The Diadenosine Hexaphosphate Hydrolases from
Schizosaccharomyces pombe and Saccharomyces cerevisiae
Are Homologues of the Human Diphosphoinositol Polyphosphate
Phosphohydrolase

OVERLAPPING SUBSTRATE SPECIFICITIES IN A MutT-TYPE PROTEIN*

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Aps1 from Schizosaccharomyces pombe (Ingram, S. W., Stratemann, S. A., and Barnes, L. D. (1999) Biochemistry 38, 3649–3655) and YOR163w from Saccharomyces cerevisiae (Cartwright, J. L., and McLennan, A. G. (1999) J. Biol. Chem. 274, 8604–8610) have both previously been characterized as MutT family hydrolases with high specificity for diadenosine hexa- and pentaphosphates (Ap6A and Ap5A). Using purified recombinant preparations of these enzymes, we have now discovered that they have an important additional function, namely, the efficient hydrolysis of diphosphorylated inositol polyphosphates. This overlapping specificity of an enzyme for two completely different classes of substrate is not only of enzymological significance, but in addition, this finding provides important new information pertinent to the structure, function, and evolution of the MutT motif. Moreover, we report that the human protein previously characterized as a diphosphorylated inositol phosphates may competitively inhibit this process.

Following the discovery of dinucleotide polyphosphates in biological systems over 30 years ago (1), these compounds have been studied extensively in prokaryotic and eukaryotic organisms. Several important intracellular and extracellular signaling functions have now been ascribed to the diadenosine com-

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pounds, Ap6A, Ap5A, and Ap4A (2–4). Indeed, the ultimate fate of cell lineages and the very survival of an organism may depend upon the tight control of cellular diadenosine polyphosphate metabolism. For example, the intracellular level of Ap6A has long been known to be associated with cell proliferation (5). Moreover, it was recently proposed (6) that Ap6A has an antiproliferative role when complexed with the putative tumor suppressor Fhit protein, an Ap6A hydrolase (7, 8). Thus, the Ap6A/Ap5A ratio may be an important factor in determining the alternative cellular fates of proliferation, differentiation, and apoptosis (3, 9). In higher eukaryotes, Ap6A appear also to be intracellular mediators of certain extracellular stimuli; they respond to glucose in pancreatic β-cells (10). Ap6A may also regulate ATP-sensitive K+ channels in β-cells and cardiac muscle (11–13) and intracellular ryanodine-binding Ca2+-release channels in cardiac and skeletal muscle, and in the brain (14, 15). Finally, extracellular Ap6A and Ap5A have also been identified as neurotransmitters (2) and vasomodulators (16, 17).

In addition to these physiological functions, Ap6A respond to heat shock and oxidative stress with an increase in concentration (18). If allowed to accumulate, they could prove toxic through their ability to inhibit nucleotide kinases (19, 20), protein kinases (21, 22), and other enzymes (23). Thus, there is considerable interest in the enzymes that control the synthesis and catabolism of diadenosine polyphosphates.

The MutT/Nudix motif represents one general solution to the challenge of regulating the levels of metabolic intermediates that either act as cellular signals, or can be deleterious to cell function (24). This motif, which appears in a number of proteins from across the phylogenetic spectrum, is characterized by the following (or closely related) sequence: GXGU, where U is usually either I, L, or V (24). There is a family of so-called “Ap6A” hydrolases, which contain the MutT motif; these enzymes hydrolyze Ap6A in preference to Ap4A, Ap5A, Ap4A, and Ap6A (2–4). The abbreviations used are: ApnA, diadenosine 5’-n-P’-oligophosphate (n = 3–6); Ap4A, Ap5A, Ap6A; pA, adenosine 5’-tetraphosphate; pA, adenosine 5’-pentaphosphate; PP-InsP5, diphosphoinositol pentakisphosphate; [PP]2-InsP4, bisdiphosphoinositol tetraakisphosphate; InsP6, inositol hexakisphosphate; DIPP, diphosphoinositol polyphosphate phosphohydrolase; HT, histidine triad; Fhit, fragile histidine triad; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonate; E-64, trans-epoxysuccinyl-l-leucylamido(4-guanidino)-butane; hMTH1, human MutT homologue, type 1; k-1 is the first-order rate constant in the rate equation, [S] = [S]e- k-1 HPLC, high performance liquid chromatography.
other dinucleotides (25–28). Distinct MutT-type hydrolases that prefer Ap5A and Ap6A as substrates have recently been identified in Schizosaccharomyces pombe (Aps1; see Ref. 29) and Saccharomyces cerevisiae (YOR163w; see Ref. 30). However, no Ap5A/Ap6A hydrolases have previously been found in higher eukaryotes.

The search for mammalian homologues of the yeast Aps1 and Ap6A hydrolases has now drawn us to the observation that there is a human MutT-type protein with some limited sequence similarity to both Aps1 and YOR163w (Fig. 1). However, this particular human enzyme has not previously been shown to have any significant activity toward nucleoside phosphates: for example, dATP (31) and dGTP2 are not physiologically significant substrates (6). Instead, this enzyme has been shown to have a quite different catalytic activity; it was identified (31) as a diphosphoinositol polyphosphate phosphohydrolase (DIPP). DIPP’s substrates, PP-InsP5 and [PP]2-InsP4 (31), which are the most highly phosphorylated members of the inositol-based cell-signaling family, are metabolically unrelated to the diadenosine polyphosphates. However, it is of interest that PP-InsP5 and [PP]2-InsP4 have themselves been strongly implicated as playing important roles in signal transduction. For example, cellular levels of PP-InsP5 act as a sensor for the diadenosine polyphosphates. We therefore determined if DIPP and YOR163w (30), have been shown to metabolize nucleoside phosphates, and neither Aps1 nor YOR163w has been shown to metabolize diphosphoinositol polyphosphates.

**Materials—** For the analysis of Aps1, YOR163w, and DIPP hydrolases, namely Aps1 from S. pombe (accession no. Q09790) and YOR163w from S. cerevisiae (Z75071), were aligned with human DIPP (AF062529), using the PILEUP algorithm (with the caveat that the only gap permitted within the MutT motif was to accommodate an additional asparagine residue in YOR163w). The MutT motif is shaded gray. Stars denote amino acids in either of the two yeast proteins that were identical to corresponding residues in DIPP; physicochemically similar residues are marked with a cross.

**Enzyme Assay—** For the analysis of Aps1 (n = 3–6) hydrolysis, the substrate was incubated with enzyme in buffer containing 50 mM HEPES (pH 7.6), 1 mM MnCl2, and 100 μg/ml bovine serum albumin at 37 °C. The mass of enzyme, incubation time, and substrate concentration were varied as described previously to determine substrate specificity, time courses, and substrate saturation curves (29). Assay solutions were analyzed by HPLC to resolve individual nucleotides as described above. For the analysis of PP-InsP5 and [PP]2-InsP4 hydrolysis, the substrate was incubated with enzyme in buffer containing 50 mM KCl, 50 mM HEPES (pH 7.2), 4 mM CHAPS, 50 μg/ml bovine serum albumin, 1 mM Na2EDTA, 2 mM MgSO4. At the appropriate time, aliquots were quenched, neutralized and subsequently analyzed by HPLC as described above, except that the gradient was generated by mixing buffer A (1 mM Na2EDTA) and buffer B, which also contains 1 mM dithiothreitol, 20 mM Hepes (pH 7.6), 4 mM CHAPS, 20 Sigma units/ml creatine phosphokinase. The resulting [PP]2-[3H]InsP4 (80% conversion) was purified by HPLC (23) and desalted (37) with 17% recovery. Pure, recombinant preparations of Aps1 (29), YOR163w (30), Flt (38), human Ap6A hydrolase (25), Ina1 protein from Bartonella bacilliformis (28), and DIPP (31) were all obtained as described previously.

**RESULTS**

**Structural Similarities among Aps1, YOR163w, and DIPP—** Fig. 1 compares the sequences of three enzymes that each contain the MutT motif. Two of these proteins, Aps1 from S. pombe (29) and YOR163w from S. cerevisiae (30), have been characterized as Ap6A/Ap5A hydrolases. The third protein is from Homo sapiens and has been characterized as a PP-InsP5/ [PP]2-InsP4 phosphohydrolase (31). Much of the DIPP sequence (around 70%) showed no significant similarity to either of the two yeast proteins (Fig. 1). However, the region from Val44 to Glu65 in DIPP, which includes the MutT motif and short flanking regions, is 46% identical to the corresponding regions of YOR163w (Val47 to Glu68) and Aps1 (Val57 to Lys108). This comparison was of particular interest because DIPP has not been shown to metabolize nucleoside phosphates, and neither Aps1 nor YOR163w has been shown to metabolize diphosphoinositol polyphosphates. Therefore determined if DIPP and the Ap6A/Ap5A hydrolases could metabolize the other enzyme’s

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**EXPERIMENTAL PROCEDURES**

Materials—Aps1, Aps6A, and Ap6A hydrolases were prepared as described previously (29). Ap6A, Ap5A, and Ap6A were purchased from Sigma. Non-radiolabeled PP-InsP5 was synthesized as described previously (34). The sources of PP-InsP4, [3H]InsP5, and PP-InsP4 were all as described previously (31). PP-InsP4 was prepared as follows: three rat brains were homogenized in 2 volumes of buffer A (20 mM HEPES, pH 6.8, 2 mM CHAPS, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol) plus 6.25 μg/ml pepstatin, 25 μg/ml aprotinin, 5 μg/ml leupeptin, 25 μg/ml E-64. A 100,000 × g supernatant was loaded onto a heparin-agarose column (5 mm × 5 cm) in buffer A. Proteins were eluted with a gradient generated by mixing buffer A with buffer B (buffer A plus 1 M KCl): 0–10 min, 0% buffer B; 10–20 min, 10–50% B; 20–30 min, 100% B. The InsP6 kinase (35) and PP-InsP5 kinase (36) eluted from the column. Aliquots of peak fractions were incubated with [3H]InsP5 in buffer containing: 0.75 mM EGTA, 1.5 mM EDTA, 9 mM MgSO4, 7.5 mM ATP, 10 mM NaF, 20 mM phosphocreatine, 1 mM dithiothreitol, 20 mM HEPES (pH 7.6), 4 mM CHAPS, 20 Sigma units/ml creatine phosphokinase. The resultant [3H]InsP4 (80% conversion) was purified by HPLC (37) with 17% recovery. The pure, recombinant preparations of Aps1 (29), YOR163w (30), Flt (38), human Ap6A hydrolase (25), Ina1 protein from Bartonella bacilliformis (28), and DIPP (31) were all obtained as described previously.
substances. For these experiments, we used pure, recombinant preparations of each enzyme (see “Experimental Procedures”).

Reactivity of DIPP toward Diadenosine Polyphosphates—

Ap5A was found to be actively metabolized by DIPP (Fig. 2), with multiple products being formed (Fig. 3A). In fact, when incubated with 100 μM Ap5A, DIPP was virtually as efficient as an Ap5A hydrolase (1.1 μmol/min/mg) as is either Aps1 or YOR163w, both of which hydrolyze 100 μM Ap5A at a rate of 1–2 μmol/min/mg (29, 30). The rank order of relative specific activities of DIPP toward the various diadenosine polyphosphates (Ap5A > Ap6A > Ap7A > Ap8A; Fig. 2) mirrored that for Aps1 (29). In terms of relative substrate affinities, YOR163w is slightly different from both Aps1 and DIPP, since YOR163w does not hydrolyze Ap5A, possibly due to the insertion of an extra asparagine residue within the putative substrate-binding MutT motif (Fig. 1) (30). It is significant that DIPP provides the first example of a hydrolase in the animal kingdom that expresses a preference for Ap5A and Ap6A over other diadenosine polyphosphates.

We also determined km and km values for the hydrolysis of Ap5A and Ap6A by DIPP (Table I). The affinities of DIPP for these particular substrates (Km = 6–8 μM) were slightly higher than the affinity of Aps1 (approximately 20 μM; Ref. 29) and YOR163w (approximately 60 μM; Ref. 30). The km values for DIPP (0.5 s−1, Table I) were intermediate between those for Aps1 (approximately 2 s−1; Ref. 29) and YOR163w (0.06 s−1 for Ap5A and 0.4 s−1 for Ap6A; Ref. 30). It is striking that the Ap5A/Ap6A hydrolase activity of DIPP is kinetically very similar to that of both yeast enzymes.

The reaction mechanisms of Aps1, YOR163w, and DIPP promise to be interesting to unravel and compare. For the yeast enzymes, it has been noted that Ap5A can be hydrolyzed to three different sets of products. Aps1 primarily hydrolyzes Ap5A asymmetrically to yield p4A and ADP, but there is also some symmetrical hydrolysis to yield 2 ATP as a minor product (29). YOR163w also generates p4A and ADP as major products from Ap5A, but p5A and AMP are also formed as minor products (30). These different modes of Ap5A hydrolysis have been attributed to Aps1 and DIPP having some mobility within the active site (30).

The time course of Ap5A hydrolysis by DIPP (Fig. 4A) provides insight into this enzyme’s reaction mechanisms. In these assays, the rate of consumption of Ap5A was matched by the rate of accumulation of AMP plus ADP, at a ratio of about 4:1 (Fig. 4A). These results suggest that the predominant route of Ap5A hydrolysis is to AMP plus p5A, with the formation of ADP plus p4A being a more minor reaction. Although we estimate that approximately 80% of the Ap5A was degraded to p5A plus AMP, the p4A did not accumulate, other than for a small amount after 2 min (Fig. 4A). Presumably, p4A is dephosphorylated almost as rapidly as it is formed; p4A that was generated from Ap5A was also rapidly hydrolyzed to p3A plus P1 in previous experiments with YOR163w (30). DIPP also hydrolyzed the p5A that was formed during Ap5A hydrolysis (Fig. 4A). Between the 15- and 35-min time points, the decrease in p5A levels was matched by a corresponding increase in ATP levels. Thus, we conclude DIPP hydrolyzed p5A to ATP and P1. Furthermore, once all the original Ap5A had been consumed (after approximately 15 min), there were only relatively minor increases in the levels of ADP and AMP. Thus, we also conclude that there is relatively little hydrolysis of either p5A or ATP to either ADP or AMP. Finally, by the end of the time course, the total amount of [AMP + ADP] that was formed was approximately equivalent to the amount of Ap5A added (Fig. 4A). Thus, all the ATP that accumulated can be accounted for by further

## Table I

| Substrate | Km (μM) | km (μM) | km/Km (μM s−1) |
|-----------|--------|---------|----------------|
| Ap5A      | 5.9 ± 3.0 (5) | 0.50 ± 0.15 (5) | 0.85           |
| Ap6A      | 7.7 ± 2.2 (3) | 0.42 ± 0.13 (3) | 0.55           |
dephosphorylation of p₄A and p₅A, rather than direct, symmetrical cleavage of Ap₅A.

With regards to Ap₅A, both Aps1 and YOR163w degrade this substrate to two sets of products, namely ADP plus ATP, and p₅A plus AMP (29, 30). However, one difference between these two enzymes is that ADP plus ATP are the major reaction products for Aps1 (29), whereas for YOR163w, p₅A and AMP are the major products (30). When DIPP was incubated with Ap₅A, this substrate was primarily hydrolyzed to AMP plus p₄A (Figs. 3B and 4B). ADP accounted for no more than 4% of total nucleoside products (Fig. 4B), indicating that there was relatively little cleavage of Ap₅A to ADP plus ATP. Thus, the ATP that did accumulate in these reactions (Fig. 4B) must have arisen from the further hydrolysis of p₅A.

Reactivity of Aps1 and YOR163w toward Diphosphorylated Inositol Polyphosphates—We next discovered that Aps1 and YOR163w expressed phosphohydrolase activity toward PP-InsP₅ and [PP]₂-InsP₄. We have also found [PP]₂-InsP₄ to be metabolized by Aps1 and YOR163w (Fig. 5A). Reaction products were identified by HPLC. InsP₅ was formed (Fig. 5A), which indicates that both yeast enzymes cleaved the β-phosphate from the diphosphate group of PP-InsP₅, as is the case for DIPP (31). No InsP₆ was produced (Fig. 5A), indicating that the γ-phosphate group was not removed from PP-InsP₆; the absence of InsP₆ in these reactions also demonstrates that InsP₆ was not a substrate. The yeast enzymes also did not hydrolyze any of the monoester phosphates of PP-InsP₅, since no PP-InsP₄ was formed (Fig. 5A). Both Aps1 and YOR163w had only an 8-fold lower substrate affinity for PP-InsP₅ compared with DIPP (Table II). $k_{\text{cat}}$ values for the three enzymes were also quite similar (Table II).

[PP]₂-InsP₄ is another diphosphorylated inositol phosphate that is hydrolyzed by DIPP (31). We have also found [PP]₂-InsP₄ to be metabolized by Aps1 and YOR163w (Fig. 5B). Under first-order conditions, Aps1 hydrolyzed [PP]₂-InsP₄ 5-fold more rapidly ($k^{-1} = 8.3 \pm 1.1 \mu \text{g}^{-1} \text{min}^{-1}$, $n = 5$) than was the case for YOR163w ($k^{-1} = 1.7 \pm 0.5 \mu \text{g}^{-1} \text{min}^{-1}$, $n = 3$). In fact, the rate achieved by Aps1 was only 8-fold less than that for DIPP ($k^{-1} = 70 \pm 20 \mu \text{g}^{-1} \text{min}^{-1}$, $n = 4$). We were unable to generate more detailed kinetic data for [PP]₂-InsP₄ hydrolysis, because we do not have sufficient mass amounts of this particular substrate.

Reactivity of Other Ap₅A Hydrolases toward PP-InsP₅ and [PP]₂-InsP₄—The results described above indicate that two MutT motif Ap₅A/Ap₅A hydrolases from yeasts can also hydrolyze PP-InsP₅ or [PP]₂-InsP₄. We therefore examined if this overlapping substrate specificity was also a feature of other MutT-type Ap₅A hydrolases. In Fig. 1, we noted a region of DIPP (from Val₁³⁴ to Glu₈⁵) that was 46% identical in sequence to corresponding domains in the yeast Ap₅A/Ap₅A hydrolases.
This same region of DIPP was only 33% identical to a human Ap₆A hydrolase (Fig. 6). Although the latter enzyme did hydrolyze PP-InsP₅ and [PP]₂-InsP₄, the reactions proceeded relatively slowly (for PP-InsP₅ hydrolysis, \( k^{-1} = 0.005 \, \text{µmol}^{-1} \cdot \text{s}^{-1} \) (0.0002% of DIPP activity)²; for [PP]₂-InsP₄ hydrolysis, \( k^{-1} = 0.006 \, \text{µmol}^{-1} \cdot \text{s}^{-1} \) (0.007% of DIPP activity, see above)).

The IaLA invasion protein of *B. bacilliformis* is another MutT motif protein, and this enzyme expresses broadly similar catalytic activities toward Ap₆A, Ap₅A and Ap₄A (27, 28). The IaLA protein, when incubated at up to a 120-fold higher protein concentration than was used for Aps1, showed no detectable hydrolysis of diphosphorylated inositol polyphosphates (data not shown). Indeed, outside the MutT motif, IaLA has no significant similarity to DIPP (Fig. 6). Further evidence that the MutT motif does not generally impart the capacity to metabolize PP-InsP₅ and [PP]₂-InsP₄ comes from experiments showing that these compounds are not significant substrates of hMTH₁, a human homologue of the prototypical MutT protein (which instead hydrolyzes 8-oxo-dGTP; Ref. 39).

Nature has developed more than one solution to the problem of how to regulate cellular levels of diadenosine polyphosphates. As well as Aps1, YOR163w, and DIPP, which belong to the MutT motif family (see above), some Ap₆A hydrolases belong to the HIT protein family and are characterized by the MutT motif (see above), some Ap₆A hydrolases belong to the MutT motif family (see above), some Ap₆A hydrolases belong to the HsXH motif, HxHxH (7, 8, 40). We incubated one such enzyme, the human Fhit Ap₆A hydrolase (7) with either PP-InsP₅ or [PP]₂-InsP₄. Neither substrate was metabolized by a concentration of Fhit that was 1000-fold greater than was used for Aps1 (data not shown).

In view of these results, we conclude that the remarkable overlapping substrate specificity seen in Aps1, YOR163w, and DIPP is neither a general property of MutT-type proteins, nor is it a general feature of Ap₆A metabolizing enzymes.

**DISCUSSION**

The MutT paradigm is based on this motif recurring in a series of nucleoside phosphate hydrolases that act as “guardians” of cellular integrity (24). Members of this enzyme family have been shown to hydrolyze nucleoside phosphates that are directly hazardous, such as the mutagenic 8-oxo-dGTP (39), and they may also protect the cell against the potentially dangerous consequences of inappropriate increases in the levels of intracellular signals, such as dATP (41). To achieve efficiency in this role, it has previously seemed that individual MutT motif proteins have each evolved exclusive catalytic specificities toward restricted groups of nucleoside phosphates (24). Our results with Aps1, YOR163w, and DIPP are therefore exceptional, because the diadenosine polyphosphates, Ap₆A and Ap₅A, and the diphosphorylated inositol phosphates, PP-InsP₅ and [PP]₂-InsP₄, are two unrelated classes of substrates. Furthermore, these two groups of metabolites have independently emerged as participants in various aspects of signal transduction (see the Introduction). Since specificity of action is paramount for an intracellular signal, it is remarkable that one enzyme can hydrolyze these different molecules, thereby potentially regulating their levels and affecting their signaling strength. Thus, our observations not only draw an unexpected link between two disparate areas of signal transduction, but they are also pertinent to the evolution and functions of the MutT motif.

Evidence has accumulated that Ap₆A and Ap₅A are cellular signals that may act both outside (2, 16, 17, 42) and inside (12–15) the cell. There is therefore considerable interest in characterizing the enzymes that are responsible for metabolizing these two diadenosine polyphosphates. DIPP represents the first hydrolase in the animal kingdom shown to prefer Ap₆A and Ap₅A over Ap₄A. In fact, the kinetic parameters associated with DIPP-dependent Ap₆A/Ap₅A hydrolase activity are not only similar to those of Aps1 and YOR163w (Tables I and II), they are also in line with the characteristics of other MutT motif Ap₆A hydrolases (25–28, 30). In that sense, DIPP is a typical Ap₆A/Ap₅A hydrolase. This conclusion raises the important question as to whether this is a function of DIPP in vivo. Our *in vitro* data suggest that PP-InsP₅ is preferred as a substrate over either Ap₆A or Ap₅A (Tables I and II). However, we do not know the subcellular compartments in which DIPP is present, nor do we have reliable estimates of the concentrations, in these same compartments, of any of DIPP's substrates. Nevertheless, if DIPP were closely associated with the secretory vesicles in which Ap₆A and Ap₅A appear to be concentrated, at least in platelets, chromaffin cells and synapses (2), this enzyme could regulate the signaling strength inside the vesicles. If some DIPP were present on the cell surface, it could contribute to terminating the interactions of Ap₆A and Ap₅A with purinergic receptors (2). Finally, DIPP that is free in the cytosol may be a site for metabolic competition between Ap₆A, Ap₅A, Ap₇A, PP-InsP₅, and [PP]₂-InsP₄.

The fact that, to date, yeasts have not been reported to contain either Ap₆A or Ap₅A has raised questions concerning the possible biological functions of Aps1 and YOR163w (29, 30). While the search for Ap₆A and Ap₅A in yeasts continues, it is worth noting that both *S. pombe* (43) and *S. cerevisiae* (44) are known to synthesize PP-InsP₅ and [PP]₂-InsP₄. Thus, another important feature of our studies is that PP-InsP₅ and [PP]₂-InsP₄ represent a useful, new focus for further studies into the functions of YOR163w and Aps1. The identification of a PP-InsP₅/[PP]₂-InsP₄ phosphohydrolase in a genetically tractable organism such as yeast also provides us with new opportunities to rapidly increase our insight into the roles of these particular compounds. This is a particularly timely opportunity in the light of a recent observation from studies with *S. pombe*; the size of this yeast’s InsP₅ metabolic reservoir, which in turn dictates the amount of PP-InsP₅ and [PP]₂-InsP₄ inside cells (37), was recently shown to be acutely sensitive to hyperosmotic stress (43).

Another important feature of our study relates to the value of comparing related enzymes from different species, which can provide fresh insight into relationships between protein structure and function. The sequence alignment of DIPP with yeast Ap₆A/Ap₅A hydrolases (Fig. 1) identifies a number of conserved amino acid residues both inside the MutT motif, and in the immediately adjacent flanking regions. A systematic study into the contribution made by these residues, by site-directed mutagenesis for example, will be of great value in future studies into the catalytic process. Thus, our studies represent an important step toward the goal of understanding how the tertiary structures of this class of MutT-type proteins can accommodate two different substrate structures so successfully. Further in-

### Table II

| Enzyme | \( K_m \) (µM) | \( k_{cat} \) (s⁻¹) | \( k_{cat}/K_m \) (µM⁻¹s⁻¹) |
|--------|----------------|-----------------|----------------------------|
| Aps1   | 31 ± 3 (4)     | 0.17 ± 0.02 (4) | 53                         |
| YOR163w| 31 ± 6 (3)     | 0.06 ± 0.01 (3) | 20                         |
| DIPP   | 4.2 ± 0.4 (5)  | 0.2 ± 0.01 (8)  | 474                        |

H. Hayakawa, S. T. Safrany, S. B. Shears, and M. Sekiguchi, unpublished data.
formation on structure/function relationships will also come from the comparison of the sequence of DIPP with the sequences of other MutT-type enzymes that we have now shown not to attack PP-InsP₃ and [PP]₂-InsP₄ (see Fig. 6 and "Results").

A popular viewpoint concerning the evolution of catalytic motifs envisages relatively nonspecific progenitors becoming duplicated and then adapted to perform specific tasks (45). The MutT motif has been proposed to exemplify this evolutionary process (24). It has been proposed that, from a primordial family, a protein with proteins of MutT motifs each have evolved subtly different phosphohydrolase specificities toward particular nucleoside phosphates (24). A contrary view states that a protein motif that provides a particular advantage can arise independently, in otherwise unrelated proteins, by convergent evolution (46). It is argued that the latter idea is supported by the considerable divergence in the protein sequences that lie outside the eponymous motif of the MutT-related proteins (see Figs. 1 and 6 and Ref. 24). However, sequence is less tightly conserved during divergent evolution than is structure (47). In view of these conflicting ideas, Ap₅₁, and DIPP represent models of particular interest for further studies into the evolution of functions and tertiary structures of the MutT family.

It has been suggested that "Nudix" is a more accurate name for the "MutT" motif, in part because of the earlier data indicating that this motif imparted catalytic specificity toward compounds containing a nucleoside diphosphate attached to another moiety, "p" (24). Thus, the original discovery of a MutT motif in DIPP created an important precedent; DIPP became the first member of this class of enzymes shown to actively metabolize substrates that do not conform to the above structure. Our new data with Ap₅₁ and YOR163w extend this exception. The unexpected catalytic promiscuity of this group of enzymes is an important new factor that will have to be taken into account in our ongoing efforts to understand how cells regulate the cellular levels and the biological functions of both diadenosine polyphosphates and diphosphorylated inositol phosphates.

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