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HemK2 protein, encoded on human chromosome 21, methylates translation termination factor eRF1

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Abstract The ubiquitous tripeptide Gly-Gly-Gln in class 1 polypeptide release factors triggers polypeptide release on ribosomes. The Gln residue in both bacterial and yeast release factors is N5-methylated, despite their distinct evolutionary origin. Methylation of eRF1 in yeast is performed by the heterodimeric methyltransferase (MTase) Mtq2p/Trm112p, and requires eRF3 and GTP. Homologues of yeast Mtq2p and Trm112p are found in man, annotated as an N6-DNA-methyltransferase and of unknown function. Here we show that the human proteins methylate human and yeast eRF1.eRF3.GTP in vitro, and that the MTase catalytic subunit can complement the growth defect of yeast strains deleted for *mtq2*.

Structured summary:
MINT-6571489:
HemK2α (uniprotkb:Q9Y5N5) *binds* (MI:0407) to *hTrm112* (uniprotkb:Q9UI30) by *pull down* (MI:0096)

Keywords: HemK2 methyltransferase; eRF1; N5-methylglutamine; hTrm112; Human chromosome 21

1. Introduction

Translation termination is an essential step in all three living kingdoms, leading to polypeptide release from the ribosome. This reaction is catalyzed by class 1 release factors (RFs) after recognition of an in frame stop codon in the ribosomal A site [1]. In eubacteria, two class 1 RFs are present: RF1 recognizing UAA and UAG, and RF2 recognizing UAA and UGA. In eukaryotes, a single RF, eRF1 is able to promote termination at all three stop codons. Despite a similar function, these RFs evolved independently, and have only one motif in common, a GGQ tripeptide that is essential for interaction with the peptidyl transferase center of large ribosomal subunit [2,3]. Strikingly, in both bacteria and *Saccharomyces cerevisiae*, not only is this peptide sequence conserved, but also the post-translational N^5-methylation on the Gln residue [4,5]. Recently, we identified PrmC [6] and Mtq2p (Ydr140wp) [5] as the methyltransferases (MTases) acting respectively on *Escherichia coli* RF1/2 and *S. cerevisiae* eRF1. In the latter case, eRF1 methylation occurs in the presence of eRF3, a class 2 RF forming a complex with eRF1 in the absence of ribosomes. The enzyme itself is an heterodimer made of two subunits: Mtq2p carrying the catalytic and AdoMet binding sites, and Trm112p (Ynr046wp), a small zinc finger protein, necessary for the solubility and activity of the catalytic subunit [7].

Mammalian genomes encode proteins sharing homology with Mtq2p, the function of which is unknown although they are annotated as N6-adenine MTases. A recent analysis of PRED28, the murine homolog Mtq2p, failed to show any evidence for adenine-MTase activity [8].

Here we describe the characterization of these enzymes as MTases responsible for mammalian eRF1 methylation. Human eRF1 (heRF1) is methylated in the presence of eRF3 by human orthologues of Mtq2p/Trm112p. Moreover, the overexpression of the mammalian MTase catalytic subunit is able to complement the growth defect of a yeast strain bearing a deletion of the *mtq2* gene.

2. Materials and methods

2.1. Bacteria and yeast growth

Bacteria were grown in LB medium supplemented according to requirements. Antibiotics were added at the following final concentrations: kanamycin, 50 μg/ml; ampicillin, 200 μg/ml; chloramphenicol, 15 μg/ml. *S. cerevisiae* was grown in liquid or solid YPDA medium. Cells were transformed by the LiAc method [9] before plating on selective HC medium lacking uracil.

2.2. Recombinant DNA manipulations

General procedures for DNA recombinant techniques, plasmid extraction, etc. were performed as described by Sambrook et al. [10].

2.3. Strains and plasmids

Wild-type strain BY4741 from EUROSCARF was deleted for YDR140w ORF with an histidine knockout cassette (pRS303), creating strain FF12 (*MATa*, *his3Δ1*, *leu2Δ0*, *met15Δ0*, *ura3Δ0*, *mtq2::HIS3*). Primers used in this study are listed in Table 1. The gene encoding heRF1 was amplified from human cDNA (CAMA1) into two steps. The first PCR was performed with Oligo1 and Oligo2 introducing, respectively, NdeI and BglII restriction sites. This purified PCR product was then used for a second amplification using Oligo1 and Oligo3 thereby adding a His_6 tag to the C-terminus of the protein. After digestion, this product was inserted between the NdeI/BamHI sites of a pET11a expression vector, creating plasmid pFFh1A. The DNA encoding truncated heRF3a (amino acids 139–637) with a C-terminal His_6 tag was amplified as described above in two steps with Oligo4, Oligo5 and Oligo6. The PCR fragment digested by NdeI and BamHI was cloned into pET11a, creating pFFh3. The gene encoding HemK2α was amplified in the same way using Oligo7, Oligo8 and Oligo9, and then cloned into plasmid pET11a, giving pFFh2. This plasmid was used as template to amplify *HemK2α* with Oligo10 and Oligo11. The PCR product was inserted between BamHI and EcoRI

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Abbreviations: RF, release factor; MTase, methyltransferase

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Table 1
Oligonucleotide primers

Oligo	Sequence
1	5′-AGGAGGAGGCGAG**CAT*ATG***GCGGACGACCCCAGTGCTGCCGACAGGAACGTGGAAATCTGGAAG-3′
2	5′-TTGCCGGACCC**AGATCT**ACTAC*CTA*-3′
3	5′-TGCCGGACCC**AGATCT**ACTAC*CTA***GTGGTGGTGGTGGTGGTG**GTAGTCATCAAGGTCAAA-3′
4	5′-GGTTCAAATTCAGCTGTT**CAT*ATG***GAACTTTCA-3′
5	5′-TTGTGCAGGG**GGATCC**AGAAAATG-3′
6	5′-TGCAGGG**GGATCC**AGAAAATGC*TTA***GTGGTGGTGGTGGTGGTG**GTCTTTCTCTGGAAC-3′
7	5′-CAGCGAAGG**CAT*ATG***GCAGGGGAGAAC-3′
8	5′-GTTCTGGGCACACACT**GGATCC*CTA***AGACTTG-3′
9	5′-GTTCTGGGCACACACT**GGATCC*CTA***GTGGTGGTGGTGGTGGTG**AGACTTGGTGAACTTGAG-3′
10	5′-GAGATATACATC**GGATCC*ATG***GCAGGGGAGAA-3′
11	5′-AGCAGCCGGATCGCACT**GAATTC*CTA***AGACTTGGTGAACTTGAGGAC-3′
12	5′-CACATTCAACCAGTTATTACAGATTTGGTAGGAAGTCACGGAATAGAGGCAGCTTGGGCTGGTGGCAAAAATGGTC-3′
13	5′-CTATTCCGTGACTTCCTACCAAATCTGTAATAACTGGTTGAATGTGAACTTTGTTACAGCGTGCTGTCTC TAGGGTAC-3′
14	5′-GTTTGTGCGGC**CAT*ATG***AAACTGC-3′
15	5′-AACAAGA**AGATCT**GGCGCCTGGCACAA*TCA*AC-3′
16	5′-ACAAGA**AGATCT**GGCGCCTGGCACAA*TCA***GTGGTGGTGGTGGTGGTG**ACTCTCAGTTTCCTCTTCA-3′

sites of the yeast expression vector pYeDP60, giving pFF2A. To clone *Hemk2β* into pYeDP60, a three step PCR strategy was applied to pFF2A to delete part of *Hemk2α*. Two PCR reactions were performed, one with Oligo10 and Oligo12, and another with Oligo13 and Oligo11. As Oligo12 and Oligo13 overlapped, these two PCR products were used as a matrix with Oligo10 and Oligo11 to amplify the short form of *HemK2*. This was cloned into pYeDP60, yielding pFF2C. Plasmids pFFh5 and pFF6, which carry the gene encoding the human Trm112 homolog without or with a His_6 tag were constructed by PCR amplification from the same human cDNA with Oligo14 in each case and respectively Oligo15 or Oligo16. These PCR fragments were cloned between the NdeI/BglII restrictions sites of the expression vector pACYCDuet-1 (Novagen).

2.4. Protein expression and purification

After transformation of BL21 DE3-Gold (Stratagene) by the appropriate plasmid, the expression of His tagged proteins: heRF1, heRF3Cter, HemK2 and hTrm112 expressed alone or co-expressed (in this case only HemK2 was His-tagged) was induced by IPTG 1 mM at an optical density (600 nm) of 0.5 in selective LB medium, followed by overnight growth at 23 °C. Yeast proteins (eRF1, eRF3Cter, Mtq2p and Trm112p) and their human homologs were purified on Ni-NTA column (Sigma) as described previously [7].

2.5. In vitro methylation assays

Methylation assays were performed as described before [7]. Each test corresponds to 15 pmol of eRF1 (Human or Yeast), 15 pmol of heRF3 (Human or Yeast) and 5 pmol of methylation complex.

3. Results

3.1. In silico mammalian eRF1 MTase identification

We showed previously that *S. cerevisiae* Mtq2p MTase was not sufficient to methylate the ternary complex eRF1.eRF3.GTP, but that the yeast protein Trm112p, predicted by 2-hybrid experiments to interact with Mtq2p, was required to solubilize and activate the MTase. A search in protein sequence databases showed that higher eukaryotes encode homologs to yeast Mtq2p and Trm112p proteins. The Human genome contains a gene annotated N6AMT1 on chromosome 21 giving transcripts encoding two distinct isoforms (HemK2α and HemK2β). HemK2α shares 34% identity with Mtq2p going beyond the GxGxG motif essential for AdoMet binding and containing also the NPPY motif essential to bind the planar amide side chain of Gln. HemK2β results from an alternative splicing of exon IV, eliminating the NPPY motif. In mice, two protein forms are also encoded. PRED28α shares 33% identity with Mtq2p. However, the short form PRED28β resulting from exon V deletion still contains the NPPY motif (see Fig. 1). A homolog of yeast Trm112p has also been identified in mammalian genomes. Human Trm112 (Q9UI30) and mouse homolog (Q9DCG9) share, respectively, 30% and 33% identity with yeast Trm112p. However, both of them lack three of the four Cys residues coordinating the Zn atom.

3.2. HemK2α interacts with hTrm112

To determine the degree of interaction between HemK2α protein and hTrm112, the two proteins were co-expressed in *E. coli*. For this purpose, we added an His_6 tag to the C-terminus of HemK2α and examined the composition of fractions eluted by imidazole from the Ni-NTA column. HemK2α expressed alone was poorly soluble, but co-expression of hTrm112 greatly increased its solubility (Fig. 2A). Data shown in Fig. 2B demonstrate that hTrm112 interacts with Ni-NTA bound HemK2α since they are coeluted with buffer containing imidazole. These two proteins form a stable complex. A control with hTrm112 alone showed that it is unable to bind Ni-NTA resin by itself. A similar interaction was shown between PRED28α and the murine homolog of Trm112p (data not shown).

3.3. Human eRF1 is methylated by the complex HemK2α/hTrm112

To determine the function of the HemK2α/hTrm112 complex, we performed in vitro methylation assays on human ternary complex eRF1.eRF3.GTP. For this purpose, RFs were overexpressed in *E. coli* and purified. Like *E. coli* RF or yeast eRF1, such heRF1 is expected to be unmodified. Fig. 3A shows that heRF1 is an effective substrate for the MTase complex HemK2α/hTrm112, since it can be methylated up to 40%. Similar experiments showed that murine eRF1 is efficiently methylated in vitro by the murine PRED28α/mTrm112 complex in presence of eRF3 and GTP (data not shown). As described above, Hemk2α is partially soluble and can be purified alone, but it is unable to methylate eRF1 (Fig. 3B). Adding separately purified His tagged hTrm112 to HemK2α is sufficient to restore eRF1 methylation activity in vitro. Methylation efficiency is proportional to the amount of hTrm112 added in the reaction (Fig. 3B), suggesting that this

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                    ..**      :  : .*******:**:*:.**     *       : *  *:*:***:*::** .   :   .::
HUMAN_HemK2α        MAGENFATPFHGHVGRGAFSDVYEPAEDTFLLLNALEAAAAELAG-----VEICLEVGSGSGVVSAFLAS---MIGPQAL   72
HUMAN_HemK2β        MAGENFATPFHGHVGRGAFSDVYEPAEDTFLLLNALEAAAAELAG-----VEICLEVGSGSGVVSAFLAS---MIGPQAL   72
MOUSE_PRED28α       MAAPSVPTPLYGHVGRGAFRDVYEPAEDTFLLLDALEAAAAELAG-----VEICLEVGAGSGVVSAFLAS---MIGPRAL   72
MOUSE_PRED28β       MAAPSVPTPLYGHVGRGAFRDVYEPAEDTFLLLDALEAAAAELAG-----VEICLEVGAGSGVVSAFLAS---MIGPRAL   72
YEAST_Mtq2          ----MLPTPYV----KCDYDKVYEPAEDSFLILDCLEKEHDFLKQKFGNRLAIVCEIGSGSGIVTTFLMQNKIIPQENSI   72
ruler               1.......10........20........30........40........50........60........70........80

                    ::..**** *  .**:**: *  : . : .  .
HUMAN_HemK2α        YMCTDINPEAAACTLETARCNKVHIQPVITDLVKGLLPRLTEKVDLLVFNPPYV-------VTPPQEVGSHGIEAAWAGG  145
HUMAN_HemK2β        YMCTDINPEAAACTLETARCNKVHIQPVITDLVG-----------------------------------SHGIEAAWAGG  117
MOUSE_PRED28α       YMCTDINPEAAACTLETARCNRVHVQPVITDLVHGLLPRLKGKVDLLVFNPPYV-------VTPPEEVGSRGIEAAWAGG  145
MOUSE_PRED28β       YMCTDINPEAAACTLETARCNRVHVQPVITDLVHGLLPRLKGKVDLLVFNPPYV-------VTPPEER------------  133
YEAST_Mtq2          HLAVDINPWALEATLDTAKLNSCKSSFLEVIQADLNSSIRNNQVDVLIFNPPYVPAECVPDVPGSREEADQWLDLALLGG  152
ruler               ........90.......100.......110.......120.......130.......140.......150.......160

                                                      * :
HUMAN_HemK2α        KNGREVMDRFFPLVPDLLSPKGLFYLVTIKENNPEEILKIMKTKGLQGTTALS-RQAGQETLSVLKFTKS  214
HUMAN_HemK2β        KNGREVMDRFFPLVPDLLSPKGLFYLVTIKENNPEEILKIMKTKGLQGTTALS-RQAGQETLSVLKFTKS  186
MOUSE_PRED28α       RNGREVMDRFFPLAPELLSPRGLFYLVTVKENNPEEIFKTMKTRGLQGTTALC-RQAGQEALSVLRFSKS  214
MOUSE_PRED28β       -------------------------------------KSLKQ--------------------------  138
YEAST_Mtq2          KDGMAITDKLLRQLEQILSPDGVAYILFCARNKPKEVIKRFVDTYKWNVKLIETRKAGWEVLSVYSFTR-  221
ruler               .......170.......180.......190.......200.......210.......220.......230
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Fig. 1. ClustalX alignments of human and mouse Mtq2p orthologs. Grey areas show the AdoMet binding site (GxGxG) and the NPPY motif of the

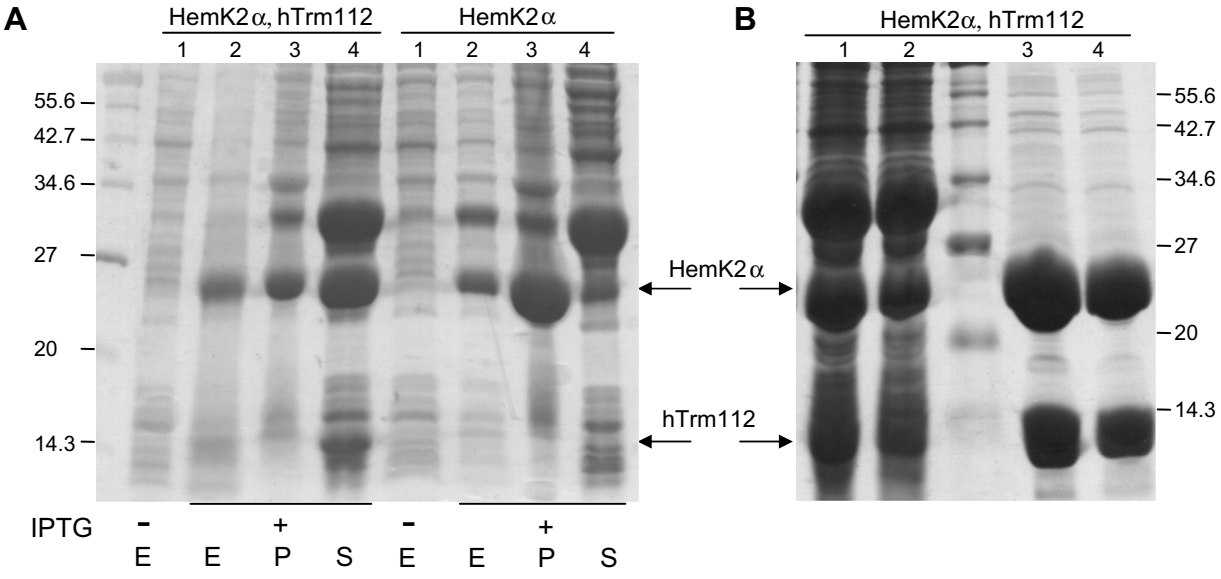

Fig. 2. Co-expression and co-purification of HemK2α together with hTrm112 in *E. coli* BL21 DE3 Gold. (A) HemK2 was overexpressed alone or with hTrm112, using compatible plasmids. Lanes 1 and 2 correspond to the extract (E) before and after IPTG induction. After passage through French press, cells were centrifuged to separate the pellet (P) from soluble proteins (S). (B) Co-elution of His-tagged HemK2 and hTrm112 on Ni-NTA column. Lane 1: total protein, lane 2: flow through, lanes 3 and 4: successive fractions eluted with 150 mM imidazole.

protein, besides promoting HemK2α solubilization, is also essential for methylation activity.

3.4. Human HemK2α is functionally similar to yeast Mtq2p

To gain insight into the biological function of HemK2, we conducted parallel in vivo and in vitro experiments. HemK2α and HemK2β were expressed in a yeast strain deleted for the *mtq2* gene. Fig. 4A shows that the growth defect of this strain can be suppressed to the same level by the expression of either Mtq2p or HemK2α. In both cases the growth becomes similar to that of a wild-type strain. In contrast, HemK2β lacking the NPPY motif does not restore growth of the deleted strain. These results suggest that HemK2α is able to interact in vivo with yeast Trm112p, as observed in vitro (data not shown). Methylation experiments in vitro also underline HemK2α function, since it is able to modify yeast eRF1 in the presence of eRF3. Data concerning heterologous complex eRF1–eRF3 methylation show that human eRF1 has the same affinity for human or yeast eRF3, in contrast to yeast eRF1 (Fig. 4B).

4. Discussion

On the basis of current knowledge, Gln methylation is a rare modification, shown up to now to affect ribosomal protein L3 and RFs in *E. coli* and many bacteria, and eRF1 in yeast. This

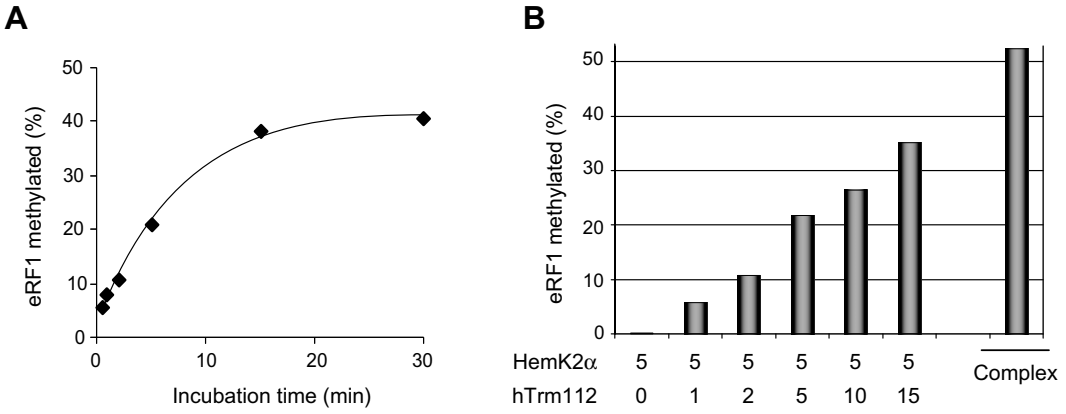

Fig. 3. In vitro methylation of recombinant human eRF1 in the presence of human eRF3Cter, [^{3}H]AdoMet and GTP. (A) In vitro methylation by the purified complex HemK2α/hTrm112. (B) In vitro methylation by HemK2α alone and with various amounts of separately purified hTrm112.

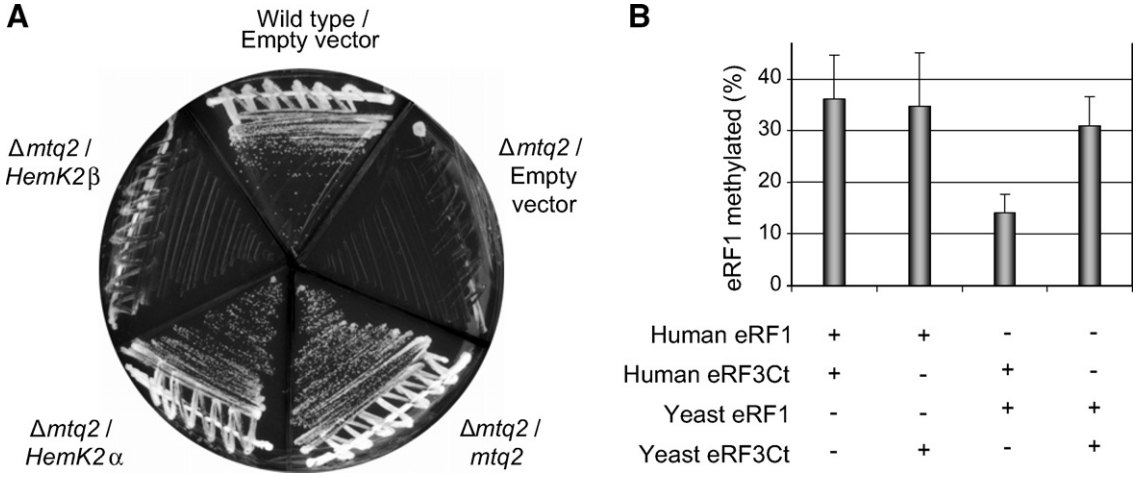

Fig. 4. Human HemK2α is a functional homolog of yeast Mtq2p. (A) Complementation of yeast *Δmtq2* growth defect: strain FF12 was transformed with plasmids carrying gene *mtq2*, *HemK2α* or *HemK2β*, or no insert (genotype/plasmid); the parental wild-type strain BY4741/empty plasmid was used as control. (B) Methylation of purified human or yeast eRF1 by the human methylation complex in the presence of human or yeast eRF3Cter.

is the first report of N5-Gln methylation in higher eukaryotes. Lack of L3 modification in *E. coli* leads to a ribosome assembly defect. Bacterial RF modification is universally conserved, and in *E. coli* is clearly necessary for efficient bacterial translation termination in vivo, since *prmC* inactivation reduces the specific termination activity of RFs by 3- to 4-fold. In *E. coli* K12 strains, which carry a mutation reducing RF2 activity, lack of methylation is no longer compatible with normal cell growth. In strains with normal RF2 activity, *prmC* inactivation considerably reduces growth on poor carbon sources, suggesting that lack of RF methylation limits the synthesis of some essential proteins under such conditions [11].

In *S. cerevisiae*, depletion of Ynl063wp, the mitochondrial MTase shown to methylate termination factor Mrf1p, leads to moderate growth defects on non-fermentable carbon sources and increases readthrough of a stop codon present in Cox2 mRNA [12]. In contrast, the deletion of *mtq2* gene affects growth more severely, decreasing by 2-fold the growth rate at 30 °C. This mutant is sensitive to translation fidelity antibiotics such as paromomycin and geneticin, indicating that eRF1 methylation affects its function and particularly its accuracy. The effects of eRF1 methylation on termination efficiency have not yet been subject to detailed study, but preliminary experiments suggest that lack of methylation may diminish stop codon readthrough, in contrast to observations in bacteria or yeast mitochondria [13]. The deletion mutant is also shown to be resistant to the anti-microtubule drugs thiabendazole and benomyl, suggesting that microtubule-related proteins may be a methylation substrate, or that eRF1 methylation may affect a role of the factor unconnected with translation termination [12].

Distinct isoforms of the catalytic subunits of both human and mouse eRF1 MTases have been identified, reflecting long and short transcription variants. We have shown that HemK2α, the long form of the human enzyme, is functional since it is able to complement the *mtq2* deletion in yeast, in contrast to the short form (HemK2β) lacking the NPPY motif. RT PCR analysis has shown the presence of both transcripts, although to different levels, in all tissues examined in mice, and some experiments suggest different relative levels of the transcripts according to stage of development [8].

Mammalian eRF1 MTase is a heterodimer, as in yeast. The precise function of the non-catalytic subunit is unknown, although the structure of the isolated yeast protein (Trm112p)

has been determined [7]. Trm112p appears to have the unusual property of being shared between several enzymes. In addition to eRF1 methylation, functional studies have demonstrated a role for Trm112p as subunit of the tRNA MTase Trm11p. Moreover, TAP-tag co-purification or two-hybrid mapping studies show interaction with a further tRNA MTase Trm9p, and Lys9p, a dehydrogenase with a Rossman fold similar to that found in RNA MTases [14]. These mutiple interactions may underlie some functional interconnectiveness between the various catalytic pathways. It is not yet know whether multiple interactions are also a characteristic of the mammalian Trm112 subunit, although Trm9p [15] and Trm11p are conserved in higher eukaryotes with at least 30% identity. The increased complexity of nucleic acid and protein modifications in higher eukaryotes in general may be reflected in the variety of interactions of the mammalian Trm112 subunit.

RF MTases present a striking case of misidentification of gene function perpetuated for many years in sequence databases. The product of the bacterial *hemK* gene was first annotated as a putative protoporphyrinogen oxidase required for heme biosynthesis, and later as a DNA adenine MTase on the basis of the (D/N)PPY motif present in identified DNA MTases, in both cases in the absence of significant biochemical data. Subsequent homologs in all three kingdoms were similarly annotated. A combination of biochemical [6,16] and structural [17] data finally led to the identification of HemK N5-glutamine MTase activity and to an understanding the role of the NPPY motif in the recognition of planar nitrogen whether in nucleic acid bases or in Gln side chains. The results presented here correct this confusion in mammalian genome databases, and open the way to studies of the structure and physiological role of the protein and the possible involvement of this activity in human disorders.

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References

[1] Kisselev, L.L. and Buckingham, R.H. (2000) Translational termination comes of age. Trends Biochem. Sci. 25, 561–566.
[2] Frolova, L.Y., Tsivkovskii, R.Y., Sivolobova, G.F., Oparina, N.Y., Serpinsky, O.I., Blinov, V.M., Tatkov, S.I. and Kisselev, L.L. (1999) Mutations in the highly conserved GGQ motif of class 1 polypeptide release factors abolish ability of human eRF1 to trigger peptidyl-tRNA hydrolysis. RNA 5, 1014–1020.
[3] Petry, S., Brodersen, D.E., Murphy IV, F.V., Dunham, C.M., Selmer, M., Tarry, M.J., Kelley, A.C. and Ramakrishnan, V. (2005) Crystal structures of the ribosome in complex with release factors RF1 and RF2 bound to a cognate stop codon. Cell 123, 1255–1266.
[4] Dincbas-Renqvist, V., Engstrom, A., Mora, L., Heurgue-Hamard, V., Buckingham, R. and Ehrenberg, M. (2000) A post-translational modification in the GGQ motif of RF2 from *Escherichia coli* stimulates termination of translation. EMBO J. 19, 6900–6907.
[5] Heurgué-Hamard, V., Champ, S., Mora, L., Merkulova-Rainon, T., Kisselev, L.L. and Buckingham, R.H. (2005) The glutamine residue of the conserved GGQ motif in *Saccharomyces cerevisiae* release factor eRF1 is methylated by the product of the YDR140w gene. J. Biol. Chem. 280, 2439–2545.
[6] Heurgue-Hamard, V., Champ, S., Engstrom, A., Ehrenberg, M. and Buckingham, R.H. (2002) The hemK gene in *Escherichia coli* encodes the N(5)-glutamine methyltransferase that modifies peptide release factors. EMBO J. 21, 769–778.
[7] Heurgue-Hamard, V., Graille, M., Scrima, N., Ulryck, N., Champ, S., van Tilbeurgh, H. and Buckingham, R.H. (2006) The zinc finger protein Ynr046w is plurifunctional and a component of the eRF1 methyltransferase in yeast. J. Biol. Chem. 281, 36140–36148.
[8] Ratel, D., Ravanat, J.L., Charles, M.P., Platet, N., Breuillaud, L., Lunardi, J., Berger, F. and Wion, D. (2006) Undetectable levels of N6-methyl adenine in mouse DNA: cloning and analysis of PRED28, a gene coding for a putative mammalian DNA adenine methyltransferase. FEBS Lett. 580, 3179–3184.
[9] Gietz, R.D. and Woods, R.A. (2002) Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. Methods Enzymol. 350, 87–96.
[10] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
[11] Mora, L., Heurgue-Hamard, V., de Zamaroczy, M., Kervestin, S. and Buckingham, R.H. (2007) Methylation of bacterial release factors RF1 and RF2 is required for normal translation termination in vivo. J. Biol. Chem. 282, 35638–35645.
[12] Polevoda, B., Span, L. and Sherman, F. (2006) The yeast translation release factors Mrf1p and Sup45p (eRF1) are methylated, respectively, by the methyltransferases Mtq1p and Mtq2p. J. Biol. Chem. 281, 2562–2571.
[13] Polevoda, B. and Sherman, F. (2007) Methylation of proteins involved in translation. Mol. Microbiol. 65, 590–606.
[14] Purushothaman, S.K., Bujnicki, J.M., Grosjean, H. and Lapeyre, B. (2005) Trm11p and Trm112p are both required for the formation of 2-methylguanosine at position 10 in yeast tRNA. Mol. Cell. Biol. 25, 4359–4370.
[15] Kalhor, H.R. and Clarke, S. (2003) Novel methyltransferase for modified uridine residues at the wobble position of tRNA. Mol. Cell. Biol. 23, 9283–9292.
[16] Nakahigashi, K. et al. (2002) HemK, a class of protein methyl transferase with similarity to DNA methyl transferases, methylates polypeptide chain release factors, and hemK knockout induces defects in translational termination. Proc. Natl. Acad. Sci. USA 99, 1473–1478.
[17] Schubert, H.L., Phillips, J.D. and Hill, C.P. (2003) Structures along the catalytic pathway of PrmC/HemK, an N5-glutamine AdoMet-dependent methyltransferase. Biochemistry 42, 5592–5599.