the role of K509 and K613 methylation in protein–protein and protein–RNA interactions, respectively.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.110.

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