The Saccharomyces cerevisiae YOR163w Gene Encodes a Diadenosine 5',5''-P1,P6-Hexaphosphate (Ap6A) Hydrolase Member of the MutT Motif (Nudix Hydrolase) Family*

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The YOR163w open reading frame on chromosome XV of the Saccharomyces cerevisiae genome encodes a member of the MutT motif (nudix hydrolase) family of enzymes of $M_1$ 21,443. By cloning and expressing this gene in Escherichia coli and S. cerevisiae, we have shown the product to be a (di)adenosine polyphosphate hydrolase with a previously undescribed substrate specificity. Diadenosine 5',5''-P1,P6-hexaphosphate is the preferred substrate, and hydrolysis in $H_2^{18}O$ shows that ADP and adenosine 5'-triphosphate are produced by attack at P$^\beta$ and AMP and adenosine 5'-pentaphosphate are produced by attack at P$^\alpha$ with a $K_m$ of 56 $\mu$M and $k_{cat}$ of 0.4 s$^{-1}$. Diadenosine 5',5''-P1,P3-pentaphosphate, adenosine 5'-pentaphosphate, and adenosine 5'-tetraphosphate are also substrates, but not diadenosine 5',5''-P1,P4-tetraphosphate or other dinucleotides, mononucleotides, nucleotide sugars, or nucleotide alcohols. The enzyme, which was shown to be expressed in log phase yeast cells by immunoblotting, displays optimal activity at pH 6.9, 50 °C, and 4–10 mM Mg$^{2+}$ (or 200 $\mu$M Mn$^{2+}$). It has an absolute requirement for a reducing agent, such as diithothreitol (1 mM), and is inhibited by Ca$^{2+}$ with an IC$_{50}$ of 3.3 mM and F$^-$ (noncompetitively) with a $K_i$ of 80 $\mu$M. Its function may be to eliminate potentially toxic dinucleoside polyphosphates during sporulation.

Recent interest in the diadenosine polyphosphates (Ap$\alpha$A)$^3$ has focused on their possible roles as regulators of cell proliferation. Ap$\alpha$A has been proposed as a component of the interferon-induced antiproliferative response in mammalian cells (1), whereas Ap$\beta$A, the intracellular level of which has long been known to be associated with proliferation (2, 3), may be an antagonist of this pathway. A key factor in this response in humans is the fragile histidine triad (FHIT) protein, an Ap$\beta$A hydrolase that is absent or defective in many common cancers (4, 5). Precisely how this protein and its substrate, Ap$\beta$A, contribute to antiproliferation is not clear, but there is little doubt that the regulation of the intracellular levels of specific diadenosine polyphosphates is of great importance.

Eukaryotic Ap$\beta$A hydrolases exhibit an approximately 10-fold preference for Ap$\beta$A over Ap$\alpha$A as substrates (6). They are members of the histidine triad (HIT) family of proteins and possess the catalytic sequence motif HITXHITXHIT in which the central histidine residue forms a covalent enzyme-AMP reaction intermediate (4). Animals and higher plants also possess an asymmetrical cleaving Ap$\beta$A hydrolase that prefers Ap$\beta$A but is also active toward higher homologues, such as Ap$\alpha$A and Ap$\beta$A, but inactive toward Ap$\alpha$A (6). This enzyme belongs to the MutT motif (or nucleoside diphosphate linked to x (nudix)) family of nucleotide hydrolases (7, 8). Together, these two enzymes are probably crucial for regulating the Ap$\alpha$A/Ap$\beta$A ratio.

Many lower eukaryotes appear unusual in possessing one or more Ap$\beta$A phosphorylases in place of Ap$\alpha$A hydrolase. For example, Saccharomyces cerevisiae has two Ap$\beta$A phosphorylases, Apa1 and Apa2, in addition to an Ap$\alpha$A hydrolase (6, 9–11). Like the Ap$\beta$A hydrolases, the yeast phosphorylases can also degrade Ap$\beta$A but not Ap$\alpha$A (6, 11). The phosphorylases appear to be distantly related to the HIT proteins, having an HXXHX motif in place of the HXXLHX histidine triad (10, 12). Although S. cerevisiae does not have an Ap$\alpha$A hydrolase, genes for five potential MutT motif proteins can be discerned in the genome sequence. Here, we report that one of these, YOR163w (GenBank™ accession no. Z75071) from chromosome XV, encodes an Ap$\beta$A hydrolase that is also active against Ap$\beta$A and the adenosine 5'-polyphosphates p$\alpha$A and p$\beta$A, but not Ap$\beta$A or Ap$\alpha$A. This is the first time that an enzyme with this substrate specificity has been described. A preliminary report of this work has appeared (13).

EXPERIMENTAL PROCEDURES

Materials

Ap$\alpha$A was synthesized by carbodiimide condensation of ATP (14). p$\beta$A was synthesized using the recombinant Lys$U$ lysyl-tRNA synthetase and tetrapolyphosphate and tetrapolyphosphate (15, 16). The plasmid pXLYS5 was a gift from P. Plateau, and the Lys$U$ protein was purified as described (16). All other nucleotides were from Sigma. The cosmid clone pUOA1258, which carries the complete YOR163w open reading frame from yeast chromosome XV, was a gift from B. Dujon. The vector pPGY1 was a gift from L. Peace. Cat intestinal alkaline phosphatase (200 units/mg) and yeast inorganic pyrophosphatase (200 units/mg) were from Boehringer Mannheim. Pfu DNA polymerase was from Stratagene. $H_2^{18}$O (97.66 at %) was from Amersham Pharmacia Biotech.

Methods

Cloning in Escherichia coli—The YOR163w gene was amplified from the cosmid clone pUOA1258 (GenBank™ accession no. U55021) using the polymerase chain reaction. The 29-mer oligonucleotide primers d(A-

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‡ The abbreviations used are: Ap$\alpha$A, diadenosine 5',5''-P1,P6-polyphosphate; Ap$\beta$A, diadenosine 5',5''-P1,P6-diphosphate; Ap$\alpha$A, diadenosine 5',5''-P1,P3-triphosphate; Ap$\beta$A, diadenosine 5',5''-P1,P3-polyphosphate; Ap$\alpha$A, diadenosine 5',5''-P1,P2-pentaphosphate; Ap$\beta$A, diadenosine 5',5''-P1,P2-hexaphosphate; p$\alpha$A, adenosine 5'-pentaphosphate; p$\beta$A, adenosine 5'-pentaphosphate; p$\alpha$A, diadenosine 5'-pentaphosphate; DTT, dithiothreitol; nudix, nucleoside diphosphate linked to x; Bis-Tris propane, 1,3-bis[(hydroxyethyl)methylamino]propane; CAPS, 3-(cyclohexylamino)propanesulfonic acid.

2 All the enzymes mentioned are active toward other dinucleoside polyphosphates in addition to diadenosine polyphosphates.
ACACACATGGGACAACCGCGGATAAT) and d(AGGAATGATCGCTATGGTGGTGGTCTG) were synthesized to provide an NcoI restriction site at the start of the amplified gene and a BamHI site at the end. After amplification with Pfu DNA polymerase, the DNA was recovered by phenol/chloroform extraction and digested with NcoI and BamHI, respectively, and the gel-purified restriction fragments were ligated into the NcoI- and BamHI sites of the PET15b vector (Novagen), thus generating the ATG initiator in the NcoI site and eliminating the His tag sequence from the vector. The resulting plasmid, pET163W, was used to transform E. coli XL1-Blue cells for propagation.

Cloning in Yeast—The YOR163w gene was amplified as above using the primers d(TGGGATCCATGCGAAGATATGGGAAAACGCCGGG) and d(AGAATCTATGCTAATGCGAATACCGGCCGCGGCTG). These primers provided an EcoRI restriction site at the start of the amplified gene and a Xhol site at the end. After amplification, the recovered DNA was digested with EcoRI and Xhol, and the gel-purified restriction fragment was ligated into the EcoRI and Xhol sites of the yeast centromere vector, pPGY1. The resulting construct, pPGY163W, generated the ATG initiator downstream of GAL1p, a galactos-inducible promoter. The plasmid was used to transform E. coli XL1-Blue cells for propagation.

Protein Expression in E. coli and Purification—E. coli strain BL21(DE3) was transformed with pET163W. A single colony was picked from an LB agar plate containing 20 µl ml ampicillin and inoculated into 10 ml of LB medium containing 60 µg/ml ampicillin. After overnight growth, the cells were harvested, washed, and resuspended in 50 ml of breakage buffer (50 mM Tris-HCl, pH 8.0, 2 mM EDTA, 0.1 mM NaCl). The cell suspension was sonicated, and the inclusion bodies were recovered by centrifugation at 10,000 × g for 20 min. After washing by resuspension in breakage buffer containing 2.5 mM urea, the inclusion bodies were dispersed in 6 ml of 6 M guanidinium-HCl, 10 mM DTT, and the extract was centrifuged at 100,000 × g for 1 h. The supernatant was applied in 1 ml aliquots to a Bio-Rad Hi-Pore RP-304 reversed phase column (250 × 3 mm). 0.75-mm 15% SDS-polyacrylamide gel. The gel was equilibrated immediately after electrophoresis in transfer buffer (10 mM Tris-acetate, pH 7.5, 0.3 M NaCl, 10 mM 2-mercaptoethanol) containing 60 µg/ml ampicillin and grown to an A_{600} of 0.9. Isopropyl-thio-β-galactopyranoside was added to 0.4 mM, and the cells were induced for 4 h. The induced cells (5.1 g) were harvested, washed, and resuspended in 50 ml of breakage buffer containing 2.5 mM urea, the inclusion bodies were dispersed in 6 ml of 6 M guanidinium-HCl, 10 mM DTT, and the extract was centrifuged at 100,000 × g for 1 h. The supernatant was applied in 1-ml aliquots to a Bio-Rad Hi-Pore RP-304 reversed phase column (250 × 3 mm). The column was equilibrated with 30 mM sodium phosphate, pH 6.8, 0.01 mM CaCl2. Homogeneous YOR163w gene product eluted at 50% (v/v) CH3CN. The dialysate (20 ml) was applied at 1 ml/min to a 250 × 4.6-mm Phenomenex Jupiter C18 column in 4 mM potassium phosphate, pH 6.1, 8% (v/v) methanol. The adenosine peak was integrated.

Protein Expression in Yeast and Purification—S. cerevisiae strain INVSc1 was transformed with pPGY163W. A single colony was picked from an SC-Ura (Synthetic Complete medium without uracil) agar plate and inoculated into 100 ml of SC-Ura medium supplemented with 5% glucose. After 36 h, the cells were harvested by centrifugation, resuspended in 1 liter of SC-Ura (5% glucose), and further grown for 24 h. The cells (4.27 g) were again recovered by centrifugation, resuspended in 1 liter of SC-Ura (2% galactose, 1% raffinose), and grown for 16 h to fully induce expression of YOR163w. The induced cells (8.1 g) were harvested, washed, and resuspended in 8 ml of breakage buffer (50 mM Tris acetate, pH 7.5, 0.3 M NaCl, 10 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 5 µM trans-epoxysuccinyl-1-leucyl-aminocyclohexane, and 1 µM Tris acetate, pH 8.0). The separated polypeptides to a nitrocellulose membrane at 150 mA and 4 °C for 2 h. The membrane was blocked for 2 h at room temperature with phosphate-buffered saline containing 3% fat-free powdered milk and 0.2% Tween-20 and then probed with a 1:5000 dilution of a whole polyclonal antibody raised against YOR163w.

RESULTS

Cloning and Expression of the YOR163w Gene Product

Open reading frame YOR163w potentially encodes a 188-amino acid protein with a molecular mass of 21,575 Da. The intronless gene was polymerase chain reaction-amplified from the cosmid clone pUOA1258 using 29-base forward and reverse primers that included NcoI and BamHI sites, respectively, and the polymerase chain reaction product was inserted into the PET15b expression vector. E. coli strain BL21(DE3) was transformed with the recombinant plasmid, and exponentially growing cells were induced for up to 3 h with isopropyl-1-thio-β-D-galactopyranoside. SDS-polyacrylamide gel electrophoresis of
cell extracts revealed the presence in both the soluble fraction (10–20%) and inclusion bodies (80–90%) of a major band migrating with an apparent molecular mass of 24 kDa, which increased with induction time and so was presumed to be the required product (Fig. 1). Inclusion bodies were then isolated after 4 h of induction and solubilized, and the protein was purified in a single step to homogeneity by reversed phase chromatography (Fig. 2). The N-terminal sequence of the purified recombinant protein was determined to be GKTADNH-PVRS by Edman degradation, and its mass was measured as 21,443 Da by electrospray mass spectrometry. These correspond exactly to the predicted sequence and mass (21,443.6 Da) for the 187-amino acid polypeptide lacking the N-terminal methionine. These data confirm the accuracy of the cloning procedure and the identity of the protein.

In order to confirm that the observed properties of the enzyme were not due to an alternative folding of the protein after reversed phase chromatography, the enzyme was also overexpressed in a soluble form in a yeast host system and purified conventionally by chromatography on a nickel affinity resin and hydroxypatite. The enzyme binds tightly to the nickel column even though it was not expressed with a histidine tag. Both procedures yielded enzyme with very similar properties. The data presented here were obtained with the bacterially expressed protein.

**Properties of the Protein**

**Substrates**—Almost all MutT motif proteins studied so far are nucleotide pyrophosphatases that hydrolyze compounds containing an NDP linked to another moiety, hence the alternative name of nudix hydrolase (8). A wide range of nucleotides was assayed to determine the substrate(s) of the YOR163w protein. Of these, only Ap6A, p5A, Ap3A, and p4A yielded significant activity (Table I). ATP was very slowly degraded to ADP + P1, whereas no activity was detectable with the following compounds in the presence of Mg2+ or Mn2+ ions: ApA, Ap5A, Ap6A, NAD+, NADH, NADP+, NADPH, desaminocasein, FAD, coenzyme A, (deoxy)nucleotide 5′-triphosphates (CTP, UTP, CTP, dATP, dGTP, dCTP, and TTP), nucleotide 5′-diphosphates (ADP, GDP, CDP, and UDP), nucleotide 5′-monophosphates (AMP, GMP, CMP, and UMP), nucleotide sugars (ADP-ribose, IDP-ribose, ADP-glucose, GDP-glucose, GDP-mannose, GDP-glucose, UDP-glucose, UDP-galactose, and UDP-N-acetylglactosamine), or nucleotide alcohols (CDP-glycerol, CDP-choline, and CDP-ethanolamine). Diguanosine polyphosphates were not tested as substrates. Table I shows the kinetic constants obtained with the active substrates. Km values were all within the range 30–70 μM and had apparent catalytic constants (kcat) below 1 s−1. According to the calculated catalytic efficiencies (kcat/Km), Ap6A is an 8-fold better substrate than ApA, the overall preference being ApA > p5A > p4A. Kinetic parameters for p4A were calculated using subsaturating substrate concentrations only because substrate inhibition by this compound was observed above 50 μM. Given the preference for ApA, we propose that this protein should be described as a diadenosine 5′,5′′-P1,P′-hexaphosphate hydrolase.

**Reaction Requirements**—With 160 μM Ap6A as substrate, the enzyme displayed optimal activity at pH 6.9, 50 °C, and 4–10 mM Mg2+, Mn2+ at 200 μM also sustained optimal activity, but Cu2+ inhibited with an IC50 of 3.3 mM. F− was also inhibitory (noncompetitive), with a KI of 80 μM. In this respect, the yeast Ap6A hydrolase is similar to but less sensitive than the plant
and animal Ap4A hydrolases, which have $K_i$ values for F2 in the ranges 2–3 and 20–30 μM, respectively (20). The enzyme had an absolute requirement for a reducing agent, such as DTT (optimal at 1 mM).

**Reaction Products**—The reaction products generated from each of the four active substrates were determined after various incubation times by ion-exchange high performance liquid chromatography (Table I and Fig. 3). From the kinetics of product formation, the following overall conclusions were drawn. Ap6A yielded mainly p4A1ADP (76%) but also p5A1AMP (24%). AMP must be a primary breakdown product because ADP is resistant to further hydrolysis, hence the enzyme displays two alternative modes of attack on the Ap6A substrate. p5A, either alone or as a product of Ap6A breakdown, generated almost exclusively p4A1Pi. The example high performance liquid chromatography profile in Fig. 3 shows only a small amount of p5A; however, assays using shorter incubation times clearly showed the generation of equimolar amounts of p5A and AMP before the p5A itself is degraded to p4A1Pi. ATP was also observed, most likely due to the breakdown of the primary p4A product (see below). Similarly, Ap5A yielded predominantly p4A1AMP (96%), but with a small amount of ATP + ADP (4%), whereas p4A broke down to ATP + P. The preferential generation of p4A from both Ap6A and Ap5A suggests a reaction mechanism similar to the plant and animal Ap4A hydrolases, which always generate ATP from ApnA substrates ($n \geq 4$). A binding pocket on these enzymes accommodates a pppA moiety, with the fourth phosphorus distal to the A being subject to nucleophilic attack by water (21, 22). By analogy, the yeast Ap6A hydrolase appears to preferentially accommodate a pppA moiety in the substrate binding site.

Regarding the generation of alternative products, this could occur in one of three ways. Using Ap6A as an example, these are (i) exclusive attack of the nucleophile (presumed to be water) on Pb, with elimination of the Pb—O(Pg) bond, yielding p4A1ADP, and elimination of the P—O(Pg) bond, yielding p4A1AMP; (ii) attack on Pb and elimination of the P—O(Pg) bond, yielding p5A1AMP; (iii) attack on Pb and elimination of the P—O(Pg) bond, yielding p1ADP, and attack on Pa and elimination of the P—O(Pg) bond, yielding p5A1AMP. These possibilities can be distinguished by carrying out the reaction in the presence of H218O and following the fate of the 18O by mass spectrometry (23) (Fig. 4). When Ap6A was hydrolyzed in the presence of H216O, the AMP and ADP

![Fig. 3. Example of product determination using Ap6A as substrate.](image)

![Table I: Products and kinetic constants](image)

![Fig. 4. Possible mechanisms for the generation of alternative products from Ap6A.](image)
products had masses of 348 and 428 Da, respectively (Fig. 5, A and C), whereas hydrolysis in the presence of H$_2^{18}$O resulted in fully $^{18}$O-labeled AMP and ADP, with masses of 350 and 430 Da, respectively (Fig. 5, B and D). In both cases, the p$_4$A product was unlabeled, with a mass of 588 Da (Fig. 5, E and F), whereas rapid degradation of the p$_5$A prevented an assessment of its labeling pattern. Because only mechanism iii leads to labeling of both AMP and ADP and lack of labeling of p$_4$A (Fig. 4), this must be the normal mode of attack and is, therefore, identical to that previously established for the Artemia and lupin Ap$_4$A hydrolases (22–24).

**DISCUSSION**

The recovery of active YOR163w protein after reversed phase chromatography and its high temperature optimum of 50 °C reflect the high stability of the MutT motif proteins as a family. This purification system has also been found to yield pure and fully active recombinant human Ap$_5$A hydrolase.$^3$ Stability can probably be attributed to the mixed $\beta$-sheet structure shown to be at the core of the E. coli MutT protein itself and other highly stable proteins (25).

So far, Ap$_6$A and Ap$_5$A have only been described in the secretory granules of certain specialized mammalian cells. It is not known whether they are present in the cytosol of eukaryotes in general, including yeast. If so, and if they are synthesized by aminoacyl-tRNA synthetases in a reaction similar to the lower order diadenosine polyphosphates (26, 27), then both p$_5$A and p$_4$A would be required as adenylate acceptors. Neither of these compounds exists at detectable levels in vegetative yeast cells; however, they are both synthesized and excreted during the latter stages of sporulation following ascospore formation, reaching 1.5 and 2% of the concentration of ATP, respectively (28). They are not produced by asporogenous a/a or a/a strains placed in sporulation medium, and so they have been proposed as signals marking the end of sporulation (28). They may be synthesized by acetyl-CoA synthetase, which is known to generate them in vitro (29). Their presence in yeast cells suggests that Ap$_6$A and Ap$_5$A might also be synthesized at low levels during sporulation. Because Ap$_6$A is a potent inhibitor (active in the nanomolar range) of the essential enzyme adenylate kinase (30), one function of the Ap$_6$A hydrolase may

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$^3$ J. L. Cartwright and A.G. McLennan, unpublished observations.
be to eliminate these potentially toxic dinucleotides during sporulation. In this context, it is of interest to note that the region upstream of the YOR163w gene contains a single copy of the stress response element 5'-AGGGG, which is known to contribute to the response to nitrogen starvation (31). The possibility that YOR163w expression is regulated by cellular stress remains to be determined. Alternatively, the accumulation of p_A and p_A during this period may reflect a higher activity of the ApA hydrolase during vegetative growth, its function being the removal of the substrates for ApA and ApA synthesis. Such functions would be in keeping with the “housecleaning” role proposed for the nudix hydrolase family (8).

In generating AMP + p_A from ApA, the reaction mechanism of the yeast ApA hydrolase is identical to that previously determined for the production of AMP + ATP by the *Artemia* and lupin ApA hydrolases, namely nucleophilic attack of water on P^v_ and elimination of the P^v—O(P^v) bond (22–24). Interestingly, the *Artemia* ApA hydrolase can be forced to switch attack to P^v when presented with substrates containing nonscissile P^v—C phosphonate linkages, such as diadenosine 5',5 '-P^1,P^4-(P^1,P^2-monofluoromethylene-P^2,P^4-monofluoromethylene) tetraphosphate (ApCHFPpApA) (21), or α-thiophosphates, such as (R,S)-diadenosine 5',5 '-P^1,P^4-(P^1,P^2-dithio)-tetraphosphate (ApDpApA) (32). This so-called frameshift mode of attack was attributed to a flexibility in the binding of the polyphosphate substrate to the enzyme, with either P^v or P^i being positioned next to a fixed catalytic center, a situation more commonly encountered with polymeric substrates (21). This flexibility is also demonstrated by the yeast ApA hydrolase with the natural substrate ApA and, to a lesser extent, ApA, with alternative sets of products being generated in each case.

Fig. 7A shows a partial sequence alignment of the YOR163w protein with other known eukaryotic dinucleoside polyphosphate hydrolases, including the YA9E protein from *Schizosaccharomyces pombe*, which shares 43% sequence identity with YOR163w. The gene encoding YA9E has recently been cloned and expressed, and the protein has ApA hydrolase activity with ApA and ApA as the preferred substrates, but with some activity toward ApA, in contrast to YOR163w, which has no activity with this substrate. Several observations can be made. First, YOR163w has an extra proline residue inserted in the MutT motif, the sequence common to all members of this protein family. This may in part explain the exclusive accommodation of the longer polyphosphate chains compared with the ApA hydrolases and the YA9E protein, which do not have this extra residue. Second, the hydrophobic patch in the fungal enzymes located just N-terminal to the MutT motif (YOR163w residues 47–50) is more similar to that in the animal ApA hydrolases than the plant enzyme sequences (Fig. 7A). However, further toward the N terminus (YOR163w residues 26–

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**Fig. 6. Western blot analysis of yeast extract.** Lane 1, 10 ng of purified recombinant YOR163w protein; lane 2, 5 μl of total yeast cell extract; lane 3, mixture of 10 ng of purified recombinant YOR163w protein and 5 μl of total yeast cell extract prepared as follows: the cells from 200 μl of an overnight culture of strain S288C in YPD (yeast extract/peptone/dextrose) medium were collected at 16,000 × g for 1 min and then resuspended in 100 μl of SDS sample buffer and boiled for 5 min. After centrifugation, the supernatant was used. Immunoblotting was carried out as described under “Experimental Procedures.”

**Fig. 7. Partial sequence alignment of YOR163w protein with other known dinucleoside polyphosphate hydrolases (A) and two possible human homologs (B).** A, the YOR163w sequence (GenBank™ accession number Y78717) was aligned with lupin (GenBank™ accession number M55083) and barley (GenBank™ accession number Z75071) ApA hydrolases, S. pombe YA9E protein (SwissProt accession number Q09790), and human (SwissProt accession number P56380) and mouse (SwissProt accession number P56380) ApA hydrolases using the CLUSTAL W program. B, the YOR163w sequence (GenBank™ accession number U55021) was aligned with the human diphosphoinositol polyphosphate phosphohydrolase DIPP (GenBank™ accession number AF062529) and the related human sequence of unidentified function (GenBank™ accession number AA916467). Amino acid identities with YOR163w are shaded gray, the MutT motif (GXXEX,REX,EEXG) is underlined, and the hydrophobic patch is overlined. Numbers to the right and left of the sequences represent the positions in the respective complete amino acid sequences.
40), both of the fungal proteins, especially YOR163w, share additional sequence similarity with the two plant Ap₄A hydrolases. This similarity is absent from the animal hydrolases, which do not align with any significance in this region. The fungal and plant enzymes also share the enzymic property of hydrolyzing both nucleoside and dinucleoside polyphosphates: the lupin Ap₄A hydrolase degrades p₄A, whereas this compound is a potent inhibitor of the animal Ap₄A hydrolases (6, 33). Thus, there may be a closer evolutionary relationship between the plant and fungal Ap₄A hydrolases. Another activity that may be related is the dinucleoside polyphosphate hydrolase purified from the green alga Scenedesmus obliquus, an organism that, like S. cerevisiae, has an Ap₄A phosphohydrolase (34). This enzyme hydrolyzes Ap₄A with the preference Ap₄A > Ap₄A > Ap₃A. No significant similarity to the E. coli orf186 gene product, an enzyme that prefers Ap₃A as substrate but that also hydrolyzes ADP-ribose and NADH (35), was detected outside the MutT motif.

With regard to possible mammalian orthologs of YOR163w, a 41-amino acid sequence of this protein encompassing the MutT motif shows 50% identity and 65% similarity with two closely related but distinct sequences that are represented by several human, mouse, and rat clones in the GenBank™ expressed sequence tag data base. The alignment with the two human sequences is shown separately from the other dinucleoside polyphosphate hydrolases in Fig. 7B for clarity. One of these, DIPP, has recently been shown to be a diphosphoinositol polyphosphate phosphohydrolase, the first MutT motif protein with activity toward non-nucleotide substrates (36). Like YOR163w, DIPP shows positional flexibility in its site of attack. It is believed to attack a different pyrophosphoryl group in its two substrates, diphosphoinositol pentakisphosphate and bis(diphosphoinositol) tetrakisphosphate. Given the broad substrate specificity of some MutT motif proteins and the similarity between these proteins, it will be of particular interest to determine whether YOR163w can also hydrolyze diphosphoinositol polyphosphates.

In conclusion, we have characterized a dinucleoside polyphosphate nudix hydrolase from S. cerevisiae with a novel substrate specificity. Given the current power of genetic manipulation in yeast, phenotypic analysis of YOR163w knockouts in appropriate genetic backgrounds (e.g. apa1 and apa2 mutants) should help determine the role(s) of this enzyme and the relative contribution of hydrodases and phosphorylases to Ap₄A catabolism and function in those organisms in which both types of enzyme exist (34).

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