The Zinc Finger Protein Ynr046w Is Plurifunctional and a Component of the eRF1 Methyltransferase in Yeast*

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Protein release factor eRF1 in Saccharomyces cerevisiae, in complex with eRF3 and GTP, is methylated on a functionally crucial Gln residue by the S-adenosylmethionine-dependent methyltransferase Ydr140w. Here we show that eRF1 methyla-
tion, in addition to these previously characterized components, requires a 15-kDa zinc-binding protein, Ynr046w. Co-expression in Escherichia coli of Ynr046w and Ydr140w allows the lat-
ter to be recovered in soluble form rather than as inclusion bod-
ies, and the two proteins co-purify on nickel-nitrilotriacetic acid chromatography when Ydr140w alone carries a His tag. The crystal structure of Ynr046w has been determined to 1.7 Å res-
olution. It comprises a zinc-binding domain built from both the N- and C-terminal sequences and an inserted domain, absent
from bacterial and archaeal orthologs of the protein, composed of three α-helices. The active methyltransferase is the het-
erodimer Ydr140w-Ynr046w, but when alone, both in solution and in crystals, Ynr046w appears to be a homodimer. The Ynr046w eRF1 methyltransferase subunit is shared by the tRNA
methyltransferase Trm11p and probably by two other enzymes containing a Rossmann fold.

Termination codons in mRNA are recognized on the ribo-
some by class I protein termination factors (or release factors (RFs)) in eubacteria, archaea, and eukaryotes (1–3). Three codons are used as stop signals in most organisms: UAA, UGA,
and UAG. In bacteria, two class I RFs are required for termina-
tion: RF1, which recognizes UAA and UAG codons, and RF2, which recognizes UAA and UGA codons. In contrast, a single RF, eRF1 or aRF1, is sufficient for termination at all three stop
codons in eukaryotes and archaea, respectively. eRF1 and aRF1 form closely related protein families but are evolutionarily distinct from the eubacterial RFs (4). Thus, despite a similar function, the only sequence element common to all RFs is a tripeptide
sequence, GGQ. Structural and mutational analysis shows that
this motif is essential for RF activity and is required to interact
with the peptidyl transferase center of the large ribosomal sub-
unit and trigger hydrolysis of the ester bond in peptidyl-tRNA
(5, 6). The Gln residue of the GGQ motif is methylated in both
bacteria (7) and Saccharomyces cerevisiae (8, 9), and probably in
mammals. Bacterial RF methylation depends on the PrmC
methyltransferase (MTase) (10, 11), the product of the gene
prnC (previously named hemK) situated in Escherichia coli,
and most other bacteria immediately downstream of the gene
prfA encoding RF1. RF methylation in E. coli strongly stimu-
lates the activity of the factors (7, 11).

It is remarkable that the modification of Gln in the GGQ
motif is conserved from bacteria to eukaryotes despite the dif-
f erent evolutionary origin of the class I RFs themselves. The S. cerevisiae genome encodes two proteins, Ydr140w and
Ynl063w, with significant similarity to bacterial PrmC that goes
beyond the motifs known to be involved in AdoMet binding (7).
Inactivation of Ydr140w was shown to lead to a loss of eRF1
methylation (8). The gene Ynl063w is required for methylation of the mitochondrial RF, Mrp1p (9). Methylation of eRF1 by
Ydr140w differs from prokaryotic RF methylation in that the
presence of the class II RF (eRF3 in yeast), and GTP was also
required. The substrate of the yeast MTase therefore appears to
be the ternary complex eRF1-eRF3-GTP rather than eRF1 alone.
The role of class II factors in eubacteria is to catalyze the recycl-
ing of the class I RFs following peptide release (12, 13); in
eukaryotic organisms the role of eRF3 seems to be closer to that of
EF1α and leaves the ribosome after peptide release (14). Thus,
a significant difference between bacterial and eukaryotic factors is
that, in the presence of GTP, the class I and class II factors (eRF1
and eRF3) bind to each other (15), whereas the eubacterial factors
do not. Archaea appear to have no class II RF.

Some observations made during the characterization of
Ydr140w as the eRF1 MTase in S. cerevisiae suggested that at least
one further component, in addition to the ternary complex
eRF1-eRF3-GTP and Ydr140w, is required for methylation of eRF1

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Only impure preparations of Ydr140w, produced in yeast, were able to methylate RFs in vitro, and with low efficiency. Overproduction of Ydr140w in E. coli led exclusively to insoluble protein, but even when resolubilized by routine renaturation methods, no MTase activity could be detected. Polevoda et al. (9) confirmed the function of Ydr140w in methylation of eRF1, but their assays were also performed with partially purified components and resulted in a low efficiency of methylation. Other studies of proteins interacting with Ydr140w in yeast, either by two-hybrid mapping or by tandem affinity purification-tagged co-purification, followed by mass spectrometry, identified Ynr046w, a 15-kDa protein, as a component interacting with Ydr140w (16, 17). Furthermore, affinity studies showed that two S. cerevisiae tRNA MTases, Trm11p and Trm9p, and a further protein, Lys9p, apparently bind to Ynr046w (16–18), and Purushothaman et al. (19) demonstrated the requirement for both Ynr046w and Trm11p for m2G10 formation in yeast tRNA. Lys9p is a dehydrogenase with a Rossmann fold similar to that in most RNA MTases. Sequence analysis has revealed the existence of Ynr046w orthologs within the three kingdoms of life (19). Interestingly, archaeal and bacterial proteins from this family are much shorter (about 55–65 residues) than eukaryotic proteins (about 130 residues; Fig. 1). Hence, Ynr046w is made of a central region specific for eukaryotic members (“insert” domain, residues 39–101) inserted within a region conserved in the three kingdoms of life (“conserved” domain, residues 1–38 and 102–135). Among fungal orthologs, this latter domain harbors a putative zinc finger signature made of the $1^{\text{1}}C_{X}C_{5}^{\text{16}}$ and $1^{\text{12}}C_{X}C_{115}^{\text{1}}$ motifs (where $X$ can be any residue; from the N- and C-terminal parts, respectively).

Here we show that Ynr046w is required as a subunit of the eRF1 MTase in S. cerevisiae. In vitro, purified Ydr140w, Ynr046w, and AdoMet are necessary and sufficient to methylate the ternary complex eRF1/H18528 eRF3/H18528 GTP. Finally, we present the 1.7 Å resolution crystal structure of this small protein and show that it comprises two domains: a domain built from both N-terminal and C-terminal sequences that contains a zinc-binding site and an inserted domain, absent from bacterial and archaeal orthologs of the protein, composed of three α-helices.

**EXPERIMENTAL PROCEDURES**

**Bacterial Growth**—LB medium was supplemented according to requirements. Antibiotics were added at the following final concentration: kanamycin, 50 μg/ml; ampicillin, 200 μg/ml;
and chloramphenicol, 15 μg/ml. When induction was necessary to overexpress proteins, 1 mM IPTG was added to liquid medium to a final concentration of 1 mM. For expression of Ynr046w, alone or as a complex with Ydr140w, ZnCl₂ was added to a final concentration of 100 μM.

**Recombinant DNA Manipulations**—General procedures for DNA recombinant techniques, plasmid extraction, etc. were performed as described by Sambrook et al. (20).

_Yeast eRF1 and Truncated eRF3 Expression Vectors—pYSC1_, a derivative of pET11a plasmid encoding eRF1 with a His₆ tag on its C terminus, was constructed by gene cloning between the Ndel and BamHI sites. Amplification was performed on chromosomal DNA from yeast strain YLPC27. The upstream oligonucleotide (5′-AATACTTCATATGGATAACGGT-3′) introduces an Ndel site. The downstream oligonucleotide (5′-CGGGATCCTTTAGTGGGTGTTGGAATGAAATCATAGCGGACCCCTCA-3′) introduces a new BamHI site, eliminating the original one upstream of the stop codon, and encodes the His₆ tag. pYSC2, also a derivative of pET11a, encodes yeast eRF3 truncated at the N terminus. Amplification of genomic DNA was done with the following oligonucleotides, introducing, respectively, the Ndel site followed by the His₆ tag (5′-GGAATTCATATGGACACCCACCAACACTTGGTTGGAATGAAATCATAGCGGACCCCTCA-3′) and the BamHI site (5′-CGGGATCCTTTACGCGGACATATTACAC-3′).

_MTase Ydr140w and Ynr046w Subunit Expression Vectors—pVH450 encodes Ynr046w with a His₆ tag at its C terminus and pVH451 encodes the same protein without tail. Both are derivatives of the pET11a vector with a kanamycin cassette and were constructed in the same way after genomic DNA amplification from yeast strain yLPC27. The upstream oligonucleotide, 5′-TAGCCTATGGTGAATGACGTGGT-3′, and the downstream primers are 5′-ACACAGGATCCCGCTTTGTTTGTGGGTGTAACAGCA-3′ for pVH450 and 5′-ACACAGGATCCCGCTTTGTTTGTGGGTGTAACAGCA-3′ for pVH451.

For co-expression experiments, Ydr140w and a His₆ tag on its C terminus was also expressed from plasmid pACYCDuet (ydrH6) compatible with pET plasmids. The gene encoding Ydr140wH6 was cloned in two steps (fragment Ndel-BamHI and fragment Ndel-NdeI) between Ndel and BsrGI in the MCS2 of pACYCDuet1 (Novagen). pACYCDuet(ydrH6) was constructed from plasmid pTrc(ydr140wH6), a derivative of pVH383 itself derived from pVH371 (8). In the first step, Ydr140w gene was cloned from pVH371 into pLV1 between Ndel and BsrGI sites to yield pVH383. A His₆ tag was then introduced by inserting phosphorylated oligonucleotides (5′-GTACACCTTTTACGCGGACATTTACAC-3′ and 5′-GATCCTATGGTGAATGACGTGGT-3′) between the BsrGI and BamHI sites to give pTrc(ydr140wH6).

**Protein Expression and Purification**—After transformation of BL21(DE3) Rosetta by the relevant plasmids, the expression of His-tagged eRF1 or truncated His-tagged eRF3 (eRF3Ct) was induced by IPTG (1 mM) at 16 °C at an optical density (600 nm) of 0.5 in LB medium with appropriate antibiotics, followed by growth overnight. For eRF1, the cells were resuspended in buffer A1 (20 mM Tris-HCl, pH 7.5, 500 mM NaCl, 6 mM β-mercaptoethanol, and 5 mM imidazole) with EDTA-free antiprotease (Roche Applied Science) and broken by passage through a French press. After centrifugation, the supernatant was loaded on Ni-NTA resin (Sigma). The column was washed with buffer A1 and eluted with buffer A1 with 150 mM imidazole but no NaCl. Fractions containing protein were dialyzed against buffer A2 (20 mM Tris-HCl, pH 7.5, 6 mM β-mercaptoethanol) and concentrated by ultrafiltration through Amicon Ultra 30 (Millipore). For eRF3, the cells were resuspended in buffer B1 (50 mM sodium phosphate, pH 7.0, 500 mM NaCl, 6 mM β-mercaptoethanol, and 5 mM imidazole) with EDTA-free antiprotease (Roche Applied Science) and broken by passage through a French press. After centrifugation, the supernatant was loaded on Ni-NTA resin (Sigma). The column was washed with buffer B1 and eluted with buffer B1 with 150 mM imidazole but no NaCl. Fractions containing protein were concentrated by ultrafiltration through Amicon Ultra 30. Imidazole was eliminated by gel filtration on Sephadex G25 in buffer B2 (50 mM sodium phosphate buffer, pH 7.0, 6 mM β-mercaptoethanol).

Ynr046wH6 was expressed alone in BL21-Gold (DE3) (Stratagene). Co-expression of Ydr140wH6 and Ynr046wH6 was done in the same strain at 23 °C after induction with 1 mM IPTG at an optical density (600 nm) of 0.5 in LB medium with the appropriate antibiotics, followed by growth overnight. Purification of Ynr046wH6 alone or the complex (Ydr140wH6-Ynr046w) was performed as described for eRF1 purification with small modifications. Buffer A1 was modified to buffer C1 (10 mM Tris- HCl, pH 8.0, 500 mM NaCl, 6 mM β-mercaptoethanol, 10 μM ZnCl₂). Elution was performed with buffer C1 with 50 mM imidazole and no NaCl. Fractions containing protein were dialyzed against buffer C2 (10 mM Tris-HCl, pH 8.0, 6 mM β-mercaptoethanol, 10 μM ZnCl₂). Protein was concentrated by ultrafiltration through Amicon Ultra 30 for the complex and Amicon Ultra 10 for Ynr046wH6 alone.

_In Vitro Recombinant Ydr140w Purification from Inclusion Bodies—Production of Ydr140w alone in_ _E. coli_ was achieved as described by Heurgue-Hamard et al. (8) from pVH371. Purification was carried out according to Vuillard and Freeman (dwb.unl.edu/Teacher/NSF/C08/C08Links/www.nwfsc.noaa.gov/protocols/inclusion.html). This procedure involves solubilization of proteins from inclusion bodies by guanidinium chloride, followed by rapid dilution in the presence of nonionic detergent sulfobetaines to limit aggregation. The pellet from 150 ml of bacterial suspension was resuspended in 50 mM HEPES-NaOH, pH 7.5, 0.5 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 5 mM DTT containing 0.35 mg/ml lysozyme and then incubated for 30 min at 20 °C. Triton X-100 was added to a concentration of 1% (v/v), and the cells were broken by passage through a French press. The extract was treated with DNase I (20 μg/l) for 1 h at 37 °C and centrifuged at 30,000 × g for 30 min at 4 °C. The pellet (inclusion bodies) was washed twice with phosphate-buffered saline containing 1% Triton X-100, centrifuged at 30,000 × g for 30 min at 4 °C, and then solubilized for 1 h at 4 °C in 2 ml of 50 mM HEPES-NaOH, pH 7.5, 6 mM guanidine HCl, 25 mM DTT. After centrifugation at 100,000 × g for 10 min, the insoluble material was removed. The protein concentration in the supernatant was adjusted to 1 mg/ml (from 30 mg/ml) using 50 mM HEPES-NaOH, pH 7.5, 6 mM guanidine HCl.
TABLE 1

| Data collection statistics | Native | SAD |
|----------------------------|--------|-----|
| Resolution (Å)            | 20-1.7 (1.75-1.7) | 45-2.0 (2.2-2.0) |
| Space group               | C 2    | C 2 |
| Unit cell parameters      | a = 92.6 Å, b = 38.5 Å, c = 45.8 Å, β = 102.2°. | a = 92.7 Å, b = 38.5 Å, c = 45.8 Å, β = 102.2°. |
| Total number of reflections | 42,338 | 57,188 |
| Total number of unique reflections | 17,182 | 10,322 |
| Rsym (%)                  | 13.3 (52.4) | 13.7 (49.2) |
| Completeness (%)          | 97.7 (99)  | 99.4 (99.5) |
| I/σ(I)                    | 6.2 (2.4) | 9 (1.3) |
| Redundancy                | 2.5     | 5.5  |

Refinement

- Resolution (Å) 20-17.0
- Rsym (%) 19.8/23.6
- Root mean square deviation
  - Bonds (Å) 0.011
  - Angles (°) 1.296
  - Mean B factor (Å²) protein/water 16.6/27
- Ramachandran plot
  - Most favored (%) 93.3
  - Allowed (%) 6.7

- Rfree = ΣΣ |Ih| − <Ih> |ΣΣ Ih|, where Ih is the ith observation of the reflection h, whereas <Ih> is the mean intensity of reflection h.
- Rfactor = Σ|Fh| − |Fe|Σ|Fe|, Rfree was calculated with a small fraction (5%) of randomly selected reflections.

Data collection statistics

Beams were collected on beamlines ID23-EH1 and ID23-EH2 (ESRF, Grenoble, France), respectively.

The structure was determined by the single wavelength anomalous dispersion method using the anomalous signal from the zinc element. The data were processed using the XDS package (23).

In Vitro Methylation Assays—Methylation assays were performed in buffer D (100 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 100 mM ammonium acetate, 2 mM DTT, 0.1 mM EDTA, 100 µg/ml bovine serum albumin) and 10 µM [³H]AdoMet (0.86 Ci/mmol). GTP, GDP, or GDPNP were added at a final concentration of 1 mM. The samples were withdrawn at different times, and the reaction was stopped by cold trichloroacetic acid (5%) precipitation, followed by filtration on Whatman GF/C filters and measurement of radioactivity by scintillation counting.

Western Blot Analysis—Western blot experiments were performed using rabbit anti-Ydr140w antibodies commercially prepared from pure protein produced as inclusion bodies in E. coli. The proteins were separated on 18% SDS-polyacrylamide gels as described by Laemmli (22). Transfer to nitrocellulose membranes and Western blotting with antibodies (diluted 5000X) were performed as described by Sambrook et al. (20) using a peroxidase-coupled secondary antibody (diluted 5000X), SuperSignal chemiluminescent substrate (Pierce), and Kodak X-Omat AR.

RESULTS

Ynr046w/Ydr140w Interaction—Previous observations showed that Ydr140w could be efficiently overproduced in E. coli using the pET expression system, but only as insoluble protein present in inclusion bodies. Attempts to produce soluble protein by reducing the level of induction, growing cells at lower temperatures or co-expressing chaperones were unsuccessful. Denaturation and renaturation of protein from inclusion bodies did yield substantial amounts of soluble Ydr140w. However, the resulting protein did not have methylation activity in vitro and appeared to be unfolded according to circular dichroism meas-
eRF1 Methyltransferase Is a Heterodimer Ydr140w·Ynr046w

FIGURE 2. Co-expression of Ydr140w and Ynr046w in E. coli. Ydr140w and Ynr046w were overexpressed in E. coli strain BL21-Gold (DE3), either separately or together, with the use of compatible plasmids. The first two lines show the presence and absence of plasmids expressing each of the proteins; (+) indicates the absence of the inducer IPTG. Except for the noninduced control cultures, cell extracts were centrifuged to separate inclusion bodies (P indicates pellet) and soluble proteins (S), as indicated on the third line. The proteins were separated by electrophoresis in an 18% polyacrylamide-SDS gel. A, proteins were stained with Coomassie Blue. Molecular mass standards are shown in the leftmost lane, with values in kDa on the left, and purified Ydr140w was applied to the lane on the extreme right. B, a similar gel was subjected to Western blotting using polyclonal antibodies raised against purified Ydr140w.

Ynr046w Restores Ydr140w Activity from Inclusion Bodies

FIGURE 3. Co-elution of His-tagged Ydr140w and nontagged Ynr046w from Ni-NTA resin with imidazole. A cell extract from cells co-expressing the two proteins was applied to a Ni-NTA column, washed, and then eluted with buffer containing 50 mM imidazole. Lane 1, total protein; lane 2, flow-through fractions; lane 3, molecular mass standards (values in kDa shown on the right); lanes 4 and 5, successive fractions containing the bulk of the proteins eluted from the column with imidazole.

Ynr046w restores Ydr140w activity from inclusion bodies

Supporting the idea that Ynr046w is an essential component of the eRF1 MTase.

eRF1 Is Efficiently Methylated by the Ydr140w·Ynr046w Complex—As described above, Ydr140w and Ynr046w form a complex when co-expressed in E. coli and may be purified by means of a His tag on Ydr140w. In vitro methylation assays were performed with the complex Ydr140w·H6·Ynr046w purified on Ni-NTA resin (Fig. 5). When the ternary complex eRF1·eRF3·GTP is present, 25% of eRF1 molecules are methylated within 5 min, reaching a maximum of 60% after 30 min (Fig. 5, filled squares). As shown previously, we confirm that eRF3 and GTP are needed for efficient methylation because no methylation occurs without eRF3 and less than 5% after 60 min in presence of GDP (Fig. 5, open circles). The kinetics of methylation of eRF1 in the presence of eRF3 and GDPNP, a GTP nonhydrolyzable analog, show clearly that the analog can substitute for the normal nucleotide (Fig. 5, filled triangles). Currently available data suggest that GDPNP, a nonhydrolyzable GTP analog, does not induce a major switch in conformation of yeast eRF3 either in the case of the isolated protein (29) or when eRF3 is present with eRF1 (30). However, our data suggest that the eRF1 MTase may promote a conformational switch of the eRF3 protein to the GTP form in the presence of GDPNP.

7 V. Heurgué-Hamard, M. Graille, N. Scrima, N. Ulryck, S. Champ, H. van Tilbeurgh, and R. H. Buckingham, unpublished data.
Ynr046w Structure—Bioinformatics analysis of the Ynr046w sequence suggested the presence of a putative zinc finger region. The Ynr046w crystal structure was determined using the anomalous scattering from the zinc atom, thereby confirming zinc binding to this protein. The structure has been refined to 1.7-Å resolution, and the excellent quality of the electron density map permitted the building of all of the Ynr046w residues. A single copy of Ynr046w is present in the asymmetric unit, but a homodimer can be generated by applying the crystal symmetry operators (see below). The Ynr046w monomer binds one zinc atom and has an α/β fold with approximate dimensions of 30 × 30 × 50 Å³. The conserved domain is made of a curved three-stranded anti-parallel β-sheet and the N-terminal helix α1 (Fig. 6A). The zinc-binding site is situated in a depression on one edge of the sheet. The helical domain is composed of three α-helices and is inserted at the opposite edge of the sheet. This insert domain packs against the helix α1 from the conserved domain.

Search for structural analogs did not provide convincing results for intact Ynr046w. However, structural homologs can be found for each domain separately. First, the zinc-binding region can be classified as a member of the “Gag knuckle” fold described by Krishna et al. (31). This motif is one of the 8-fold groups determined from the systematic analysis of the different zinc finger structures. However, when the zinc-binding motif of Ynr046w is compared with the prototype member of this family, the F1 zinc knuckle domain from HIV-1 nucleocapsid protein that plays a major role in the recognition and packaging of the retroviral genome (Ref. 32 and Fig. 6B), there is an inversion in the zinc knuckle topology. In the HIV-1 nucleocapsid protein, the two N-terminal zinc ligands come from two short strands connected by a turn (zinc knuckle) and the two C-terminal ligands from a loop. In Ynr046w, the two N-terminal zinc ligands (Cys11 and Cys16) are located in the long loop connecting helix α1 to strand β1, whereas the two C-terminal ligands (Cys112 and Cys115) are from the turn connecting strands β2 and β3. The α-helical insertion of Ynr046w has structural similarities to the Pit-1 POU domain, a helix bundle that recognizes defined DNA sequences (root mean square deviation of 2.9 Å over 50 of 60 Cα positions (33)). However, this is unlikely to reflect functional similarity, because the helical bundle in Ynr046w is charged negatively rather than positively, as would be expected for a DNA-binding module.

Analytical gel filtration chromatography shows that in the absence of sodium chloride, Ynr046w exists in solution as an equilibrium between a high molecular mass species (more than 200 kDa, major population) and a homodimer (34 kDa, minor population). However, when the ionic force is increased up to 0.5 M NaCl, the protein elutes exclusively as a homodimer (data not shown). The asymmetric unit of the crystal contains only one copy of the Ynr046w protein, but careful analysis of the crystal packing reveals that an asymmetric homodimer can be obtained by a crystallographic 2-fold screw axis (Fig. 6C). In the crystal, this homodimer is arranged so as to form parallel fibers along the crystallographic axis. This homodimer very likely corresponds to the solution dimer, and the formation of long fibers in the crystal could be driven by crystal contact forces. The homodimer buries in total 1400 Å² of solvent-accessible surface area, a value comparable with the average calculated for other stable protein-protein complexes (34). It has approximate dimensions of 30 × 45 × 65 Å³. Ten residues from each monomer are involved in homodimer formation (Fig. 1). Eight of ten residues from monomer A correspond to the Ynr046w peptide Asn123–Leu130 (Fig. 1, closed circles), whereas residues from monomer B are mainly contributed by helix α2 from the insert.
domain and the C-terminal peptide Pro^{132}–Leu^{134} (Fig. 1, closed squares). Five hydrogen bonds are involved in homodimer formation (Fig. 6D). These occur between the Asn^{123} carbonyl group, the Leu^{129} and Leu^{130} amides from monomer A with the Leu^{134} amide, Glu^{45} O/H and Asn^{49} O/H atom from monomer B, respectively. The two remaining H-bonds are between the Pro^{126} and Leu^{128} carbonyl groups from monomer A and the Asn^{49} N/H atom from monomer B. In this asymmetric homodimer, the residues from monomer A involved in homodimer formation are located within the zinc-binding domain, whereas those from monomer B are mainly from the insertion as well as the C-terminal part. Because the insert domain is absent from archaeal and bacterial Ynr046w orthologs, this quaternary structure organization should be specific for the eukaryotic orthologs.

**DISCUSSION**

Previous work has shown that the universally conserved GGQ motif in class 1 RFs is methylated both in eubacteria and eukaryotes, and the AdoMet-dependent MTases have been identified. Here we show that yeast eRF1 methylation requires the presence of a heterodimer constituted by the MTase itself, Ydr140w, and a zinc-binding protein, Ynr046w. We have been able for the first time to reconstitute in vitro eRF1 methylation with purified proteins overproduced in *E. coli*. Previous in vitro experiments, which allowed the eRF1 MTase activity to be demonstrated, presumably depended on the fact that Ydr140w was prepared from yeast cells and that Ynr046w was present in the MTase preparation because of the affinity between the two proteins. We also confirm the need for eRF3 in its GTP conformation and show that GDPNP can substitute for GTP in the methylation reaction. Previous structural and biochemical studies of *Schizosaccharomyces pombe* eRF3, alone or in complex with GDP or GDPNP, left some doubt as to whether GDPNP was able to switch the conformation of eRF3 to the GTP form, either when present alone (29) or in complex with eRF1 (30). Our results show that the heterodimeric MTase Ydr140w/Ynr046w is able to methylate the eRF1/eRF3 complex in the presence of GTP or GDPNP but not GDP. This suggests that in the presence of the heterodimeric MTase, GDPNP is able to promote the switch of eRF3-eRF1 to the GTP conformation.

We here showed that co-expression in *E. coli* of Ynr046w and Ydr140w allows the latter to be recovered in soluble form rather than from inclusion bodies. In addition, analytical size exclusion chromatography suggests that in the presence of 0.5 M NaCl, the Ynr046w-Ydr140w complex is a heterodimer in solution, whereas Ynr046w alone forms a homodimer. This implies that binding of Ydr140w to Ynr046w hinders the formation of Ynr046w homodimer. Hence, this suggests that the Ynr046w region involved in Ydr140w binding is in close proximity or directly overlaps with the Ynr046w region involved in
homodimer formation. As shown in Fig. 6, the regions of the Ynr046w monomer involved in homodimer formation (Fig. 6F, green and blue patches) are very well conserved (Fig. 6G, red patch) and highly hydrophobic (Fig. 6G, red patch). In addition to being responsible for homodimer formation, they are implicated in the generation of parallel fibers within the crystal. This could indicate that this region is prone to interact with other protein partners. Hence, we suspect that this particular region of Ynr046w directly interacts with Ydr140w, thereby reducing its solvent-accessible hydrophobic surface and hence reducing its tendency to aggregate. Ynr046w may also have a more active role in Ydr140w folding.

Global analyses of protein complexes in yeast (17), based on affinity data, suggest that, in addition to its interaction with Ydr140w, Ynr046w co-purifies with three other proteins: Trm11p, Trm9p, and Lys9p. Interestingly, these four proteins all share a common Rossmann-fold. Previous to this report, the interaction between Trm11p and Ynr046w has been the most extensively studied (19). Trm11p has been identified as a yeast tRNA MTase, specific for guanosine methylation. This activity is completely lost in the absence of Ynr046w, and subunit association in vivo has been confirmed by immunoprecipitation. However, co-expression of Trm11p and Ynr046w in E. coli does not seem to be sufficient to recover specific tRNA methylation activity (19). The precise function of Ynr046w in this reaction is not known, but it does not seem to play a role in Trm11p protein synthesis or life span. Ynr046w also does not directly catalyze methyl transfer on tRNA. Indeed, Trm11p possesses in principle the full functionalities to do this: a tRNA-binding site on its N-terminal domain and a MTase domain on its C-terminal domain (19).

Trm9p was identified as a novel tRNA MTase catalyzing methyl esterification of modified uridine nucleotides, resulting in the formation of mc^m^5U and mc^m^3s^2U (18). In this case, Trm9 fused to a glutathione S-transferase tag was purified in E. coli and shown to be active for yeast tRNA methylation. Thus, Ynr046w is not essential for the catalytic reaction, but it has not been excluded that the protein might enhance catalytic activity. Lys9p is a dehydrogenase involved in the lysine biosynthetic pathway. The enzyme was purified from yeast by Storts and Bhattacharjee (35) as a 50-kDa protein. The authors concluded that Lys9p was a monomeric enzyme, but gel filtration chromatography seemed to indicate a higher molecular mass of around 67 kDa, which would be consistent with the presence in solution of a heterodimer of Lys9p and Ynr046w. The effect of Ynr046w on the enzymatic reaction of Lys9p has not been further investigated.

The best characterized example concerning a MTase acting with a partner concern tRNA MTases. For instance, yeast tRNA m^7G MTase Trm8p possesses AdoMet- and tRNA-binding sites and is in interaction with Trm82p. This partner seems to control the intracellular quantity of Trm8p at the protein level. The exact mechanism by which Trm82p exerts these effects is unclear. Current models propose that Trm82p possesses a chaperone-like function that protects Trm8p from degradation and/or stabilizes the MTase in an active conformation (36). Trm6-Trm61p constitutes another example of a complex involved in specific tRNA methylation. Here each partner seems to have distinct functions in catalysis; the Trm61p subunit is required for tRNA binding, whereas Trm6p binds to AdoMet and catalyzes the methylation reaction (37).

Considering the crucial role played by Ynr046w in the methylation of eRF1 and tRNA by Ydr140w and Trm11p, respectively, its precise function needs to be investigated in more detail. Orthologs of Ynr046w are widely distributed throughout the bacterial, archaeal, and eukaryotic kingdoms (19). However, the central α-helical region of the Ynr046w (residues 39–101) is found only in eukaryotic members of the extended family, whereas the N-terminal and C-terminal parts, residues 1–38 and 102–135, respectively, corresponding to the zinc finger domain, are common to all orthologs. The presence of this insert in eukaryotic proteins raises the question as to whether this domain has any specific function.

The Ynr046w zinc finger signature is located within the domain conserved in the three kingdoms of life. Sequence alignment reveals that the four Cys residues involved in zinc binding in the Ynr046w protein are strictly conserved among yeast proteins and Methanococcales archaea (Fig. 1). In bacteria and some archaea (Halobacterium marismortui and Halobacterium salinarum), one Cys residue is replaced by an Asp side chain (Cys^146 for archaea and Cys^115 for bacteria). Considering that an acidic residue is found as a zinc ligand in structural zinc sites at a frequency of 15% (38), this zinc-binding site should be conserved in bacteria and archaea. The presence of the zinc-binding site in pluricellular eukaryotes is uncertain because only Cys^112 is conserved (Fig. 1). Cys^111 and Cys^115 are substituted by Ser and/or Thr, whereas Cys^16 is replaced by Val in Ynr046w, this zinc-binding domain has structural similarity to zinc finger regions involved in the recognition of nucleic acids (e.g. HIV-1 Gag nucleocapsid protein). Hence, although Trm11p possesses its own tRNA-binding site on its N-terminal domain, and Trm9p alone has been shown to be active for yeast tRNA methylation, the Ynr046w zinc finger domain could improve their affinity and specificity toward tRNA. Interestingly, a fusion of a zinc finger domain with a C-terminal MTase domain was found in another family of RNA modification enzymes, RlmAI/RlmAII, which catalyzes a specific methylation on 23 S rRNA (21). In this case, structural data showed clearly that the zinc-binding domain was involved in specific binding of the rRNA substrate. Despite the lack of structural resemblance between Ynr046w and RlmA zinc finger domains, they could have similar roles in nucleic acid binding.

In conclusion, we demonstrate that Ynr046w interacts with Ydr140w to form a stable 1:1 complex able to transfer a methyl group to the glutamine side chain of the universally conserved GGQ motif present in eukaryotic class I RFs. We show that this complex is only active on the eRF1-eRF3-GTP complex. The small Ynr046w protein (15 kDa) is built from a conserved zinc finger domain and an insert α-helical domain, the latter being present only in Ynr046w eukaryotic orthologs. Gel filtration experiments reveal that Ynr046w alone associates as a homodimer under high salt conditions, whereas it forms a heterodimer with Ydr140w. This suggests that the Ynr046w regions involved in homodimer formation and in Ydr140w formation are either overlapping or in close proximity. However, more detailed studies of complexes between Ynr046w and its
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different MTases partners using a combination of structural and biochemical approaches will be required for a better understanding of its role for eRF1 methylation by Ydr140w and for tRNA methylation by Trm9p and Trm11p.

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