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# Sterol Uptake Induced by an Impairment of Pyridoxal Phosphate Synthesis in *Saccharomyces cerevisiae*: Cloning and Sequencing of the *PDX3* Gene Encoding Pyridoxine (Pyridoxamine) Phosphate Oxidase

ADIL LOUBBARDI,† CHRISTOPHE MARCIREAU,‡ FRANCIS KARST, AND MICHEL GUILLOTON*

*Laboratoire de Biochimie et Génétique des Microorganismes, Institut de Biologie Moléculaire et d'Ingéniérie Génétique, Université de Poitiers, Poitiers, France*

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**Exogenous sterols do not permeate wild-type *Saccharomyces cerevisiae* in aerobic conditions. However, mutant strain FK*erg7*, affected in lanosterol synthase, is a sterol auxotroph which is able to grow aerobically in the presence of ergosterol. Viability of this strain depends on the presence of an additional mutation, *aux30*, that leads to sterol permeability. Cells bearing the *aux30* mutation fail to grow in standard yeast nitrogen base medium containing pyridoxine but grow normally if pyridoxine is replaced by either pyridoxal or pyridoxamine. These mutants are characterized by a lack in pyridoxine (pyridoxamine) phosphate oxidase [P(N/M)P oxidase] (EC 1.4.3.5) activity. The pleiotropic phenotype induced by the *aux30* mutation includes a strong perturbation in amino acid biosynthesis. Strains bearing the *aux30* mutation also display atypic fatty acid, sterol, and cytochrome patterns. Transformation of an *aux30* strain with a replicative vector carrying the wild-type *PDX3* gene encoding P(N/M)P oxidase restored wild-type fatty acid, sterol, and cytochrome patterns and suppressed exogenous sterol accumulation. It is proposed that sterol permeation of *aux30* strains is mainly the consequence of their leaky Hem$^-$ character. The amino acid sequence of *S. cerevisiae* P(N/M)P oxidase inferred from the nucleotide sequence of *PDX3* shows a high percentage of homology with the corresponding enzymes from *Escherichia coli* and *Myxococcus xanthus*. Several putative Gcn4p binding sequences are present in the *PDX3* promoter region, leading to the assumption that transcription of this gene is under the general control of nitrogen metabolism.**

When grown anaerobically, wild-type *Saccharomyces cerevisiae* is unable to synthesize ergosterol but is able to take up sterols from the culture medium (1). Conversely, aerobic yeast cells do synthesize ergosterol but do not take up exogenous sterols (51, 59). Sterol uptake by wild-type *S. cerevisiae* (36, 43, 51) is induced by heme deprivation (32, 54), and hemes are not formed under anaerobic conditions (48, 49).

Ergosterol synthesis is an aerobic process that requires molecular oxygen and that is also dependent on heme. The first $O_2$-requiring step in sterol biosynthesis is the formation of 2,3-oxidosqualene, the direct precursor of lanosterol in yeasts and animals (57). Heme-dependent enzymes are responsible for the processing of the sterol nucleus, for demethylation (14-demethylase [2] and 4,4-demethylases [3]), and probably $\Delta^{22}$ desaturation (23). Heme is also involved in the regulation A of ergosterol biosynthesis. Heme activates HMG coenzyme reduction (35), and its effect is mediated through a complex with Hap1p that enhances transcription of *HMG1* (58); a complex between heme and Hap1p was also shown to be involved in the enhancement of transcription of *ERG11*, the gene encoding lanosterol 14-demethylase (61).

Sterol auxotrophic yeast mutants must take up sterols from their surroundings and can grow in anaerobiosis in the presence of exogenous sterols. However, to grow aerobically, those blocked in sterol biosynthesis downstream of farnesyl diphosphate formation require additional mutations that allow sterols to permeate the cell (9). Certain of these mutations are known to affect heme biosynthesis (18, 32). Other mutations allow sterol permeation in a heme-competent background (33). One of these is *aux30*, present in the lanosterol synthase (EC 5.4.99.7) mutant FK*erg7*, allowing the mutant to grow aerobically in the presence of ergosterol (27). Strains bearing the *aux30* mutation decompose hydrogen peroxide, and their associated phenotypes are not reversed by hemin. This article shows that the properties of *aux30* strains are the consequences of a lack of pyridoxine (pyridoxamine) phosphate [P(N/M)P] oxidase activity. Transformation of *aux30* strains by a replicative vector carrying a yeast gene encoding P(N/M)P oxidase restores a wild-type phenotype. The sequencing of this gene and the physiology of both mutant and transformed cells are described below. We propose for this gene the designation *PDX3*, since the designation *pdx2* has been given to a locus harboring a mutation leading to pyridoxine auxotrophy (40).

## MATERIALS AND METHODS

**Strains and culture conditions.** The bacterial strains used to make the constructs were derivatives of *Escherichia coli* K-12 (Table 1). The yeast strains used in this study (Table 2) are derived from *S. cerevisiae* FL100 (ATCC 28383), haploid (**a**) mating type, and the isogenic strain FL200 (ATCC 32119), haploid ($\alpha$) mating type (26).

The complete YPD medium consisted of 1% yeast extract (Biokar), 1% peptone pepsique de viande (Biokar), and 2% glucose. The minimal medium used was yeast nitrogen base (YNB) (Difco). Synthetic minimal medium used for the growth of *aux30* strains contained (per liter) 8 ml of a salts solution ($KH_2PO_4$, 25 g; $MgSO_4$, 12.5 g; NaCl, 2.5 g; $CaCl_2$ 2.5 g); 10 ml of a trace elements solution (boric acid, 0.5 g; $CuSO_4$, 40 mg; KI, 100 mg; $FeCl_3$, 200 mg; $MnSO_4$, 400 mg;

* Corresponding author. Present address: Institut de Biotechnologie, Université de Limoges, 123, Ave. Albert Thomas, 87060 Limoges Cédex, France. Phone: 33 55 45 76 55. Fax: 33 55 45 76 53.

† Present address: Institut de Biotechnologie, Université de Limoges, Limoges, France.

‡ Present address: Centre de Recherches Rhône-Poulenc Rorer, Département des Biotechnologies, Vitry sur Seine, France.

TABLE 1. Bacterial strains and plasmids used in this study

| Strain or plasmid | Genotype or phenotype | Reference |
|---|---|---|
| Strains | | |
| DH5α | *supE44 hsdR17 recA1 endA1 gyrA96 thi-1* Δ*lacU169* (ϕ80 *lacZ*ΔM15) | 50 |
| XL1-Blue | *supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F′(*proA*$^+$*B*$^+$ *lacI*$^q$ *lacZ*ΔM15 Tn*10*) | 8 |
| Plasmids | | |
| pFL44L | Yeast-*E. coli* replicative shuttle vector (Amp$^r$ *URA3*) | 6 |
| pAL1 | Yeast 3.7-kb insert cloned in pFL44L | This study |
| pAL1K | *Kpn*I-*Bam*HI 1.7-kb fragment of the pAL1 insert cloned in pFL44L | This study |

$Na_2MoO_4$ 200 mg; $ZnSO_4$, 400 mg); 25 ml of 5% ammonium sulfate; 10 g of glucose; 1 ml of a vitamin solution containing (per liter) biotin (20 mg), folic acid (20 mg), inositol (20 g), niacin (4 g), calcium panthotenate (4 g), thiamine (4 g), and 1-amino-4-benzoic acid (2 g); and 10 ml of a riboflavin solution (200 mg). The three different B6 vitamers—pyridoxal (PL), pyridoxamine (PM), and pyridoxine (PN)—were added at 1 mg/liter.

**Biochemical methods.** Sterol extraction and analysis were performed as described previously (38). Crude $\Delta^{5-7}$ sterol contents of wild-type and *aux30* strains were measured on four independent cultures of each strain in YPD. Analyses of sterols by high-performance liquid chromatography (HPLC) were conducted as described by Xu et al. (63). Cholesterol uptake was assayed as described by Lewis et al. (33). Fatty acids were extracted by diethylether from saponified yeast cells, transformed into the corresponding methyl esters either by diazomethane (Aldrich) or by the instant methanolic HCl method (Alltech), and analyzed by gas-liquid chromatography. P(N/M)P oxidase was measured by a previously described method (28). Protein contents of cell extracts were determined by the Bradford procedure (7). Triphenyl tetrazolium chloride overlay was used to test the respiration deficiency of *aux30* strains, by the method of Ogur et al. (46).

**Characterization of molecules excreted by *aux30* strains in minimal medium.** Cells harboring the *aux30* mutation were grown in liquid YPD, transferred into YNB ($A_{700}$, 0.4), and incubated at 28°C with shaking for 24 h. UV fluorescent products that appeared in the culture medium were extracted with *n*-butanol and purified by thin-layer chromatography on a silica gel (elution with tertamyl alcohol–acetone–water–33% ammonia [40/40/15/5, vol/vol/vol/vol]). The main component, extracted with diethyl ether, analyzed by thin-layer chromatography, UV, and FTIR spectroscopy was identified with 2-aminobenzoic acid (anthranilic acid). Indole was identified by thin-layer chromatography analysis and Erlich reagent (dimethylaminobenzaldehyde). α-Keto acids were transformed into the corresponding 2,4-dinitrophenylhydrazones. Pyruvic and oxalacetic acids were tentatively identified by comparison with known standards. The pyruvic acid content of the culture fluid was assayed photometrically (340 nm) by reduction with NADH in the presence of lactate dehydrogenase.

**DNA sequencing.** DNA was sequenced either manually by the dideoxy chain termination method (53) using the T7 DNA polymerase sequenase (U.S. Biochemical Corporation) or automatically with an ALF sequencer (Pharmacia) using the Pharmacia AutoRead kit. Plasmid DNA from *E. coli* was obtained by the CTAB minipreparation procedure (11).

**DNA techniques and plasmid constructions.** The procedures for construction, isolation, and analysis of plasmids and for transformation were based on those of Sambrook et al. (52). In order to disrupt the *PDX3* gene, an *Xho*I-*Sac*II DNA fragment containing the *URA3* gene was subcloned into plasmid pAL1, in which the 0.5-kb *Xho*I-*Sac*II fragment (see Fig. 3) was removed. A *ura3* strain was transformed to Ura$^+$ by the linearized construct. The resulting AL2 strain was crossed with a *ura3* strain; the resulting diploid was sporulated, and tetrads were dissected.

**Nucleotide and protein sequence accession numbers.** The nucleotide sequence data reported in this article were deposited in the EMBL, GenBank, and DDBJ nucleotide sequence databases under the accession number X76992 SCPDX3.

TABLE 2. *S. cerevisiae* strains used in this study

| Strain | Genotype or description | Reference |
|---|---|---|
| FL100[a] | *MAT***a** | 26 |
| FL200[a] | *MAT*α | 26 |
| FK*aux30* | *MAT*α *aux30* | 27 |
| 6034 | *MAT*α *aux30 ura3* | This study |
| AL2 | *MAT***a** *ura3 pdx3*::*URA3* | This study |
| AL17 | Diploid obtained by crossing AL2 and 6034 | This study |
| AL18 | *MAT*α/*MAT*a *ura3*/*ura3 PDX3*/*pdx3*::*URA3* | This study |
| AL114 | *MAT*α *aux30 ura3* (pAL1K) | This study |

[a] Wild type.

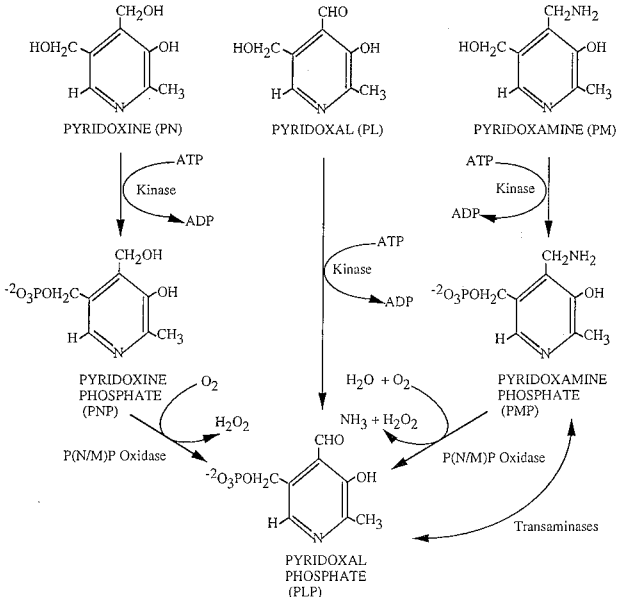


FIG. 1. Biosynthesis of PLP from PN, PM, and PL. This scheme is based on data presented in references 13 and 31. Phosphorylation of PN, PM, and PL is believed to be catalyzed by a single enzyme, PN kinase (EC 2.7.1.35), and P(N/M)P oxidase (EC 1.4.3.5) achieves the last step by oxidizing either PNP or PMP. Amino acid transaminases, or a specific transaminase (56), can also transform PMP into PLP.

## RESULTS

**Nature of the metabolic step affected in *aux30* strains.** Strains containing the recessive *aux30* mutation were obtained as segregants from crosses between FK*erg7* and the wild-type strain. Cells carrying the *aux30* mutation are unable to grow on standard YNB but grow on YPD. Supplementation of YNB with amino acids or nucleic acid bases, either individually or in combination, as well as addition of casein hydrolysate failed to restore the growth of *aux30* strains. In order to further characterize the growth behavior and the physiology of *aux30* mutants, these cells were grown in liquid YPD medium and then transferred into standard YNB as described in Materials and Methods. Growth progressively slowed down and virtually stopped at 24 h. After approximately two doublings of cellular mass, the culture medium developed a strong blue fluorescence mainly due to the accumulation of anthranilic acid (see

TABLE 3. P(N/M)P oxidase specific activity in *S. cerevisiae*

| Strain | Sp act[a] |
|---|---|
| FL100 | 0.26 |
| FK*aux30* | ND |
| 6034[b] | ND |
| AL114[c] | 1.72 |

[a] Nanomoles of PLP-Tris-imine formed per milligram of protein in 1 h. ND, not detectable.
[b] *aux30.*
[c] *aux30* (pAL1K).

TABLE 4. Fatty acid composition of *S. cerevisiae* strains[a]

| Strain | % of total fatty acids[b] | | | | | % Saturated fatty acids |
|---|---|---|---|---|---|---|
| | 14:0 | 16:0 | 16:1 | 18:0 | 18:1 | |
| FL100 | 2.3 ± 0.7 | 21.6 ± 4.0 | 55.3 ± 9.5 | 1.5 ± 0.7 | 19.3 ± 0.7 | 25.4 |
| FL200 | 2.6 ± 0.4 | 22.4 ± 0.7 | 52.9 ± 1.3 | 2.9 ± 0.9 | 19.2 ± 1.8 | 27.9 |
| FK*aux30* | 4.8 ± 0.4 | 35.2 ± 3.2 | 42.7 ± 2.3 | 2.8 ± 0.7 | 15.3 ± 1.6 | 42.8 |
| 6034[c] | | | | | | |
| Without PL | 5.9 ± 2.0 | 34.2 ± 3.7 | 37.5 ± 1.9 | 2.8 ± 0.2 | 19.1 ± 2.8 | 42.9 |
| With PL[d] | 2.0 ± 0.7 | 24.0 ± 2.5 | 51.0 ± 1.5 | 1.2 ± 0.6 | 22.8 ± 3.0 | 26.2 |
| AL114[e] | 1.8 ± 0.5 | 17.2 ± 0.9 | 55.8 ± 1.3 | 2.9 ± 0.2 | 22.3 ± 0.2 | 21.9 |

[a] Grown in YPD to 1 to 3 units of optical density at 700 nm. Fatty acids were extracted and analyzed as described in Materials and Methods.
[b] The data are means for three independent experiments.
[c] *aux30*.
[d] One milligram of PL per liter of YPD.
[e] *aux30* (pAL1K).

Materials and Methods); unidentified fluorescent compounds were also detected in the culture medium, along with indole and α-keto acids, mainly pyruvic and oxalacetic acids. These compounds were not detected in cultures of wild-type cells treated in the same way. The slowing down of the growth of *aux30* mutant strains was evidently accompanied by a perturbation of the synthesis of amino acids, probably at the level of transamination steps.

Since anthranilic acid and indole were also detected in culture supernatants, our rationale focused on tryptophan biosynthesis. Tryptophan synthase activity requires pyridoxal phosphate (PLP). In the absence of its coenzyme, this enzyme splits indole glycerol phosphate into indole and glyceraldehyde phosphate (GAP) (16, 37):

$$\text{indoleglycerolphosphate} + \text{serine} \xrightarrow{\text{PLP}} \text{tryptophan} + \text{GAP}$$

$$\text{indoleglycerolphosphate} \xrightarrow{\text{no PLP}} \text{indole} + \text{GAP}$$

Impairment of tryptophan biosynthesis would lead to a loss of feedback inhibition of anthranilate synthase by tryptophan and to the accumulation of anthranilic acid (15). Since a single mutational event was the cause of this pleiotropic phenotype, we supposed that the *aux30* mutation might in some way affect the cellular pool of PLP.

Growth of *aux30* strains on solid YPD medium was strongly inhibited by $10^{-2}$ M hydrazine or isoniazid, whereas the growth of wild-type strains was little affected by these carbonyl reagents in the same conditions. That *aux30* mutation actually affects the synthesis of PLP was further implied by the fact that *aux30* strains failed to grow on a PN-supplemented synthetic medium but grew normally with PM or PL. It is generally assumed that only one kinase is responsible for the phosphorylation of all the three vitamers and that one oxidase, namely, P(N/M)P oxidase is able to oxidize either PNP or PMP into PLP (Fig. 1) (31). If this scheme also applies to *S. cerevisiae*, the step affected in *aux30* strains might be the oxidase, and assay of P(N/M)P oxidase revealed in fact that this activity was absent, or undetectable, in these strains (Table 3). According to its known catalytic properties (60), P(N/M)P oxidase transforms PMP into PLP, so its absence might have been expected to also prevent use of PM as supplement. However, as indicated in Fig. 1, PLP could be obtained as a by-product of amino acid transaminases (13), or PMP might also be converted into PLP by a specific transaminase, as in clostridia (56).

**Cloning the *PDX3* gene.** Strain 6034 (*MAT*α *aux30 ura3*) was transformed by a yeast genomic library constructed in pFL44L (6). A clone growing on YNB was isolated and was shown to harbor a plasmid (pAL1) carrying a 3.7-kb insert. Reduction of this insert by *Kpn*I to 1.7 kb (plasmid pAL1K) was obtained without change of the phenotype. As shown in Table 3, P(N/M)P oxidase was not detectable in *aux30* strains whereas specific activity in strain AL114 [6034(pAL1K)] was 6.7 times higher than that in wild-type strains. Further study of *aux30* strains harboring plasmid pAL1K showed that transformation of mutants led to the reversal of the pleiotropic traits of the mutated strains.

**Lipid and cytochrome patterns and sterol uptake in *aux30* and *aux30* (pAL1K) cells.** Lipid patterns of FK*aux30* are dif-

TABLE 5. Comparison of sterol and squalene distributions in *S. cerevisiae* strains[a]

| Strain | %[b] | | | | | |
|---|---|---|---|---|---|---|
| | Ergosterol | $\Delta^{5,7}$ Ergostadienol | Zymosterol | Fecosterol | Lanosterol | Squalene |
| FL100 | 58.9 ± 1.2 | 22.2 ± 1.1 | 12.7 ± 0.9 | 2.3 ± 2.3 | 5.6 ± 0.6 | 1.3 ± 1.1 |
| FL200 | 55.7 ± 3.2 | 24.0 ± 1.8 | 12.3 ± 0.4 | Trace | 6.5 ± 2.0 | Trace |
| FK*aux30* | 14.6 ± 0.7 | 42.3 ± 2.6 | 3.9 ± 2.7 | 10.5 ± 0.4 | 28.8 ± 1.9 | 10.7 ± 5.8 |
| 6034[c] | | | | | | |
| Without PL | 26.9 ± 1.4 | 41.4 ± 4.0 | 9.6 ± 0.12 | 6.4 ± 0.4 | 13.5 ± 2.4 | 2.2 ± 0.5 |
| With PL[d] | 50.9 ± 3.4 | 19.2 ± 2.0 | 16.7 ± 2.3 | 4.4 ± 1.3 | 7.4 ± 2.0 | 1.4 ± 1.1 |
| AL114[e] | 49.1 ± 6.3 | 11.6 ± 2.1 | 15.4 ± 0.2 | 3.1 ± 0.7 | 14.4 ± 4.9 | 6.9 ± 4.0 |

[a] Grown in YPD to 2 to 6 units of optical density at 700 nm. Sterols were extracted and analyzed as described in Materials and Methods.
[b] Of total area after integration of gas-liquid chromatography peaks. The data are means for three independent experiments.
[c] *aux30*.
[d] One milligram of PL per liter of YPD.
[e] *aux30* (pAL1K).

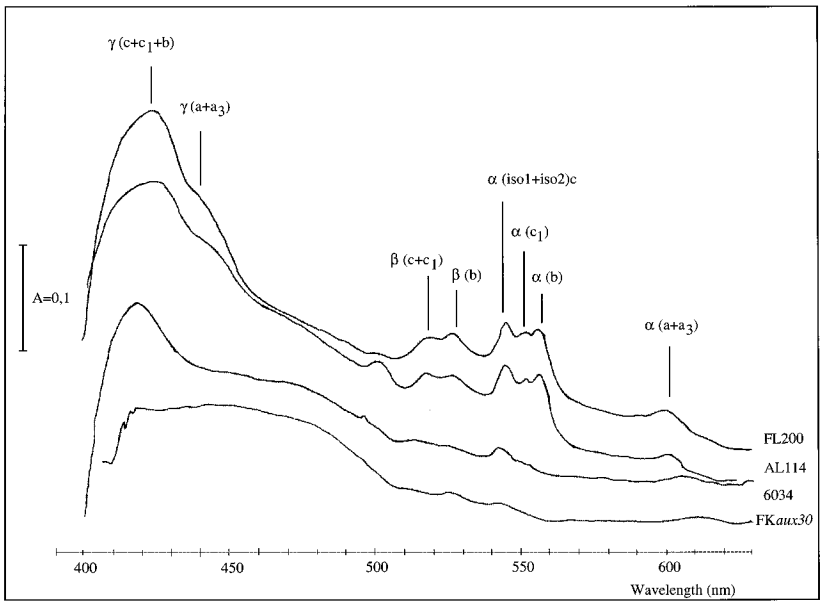


FIG. 2. Low-temperature visible spectra of wild-type and *aux30* yeast cells. Cells were grown for 48 h on YPD medium. Spectra were determined at −196°C by the method of Labbe and Chaix (29). Absorption maxima of α, β, and γ bands of respiratory cytochromes are indicated. An unidentified absorption band ($\lambda_m$ = 502 nm) appeared in AL114 cells.

ferent from those of the wild type with respect to fatty acid and sterol composition. Analysis of tetrads from the cross FK*aux30* × FL100 showed that the modified patterns cosegregate with the other phenotypic traits related to the *aux30* mutation. Fatty acid composition of *aux30* strains is characterized by a high percentage of saturated fatty acids, mostly due to a decrease in $C_{16:1}$ content and an increase in $C_{16:0}$ content (Table 4). Strain AL114, which harbors pAL1K plasmid, displays the wild-type distribution of fatty acids.

Sterol patterns of *aux30* strains also differed from those of the wild type in several ways. Crude $\Delta^{5-7}$ sterol content estimated by UV spectroscopy was 0.71% ± 0.05% (grams of sterol per 100 g [dry weight]) in wild-type strains, but 0.42% ± 0.05% was detected in strains carrying the *aux30* mutation. Analysis of $\Delta^{5-7}$ sterols by HPLC (data not shown) and gas-liquid chromatography (Table 5) showed that the ergosterol/$\Delta^{5-7}$ ergostadienol ratio, which is roughly 2.5 in wild-type cells, is reduced to 0.4 in FK*aux30*; in addition, the *aux30* mutation increased the proportion of lanosterol (Table 5). The modifications in *aux30* strains were reversed by the pAL1K plasmid since the transformed strain AL114 behaved essentially like the wild-type strains.

The various modifications of lipid patterns in *aux30* strains might be the consequences of a low level in heme-dependent enzyme activities. For instance, fatty acid desaturation is carried out by a cytochrome $b_5$-containing enzyme complex (5), lanosterol demethylase is the main cytochrome P450 in *S. cerevisiae* (25), and $\Delta^{22-23}$ desaturation of the side chain of ergosterol is also believed to be effected by a heme-dependent enzyme (22, 23). Furthermore, according to the triphenyl tetrazolium chloride overlay method, it appeared that *aux30* cells have a low respiration rate compared with wild-type cells or strain AL114 (*aux30* [pAL1K]). Figure 2 shows that the *aux30* cells have a low content of cytochrome restored to the wild-type level by pAL1K. Cholesterol uptake experiments (Table 6) showed that *aux30* mutants accumulated 10 times more cholesterol than wild-type strains and that plasmid pAL1K restored wild-type uptake. Analogous experiments with the *aux30* strains grown in reconstituted YNB containing either PL or PM gave the same results as the wild-type strain: no signif-

TABLE 6. Comparison of the rates of [$^{14}$C]cholesterol uptake in *S. cerevisiae* strains

| Strain | [$^{14}$C]cholesterol incorporation (μg/mg [dry wt])[a] |
|---|---|
| FL100 | 0.28 ± 0.09 |
| FL200 | 0.32 ± 0.05 |
| 6034[b] | |
| Without supplements | 3.35 ± 0.03 |
| With PL[c] | 0.39 ± 0.03 |
| With δ-ALA[d] | 0.70 ± 0.13 |
| AL114[e] | 0.36 ± 0.03 |

[a] The data are means ± standard deviations for three independent experiments.
[b] *aux30.*
[c] One milligram of PL per liter of YPD.
[d] One hundred milligrams of δ-ALA per liter of culture medium.
[e] *aux30* (pAL1K).

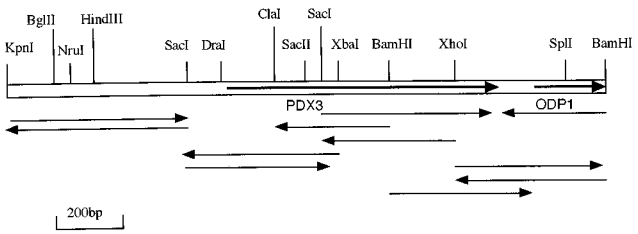


FIG. 3. Restriction map of the 1.7-kbp insert of plasmid pAL1K and the sequencing strategy for the cloned DNA fragments. The locations and orientations of the *S. cerevisiae* P(N/M)P oxidase gene *PDX3* and the *ODP1* gene are indicated by thick arrows. The thin arrows indicate the directions of fragment sequencing.

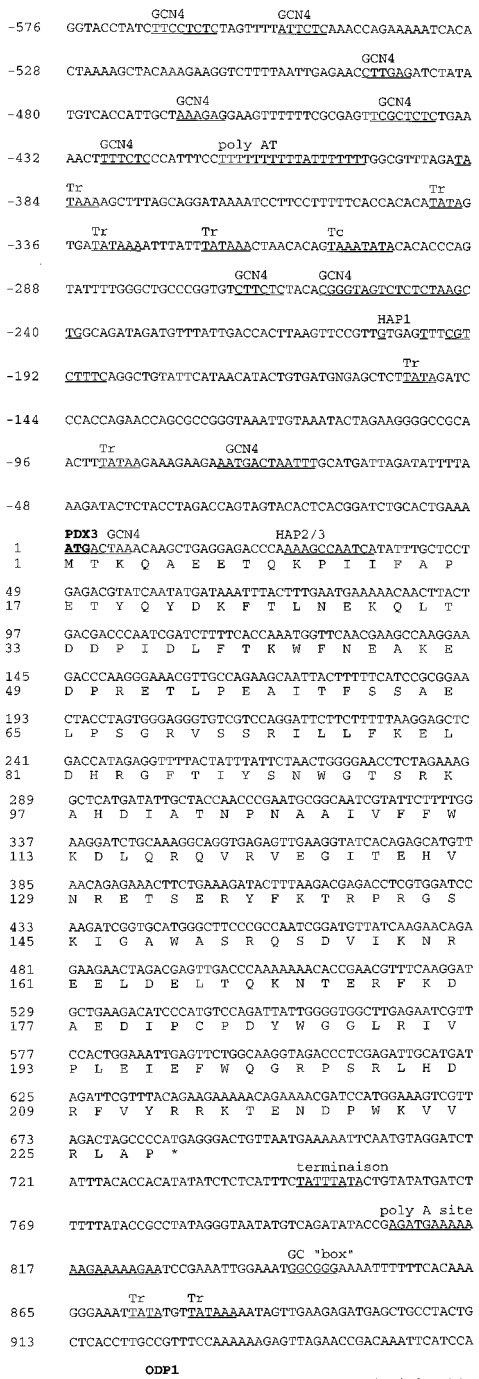


FIG. 4. Nucleotide sequence of the *S. cerevisiae* P(N/M)P oxidase gene *PDX3* and the unknown-function *ODP1* gene with their respective deduced amino acid sequences. Tr, regulative TATA box; Tc, constitutive TATA box. Other typical promoter sequences in this sequence are the GC box for transcription, putative Gcn4p binding sites (4) involved in the general control of nitrogen metabolism, putative Hap1p and Hap2/3p (47) binding sites controlled by oxygen and heme, and poly(A · T) as a constitutive promoter. Terminaison, 3′-end-forming signal. The potential initiation methionine codon (ATG) in the *PDX3* sequence (AAAATGACTAAA) is flanked by nucleotides that satisfy the consensus sequences (A/Y)A(A/T)AATGTCT (10), (A/C)A(A/C)AATGTCT (20), and AxATGxxT (14), reported for optimizing initiation in *S. cerevisiae*. The beginning of a second reading frame, *ODP1*, was found downstream from *PDX3*; the partial encoded protein displays a high percentage of homology with identified sequences from *Caenorhabditis elegans* CE13E12 (62) and M80085 (39).

icant accumulation of exogenous cholesterol (data not shown). In YPD medium, sterol uptake abnormality and the altered sterol and fatty acid patterns of *aux30* strains all appear to be merely consequences of a limitation in PLP, with addition of PL (1 mg/liter) resulting in reversal of the mutant properties (Tables 4 to 6).

Addition of δ-aminolevulinic acid (δ-ALA) to *hem1* mutants, deficient in δ-ALA synthesis, complements the enzymatic defect and reverses the $Hem^-$ phenotype (36, 54). Also, sterol uptake by *hem1* cells is reduced to a wild-type level in the presence of δ-ALA (54). However, addition of δ-ALA to cultures of *aux30* strains only repressed cholesterol accumulation by 80% (Table 6). This residual accumulation appeared to be significant, since *aux30* strains which are resistant to fenpropimorph, a sterol biosynthesis inhibitor, in the presence of ergosterol (since sterols are taken up [38]), were sensitive to the inhibitor when transformed with plasmid pAL1K but remained resistant when the growth medium was supplemented with 100 μg of δ-ALA per ml (data not shown). These observations evidenced that transformation of *aux30* with a functional *PDX3* gene totally reverses the mutant phenotype to the wild type but that feeding δ-ALA is not sufficient to completely suppress the uptake properties. Therefore, heme deficiency is not sufficient to explain the sterol uptake properties of *aux30* mutants; unknown metabolic modifications, unlinked to δ-ALA and heme synthesis, must therefore contribute to this property.

**Characterization of *PDX3* disruption.** *PDX3* was disrupted as described in Materials and Methods. Strain AL2 (*pdx3*::*URA3*) behaved as *aux30* strains on YNB supplemented with different B6 vitamers. Analysis of tetrads from AL18 showed that the Aux30 phenotype segregated 2:2. Southern blot analysis of AL2 and of tetrads from AL18 showed that the phenotype associated with the *aux30* mutation was linked to the *URA3* marker. AL2 (*pdx3*::*URA3*) was crossed with 6034 (*aux30 ura3*); the resulting AL17 diploid displayed the phenotype of *aux30* strains. These results demonstrate that *aux30* is allelic to *PDX3*.

**DNA sequence and analysis of *PDX3*.** We determined the DNA sequence of the insert carried by pAL1K on both DNA strands by the strategy described in Materials and Methods (Fig. 3). DNA sequence analysis delineated the genetic organization of *PDX3* and adjacent loci. The insert contains a 684-bp open reading frame (Fig. 4) that encodes a potential 228-amino-acid polypeptide; this protein was identified as the P(M/N)P oxidase on the basis of the physiological, biochemical, and genetic criteria. The calculated $M_r$ of the resulting polypeptide chain is 26,762, which is close to the value (27,000) reported for a subunit of P(N/M)P oxidase from *S. cerevisiae* (60). FASTA and TFASTA searches in the EMBL library (34) revealed a remarkable homology between *S. cerevisiae PDX3*, *E. coli pdxH* (31), and *Myxococcus xanthus fprA* (19) gene products. Identities are about 41.6% between Pdx3p and PdxHp, 39.5% between Pdx3p and FprAp, and 42.7% between PdxHp and FprAp, over the entire lengths of encoded polypeptides.

## DISCUSSION

The pleiotropic phenotype of *aux30* strains is likely to be the consequence of the shortage in PLP and of its effects on the

numerous reactions depending on this coenzyme. These mutants cannot grow in synthetic minimal medium unless PM or PL, but not PN, satisfies the demand for vitamin B6. This phenotype is similar to that of *pdxH* strains of *E. coli* deficient in P(N/M)P oxidase (12, 13, 30). Yeast cells harboring the *aux30* mutation cannot synthesize certain amino acids and excrete intermediates. All the observed metabolic effects of the *aux30* mutation are reversed after transformation with plasmid pAL1K harboring the *PDX3* gene. Similarly, *aux30* strains behave as wild-type strains when grown in presence of PL or PM in minimal medium. Another secondary mutation required for sterol uptake, the *aux32*(Ts) mutation in strain FK*erg9* is known to cause a temperature-sensitive glycine auxotrophy (27). The phenotype was found to be reversed by PM and PL (but not PN), and *aux32*(Ts)/*aux30* diploids remained *aux32*(Ts) (data not shown). Thus, *aux32*(Ts) and *aux30* likely affect the same gene.

The phenotypic traits of *aux30* strains in relation with lipids are shared with yeast strains starved in PN and yeast leaky *hem* mutants. A large decrease in palmitoleic acid content was observed in *Hanseniaspora valbiensis* grown in a vitamin B6-deficient medium (21). Vitamin B6 deficiency induced by thiamine in *Saccharomyces uvarum* 4228 led to a large modification of the lipid pattern, including a dramatic reduction in unsaturated fatty acid and sterol contents (41, 44, 45). More precisely, the modification of the sterol pattern was characterized by a decrease in ergosterol associated with a large increase in squalene, lanosterol, and $\Delta^{5-7}$ ergostadienol. Thiamine-induced inhibition of PLP synthesis also provoked a dramatic decrease in heme content in *S. uvarum* 4228 (42). All these changes were also found in *S. cerevisiae* bearing the *aux30* mutation. The heme dependency of fatty acid desaturase (5), 14-sterol demethylase (2), and $\Delta^{22}$ sterol desaturase (22, 23) can thus account for the similar lipid patterns in PN-starved yeast cells and *aux30* mutants as well.

Sterol uptake by *aux30* strains was found to be an unstable property. While strains bearing the *aux30* mutation display a low respiration rate, metabolically suppressed cells displaying a high respiration rate may appear and outnumber the former cells after a small number of subcultures. The suppressed cells always show the auxotrophy for PM or PL, characteristic of *aux30* strains, but display a wild-type phenotype with respect to lipid patterns and sterol uptake. This genetic instability of *aux30* strains is radically different from the stability of FK*erg7*. This difference can be understood because a low heme content is a prerequisite for sterol uptake and viability of FK*erg7*. This combination of mutations is well documented for *erg7* mutants harboring additional *hem* mutations (18, 32, 35).

Several sequences found upstream of ATG may indicate that transcription of *PDX3* is subjected to regulation. No fewer than 10 putative Gcn4p binding sites (24, 55) can be found in the promoter sequence (Fig. 4). The exact metabolic role of these sequences in relation to PLP biosynthesis remains to be elucidated. However, it can be assumed that the demand for PLP is stronger when amino acid biosynthesis increases. Thus, a Gcn4p-mediated activation of *PDX3* transcription reasonably fits the general control of amino acid synthesis (17).

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