1-Acylhydroxyacetone-phosphate Reductase (Ayr1p) of the Yeast Saccharomyces cerevisiae Encoded by the Open Reading Frame YIL124w Is a Major Component of Lipid Particles*

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Biosynthesis of phosphatidic acid through the dihydroxyacetone phosphate pathway requires NADPH-dependent reduction of the intermediate 1-acyldihydroxyacetone phosphate before the second step of acylation. Studies with isolated subcellular fractions of the yeast Saccharomyces cerevisiae revealed that lipid particles and the endoplasmic reticulum harbor 1-acyldihydroxyacetone-phosphate reductase (ADR) activity. Deletion of the open reading frame YIL124w (in the following named AYR1) abolished reduction of 1-acyldihydroxyacetone phosphate in lipid particles, whereas ADR activity in microsomes of the deletion strain was decreased approximately 3-fold as compared with the wild-type level. This result indicates that (i) both lipid particles and microsomes harbor Ayr1p, which was confirmed by immunological detection of the protein in these two cellular compartments, and (ii) microsomes contain at least one additional ADR activity. As a consequence of this redundancy, deletion of AYR1 neither results in an obvious growth phenotype nor affects the lipid composition of a haploid deletion strain. When a heterozygous AYR1"/ayr1" diploid strain was subjected to sporulation; however, spores bearing the ayr1 defect failed to germinate, suggesting that Ayr1p plays an essential role at this stage. Overexpression of Ayr1p at a 5- to 10-fold level of wild type caused growth arrest. Heterologous expression of Ayr1p in Escherichia coli resulted in gain of ADR activity in the prokaryote, confirming that YIL124w is the structural gene of the enzyme and does not encode a regulatory or auxiliary component required for reduction of 1-acyldihydroxyacetone phosphate. Taken together, these results identified Ayr1p of the yeast as the first ADR from any organism at the molecular level.

Phosphatidic acid (PA) is a key intermediate in the formation of glycerophospholipids and triacylglycerols. Two different pathways of PA biosynthesis are known: (i) the glycerol 3-phosphate (Gly-3-P) pathway and (ii) the dihydroxyacetone phosphate (DHAP) pathway (1). In the former pathway, Gly-3-P is first acylated to 1-acylglycerol 3-phosphate (lysophosphatidic acid, LPA) and further converted to PA by a second acyltransferase. In the DHAP pathway, the precursor DHAP is acylated to 1-acyl-DHAP. This intermediate has to be reduced to LPA in an NADPH-dependent reaction before its conversion to PA (Fig. 1). Whereas in plants and bacteria, PA formation occurs only through the Gly-3-P pathway (2, 3), both pathways are active in mammals (2, 4, 5) and yeast (6–8).

In mammalian cells, enzymes catalyzing PA formation were reported to be localized to mitochondria, the microsomal fraction, and peroxisomes (2, 4, 5). Whereas in mitochondria only Gly-3-P and, in peroxisomes, only DHAP serve as precursors, both substrates can be acylated in the microsomal compartment (9). In the yeast Saccharomyces cerevisiae, the highest specific activity of glycerol-3-phosphate acyltransferase was detected in lipid particles (10–12). The second site of glycerol-3-phosphate acyltransferase activity in the yeast is the endoplasmic reticulum (ER), whereas other organelles, e.g. mitochondria or vacuoles, are largely devoid of enzymes forming LPA from the precursor Gly-3-P (11, 12). The highest specific activity of DHAP acylation was found in lipid particles followed by microsomes. Surprisingly, 1-acyl-DHAP is also formed with mitochondria as the enzyme source although at a reduced specific activity (8). It was shown that the same enzyme, the hypothetical Gat1p, catalyzes acylation of both precursors, Gly-3-P and DHAP, in lipid particles (8). Gat1p is also present in the ER, but this compartment contains at least one additional set of acyltransferases involved in PA formation. DHAP acyltransferase of mitochondria, however, appears to be an enzyme distinct from Gat1p (8, 12). Collectively, different organelles of the yeast contribute to PA formation and may interact during this process.

1-Acyl-DHAP formed during PA synthesis through the DHAP pathway is reduced to LPA by 1-acyl-DHAP reductase (ADR) (13). In animal cells, ADR is a key enzyme for the formation of ether lipids and acylglycerolipids via the DHAP pathway (14). Localization studies demonstrated that acyl/alkyl-DHAP reductase of guinea pig liver cells, like other enzymes of the DHAP pathway, is mainly present in peroxisomes, although some activity of this enzyme was also detected in the ER (14). Amino acid analysis of acyl/alkyl-DHAP reductase purified from guinea pig liver peroxisomes revealed that hydrophobic amino acids composed 27% of the molecule; the amino acid sequence of this protein, however, was not determined. In the yeast S. cerevisiae ADR has been detected by its enzymatic activity (13), but neither the gene nor the gene product was characterized at a molecular level. Yeast ADR activity is present in lipid particles and the ER (30,000 × g microsomes), whereas mitochondria appear to be devoid of this enzyme (8).

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* The abbreviations used are: PA, phosphatidic acid; Gly-3-P, glycerol 3-phosphate; DHAP, dihydroxyacetone phosphate; LPA, lysophosphatidic acid; ER, endoplasmic reticulum; ADR, 1-acyldihydroxyacetone-phosphate reductase; YPD, yeast extract/peptone/dextrose; ORF, open reading frame; PCR, polymerase chain reaction.

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The present paper describes identification of AYR1, the structural gene encoding the major ADR of yeast. Dual localization of Ayr1p in lipid particles and the ER and the existence of at least one additional ADR activity in the ER are demonstrated. Phenotypic consequences of an ayr1 deletion are discussed.

**EXPERIMENTAL PROCEDURES**

**Strains and Culture Conditions**—The wild-type yeast strains S. cerevisiae DBY746 (MATa, his3Δ1, leu2–3, 112, ura3–52, trp1–289, hap1::kanMX4), FY1679 (MATa, leu2–3, 112, ura3–52, leu2–3Δ1/EU1, his3Δ200/ HIS3, trp1Δ63/TRP1, GAL2/GAL1) and mutants deleted of YIL124w (MATa, his3Δ1, leu2–3, 112, ura3–52, trp1–289, ayr1::kanMX4), YIR036c (MATa, his3Δ1, leu2–3, 112, ura3–52, trp1–289, yir036c::kanMX4), and YMR228c (MATa his3Δ1, leu2–3, 112, ura3–52, trp1–289, ymr228c::kanMX4) in the DBY746 background were used throughout this study.

**Construction of Deletions**—Cells were grown aerobically in 2-liter Erlenmeyer flasks to the late logarithmic phase at 30 °C in YPD medium, pH 5.5, containing 1% yeast extract (Oxoid), 2% peptone (Oxoid), and 2% glucose (Merck). Five hundred ml of culture medium were inoculated with 0.5 ml of a preculture grown aerobically for 48 h in YPD medium.

For the heterologous expression of yeast 1-acyl-DHAP reductase, the strain TOP10F (E. coli) was used as recipient for the transformation of pYES2 (see “Experimental Procedures”).

**Deletion, Overexpression, and Heterologous Expression of YIL124w**—A dominant resistance marker module, kanMX4, containing the kan′ gene of the E. coli transposon Tn903, which encodes aminoglycoside phosphotransferase (15), was included in vector pFA6a (16) and used to replace yeast ORFs. Aminoglycoside phosphotransferase activity renders S. cerevisiae resistant to the drug geneticin (G418, Calbiochem) (17). A replacement strategy making use of short flanking homology regions to the target locus (Fig. 2A) was used to construct deletion cassettes by polymerase chain reaction (PCR) (16, 18). Deletion cassettes contained the ATG codon of the ORF to be deleted, the kanMX4 gene, and the stop codon of the ORF, thus eliminating 100% of the target ORF. All deletions were made in the DBY746 or FY1679 background.

To generate marker DNA flanked by short homology regions, a pair of oligonucleotide primers with 70 nucleotides homologous to the target gene, and the stop codon of the ORF to be deleted, the kanMX4 gene, and the stop codon of the ORF, thus eliminating 100% of the target ORF. All deletions were made in the DBY746 or FY1679 background.

**Generation of Plasmids**—Aminoglycoside phosphotransferase activity renders S. cerevisiae resistant to the drug geneticin (G418, Calbiochem) (17). A replacement strategy making use of short flanking homology regions to the target locus (Fig. 2A) was used to construct deletion cassettes by polymerase chain reaction (PCR) (16, 18). Deletion cassettes contained the ATG codon of the ORF to be deleted, the kanMX4 gene, and the stop codon of the ORF, thus eliminating 100% of the target ORF. All deletions were made in the DBY746 or FY1679 background.

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site of the plasmid, which was used for transformation of yeast and E. coli Top10 (21).  

Isolation and Characterization of Subcellular Fractions—Lipid particles were obtained from yeast cells using a 1500 Tricarb scintillation counter. For the analysis of individual lipids the extract was measured by liquid scintillation counting using a 1500 Tricarb. Nonpolar lipids were extracted with 2 volumes of diethyl ether, the aqueous phase as described above. To study overexpression of Ayr1p in yeast, cells transformed with pYES2 bearing YIL124w under a GAL+ promoter were grown on yeast extract, peptone, 2% galactose. At different time points, aliquots of the culture corresponding to an A600 of 3.0 were harvested by centrifugation. Samples were suspended in 0.5 ml of medium and incubated with 50 μl of 1.85 M NaOH for 10 min on ice. Then, 50 μl of trichloroacetic acid (50%) was added for a further incubation of 1 h on ice. The resulting precipitate was isolated by centrifugation, dissolved in 70 μl of sample buffer (24), and heated for 30 min at 37 °C. Aliquots were subjected to SDS-polyacrylamide gel electrophoresis and Western blot analysis as described below.

Homogenate of E. coli was obtained from cultures grown overnight at 37 °C. Cells were harvested and disintegrated by glass bead using a Merckenschläger homogenizer (25).

Protein Analysis—Protein was quantified by the method of Lowry et al. (26) using bovine serum albumin as a standard. Proteins were precipitated with trichloroacetic acid at a final concentration of 10%. The protein pellet was solubilized in 0.1% SDS, 0.1% NaOH. Before protein analysis samples of the lipid particle fraction were delipitated. Nonpolar lipids were extracted with 2 volumes of diethyl ether, the organic phase was withdrawn, residual diethyl ether was removed under a stream of nitrogen, and proteins were precipitated from the aqueous phase as described above. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out by the method of Laemmli (24). Samples were dissociated at 37 °C to avoid hydrolysis of polypeptides, which may occur at higher temperature. Western blot analysis was performed as described by Haid and Suissa (27). Immunoreactive proteins were detected by enzyme-linked immunosorbent assay using rabbit antiserum as the first antibody and goat anti-rabbit IgG linked to peroxidase as the second antibody. For the generation of antibodies against Ayr1p, the polypeptide was purified from lipid particles by SDS-polyacrylamide gel electrophoresis and eluted from gel slices using an Electro Luter model 422 (Bio-Rad). This protein solution was used to immunize rabbits by standard procedures.

Amino acid sequence analysis of proteins solubilized by SDS-polyacrylamide gel electrophoresis was performed by Protana (Odense, Denmark) following the procedures of Shevchenko et al. (28). Briefly, proteins in gel slices were reduced with dithiothreitol and alkylated with iodoacetamide followed by overnight digestion with trypsin. Peptides generated by this procedure were extracted with 50% acetonitrile and 5% formic acid. The mixture of tryptic peptides was purified on micro-columns with POROS R2 perfusion chromatography material and on aphenyl LCG ion trap mass spectrometer (Finnigan, San Jose, CA) equipped with a nanoelectrospray source. Proteins were identified by querying a nonredundant sequence data base containing more than 300,000 entries with partial amino acid sequences (peptide sequence tags) derived from mass spectrometry spectra. The software used for this search was PepSea, Version 1.0 (Protana A/S, Odense, Denmark).

Molecular data about proteins were obtained from the yeast protein data base, Saccharomyces genome data base, SwissProt, and Munich Information Center for Protein Sequences. Homology searches were performed with BLAST Search (29).

Enzyme Analysis—Enzyme activity of ADR was measured as described by Bates and Saggerson (30). Yeast homogenate (2–3 mg of protein), lipid particles (40–80 μg of protein), or microsomes (0.5–1.0 mg of protein) were used as the enzyme sources. Samples were incubated at 30 °C in a final volume of 1 ml of 120 mM KCl, 50 mM Tris/Cl–, pH 7.5, 4 mM MgCl2, 8 mM NaF, 4 mg of bovine serum albumin, 65 nmol of oleoyl-CoA, 80 mM NADPH, 500 nmol of [U-14C]fructose 1,6-bisphosphate (0.4 μCl). 0.44 μM of aldolase and 28 units of triose phosphate isomerase. A 1% portion of the reaction mixture was preincubated for 16 min at 35 °C before the addition of the respective enzyme. Incubations were carried out for 10 min at 30 °C and terminated by the addition of 3 ml of chloroform/methanol (1:2, v/v) and 0.7 ml 1% perchloric acid. The organic phase was washed three times with 2 ml of 1% perchloric acid each, and total radioactivity was measured by liquid scintillation counting using a 1500 Tri carb Beckman scintillation counter. For the analysis of individual lipids the extract was applied to high performance TLC plates (silica gel 60, Merck), and chromatograms were developed in an ascending manner using the solvent system chloroform, methanol, acetic acid, 5% sodium metabisulfate (100:40:12:4); vol.). After chromatographic separation, the radioactively labeled lipids formed during the assay were detected by thin layer chromatography scanning using a TraceMaster 20 automated TLC linear analyzer (Berthold). In addition, lipids were visualized on high performance TLC plates by staining with iodine vapor, bands were scraped off, and radioactivity was measured by liquid scintillation counting using LSC Safety (Baker, Deventer, The Netherlands) + 5% water as a scintillation mixture.

For an alternative assay, 1-acyl-DHAP was generated with lipid particles from the wild-type strain following the procedure described above but without the addition of NADPH to the assay mixture. The resulting 1-acyl-DHAP was extracted with chloroform/methanol (1:2, v/v) and used as substrate for conversion to PA in an assay mixture containing 120 mM KCl, 50 mM Tris/Cl–, pH 7.5, 4 mM MgCl2, 8 mM NaN3, 4 mg of bovine serum albumin, 65 nmol of oleoyl-CoA, and 80 nmol NADPH in a final volume of 350 μl. After the addition of the enzyme source (40–80 μg of lipid particle protein or 2–4 μg of E. coli homogenate, respectively), incubations were carried out for 10 min at 30 °C. Termination of the assay by lipid extraction and analysis of products was carried out as described above.

Lipid Analysis—Lipids of whole yeast cells were extracted after disruption of cells with glass beads by the procedure of Folch et al. (31). Individual phospholipids were separated by two-dimensional thin layer chromatography (8) and quantified by the method of Broekhuyse (32). Triacylglycerols, ergosterol, and ergosteryl esters were quantified as described by Athenaestadt et al. (8). Individual sterols were analyzed after alkylidine hydrolysis (33) of the lipid extract by gas liquid chromatography. Gas liquid chromatography was performed on a Hewlett-Packard 5890 equipped with a flame ionization detector operated at 320 °C using a capillary column (Hewlett-Packard 5, 30m × 0.25 mm × 0.25-μm film thickness). After a 1-min hold at 150 °C, the temperature was increased to 310 °C at 10 °C/min. The final temperature was held for 10 min. Nitrogen was used as the carrier gas and 1-μl aliquots of samples were injected onto the column. Relative retention times of sterols were similar as described previously (34, 35).

Fatty acids were also analyzed by gas liquid chromatography. Lipids extracted as described above were subjected to methanolysis using BF3/methanol (14%) and converted to methyl esters (36). Fatty acid methyl esters were separated by gas liquid chromatography using the same equipment as described above. A temperature program of 2 min at 150 °C, then 10 °C/min to 300 °C was used. Fatty acids were identified by comparison to commercial fatty acid methyl ester standards (Nu-Check, Inc., Elysian, MN).

RESULTS

Two lines of evidence led us to identify AYR1, the gene encoding a 1-acylhydroxyacetone-phosphate reductase (ADR) of the yeast S. cerevisiae. First, enzymatic analysis using isolated subcellular fractions revealed that ADR activity is present in lipid particles and the ER (30,000 × g microsomes) (8). Second, systematic amino acid sequence analysis of yeast lipid particle proteins by mass spectrometry resulted in the identification of 15 ORFs encoding polypeptides associated with this compartment (37). One of these gene products has an apparent molecular mass of 33 kDa (Fig. 3), which is encoded by the ORF YIL124w, exhibits homology to an insect-type alcohol/ribitol dehydrogenase and was the only putative protein of yeast lipid particles with an oxidoreductase motif. At the N terminus of Ayr1p (amino acids 13–37), an NADPH binding domain was identified.
site, was identified by analogy to NADPH-dependent enzymes of other cell types. The tyrosine residue 157 (Y) of the protein may be part of the active site of this enzyme.

Fig. 4 shows the hydropathy blot of the polypeptide encoded by AYR1. This protein does not contain transmembrane-spanning domains but two hydrophobic stretches (hydrophobicity index $\geq 2$) (38), one in the middle of the molecule and one at the C terminus. Lack of transmembrane domains and the presence of only few hydrophobic domains had been demonstrated to be a common feature of lipid particle proteins identified so far (37).

To test whether AYR1 indeed encodes ADR, a mutant deleted of this ORF was constructed. When the gene deletion was carried out in a diploid wild-type strain, subsequent tetrad analysis yielded two colony-forming spores per tetrad, whereas two spores failed to germinate. The viable colonies lacked the kanMX4 marker (data not shown), indicating that the germination defect was caused by deletion of AYR1. When deletion of AYR1 was performed with a haploid wild-type strain, transformants were viable, grew like wild type on glucose, glycerol, ethanol, and lactate and neither exhibited temperature nor cold sensitivity. Phospholipid pattern, neutral lipid composition, fatty acid composition, and sterol pattern of total cell extracts of the deletion strain were also the same as in the corresponding wild type (data not shown). To confirm the germination defect of ayr1, the haploid deletion strain was backcrossed to wild type. When the heterozygous (AYR1/$^{+}$/ayr1$^{-}$) diploid strain was sporulated and subjected to tetrad analysis, only those spores were able to germinate that were bearing the wild-type AYR1 allele. The same result was obtained with the wild-types DBY746 and FY1679, thus indicating that the observed effect was not strain-dependent. Thus, AYR1 is essential for spore germination but not for vegetative growth of yeast cells.

Comparison of the protein patterns of lipid particles isolated from the ayr1 deletion strain and the corresponding wild type showed that a protein with an apparent molecular mass of 33 kDa was missing in the mutant (data not shown). This result was confirmed by Western blot analysis using a monospecific antibody raised against Ayr1p (Fig. 5). The presence of Ayr1p, however, is not only restricted to lipid particles, but this protein was also detected in the ER (30,000 $\times$ g microsomes) of wild type (39). Ayr1p was also detected in the ER (30,000 $\times$ g microsomes) of the deletion strain, indicating that residual ADR activity was still present in this organelle. Thus, AYR1 encodes a protein that is the only ADR of lipid particles but only one isoenzyme with ADR activity of the ER. This result is reminiscent of the dual localization and the redundancy of glycerol-3-phosphate acyltransferase (Gat1p) and 1-acylglycerol-3-phosphate acyltransferase (Scl1p) (12). Similar observations with squalene epoxidase, Erg1p, had led us previously to speculate about a relationship of these two compartments and the hypothesis that lipid particles might originate from the ER (39).

Searches for yeast homologues of Ayr1p, which might catalyze the residual ADR activity in the ER of the ayr1 deletion strain, identified two ORFs of unknown function, namely YMR226c and YIR036c, as candidates to encode ADR isoenzymes. YMR226c encodes a hypothetical protein with similarity to ketoreductases and has 35% identity to YIL124w. YIR036c encodes another hypothetical protein that is a probable member of the short chain family of alcohol dehydrogenases and exhibits 28% identity to YIL124w. Deletions of YMR226c and YIR036c, however, did not affect ADR activity in the microsomal fraction (data not shown). Thus, it is unlikely that YMR226c and YIR036c encode enzymes that significantly contribute to ADR activity of the yeast. Further studies, however, will be needed to establish whether these genes encode ADR isoforms with minor enzymatic activity.

As an alternative to the occurrence of ADR isoforms in different cellular compartments, residual ADR activity in microsomes of the ayr1 deletion strain may result from a bypass reaction. In such a pathway 1-acyl-DHAP may be dephosphorylated followed by reduction of 1-acyldihydroxyacetone to 1-acylglycerol. Then, a kinase may phosphorylate this intermediate to 1-acylglycerol 3-phosphate (LPA), which can re-enter the regular pathway of PA formation. The enzyme activities required for such a bypass are present in animal cells (40) and may also exist in yeast.

Results presented so far did not prove unambiguously that YIL124w is the structural gene encoding ADR. To distinguish between the possible role of Ayr1p as ADR enzyme or effector of ADR activity, the protein was heterologously expressed in E. coli. As mentioned in the Introduction, plants and bacteria lack the DHAP pathway for PA biosynthesis, and DHAP is converted to Gly-3-P in an NADH-dependent reaction before the
two steps of acylation that yield PA. For this reason radioactively labeled 1-acyl-DHAP had to be used as a substrate for the enzyme assay with E. coli homogenate as an enzyme source (see “Experimental Procedures”). As shown in Fig. 7, yeast Ayr1p expressed in E. coli can convert 1-acyl-DHAP to 1-acyl-Gly-3-P (LPA) which is further metabolized to PA by the bacterial acyltransferase. The positive control with lipid particles of the yeast wild-type strain demonstrated that assay conditions were appropriate. The negative control with E. coli bearing the empty plasmid showed that ADR activity in E. coli was indeed due to the presence of the AYR1 gene product. In the absence of NADPH, only background activity was observed in all three assays.

To study overexpression of Ayr1p in yeast, an ayr1 deletion strain was transformed with a multicopy plasmid bearing AYR1 under control of a GAL7 promoter. When these transformants were shifted from glucose- or raffinose-containing media to inducing conditions (galactose-containing medium), cells stopped growing within 1 h. During the period of induction, the amount of Ayr1p increased 5- to 10-fold as compared with noninducing conditions and remained constant for more than 50 h. When transformants were shifted back from inducing to noninducing conditions at any time point during this period, cells recovered and formed growing colonies. Thus, either the increased amount of Ayr1p or accumulation of metabolic intermediates formed upon the induction caused a reversible growth arrest.

**DISCUSSION**

Only a few genes and gene products involved in the biosynthesis of PA in the yeast have been identified at a molecular level. The two components characterized so far are Sic1p, a 1-acylglycerol-3-phosphate acyltransferase (12, 24), and Ayr1p, a reductase catalyzing the conversion of 1-acyl-DHAP to LPA, described in this study. Most noteworthy, Ayr1p of the yeast is the first enzyme of this type characterized at a molecular level.

The redundancy of enzymes involved in PA biosynthesis and their localization to different subcellular compartments (6, 8), namely lipid particles, the ER, and mitochondria, raises the question as to the interplay of organelles during synthesis of this key intermediate of lipid metabolism. As an example, 1-acyl-DHAP formed through acylation of DHAP in mitochondria cannot be further metabolized in this compartment, because mitochondria lack ADR. As a consequence, 1-acyl-DHAP has to be transported to a site of ADR activity, namely lipid particles or the ER, to get converted to LPA. This process may not require a specific transport mechanism, since 1-acyl-DHAP is assumed to be largely water-soluble and might reach the site of reduction by diffusion. As an alternative, translocation of 1-acyl-DHAP may occur through membrane contact between the ER and mitochondria. A specific subfraction of the ER that associates with mitochondria (MAM, mitochondria associated membrane) of the yeast (41, 42) may be involved in this process.

The obvious reason for a missing growth defect caused by an ayr1 deletion is the presence of additional ADRs or a bypass pathway to form PA in the ER. Thus, involvement of Ayr1p in PA formation through the DHAP pathway is not essential for vegetative growth of the yeast. The notable property of the ayr1 deletion strain, however, is its germination defect. This result suggests that a certain level of intermediates of the DHAP pathway may be required for spores to germinate.

Overexpression of AYR1 in yeast resulted in growth arrest but left cells viable. Thus, larger quantities of lysophospholipids accumulating in an overexpressing strain may disturb membrane proliferation. As a hypothetical alternative, ADR present in excess may act as a reductase of other substrates. The respective products might inhibit cell proliferation or prevent the formation of intermediates that are required for cell growth.

In animal cells, the DHAP pathway for PA formation is not only used for the synthesis of diacylglycerolipids but is also obligatory for the formation of ether lipids. In yeast, incorporation of DHAP appears to be restricted to glycerolipids in *vivo* (20) and *in vitro* (6, 8). The possible occurrence of ether lipids in this microorganism has been a long-standing matter of dispute. Most recently it was shown by analysis of isolated organelle...
membranes that alkyl ether lipids may be present at trace amounts in the yeast S. cerevisiae (43). A mutant lacking total ADR activity would be required to determine whether or not yeast is able to synthesize alkyl ether lipids from DHAP as a precursor. Such a mutant would also allow the study of the possible physiological role of alkyl ether lipids and the contribution of the DHAP pathway to overall glycerolipid synthesis in yeast.

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