Adenine Phosphoribosyltransferase Mutants in *Saccharomyces cerevisiae*

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Mutants of *Saccharomyces cerevisiae* deficient in adenine phosphoribosyltransferase (A-PRT, EC 2.4.2.7) have been isolated following selection for resistance to 8-azaadenine in a prototrophic strain carrying the *ade4-su* allele of the gene coding for amidophosphoribosyltransferase (EC 2.4.2.14). The mutants were recessive and defined a single gene, *apt1*. They did not excrete purine when combined with *ade4*⁺. The mutants appeared to retain some A-PRT activity in crude extracts, and strains of the genotype *ade2 apt1* responded to both adenine and hypoxanthine. Mutants deficient in adenine aminohydrolase (EC 3.5.4.2) activity, *aah1*, and hypoxanthine:guanine phosphoribosyltransferase (EC 2.4.2.8) activity, *hpt1*, were used to synthesize the genotypes *apt1 hpt1 aah*⁺ and *apt1 hpt*⁺ *aah1*. The absence of A-PRT activity in strains with these genotypes confirmed the hypothesis that the residual A-PRT activity of *apt1* mutants was due to adenine aminohydrolase and hypoxanthine:guanine phosphoribosyltransferase acting in concert.

INTRODUCTION

The purine phosphoribosyltransferases (PRTs) catalyse the conversion of free purine bases into their corresponding nucleotides, allowing organisms to utilize purines derived from nucleotide catabolism or available exogenously. The relationship of the reactions catalysed by these 'salvage pathway' enzymes to the pathway of purine synthesis *de novo* is shown in Fig. 1. Deficiency of H:G-PRT in man results in the Lesch-Nyhan syndrome (Lesch & Nyhan, 1964), indicating the importance of this salvage pathway of purine nucleotide synthesis.

Resistance to purine analogues has been used successfully to isolate PRT mutants in *Salmonella typhimurium* (Kalle & Gots, 1961) and *Schizosaccharomyces pombe* (De Groodt *et al.*, 1969). In both organisms 8-azaguanine was used to select for H:G-PRT deficiency and 2,6-diaminopurine for A-PRT deficiency. Mutants of *Drosophila melanogaster* which are resistant to the toxic effects of exogenous purine have been shown to lack activity of A-PRT (Johnson & Friedman, 1981). In studies with animal cells in culture 8-azaguanine has been used to select for H:G-PRT deficiency (Littlefield, 1963; Lever *et al.*, 1974) and both 8-azaadenine and 2,6-diaminopurine to select for A-PRT deficiency (Chasin, 1972, 1974; Jones & Sargent, 1974). In contrast, failure to utilize exogenous purines was used to select PRT mutants in *Escherichia coli* (*hpt* – Chou & Martin, 1972; *apt* – Jochimsen *et al.*, 1975; *gpt* – Holden *et al.*, 1976).

The PRTs of *Saccharomyces cerevisiae* were first investigated by Kornberg *et al.* (1955) who demonstrated biochemically separable A-PRT and H-PRT activity and also showed activity

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Abbreviations: PRT, purine phosphoribosyltransferase; A-PRT, adenine phosphoribosyltransferase; H:G-PRT, hypoxanthine:guanine phosphoribosyltransferase; AAH, adenine aminohydrolase; PRPP, 5'-phosphoribosyl-l-pyrophosphate; PRPPAT, amido phosphoribosyltransferase.

Fig. 1. Purine metabolism: *de novo* synthesis and the salvage pathway in *S. cerevisiae*. The enzymes and genes involved are: (1) amidophosphoribosyltransferase, *ade4*, EC 2.4.2.14; (2) A-PRT, *apt1*, EC 2.4.2.7; (3) H:G-PRT, *hpt1*, EC 2.4.2.8; (4) AAH, *aah1*, EC 3.5.4.2. The pathway intermediates are as follows: PRPP, 5'-phosphoribosyl-1-pyrophosphate; PRA, phosphoribosylamine; S-AMP, succinyl-AMP; XMP, xanthosine monophosphate; HYP, hypoxanthine; ADE, adenine; GUA, guanine.

with guanine as a substrate. H:G-PRT has been investigated in more detail by Miller & Bieber (1968, 1969), Schmidt *et al.* (1979) and Nussbaum & Caskey (1981). Woods *et al.* (1983) reported that mutants defective in the activity of H:G-PRT were resistant to 8-azaguanine and that they excreted purines. Pickering & Woods (1973) attempted to isolate A-PRT mutants by selecting for resistance to the adenine analogue 4-aminopyrazolo-(3,4-d)-pyrimidine; the resistant mutants defined six genes, one of which lacked a purine permease (Pickering & Woods, 1972), but all of them retained A-PRT (Pickering, 1972). Studies by Lomax & Woods (1969) and Pickering & Woods (1973) have shown that 8-azaadenine is an effective inhibitor of yeast growth. We report here the use of this analogue to isolate A-PRT deficient mutants of yeast. We also show that adenine aminohydrolase (AAH) and H:G-PRT can combine to form purine nucleotides from exogenous adenine and mask the metabolic consequences of A-PRT deficiency.

METHODS

Yeast strains. The genotypes and phenotypes of the primary strains of *Saccharomyces cerevisiae* used in this investigation are listed in Table 1. All were from the collection of R. A. Woods.

Media and growth conditions. The complete (YCM), minimal (YMM) and sporulation (YSM) media have been described previously (Lomax *et al.*, 1971). Nitrogen-free medium (YCB) contained 11.7 g Difco Yeast Carbon Base l⁻¹. Purines and purine analogues were added to give final concentrations as stated. Media were solidified with 1.5% Difco Bacto agar. All cultures were incubated at 30 °C. Qualitative growth responses were determined using 5 ml cultures in 15 × 150 mm test tubes inoculated with 10⁵ cells ml⁻¹. The cultures were incubated on a rotary shaker at 200 r.p.m. Samples were taken after 24 h, diluted and the OD₅₄₅ recorded.

Table 1. *Genotypes and phenotypes of S. cerevisiae strains*

Strain	Genotype	Phenotype
RW1-3B	▲ <i>ade4-su</i>	Prototroph, reduced amidophosphoribosyltransferase activity*, sensitive to purine analogues†, epistatic to purine excretion by <i>pur</i> mutants‡ and <i>hpt1</i> §
RW1-1C	α <i>ade4</i> *	Prototroph
RW1-1D	α <i>ade4-su</i>	As for strain RW1-3B
RW1-4D	▲ <i>ade2 ade4-su</i>	Responds to adenine or hypoxanthine
W2-1C	▲ <i>ade4-su hpt1.27</i>	Prototroph, lacks activity of H:G-PRT§

* Nieto & Woods (1983).

† Lomax & Woods (1969).

‡ Armitt & Woods (1970).

§ Woods *et al.* (1983).

Genetic techniques. Matings and ascus dissections were carried out as described by Ahmed & Woods (1967). Segregants were tested for analogue resistance and nutritional requirement by replica plating. Purine excretion was detected by heavy point inoculation of strains under test onto plates of YMM agar containing a suspension of an *ade2* strain. The test plates were scored for growth of the *ade2* strain around the inocula after 2 to 4 d. Mutants were induced by UV irradiation of RW1-1D (α *ade4-su*) to 10% survival.

Detection of AAH activity. AAH activity was scored by inoculating 5 ml YCB plus adenine (0.3 mM) with a loopful of cells and incubating cultures as above for 24 to 36 h. The cultures were then centrifuged and the optical density of the supernatant scanned over the range of 210 to 300 nm. Strains with AAH activity hydrolyse adenine ($\lambda_{\max}$, 260 nm) to hypoxanthine ($\lambda_{\max}$, 248 nm).

Preparation of cell-free extracts and assay of PRT activity. Cultures were grown from an initial inoculum of 10^5 cells ml⁻¹ in 250 ml triple-baffled conical flasks containing 50 ml YMM and harvested in mid-exponential phase by centrifugation after 18 h. The cells were washed once in cold extraction buffer [10 mM-potassium phosphate (pH 6.8); 0.25 M-sucrose; 10 mM-dithiothreitol; 1% (v/v) isopropanol] and resuspended in the same buffer at 1.0 ml per 20 ml starting culture. An equal volume of glass beads (400 μ m diameter) was added and the mixture vortexed at top speed on a table-top vortex mixer for four periods of 30 s separated by 30 s cooling on ice. The homogenates were pipetted off and centrifuged at 12000 r.p.m. for 12 min in a Beckman Microfuge. The supernatants were assayed for PRT activity by the procedure of Mulligan & Berg (1980). The assay mixture contained 100 mM-Tris/HCl (pH 8.0), 10 mM-MgCl₂, 1.5 mM-cold purine, 0.5 μ Ci (18.5 kBq) [¹⁴C]purine, 1 mM-dithiothreitol, 0.5 mg ml⁻¹ bovine serum albumin (Sigma, fraction V), and 1.0 mg ml⁻¹ 5'-phosphoribosyl-1-pyrophosphate (PRPP). The reaction was started by adding 25 μ l extract to 100 μ l assay mixture, incubated at 37 °C for 30 min, and terminated by spotting 25 μ l samples onto 2.5 cm discs of DE-81 paper. Discs were washed six times in 2 l 10 mM-NaCl to remove unreacted purine, leaving the nucleoside monophosphates bound to the disc. Dried discs were counted in 10 ml Scinti-Verse (Fisher) at 76% efficiency in a Beckman LS 7500 liquid scintillation counter. Enzyme activities were determined as nmol purine nucleoside monophosphate formed min⁻¹ (mg protein)⁻¹. Protein concentrations were determined according to the procedure of Bradford (1976), using the Bio-Rad protein-assay reagent.

Electrophoresis and assay of PRT activity. Extracts were electrophoresed on slab gels containing 8.0% (w/v) acrylamide, 0.4% (w/v) *N,N*-methylenebisacrylamide, 0.2 M-Tris/HCl (pH 8.6) and 5% (v/v) glycerol at 4 °C. The running buffer was 0.2 M-Tris/HCl (pH 8.6), 1 mM- β -mercaptoethanol. PRT activity was detected by overlaying the gels with Whatman 3MM paper soaked in a reaction mixture containing 100 mM-Tris/HCl (pH 8.0), 10 mM-MgCl₂, 2 mM-PRPP, 1 mM-dithiothreitol, 5 μ M-purine and 0.2 μ Ci [¹⁴C]purine ml⁻¹. The gel and paper were wrapped in plastic wrap and incubated for 1 h at 30 °C. The paper was then removed and the gels soaked in 100 mM-LaNO₃, 100 mM-Tris/HCl (pH 7.0) for 1 h. They were then washed in deionized water for 1.5 h and processed for gel fluorography by the procedures of Bonner & Laskey (1974) and Laskey & Mills (1975).

Assay of AAH. AAH activity in cell-free extracts was measured by a modification of the method described by Abbondandolo *et al.* (1971). Cultures were grown for 18 h in YMM medium from an initial inoculum of 10^5 cells ml⁻¹, harvested and resuspended in homogenizing buffer (pH 7.0) containing 0.1 M-phosphate buffer, 1.0 mM-EDTA and 1 mM- β -mercaptoethanol. The procedure for homogenization was as for the PRT assays. The assay mixture (100 μ l) consisted of homogenizing buffer containing 1.5 mM-adenine and 1.0 μ Ci [¹⁴C]adenine ml⁻¹. The reaction was initiated by the addition of 20 μ l cell-free extract, and terminated after 20 min at 37 °C by spotting 5 μ l samples onto sheets of 13255 cellulose (Eastman Kodak) with cold carrier adenine and hypoxanthine. The chromatograms were developed in 0.1 M-sodium phosphate buffer (pH 6.8)/saturated aqueous (NH₄)₂SO₄/*n*-propanol (100:60:2, by vol.) (solvent C, Ciardi & Anderson, 1968). The purines were visualized under UV light, cut out and counted in 10 ml Scinti-verse.

Chemicals and supplies. Purines, purine analogues, dithiothreitol, PRPP, Tris/HCl and bovine serum albumin were purchased from Sigma. [¹⁴C]Adenine was purchased from New England Nuclear and Amersham; [¹⁴C]hypoxanthine was purchased from New England Nuclear. Scinti-Verse scintillation fluid and DE-81 discs were purchased from Sigma. [¹⁴C]Adenine was purchased from New England Nuclear and Amersham; Rad.

RESULTS AND DISCUSSION

Isolation and characterization of mutants

Strains carrying the variant form of amidophosphoribosyltransferase specified by the allele *ade4-su* (Nieto & Woods, 1983) have been shown to be more sensitive to purine analogues, including 8-azaadenine, than those carrying *ade4+* (Pickering & Woods, 1973). Accordingly, strain RW1-1D was mutagenized with UV light and plated onto YMM agar supplemented with 0.37 mM-8-azaadenine. Forty eight resistant mutants were isolated. The mutants were tested for growth over 24 h in YMM plus 8-azaadenine and YMM plus 8-azaguanine; it was expected that

A-PRT deficiency would result in resistance to the adenine analogue only. Twelve mutants which were resistant to 8-azaadenine but sensitive to 8-azaguanine were selected for further analysis.

All of the mutants were crossed to RW1-3B (α *ade4-su*). They were all clearly recessive, since on YMM agar supplemented with 0.37 mM-8-azaadenine the heterozygous diploid grew less vigorously than the haploid mutants. Tetrad analysis was carried out on the cross involving mutant 9 (cross 1, W6, in Table 2). Resistance to 8-azaadenine segregated 2:2 in five complete tetrads. An 8-azaadenine resistant segregant, W6-1A, was crossed to the other 11 mutants. All of the diploids were resistant to 8-azaadenine. Eight complete tetrads from the crosses of W6-1A to each of the mutants 8, 10, 11 and 23 were dissected and analysed; all of the progeny were resistant to 8-azaadenine. Thus the 12 mutants represent a single gene, *apt1*.

Genetic and biochemical analysis

The 12 *apt1* mutants were assayed for activity of A-PRT as described in Methods. The results (Table 3) show that all appeared to retain some A-PRT activity. Relative to that in the parent strain, the levels ranged from 20% in *apt1.9* to 48% in *apt1.10*.

In other systems, resistance to the adenine analogues 2,6-diaminopurine and 8-azaadenine results in complete loss of A-PRT activity (Kalle & Gots, 1961; De Groodt *et al.*, 1969). We hypothesized that the residual activity in our mutants could be due to adenine aminohydrolase and H:G-PRT acting in concert as follows:

The IMP formed would bind to the DE-81 discs used in the radioassay and result in apparent A-PRT activity.

The hypothesis was tested in three ways. (a) Production and characterization of a strain with the genotype *apt1 hpt1*. (b) Isolation of an AAH deficient mutant, *aah*, and construction of the genotype *apt1 aah*. (c) *In situ* assay of PRT activities following electrophoresis of cell-free extracts of appropriate genotypes.

A strain of the genotype *ade4-su hpt1* is resistant to 8 azaguanine but not to 8-azaadenine (Woods *et al.*, 1983). The segregant W6-2B (α *ade4-su apt1.9*) was crossed to W2-1C (α *ade4-su hpt1.27*). Analysis of eight tetrads from this cross (cross 2, W20, in Table 2) showed that eight segregants were resistant to both analogues, eight to 8-azaadenine and eight to 8-azaguanine; the remaining eight were sensitive to both compounds. The genotype of a doubly resistant segregant, presumed to be *apt1 hpt1*, was confirmed by backcrossing to RW1-3B (*ade4-su*) (cross 3, W32, in Table 2). The results of these two crosses indicated that *apt1* was not linked to *hpt1*; the recombination frequencies were 50% and 54%, respectively.

The results of assaying for PRT activities in appropriate genotypes derived from the cross W20 are listed in Table 4. It can be seen that A-PRT activity was reduced in *apt1.9 hpt⁺ aah⁺*, and absent in *apt1.9 hpt1.27 aah⁺*. The pattern of H:G-PRT activity was as expected. Thus, H:G-PRT activity is necessary for the *apt1* mutants to show residual A-PRT activity.

The strain W6A-3B (α *ade2 apt1.9*), a segregant obtained from cross 5 in Table 2, was used as the starting point for the isolation of an *aah* mutant. It was mutagenized and plated onto YMM agar plus hypoxanthine and, after 24 h incubation, replica-plated onto YMM agar plus adenine. One mutant which responded to hypoxanthine but not to adenine was obtained. It was crossed to W6-2C (α *ade4-su*) and six complete tetrads were tested for the segregation of *ade2*, *apt1* and *aah1* (cross 4, DS1, in Table 2). In each tetrad two segregants showed the expected phenotype of AAH deficiency, failure to reduce the λ_{max} of YCB medium supplemented with adenine from 260 nm to 248 nm. Segregants with the presumptive genotypes *apt1.9 aah1* and *aah1* were backcrossed to *ade4-su* and their identities confirmed.

Table 2. Genetic analysis of *apt1*

Cross	Genotype	No. of tetrads analysed	No. of segregants of genotype:										
			wt*	ade	apt	ade apt	hpt	apt hpt	aah	ade aah	apt aah	ade apt aah	
1 (W6)	■ <i>apt1.9 ade4-su</i> x α <i>ade4-su</i>	5	10	—	10	—	—	—	—	—	—	—	
2 (W20)	α <i>apt1.9 ade4-su</i> x ■ <i>hpt1.27 ade4-su</i>	8	8	—	8	—	8	8	—	—	—	—	
3 (W32)	α <i>apt1.9 hpt1.27 ade4-su</i> x ■ <i>ade4-su</i>	12	11	—	13	—	13	11	—	—	—	—	
4 (DS1)	■ <i>ade2 apt1.9 aah1 ade4-su</i> x α <i>ade4-su</i>	6	5	1	2	4	—	—	2	4	3	3	
5 (W6A/W21)	■ <i>apt1.9 ade4-su</i> x α <i>ade2 ade4-su</i>	16	20	12	12	20	—	—	—	—	—	—	
6 (W9)	■ <i>ade2 apt1.9</i> x α <i>ade4-su</i>	12	15	9	9	15	—	—	—	—	—	—	
7 (W19)	■ <i>apt1.9 ade4-su</i> x α <i>ade4*</i>	9	18	—	18	—	—	—	—	—	—	—	

* wt indicates segregants carrying only *ade4** or *ade4-su*.

Table 3. Adenine phosphoribosyltransferase activity in mutants allocated to *apt1*

Genotype	Specific activity*	Activity relative to <i>ade4-su</i> (%)
<i>ade4-su</i>	24.1	100
<i>ade4</i> ⁺	28.7	119
<i>apt1.8</i>	8.4	35
<i>apt1.9</i>	4.9	20
<i>apt1.10</i>	11.7	48
<i>apt1.11</i>	10.9	45
<i>apt1.23</i>	10.4	43
<i>apt1.27</i>	7.7	32
<i>apt1.30</i>	5.1	21
<i>apt1.40</i>	6.1	25
<i>apt1.41</i>	10.3	43
<i>apt1.42</i>	8.8	36
<i>apt1.44</i>	10.2	42
<i>apt1.46</i>	10.3	43

* Activities are expressed as nmol AMP formed min⁻¹ (mg protein)⁻¹.

Table 4. Purine phosphoribosyltransferase and adenine aminohydrolase activities

Genotype	Specific activity*		
	A-PRT	H:G-PRT	AAH
<i>apt</i> ⁺ <i>hpt</i> ⁺ <i>aah</i> ⁺	11.8	11.1	122.5
<i>apt1.9 hpt</i> ⁺ <i>aah</i> ⁺	3.4	13.7	122.9
<i>apt1.9 hpt1.27 aah</i> ⁺	0.1	0.0	109.2
<i>apt1.9 hpt</i> ⁺ <i>aah1</i>	0.0	—	—
<i>apt</i> ⁺ <i>hpt1.27 aah</i> ⁺	9.1	0.1	144.0
<i>apt</i> ⁺ <i>hpt</i> ⁺ <i>aah1</i>	7.8	—	0.0

* Activities are expressed as nmol product formed min⁻¹ (mg protein)⁻¹.

The results of assaying for AAH activity in appropriate strains are listed in Table 4. It can be seen that the *aah1* mutant lacked activity of the enzyme. Previous studies (Roush & Saeed, 1960; Medhat, 1965) have indicated that AAH is an inducible enzyme in yeast. Our results show that activity is high in extracts prepared from cultures in mid-exponential phase; further studies (R. A. Woods & B. P. May, unpublished results) suggest that activity is negligible in nitrogen-starved cultures and is induced by both adenine and ammonium.

The results of assay for A-PRT in strains of the genotypes *apt1.9 hpt*⁺ *aah1* and *apt*⁺ *hpt*⁺ *aah1* are listed in Table 4. It can be seen that AAH is also necessary for expression of residual A-PRT activity in the *apt1* mutants.

A comparison of the electrophoretic mobility of the residual A-PRT activity in the mutant *apt1.9* with bona fide A-PRT from the parent strain *apt*⁺ *ade4-su* is shown in Fig. 2. The lower mobility of the residual activity was also demonstrated in *apt1.8*, *apt1.23*, *apt1.27*, and *apt1.30* strains (not shown). It is unlikely that this residual activity is due to mutational alteration of A-PRT since it showed the same mobility relative to A-PRT from *ade4-su* in all five mutants.

Assays for A-PRT, H:G-PRT, and H:G-PRT + A-PRT were then carried out on the same gel. H:G-PRT had a lower mobility than A-PRT, as shown by the simultaneous assay of both enzymes with an extract from an *apt*⁺ *hpt*⁺ *aah* strain. An A-PRT activity band was detectable in *apt*⁺ *hpt1 aah*⁺ and *apt*⁺ *hpt*⁺ *aah1* strains but not in *apt1 hpt1 aah*⁺ or *apt1 hpt*⁺ *aah1* (data not shown). These results are consistent with our hypothesis and suggest that the residual A-PRT activity in the *apt1* mutants is probably the result of H:G-PRT using hypoxanthine formed from adenine by the action of AAH.

The phenotypic characteristics of the *apt1* mutants were checked by crossing *ade4-su apt1.9* to RW1-4D (a *ade2 ade4-su*). This is listed as cross 5 (W6A/W21) in Table 2. The accumulation of

Fig. 2. Electrophoresis and the *in situ* assay of A-PRT in extracts from *apt1-9* and *apt⁺ ade4-su* strains. Tracks 1, 2, and 5, *apt⁺ ade4-su* (3, 7.6 and 30 μ g, respectively); tracks 3 and 4, *apt1-9* (each 344 μ g). Extracts were applied to the gel in a total volume of 20 μ l.

the red pigment characteristic of *ade2* mutants is reduced by adenine supplementation (Armitt & Woods, 1970). The phenotypes of *ade2* and *ade2 apt1-9* could be distinguished on YMM and YCM agar media supplemented with 0.15 mM-adenine. The *ade2* mutant grew well on these media and was pink whereas *ade2 apt1-9* grew poorly and was not pigmented. Burns (1964) has shown that pigment accumulation is dependent on purine nucleotide levels and has suggested that IMP is the most important inhibitor of PRPPAT *in vivo*. A-PRT deficiency would be expected to result in reduced levels of AMP and continued pigment accumulation. However, the combined activities of AAH and H:G-PRT would result in high levels of IMP in an *ade2 apt* mutant since these enzymes would be the only route for adenine utilization. This, coupled with the poor growth of such mutants, most probably accounts for the lack of pigment accumulation.

To confirm the genotype assignments the tetrads were grown in YMM and YMM plus 1.5 mM-adenine. In the latter medium the *ade2* mutant gave a tenfold greater yield of cells than the *ade2 apt1* mutant after 24 h; however, after 48 h, by which time the cultures were in stationary phase, the yields of both mutants were the same. In those tetrads in which both of the adenine-requiring segregants were identified as *ade2 apt1* neither of the prototrophs was resistant to 8-azaadenine. The identity of the double mutants was confirmed by the presence of 8-azaadenine-resistant segregants in tetrads obtained from a backcross of a presumptive *ade2 apt1* to *ade4-su* (cross 6, W9, in Table 2). The results of these reciprocal crosses show that *ade2* and *apt1* are not linked. The frequency of recombinant genotypes was 63% in cross 5 and 38% in cross 6.

Purine excretion has been shown to be a consequence of H:G-PRT deficiency in man

(Rosenbloom *et al.*, 1968), *Schizosaccharomyces pombe* (De Groodt *et al.*, 1969) and yeast (Woods *et al.*, 1983). A-PRT deficiency in man results in the accumulation of 2,8-dihydroxyadenine (Cartier & Hamet, 1974; Doppler *et al.*, 1981) but purine excretion has not been reported as a characteristic of the genetic lesion in either mammalian cell lines (Chasin, 1972; Jones & Sargent, 1974) or *Schizosaccharomyces pombe* (De Groodt *et al.*, 1969). The *apt* mutants did not appear to excrete purines when tested for crossfeeding to an *ade2* strain. However, they had been induced in *ade4-su*, which suppresses purine excretion by other mutants (Armitt & Woods, 1970; Woods *et al.*, 1983). Accordingly, W6-1A (a *ade4-su apt1.9*) was crossed to RW1-4A, which carries the allele *ade4⁺*, and the progeny were tested for analogue resistance and purine excretion (cross 7, W19, in Table 2). Resistance to 8-azaadenine segregated 2:2 in the nine tetrads tested. None of the segregants excreted purines and none were resistant to 8-azaguanine.

Most of the previous studies involving the isolation of A-PRT mutants have been with organisms which lack activity of AAH. The enzyme has not been shown to occur in *E. coli* and is not present in animal tissues (Zielke & Suelter, 1975). Thus, direct selection of deficient mutants was possible either by selecting for analogue resistance, as in *Salmonella typhimurium* (Kalle & Gots, 1961), or failure to utilize exogenous adenine, as in *E. coli* (Jochimsen *et al.*, 1975). De Groodt *et al.* (1969) noted that a 2,6-diaminopurine-resistant, A-PRT-deficient mutant of the fission yeast, *Schizosaccharomyces pombe*, responded to exogenous adenine and ascribed this to the combined activity of adenine aminohydrolase and H:G-PRT. We have shown conclusively that the activities of these two enzymes can interfere with the identification of A-PRT-deficient mutants in *Saccharomyces cerevisiae*.

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