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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

Karolina L. Tkaczuk

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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′-O-methylate 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 · Protein fold-recognition · RNA modification · Rossmann-fold methyltransferase · Zn-finger domain

K. L. Tkaczuk
Laboratory of Bioinformatics and Protein Engineering,
International Institute of Molecular and Cell Biology,
Trojdena 4,
02-109 Warsaw, Poland

K. L. Tkaczuk (✉)
Institute of Technical Biochemistry, Technical University of Lodz,
B. Stefanowskiego 4/10,
90-924 Lodz, Poland
e-mail: karolina_tkaczuk@go2.pl

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:m_2^6A 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

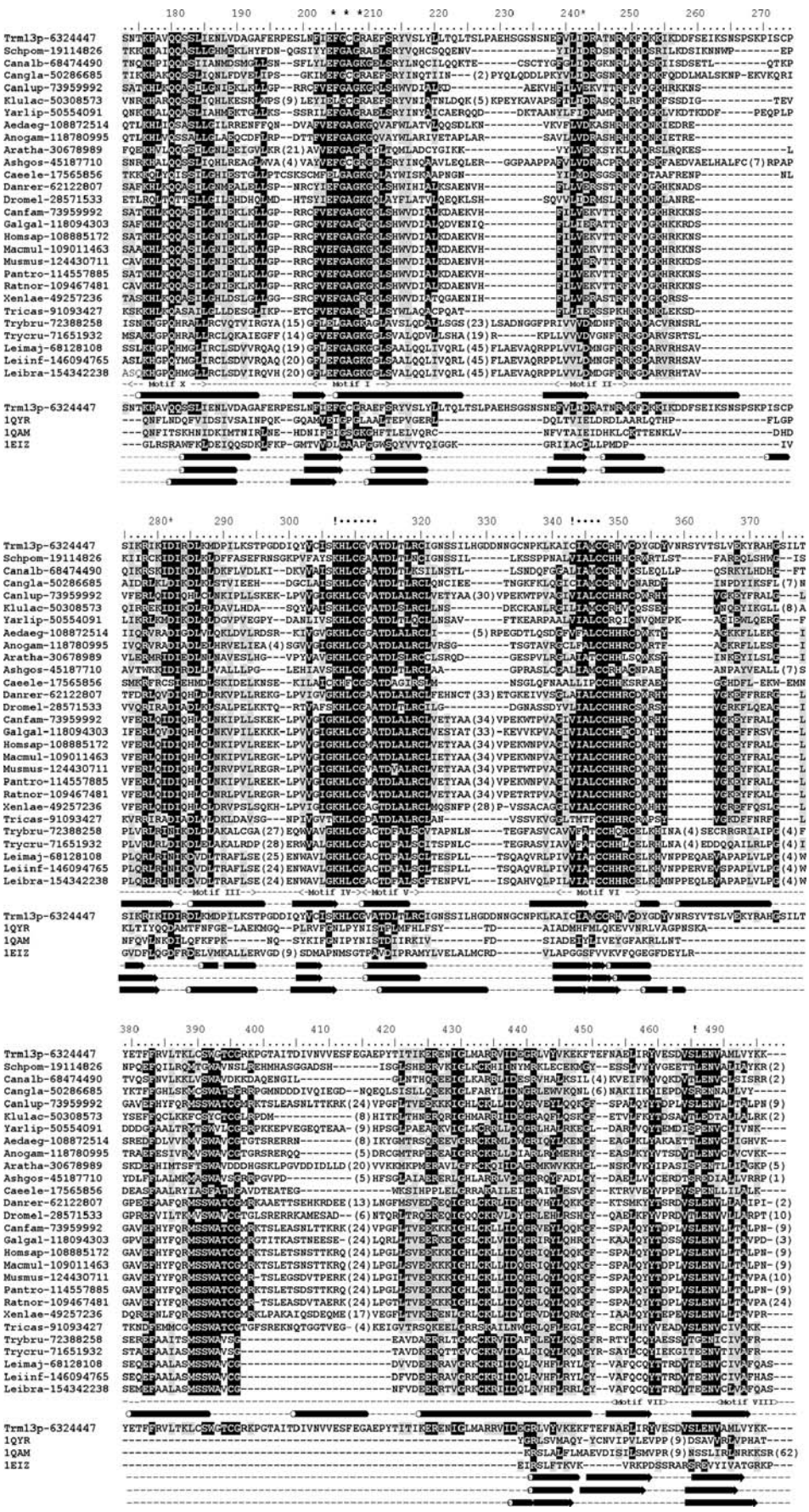

Fig. 1 Multiple alignment of representative sequences from the Trm13 family and the known RFM methyltransferase structures identified by the fold-recognition. Conserved motifs are indicated. Residues predicted to be involved in AdoMet-binding "*", RNA binding "•", and catalysis "!" are indicated above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

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 $m_{(2)}{}^6A$ 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 ~3.67 Å 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 $m_{(2)}{}^6A$ 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]	$m_2{}^6A$	rRNA	Sparks [44]: −2.22 pcons5 [17]: 0.7738 ffas [45]: −6.81 Hhsearch[46]: 14.94 seq.id[%]: 13	KsgA	*Escherichia coli*
1QAM [47]	$m_2{}^6A$	rRNA	pcons5 [17]: 0.6021 inbgu [48]: 9.75 3dpssm[49]: 4.3 seq.id[%]: 12	ErmC'	*Bacillus subtilis*
2ERC [50]	$m_2{}^6A$	rRNA	pcons5 [17]: 0.6133 mgenthr.[51]: 0.280 seq.id[%]: 12	ErmC'A	*Bacillus subtilis*
1YUB [52]	$m_2{}^6A$	rRNA	Inbgu [48]: 11.18 3dpssm[49]: 0.3 seq.id[%]: 16	ErmAM	*Streptococcus pneumoniae*
1IM8 [53]	unknown	unknown	pcons5 [17]: 0.6147 fugue [54]: 5.83 seq.id[%]: 13	YecO	*Haemophilus influenzae*
1Y8C [ref.NA]	unknown	unknown	pcons5[17]: 0.65 sparks [44]: −2.20 fugue [54]: 4.54 seq.id[%]: 11	Methyltransf_12 PF08242 PF01159	*Clostridium acetobutylicum*

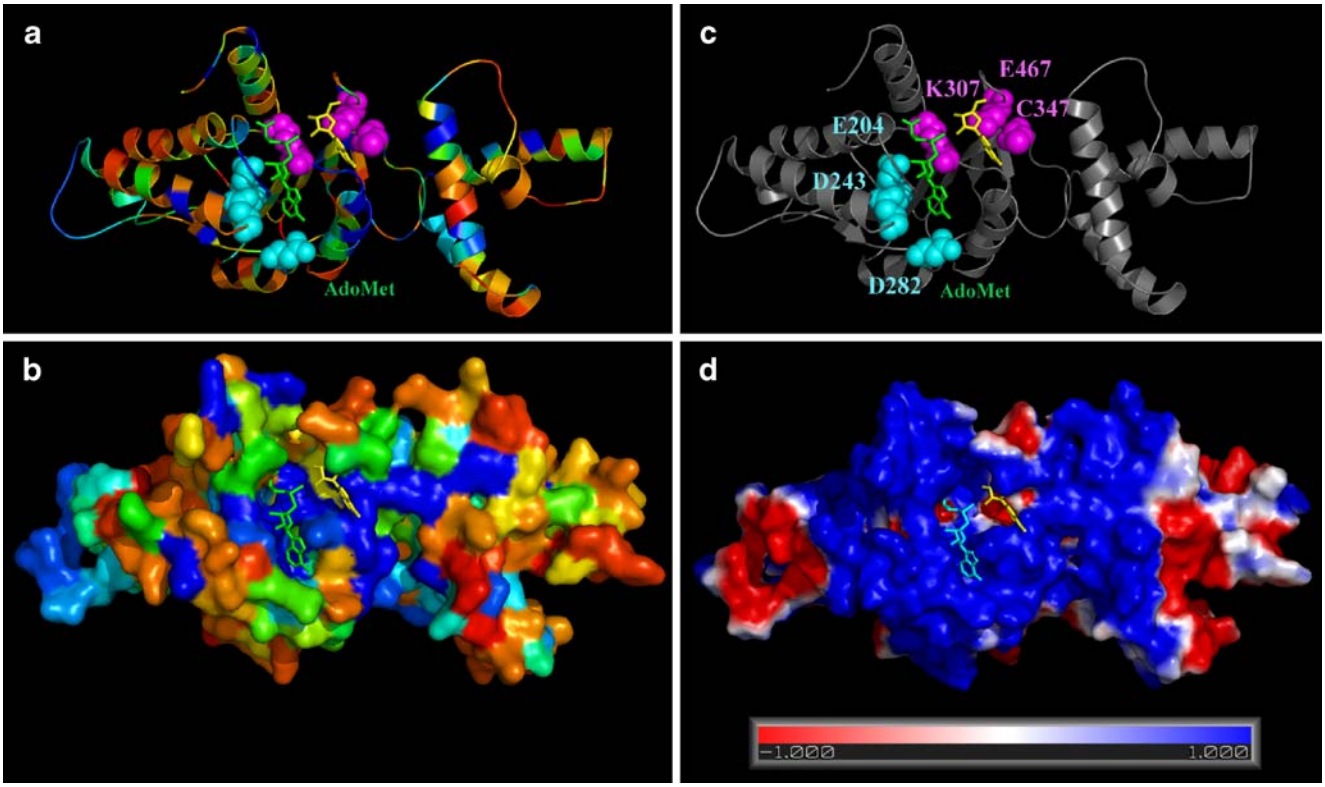

Fig. 2 A structural model of Trm13p. The Trm13p model colored according to sequence conservation in the Trm13p family, from deep blue (invariant), through light blue (conserved), to yellow/red (highly variable) in the cartoon representation (**a**) and surface representation (**b**) . A highly conserved blue patch indicates the cofactor-binding site and the predicted tRNA- binding/catalytic site. (**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 (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]

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 m^5U in RNA and m^5C 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 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 Cα 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:m^5C 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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