SHORT COMMENTS

Trm13p, the tRNA:Xm4 modification enzyme from *Saccharomyces cerevisiae* is a member of the Rossmann-fold MTase superfamily: prediction of structure and active site

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Abstract 2'-O-ribose methylation is one of the most common posttranscriptional modifications in RNA. Methylations at different positions are introduced by enzymes from at least two unrelated superfamilies. Recently, a new family of eukaryotic RNA methyltransferases (MTases) has been identified, and its representative from yeast (Yol125w, renamed as Trm13p) has been shown to 2'-Omethylate position 4 of tRNA. Trm13 is conserved in Eukaryota, but exhibits no sequence similarity to other known MTases. Here, I present the results of bioinformatics analysis which suggest that Trm13 is a strongly diverged member of the Rossmann-fold MTase (RFM) superfamily, and therefore is evolutionarily related to 2'-O-MTases such as Trm7 and fibrillarin. However, the character of conserved residues in the predicted active site of the Trm13 family suggests it may use a different mechanism of ribose methylation than its relatives. A molecular model of the Trm13p structure has been constructed and evaluated for potential accuracy using model quality assessment methods. The predicted structure will facilitate experimental analyses of the Trm13p mechanism of action.

Keywords Methyltransferase \cdot Protein fold-recognition \cdot RNA modification \cdot Rossmann-fold methyltransferase \cdot Zn-finger domain

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Abbreviations

aa	Amino acid(s)
e	Expectation
MTase	Methyltransferase
RFM	Rossmann-fold MTase
SAM	AdoMet, S-adenosyl-L-methionine

Introduction

Stable cellular RNAs, such as tRNAs, rRNAs, small nucleolar RNAs (snoRNAs), contain a large number of posttranscriptional modifications [1, 2]. The most prevalent modifications are 2'-O-ribose methylation and isomerization of uridine to pseudouridine. In Eukaryota and Archaea, methyl groups are introduced by two distinct mechanisms: either by one large enzymatic complex that is targeted to multiple sites by guide snoRNAs or by methyltransferases (MTases) that are specific for individual positions and do not require guide snoRNAs. The catalytic core of the box C/D snoRNA-guided machinery, a protein called fibrillarin [3], is evolutionarily related to a large group of site-specific MTases from the Rossmann-fold MTase (RFM) superfamily that share a common active site comprising a K-D-K triad [4]. Examples of site-specific 2'-O-ribose MTases from the RFM superfamily that share the K-D-K triad are Trm7p or TrMet (Xm32,34) that methylate positions 32 and 34 in the anticodon loop of tRNA [5] and Mrm2p or RlMet(mt-Xm2791) that modify position 2791 of the mitochondrial 21 S rRNA [6]. However, some riboses are methylated by MTases from evolutionarily, structurally, and mechanistically unrelated SPOUT superfamily [7], e.g. by Trm3p or TrMet(Xm18) which catalyze the formation of Gm18 in tRNA [8]. It should be mentioned that some positions can be methylated both by the snoRNA-guided mechanism and by a specific enzyme in the same organism [9], and by unrelated enzymes in different species [10, 11].

Recently, a new family of RNA MTases has been identified, and its representative from Saccharomyces cerevisiae encoded by an open reading frame (ORF) Yol125w has been shown to 2'-O-methylate position 4 of tRNA [12]. Consequently, it has been renamed Trm13p according to the traditional nomenclature or TrMet(Xm4) and in consonance with the MODOMICS nomenclature [2]. Trm13 is conserved in Eukaryota, but exhibits no sequence similarity to other known MTases. As indicated by its discoverers: "the lack of similarity of Trm13 family and other known methyltransferases [4][...] suggests a novel mechanism for catalysis and/or substrate recognition for this protein family or, alternatively, that subtle mechanistic or structural similarities exist between Trm13 and other known methyltransferases". Here, I used bioinformatics methods to address the question whether Trm13 is indeed unrelated to previously known MTases or whether it may be a member of the RFM superfamily, SPOUT superfamily, or one of the six unrelated AdoMet-dependent MTase superfamilies reported thus far in [13, 14]. I also predicted the three-dimensional architecture of its active site and compared it with the previously characterized 2'-O-ribose active sites that may or may not be similar regardless of homology.

Materials and methods

Sequence database searches were carried out with PSI-BLAST [15]. Protein structure prediction was carried out using a new version (http://genesilico.pl/meta2/) of the GeneSilico MetaServer [16] which is a gateway for a variety of methods for making predictions and analyzing their results. Target-template alignments reported by these methods were compared, evaluated, and ranked by the PCONS method [17] to identify the preferred modeling template and the consensus alignment.

The alignments between the sequence of Trm13p and the structure of the best template identified by PCONS were used to carry out comparative modeling. Regions predicted to be completely disordered by the MetaServer were excluded from modeling. For modeling of Trm13p sequence regions matching the structural templates I used the "FRankenstein's Monster" approach [18], while the remaining regions were modeled '*de novo*' with ROSETTA [19]. The "FRankenstein's Monster" method comprises cycles of local realignments in uncertain regions, building alternative models and their evaluation, realignment in poorly scored regions, and merging the best scoring fragments. It was found as one of the most accurate approaches to comparative modeling and FR in the rankings of CASP5 and CASP6 [20, 21]. Previously this approach was used for successful building of structural models for RNA MTases RsmC [22] and TrmB [23] that were later confirmed by crystallographic analyses [24-26]. ROSETTA is one of the best existing methods for de novo modeling of entire proteins and variable protein fragments, and has regularly ranked very high in CASP since it was introduced [27]. For Trm13p, I used ROSETTA loop modeling mode for predicting the structure of regions 245-275 and 349-451. For each region I have independently generated 50,000 decoys and selected the most representative conformation from the largest cluster. Finally, the homology-modeled core was merged with de novo-modeled insertions and optimized using Modeller.

For the evaluation of models I used PROQ [28, 29] and a MetaMQAP method recently developed in our group [30], which allows predicting the deviation of individual residues in the model from their counterparts in the native structure.

The evolutionary rates derived from the multiple sequence alignment of Trm13p homologs were mapped onto the surface of the modeled Trm13p structure using the CONSURF server [31, 32]. The electrostatic potential was calculated using APBS Tools Plug in [33] with default settings, visualized with PyMol program [34] and colored from red (-1 kT) to blue (+1 kT).

Results and discussion

Standard database searches with PSI-BLAST [15] failed to detect any similarity of Trm13p to other protein sequences apart from the previously identified eukaryotic orthologs [12] (an updated alignment of Trm13 family is shown in Fig. 1). Therefore, I submitted the S. cerevisiae Trm13p (ScTrm13) sequence (NCBI accession number 6324447) to the GeneSilico metaserver [16], to predict protein structure and search for remote homology to known protein families and structures. Methods for disorder prediction suggested that the N-terminal part of Trm13p sequence (aa 1-170) may lack stable tertiary structure. On the other hand, several fold-recognition methods aligned the C-terminal part (aa 171-476) with structures of known RFM enzymes. Therefore the C-terminal region was resubmitted for a separate analysis and obtained a confident prediction that it constitutes a separate domain with evident similarity to previously characterized MTases. Interestingly, only enzymes with specificities other than 2'-O-ribose methylation were found among fold-recognition hits. In particular, the structure of rRNA:m₂⁶A MTase KsgA (1qyr in the protein data bank) was identified as the potentially best modeling template by the PCONS consensus method (score 0.774) and

Fig. 1 Multiple alignment of representative sequences from the Trm13 family and the known RFM methyltransferase structures identified by the fold-recognition. Conserved motifs are indicated. Residues predicted to be involved in AdoMet-binding "*", RNA binding "•", and catalysis "!" are indicated above the alignment

	180 190 200 • • • • • • • • • • • • • • • • • • •
Trm13p-6324447	SNTREAVOSSHILENIVDAGAFERPESLNTINGCERAEFSRYVSLYLTOLTSLPAEHSGSNSNETVITEATNEMFERTIKDPSEIKSNSPSKPISCP
Schpom-19114826	TKRMAIOCASLLEHMEKLHYFDN-QGSIYYEFGAGRAELSRYVQHCSQQENVYILIDRDSNSTEHDSRILKDSIKNNWPEP
Canalb-68474490	TNOKEPIOONSIIANNDSMOALSN-SFLYLEFOAGKEDASRYLNQCILQQKTECSCTYGGGIDEGKNELBASSKIISDSETLQTKP
Cangla-50286685	TIKKAKQCSBIQNLFDVEFIPSGKIMEFCCERAEFSRYINQTIIN(2) PYQLQDDLPKTVHIDRGSNRAFFCKFQDDLMALSKNP-EKVKQRI
Canlup-73959992	SATKHIKOOASILGHIBKLKALGPRRCFVEFGAGKEKISHWVDIALKDAEKVHBILVEKVTTRFKVDGKHRKKNS
Klulac-50308573	VNRHIAROOSSIJQHLKESKIMPS(9)LEYIDIGCCRAEFSRYVNIJTNLDOK(5)KPEYKAVAPSBTJJDASQRLRFDNRFSSDIGTEV
Yarlip-50554091	QNRGHALQQASHIAHMSKTGAKS-SSRILJSCAGAABASRYINYMICAERQQDDKTAANYLFIDRAMPLMMDGGUVKDTKDDFPEQPLP
Aedaeg-108872514	QTLANDINGSALVETLENENFFON-DVALVAUGAGAGOVAFVLATVDQSDLLNVKVFAVBASHCHARKINGLEDRE
Anogam-118780995 Aratha-30678989	QTIANITYASSAIINGLANQCOPINE "DITIYAANACAOVAYATNIAAYITAPLAR"
Ashgos-45187710	SUBJECTION DELOTER (1/ / VIVIERCORPOSTED TO THAT TO THE STATE OF THE S
Caeele-17565856	TKKGOLYOISSILCHIESTGLPTCSKSCMPELCAGKOLAYWISKAAPNGN-YILMORSGSRUSPDTAAFRENP
Danrer-62122807	SAFKHLKOCASILENMSALELISPNRCYIBEGAGKEKLSHWIHIALKSAENVH
Drome1-28571533	ETLROITOTALEILEHDHOMD-HTSYLEYGAGKCOLAYFLATVUQEQKLSHSQVVUIDEMSLEHKONKLANRE
Canfam-73959992	SATKHEROOASILCNIERLEALGP-RRCEVEFGAGKCKASHWVDIALEDAEKVHGILMEKVTTREWVGKHRKKNS
Galgal-118094303	SAFKHEKOOASILGNMEKLHALGPGRCEVEFGACRCKASHWYDIALODVENIQGLLIERATTREWOGKHKKRDS
Homsap-108885172	SATKHIKOOASILENIDNLKIAGPRRCEVEFGAGKERISHWVDIALKDAEKVHDIAVEKVTTREWVDGHRKKNS
Macmul-109011463	SAAKHEKOOASIINEETIDKLKUNEGPRRCEVERGAGKEKONSHWVDIANKOAEKVHGINVERVTTEFEVV)GRHRKKNS
Musmus-124430711 Pantro-114557885	CAVMING VISION SIDE SIDE AND A CONSISTING AND A SAVE AND A SA
Ratnor-109467481	CAVINIES DESIGNED A DESIGNED AND A DESIGNED AND A DESIGNED A DESIGNEDA DESIGNEDA DESIGNED A DESIGNED A DESIGNEDA
Xenlae-49257236	TASKILKODASILCHIDSLGHGG-SRC-VERCHERKISHWUDIATCGAENIH
Tricas-91093427	KSKNIJKCASAIJACLIDESGIIKP-ETCZVEIGAERCLISYWLAOACPOAT
Trybru-72388258	ISNE GPOHRAL RCVQTVIRGYA (15) GELELCAGE AGE AGE AGE AGE AGE AGE AGE AGE AGE
Trycru-71651932	MSAKEGPOHRAALRCLQKAIEGFF(14)GEVEFGAGKCGLSVALQDVLLSHA(19)RKPLLVVVDVGNFRRGDARVSRTSL
Leimaj-68128108	SSLKHGPOHMGHIRCLSDVVRQAQ(19)GTUBEGAGKCGLSAALQQLIVQRL(45)FLAEVAQRPPLVVLDMMGFRRSDARVRHSAV
Leiinf-146094765	ASLINGPOYMGDIRCLSDVVRQAQ(20)GELECCAGKCGASAALQQLIVQRL(45)FLAEVAQRPPLVVLDMNGFERSSARVRHSAV
Leibra-154342238	ASOMHOPOHMGHIRCLSDVIRQVH(20)GELHATATEKEGASVALQQLIVQRL(45)FLAEVAQRPPLVVLDMDGFERSGJARVRHTAV
	<pre>c< Notif I</pre>
Trm13p-6324447	<pre>Will X >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>></pre>
1QYR	QNFLNDQFVIDSIVSAINPQKGQAMVEIEPCLAAUTEPVGERLDQLTVIELDRDLAARLQTHPFLGP
1QAM	QNFITSKHNIDKIMTNIRINEHDNIFEIESOKEHFTLELVQRCNFVTAIEIDHKLCETTENKLVDHD
1EIZ	GLRSRAWFKIDEIQQSDKIFKP-GMTVIDLARAPGGWSQYVVTQIGGKGRIIACLLPMDPIV
	200 290 300 1 320 330 340 1 350 360 370 I I I I I I I I.
Trm13p-6324447	SIKRIKIDIRDIRMOPILKSTPGDDIQYACISKHACCVATOLTIRCIGNSSILHGDDNNGCNPKLKAICIAKCCRHVODYGDAVNRSYVTSLVEKYRAHCSILT
Schpom-19114826	KIIRCKIDIKDLKLDFFASEFRNSGKPVFAYSKHLCFATDLTINGIGNSSILLKSSPPNALVIALCCHHCRARTLSTFARBOLSHWFIS
Canalb-68474490	QIRSSRIDHKDAMADRELVDLKIDRVWARSRHAGGAATDITTASILMSTLLSNDQFGGALIAMCORIVGSLEQLLPQSRKYLHDHGFT
Cangla-50286685 Canlup-73959992	ALDRIKLDIKOVOSTVIJEEHDGCLAISKHLGEVANDTIKGEORCIEETNGKFKLGELCIAGCORINGNARDYINPDIKSEL(7) H
Klulac-50308573	
Yarlip-50554091	LIKEBERGOIKOIKOIKOVPEREPY-DANLIVSKURGCOVO/THOGLUSAVFTKEARDALIVIAGOROICOVO/FPKATIB/LOEREF
Aedaeg-108872514	IIOSVRADICD/VHOKLDVLRDSRKINGVESKHORGAVDIA/HEG/I(5) RPEGDTLOSDEFWFA/GCHIRCDXKTVARKEFLEKEI
Anogam-118780995	IVOSVRADIAD LEUHRVELIEA (4) SGVVGIGKHLCEAATDLALRGLVRSGTSGTAVRECLFALCCHHRCDWRTFAEKRFLLESGI
Aratha-30678989	VLERMRIDIEDINENAVESLIGVPYVAVEKHLCEPATDLSLRECLSRQDGESPVLRELANTCCHHLSQNKSHINKEYILSLEI
Ashgos-45187710	AVTWERIDERDELEVALLEPGLEHIAVSHLGEVATOLTURGHAAGPRASLCEALHANCERHAENPAENAMPATVEALL (7) S
Caeele-17565856	SMKRFRCSHEHNDESKIDELKNSEKILAHCKHFCCSATDAGIRSHNSGLQFNAALLIPCCHHKSREAE
Danrer-62122807	TPDRIQUDIQHDDRKVPLLREKG-LPVIGVGKHLCGATDLALRGLFEHNCT (33) ETGKEIVVSGLAIALCGHRCDARHYVCKEFFREREL
Drome1-28571533	VVQGIRADIADFOISALPELKKTQRTYAFSKHLGEAATDITIRGILGDGNASSOYVLIALCEHHRGESRSYVERKFLQEAEI
Canfam-73959992	VF BEITOLING DER LINKLY LISKEK-LPVVGLEIKELGEVANDA ALKELVETYA (34) VPERVTPVACIV FACHERICOXRET
Galgal-118094303	1F BATAY DAVID JERVET LEBRAR-LEVIG IGAII GCAVIDALISCU VSIALISCU VSIALISCU VIACGILISCU TAVOLINGUM TAV
Homsap-108885172 Macmul-109011463	
Musmus-124430711	VFBMORITOHIC/INRVPULREGR-LPWG/GKHIGC/WWWWARGPVETYAA(34) VPETWFPVAEIW-MAGHIRGOARW
Pantro-114557885	VFERIONDIOHICINKI PVLREEK-LPVWG (GKHLGGOANDIALRGI/VETYAA (34) VPEKWNPVACIW FAKGHLRGDARH
Ratnor-109467481	VFERLOIDIOHUCLWRLPVLREGR-LPVVGIGKHLCCVATDLALRCLVETYAA (34) VPETRTPVAGIVIALCCHHROMRHYVCREYFKALEL
Xenlae-49257236	VFBRIGIDIQHICHDRVPSLSQKH-LPVIGIGKHLGCAGTDLAARGLNQSNFP(28)P-VSSACAGCIVIALCCHHRGDAHHYVCRSFQSLCL
Tricas-91093427	KVRSTRADIADIVIDKLDAVSGNPTWGVTSHAGEDAVDIAASELANVSSVKVGELTMTFSGHEBGRAPSYVEKDFFNRFEL

FIRCHER TOPOTTADD	
Musmus-124430711	VFERLOIDIOHDCDNRVPVLREGR-LPVVGIGKHLCGVATDVALRCLVETYAA(34)VPETWTPVAEIVIALCCHHRCDARHYVGEEYFKALGL
Pantro-114557885	VFERLOIDIOHUCLNKIPVLREEK-LPVWGIGKHLCGMATDLALRCLVETYAA (34) VPEKWNPVAGIVIALCCHHRCDWRHYVGREYFRALGL
Ratnor-109467481	VFERLOIDIOHUCUNRLPVLREGR-LPVWGIGKHLCGVATDLALRCLVETYAA (34) VPETRTPVAGIVIALCCHHRCDURHYVEREYFKALEL
Xenlae-49257236	VFERLOIDIOHUCDRVPSLSoKH-LPVIGIGKHLCGAGTDLALRCLMOSNFP(28)P-VSSACAGETVIALCCHHRCDXHHYVGREFFOSLGL
Tricas-91093427	KVRRIRADIADIVIDKLDAVSGNPIVGVTKHLCEDATDLALRCLAHVSSVKVGELTMTFCCHHRCRAPSYVEKDFFNRFEL
Trybru-72388258	PLVRERENEKOLDUAKALCGA (27) EQWAVEKHLCGACTOFALSOVTAPNLNTEGFASVCAVWERTGCHORCELKEINA (4) SECREGRIAIPE (4) F
Trycru-71651932	PLVRPRLDHKDLELAKALRDP (28) ERWALEKHLCGACYDPALSCITSPNLCTEGRASVIAVWPATCCHILCELRELNA (4) EDDQQAILRLPG (4) I
Leimaj-68128108	PLORURENERSVDLTRAFLSE (25) ENWAVLCKHLCCACTOFALSCLTESPLLTSQAQVRLPIVWTATCCHRCELREVNPPEQAEWAPAPLVLPC (4) W
Leiinf-146094765	PLORURENERSVDLTRAFLSE (24) ENWAVLGKHLCGACTDFALSGLTESPLLTSQAQVRLPIVWIATGCHHRCELKEVNPPERVEVSPAPLVLPG (4) W
Leibra-154342238	PLORUBEINHKOVDITRAFLSE (24) ENWAVLEKHLCEACTOPALSOFTENPVLISOAHVOLPILVIATCCHHRCELKHMNPPEOLEWAPAPLVLPE (4) W
Trm13p-6324447	SIKEIKIDERDEKKDPILKSTPGDDIQYNCESKHIGEVANDETHKEIGNSSILHGDDNNGCNPKLKAICHAMCCREVODYGDYWRSYVTSLVEKYRAHESILT
1QYR	KLTIYQQDAMTFNFGE-LAEKMGQPLRVFENLPYNISNPLMFHLFSYTDAIADMHFMLQKEVVNRLVAGPNSKA
1QAM	NFQVENKTLQFKFPKNQSYKIFENIPYNISTEIIRKIYFDSIADENYLIVEYGFAKRLLNT
1EIZ	GVDFLQGFR ELVMKALLERVGD (9) SDMAPNMSGTPLV IPRAMYLVELALMCRDVLAPGGSFVVKVFQGEGFDEYLR

	380	390	400	410	420	430	440	450	460	1 490	
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Trm13p-6324447	YETFERV	LTKLCSOG	CCRKPGTAI	TDIVNVVESF	EGAEPYTITI	KERENICLMAR	RVIDEGRLV	VKEKFTEFNJ	ELIREVES	DVSLENVAM	LVYKK
Schpom-19114826	NPOREQI	LROUTGOW	NSLEEHMHA	SGGADSH	ISGUSH	DERVKIGLKCK	HINNMERL	ECERMEYES	SHVYYVGE	STTLENVAL	INYKR(2)
Cana1b-68474490	TVOSENV	LKKLVSMAN	DKKDAQENG	IL	GUNT	HOREELCLKAR	RLIDESEVH	AKSIL (4) K	EIFWYVQK	DUTLENVCL	SISRR(2)
Cangla-50286685	YKTFEGH	LSKOCSWAT	SEREPGMND	DDIVQIEGD-	-NQEQLSISL	LOCEKICLFAR	YLIDNGELE	WVKQNL(6)NJ	KIIKYIEP	DWSRENNAL	LVY
Can1up-73959992	GAVERHY	FORMSSWAT	COMRKTSLE	ASNLTTKRK (24) VPGFTTV	ESKINGLER HOTEELCLKAR LOTEELCLKAR ESKINGLFAR ESKINGHLAR NEORIGHMAR	LLIDOGRVE	DOOKEFSI	ABOYMOP	VSLENVLL	TELPN(9)
Klulac-50308573	YSEFFOC	LKKFCSYCE	CGLEPDM		(8) HITKETH	NERORICHMAR	RIDEGRAQ	FLOSKEFET	VEFRIDS.	AWTLEDTAL	LALRK(2)
Yarlip-50554091	DDDGGAA	LTROTSOVL	CCERPKKEP	VEGEQTEAA-	(9) HPSGEPA	EARKVICLKCR SOREEVCRRCK	RLLDOGRLH	ARKECLDA	RIVOYEEM	DISPENNCE	IVNK
Aedaeg-108872514	SREDEDL	VVROVSMM	CETGTSRER	RN	(8) IKYGMTR	SOREEVERRON	MLDWGRIQ	KENCEEA	GERLEAKA	ETTLENVCL	IGHVK
Anogam-118780995	TRADES	IVROVSWAV	CGTGRSRER	00	(5) DRCGMTR	PEREATORROW	RLLDIARLR	MERHEYEZ	SERVICTS	WTLENVCL	VCVKK
Aratha-30678989	SKDEEHI	MISTISAN	DDDHGSKLP	GVDDIDLLD (20) VVKKMKP	MERAVLOFICK	DINDACIMK	WVKKHELNS	KEVKHIPA	SISPERTLL	IGKP(5)
Ashgos-45187710	YDLFELA	LMRCASMAN	SEREPGVPD		(5) HESGLAI	PHEAFCRRCK MSRAVLCFKCK AMERLCHLAR HPPLELCRRAK	RLVDEGRRQ	FADLEYDA	ELVICER	SISPENTLL DTSRODIAL EVSPENLLI	LVRRP(1)
Caeele-17565856	DEASEAA	LRYIASPAT	NEAVDTEAT	EG	WKSI	HPPLELCRRAK	AILEIGRAI	WESVEFKT	RVVEWVPP	EVSPENILLI	LALK
Danrer-62122807	GPEDEAA	FORMSSWAT	CEMOKAAET						SMKYNESR	DWSHDRWLI	AMIPI-(2)
Drome1-28571533	GPREEVI	LTROVSWAV	CETGLSRER	RKAMESAD	(6) NTORITR	OBREQICOOCK	RVLDYGRLE	HIRSHEYQJ	EKFYVPR	DVTLENVVL	LARPT(10)
Canfam-73959992	GAVESHY	FORMSSWAT	COMEKTSLE	ASNLTTKRK (24) VPGFILTV	OBREQICOOCK	LLHDOGEVE	OOKEFSI	ABOYNOP	VSLENVLL	TELPN-(9)
Galgal-118094303	GPVERHY	FORUSSMAN	COMPGTITE	ASTNEESE- (24) LORLINTV	EFEKEICELOR	LVHOHESIR	LOHRCYK	ALOYYADS	SVSLENVLL	THVPD-(3)
Homsap-108885172	CAUSSIN	POPULATION	COLLEGE MOT D	monometroo/	24) T DOT LOU	DOUBLE STREET,	TIMONITO	TOOPER CT			TELPN-(9)
Macmul-109011463	GAVIDEHY	FORMSSWAT	COMPRESLE	TSNSTTKRQ (24) LPGLISV	EDKKRIGHLCK EDKKRIGHLCK EDKKRIGHLCK	LLIDOGRIO	QOKEFSE	ARQYNEDP	VSLENVLL	TELPN-(9)
Musmus-124430711	GAVESYY	FORMSSWAT	CCMR-TSLE	GSDVTPERK (24) LPGI TV	ENKKRICHLCK	LLIDOGRLO	DOOKEFSI	ABOYMOP	USLENVLL	THVPA(10)
Pantro-114557885	GAVERHY	FORMISSWAT	COMPKTSLE	TSDSTTKRQ (24) LPGLESV	EDKKKICHLCK	LLIDOGRIQ	WQQKCFSI	ABQYMDP	USLENVLL	TALPN-(9) TAVPA(24)
Ratnor-109467481	GAVESYY	FORMSSWAT	COMR-TSLE	ASDVTAERK (24) LPGTITV	EBREKICHLCR	LLIDQUELQ	QQKEFSI	ABOYNADP	VSLENVLL	TAVPA(24)
Xenlae-49257236	DORDENL	FORMSSWAT	COMEKLPAK	AIQSDEQME (17) VEGFLITV	KHRENLORLOR SQKEELORRSK	LLIDYGRVD	WQRMCYI/	ABOYMEP	VSLENVL	THVPR
Tricas-91093427	TKNDEEM	MCGMSSWAT	CETGFSREK	NOTGGTVEG-	(4) KEIGVTR	SQKEELCRRSK	AILNWEELQ	FLEGLEFEC	RHYYVEA	DVSLENVCI	VERK
Trybru-72388258	SEREAA	ITSUSSWAV	SE		EAVD	ABERLTCMCOR	RVHDAFRLE	KQSEFR-RT		SWIGENICI	
Trycru-71651932	STADEAA		SC		TAVD	KERQTTCVCCK	RVIDALRIQ	KQNEYR-SI	YNCONIEK	GITEENWTI	VMFR
Leimaj-68128108	SEQUERAA	LASUSSIA	CG		DVVD	EDERAVERKON	RIIDQLEVH	FERYLEYV	FQCQUETR	DWTEENWCI	VAFQAS
Leiinf-146094765	SEQUEAA	LASSISSIM	CG		DFVD	EDURAVERKOR	RINDOLEVH	FURYLEYVI	FOCOMTR	DWTERNUCI	VAFHAS
Leibra-154342238	SEMBRAA	LASSISSIM	CG		NEVD	EBERTVERKCK	RIIDQLEVH	FURRLEYVI	FOCOMETR	DWTEENWCL	VHFQAS
									btif VID		tif VIID
Trm13p-6324447	YETFORV	LTKLCSAG	CERKPGTAI	TDIVNVVESF	EGAEPYTITI	KERENICLMAR	RVIDEGRLV	VKEKFTEFN	ELIRAVES	WSILDIWAM	LVYKK
1QYR							YGRLS	MAQY-YCNVI	PVLEVPP (DSAVURI	VPHAT
1 QAM								FLMAEVDIS			
1EIZ								FTKVK			
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was used for the comparative modeling of the central part of Trm13p CTD. Among the top 10 templates there were also other structures from the $m_{(2)}^{6}A$ MTase family, including 2erc, 2h1r, and 1qam (see Table 1 for the summary of fold-recognition results).

Although the fold-recognition analysis revealed clear similarity of Trm13 to RFM proteins, the alignments returned by different methods were similar only in the putative cofactor-binding part (aa 171-310 Trm13p) and differed greatly in the C-terminus. Therefore, in order to build a reasonable model for the whole catalytic domain, I constructed a model of the protein core using the "Frankenstein's monster" method and subsequently added variable regions using ROSETTA (see Methods for details). In the course of the modeling, the alignment of the Trm13 protein with the structural templates was refined (Fig. 1). The final model corresponding to this alignment (Fig. 2a and b) was assessed by PROQ as 'potentially very good' (LGscore 2.586), and according to MetaMAQP was predicted to exhibit the global root mean square deviation of ~3.67Å from the native structure. Although these scores suggest that our computationally-modeled structure is of lower accuracy than a crystal structure could be, it appears to be of sufficient accuracy to infer residues that may be important for cofactor-binding, RNA binding, and catalysis.

The S-adenosyl-L-methionine (SAM) was added based on the orientation of the cofactor in the crystal structure of rRNA $m_{(2)}^{6}A$ methyltransferase from *Bacillus subtilis* (1 qam) and the adenine was added to the model based on its orientation in other adenine methylating RFM superfamily members and subsequently minimized with HyperChem program [35].

All RFM proteins bind the AdoMet molecule in nearly the same manner; therefore the availability of a structural model makes prediction of cofactor-binding residues in Trm13p straightforward. By comparison with other members of the RFM superfamily one can predict that conserved residues from motifs I, II and III of Trm13p are responsible for binding of AdoMet, in particular E204 from motif I coordinates the methionine moiety, D243 from motif II coordinates the ribose hydroxyl groups, while D282 from motif III coordinates the N6 group of the adenine moiety (Fig. 2c). On the other hand, substrate-binding and catalytic residues are not conserved between different families of RFM enzymes, and their identification is often devious in these proteins without the X-ray structure available. However, our model of Trm13p shows that invariant or conservatively substituted residues (K307 in motif IV, H350 in motif VI, E467 in motif VIII, and several Cys residues in motifs IV and VI) form a pocket adjacent to the AdoMet- binding site. This suggests that at least some of them may be involved in the

Table 1 The summary of fold-recognition results

PPB code	Asigned specificity	Substrate	MetaServer scores	Description	Organism		
1QYR [43]	m ₂ ⁶ A	rRNA	Sparks [44]: -2.22 pcons5 [17]: 0.7738	KsgA	Escherichia coli		
			ffas [45]: -6.81				
			Hhsearch[46]: 14.94				
			seq.id[%]: 13				
1QAM [47]	m ₂ ⁶ A	rRNA	pcons5 [17]: 0.6021 inbgu [48]: 9.75	ErmC'	Bacillus subtilis		
			3dpssm[49]: 4.3				
			seq.id[%]: 12				
2ERC [50]	m ₂ ⁶ A	rRNA	pcons5 [17]: 0.6133 mgenthr.[51]: 0.280	ErmC'A	Bacillus subtilis		
			seq.id[%]: 12				
1YUB [52]	m ₂ ⁶ A	rRNA	Inbgu [48]: 11.18 3dpssm[49]: 0.3	ErmAM	Streptococcus pneumoniae		
			seq.id[%]: 16				
1IM8 [53]	unknown	unknown	pcons5 [17]: 0.6147 fugue [54]: 5.83	YecO	Haemophilus influenzae		
			seq.id[%]: 13				
1Y8C [ref.NA]	unknown	unknown	pcons5[17]: 0.65 sparks [44]: -2.20	Methyltransf_12 PF08242	Clostridium acetobutylicum		
			fugue [54]: 4.54 seq.id[%]: 11	PF01159			

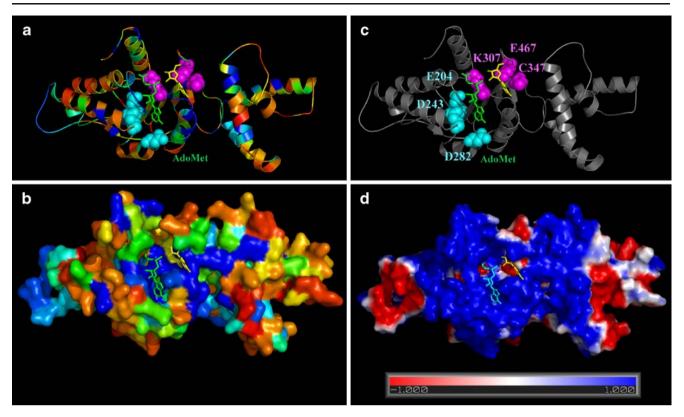


Fig. 2 A structural model of Trm13p. The Trm13p model colored according to sequence conservation in the Trm13p family, from deep blue (invariant), through light blue (conserved), to yellow/red (highly variable) in the cartoon representation (\mathbf{a}) and surface representation (\mathbf{b}). A highly conserved blue patch indicates the cofactor-binding site and the predicted tRNA- binding/catalytic site. (\mathbf{c}) The protein backbone is shown as a ribbon, the functionally important residues are shown in the wire-frame representation (magenta - catalytic residues, cyan – AdoMet binding residues), AdoMet (green), Adenine

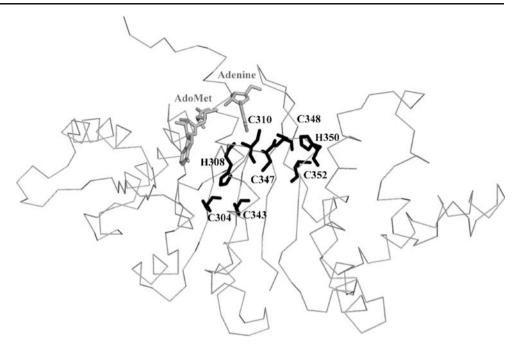
binding of the target base and/or catalysis of the methyl transfer reaction. Interestingly, the pattern of invariant residues in the Trm13 family is different from all ribose MTases studied to date. In particular, invariant K307 has never been observed in motif IV, which instead typically harbors a carboxylate residue. The K307 in Trm13 could play a similar role to that of non-homologous Lys from motif VI in the RrmJ-like family of MTases, where it has been implicated in catalytic center formation. E467 could fulfill the role of homologous E199 in motif VIII of RrmJ or of the non-homologous D124 from motif IV of RrmJ [36].

Particularly puzzling is the conservation of Cys and His residues in the vicinity of the active site of Trm13p (Fig. 3). Cys residues from motif IV and/or VI of other RFM MTases have been implicated in catalysis of C5-methylation of pyrimidines to yield m⁵U in RNA and m⁵C in RNA or DNA [37–40]. However, endocyclic carbon methylation is a chemically difficult reaction that requires formation of a covalent protein-base adduct, while there is no specific reason to employ a similar mechanism

(yellow). (d) Trm13p model colored according to the distribution of electrostatic potential, from red (-1 kT) to blue (+1 kT). AdoMet binds in a negatively-charged, red-colored cleft at the right hand side, while a blue patch in the middle of the structure suggests the localization of a tRNA-binding site around the catalytic pocket. This figure was prepared using the program PyMol [34], the electrostatic potential was calculated using the APBS tools plug in to PyMol [33], and the sequence conservation was calculated with Consurf program [31, 32]

to carry out methylation of ribose 2'O atom. In fact, known ribose MTases from the RFM superfamily such as RrmJ/fibrillarin family [4] or HEN1 family [41], as well as those from the unrelated SPOUT superfamily [7] utilize simple active sites rich in charged side-chains, but no Cys residues. The model suggests that invariant Cys residues in Trm13p may be too far from the 2'OH group of the target ribose to be directly involved in the methylation reaction. At least one pair of Cys residues, C304-C343, may form a disulfide bridge that would stabilize the protein structure. One or two pairs of disulfides may also be formed by C310-C348 and/or C347-C352. However, these residues are in such proximity to each other and to invariant H308 and H350 that it is also possible that some of these residues may together form a metal-binding site. In any case, according to the predictions made the conserved Cys and His residues in Trm13p are more likely to be involved in structural stabilization of the protein rather than directly in methylation, although an auxiliary role in catalysis cannot be excluded, e.g., such as acting as a base aiding the proton

Fig. 3 Cys and His residues located in the vicinity of the active site of the Trm13p structural model. The entire model is presented in the C α trace representation, cofactors are marked in gray and the Cys/His residues side chains are presented in black, all mentioned molecules are labeled



extraction and β -elimination postulated for Cys in motif IV of RNA:m⁵C MTases.

Analysis of the electrostatic potential distribution on the protein surface reveals a highly conserved positively charged saddle-like region on both sides of the cofactor binding patch (Fig. 2d). It suggests that this part could accommodate and position the substrate during the transfer of the methyl group from the donor to acceptor molecule. Thus, the following residues are likely to be involved in recognition of the macromolecular tRNA substrate by Trm13p: K176, K177, Q180 (the motif KKxxQ). Additionally, the entire N-terminus is positively charged (estimated charge at pH 7.00=8.2 for residues I279–I283, I243–M249, E204–E211 as calculated based on the model), suggesting that it may be involved in tRNA recognition by Trm13p.

It must be emphasized that, in addition to the modeled RFM domain, Trm13p possesses an N-terminal extension predicted to be intrinsically disordered (which therefore could not be included in the static structural model). However, the N-terminal region contains a predicted Zn-finger (residues 70-100) resembling the TRAF-type Zn-finger (Pfam 02176, MetaServer-hhsearch cdd score of 20.8, and the Hmmpfam score of 8.1) present in Eukaryotes, and AN1-like zinc finger (pfam 01428, MetaServer-hhsearch cdd score of 16.25), which is a zinc finger found at the C-terminus of An1-a ubiquitin-like protein in Xenopus laevis. Main residues predicted to be a part of this structural motif are C75, H81, H91, C95. It suggests that Trm13p apart from the SAM molecule requires zinc ion to perform methyl group transfer. Nevertheless, I was unable to model the N-terminal domain of Trm13p using comparative methods. However, in the course of this analysis there was an article published by Andreeva and Tidow [42] who confirmed our predictions concerning the Zn-finger domain and answered the question why I was unable to determine the structure of this part of Trm13p. They showed that this part of the investigated protein is a novel Zn-finger domain that has never been described previously in the literature. Moreover, they show that this domain constitutes an independent folding unit.

Conclusions

My bioinformatics analysis reveals that Trm13p belongs to the RFM superfamily of MTases despite the absence of a significant sequence similarity to previously characterized members of this superfamily. Our model reveals residues potentially responsible for cofactor binding, tRNA binding, and catalysis of the 2'-O-methylation reaction. Moreover, the model suggests a presence of one to three disulfide bonds and/or metal ion binding site next to the substrate binding pocket. Our predictions shed more light on this poorly characterized enzyme and will facilitate experimental analyses to characterize the biochemical activity of Trm13p.

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