Cloning by Metabolic Interference in Yeast and Enzymatic Characterization of Arabidopsis thaliana Sterol Δ7-Reductase*

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Eric Lecain‡§, Xavier Chenivesse§§, Roberto Spagnoli, and Denis Pompon**

From the iCentre de Génetique Moléculaire du CNRS, Laboratoire propre associé à l’Université Pierre et Marie Curie, F 91198 Gif-sur-Yvette cedex, l’Unité de Recherches Biotechnologiques, Biotechnology Department, Roussel-Uclaf, 102 route de Noisy, F 93235 Romainville cedex, France.

Reduction of the Δ7 double bond of sterols, a key biosynthetic step in higher eukaryotes, is lacking in lower eukaryotes like the yeast Saccharomyces cerevisiae, leading to terminal sterols with a Δ5,7-conjugated diene structure. Genes encoding two reductase isoforms involved, respectively, in the reduction of sterol Δ14 and Δ24(28) double bonds have been cloned to date, but no sequence information was available on the enzyme responsible for Δ7-bond reduction. This study presents the cloning of the NADPH-sterol Δ7-reductase (Δ7-red) from Arabidopsis thaliana, based on a metabolic interference approach in yeast. The principle is the functional expression of a plant cDNA library in the yeast strain FY1679-28C tolerant to sterol modifications and the selection of clones resistant to the polyene fungicide nystatin. The toxicity of this compound is dependent on the presence of Δ5,7-unaturated sterols in the yeast plasma membrane. One clone out of 10^5 transformants exhibits a cDNA-dependent alteration of cell sterol composition. The 1290-base pair cDNA open reading frame was isolated and sequenced. The corresponding protein presents a significant sequence similarity with yeast Δ14- and Δ24(28)-reductases and with human lamin B receptor. The coding sequence was extracted by polymerase chain reaction and inserted into a galactose-inducible yeast expression vector to optimize expression. Analysis using transformed wild type yeast or sterol altered mutants, indicated that Δ5,7-ergosta- and cholesterol-sterols are efficiently reduced in vivo, regardless of the structural variations on the side chain. No reductase activity was observed toward the Δ14 or the Δ5 positions of sterols. In vivo extensive Δ7-reduction of the free and esterified pools of sterols was observed upon induction of the enzyme. Ergosterol present before induction was reduced into ergosta-5,22-dieneol, whereas ergosta-5-red appeared to be a puzzling task due to the total lack of sequence information as well as of purified protein or related antibodies.

Sterols are major components of eukaryotic cell membranes. End products of the sterol biosynthetic pathway differ depending on species; cholesterol is encountered in animals, ergosterol is the most common sterol in fungi, while sitosterol, campesterol, and stigmasterol are typical plant sterols (1). Most of terminal sterols feature a similar four-ring structure including a C-5–C-6 unsaturation on ring B and a more or less branched side chain. Additional features depend on the origin; cholesterol has a C_9 side chain, whereas plant sterols bear an additional alkyl (methyl or ethyl) group on C-24. Ergosterol, in addition to a C-24 methyl group, contains two additional unsaturations on the C-7-C-8 bond of the B ring and on the C-22-C-23 bond of the side chain. These unsaturations are specific to fungal sterols and make them the target for antifungal polyene drugs like nystatin (2).

The enzymatic steps that allow conversion of lanosterol resulting from the oxidative cyclization of squalene to cholesterol have been documented (3–7). Most of steps are also found in Saccharomyces cerevisiae ergosterol biosynthesis (8, 9). Few of the involved mammalian genes like the P450 lanosterol demethylase have been isolated (10, 11). From zymosterol (cholesta-8,24-dieno) to cholesterol (cholesta-5-enol) or to ergosterol (ergosta-5,7,22-trienol), there are multiple alternative pathways leading to the terminal sterol. In mammals, the main pathway involves the following sequence: isomerization of the double bond from C-8 to C-7, introduction of a double bond at C-5, and two reduction steps at the C-24 and C-7 double bonds. These reduction steps are absent in yeast but are catalyzed by the sterol Δ24-reductase and the Δ7-red, respectively, in higher eukaryotes. Very little is known about these two reactions excepted that they can be reproduced in vitro using a reconstituted system involving partially purified fractions from rat liver (12, 13) or plant (14) microsomes. Indeed, inhibitors of the Δ7-red have been tested as therapeutic drugs against hypercholesterolemia (15), and it was recently reported that a recessive autosomal disorder, the Smith–Lemli–Opitz syndrome responsible for multiple congenital anomalies, corresponds to a deficient activity of this enzyme (16). Lipoproteins of affected patients are enriched in Δ7-cholesterol (cholesta-Δ5,7-dienol) and are highly depleted in cholesterol (17). Despite its interest, identifcation of the gene encoding the Δ7-red appeared to be a puzzling task due to the total lack of sequence information as well as of purified protein or related antibodies.

Here we present the cloning of the Arabidopsis thaliana Δ7-red based on the observation that nystatin toxicity is highly dependent on the presence of sterol carrying a Δ5,7-dienic structure. Selection for functional expression in yeast of a plant...
cDNA encoding \( \Delta 7 \)-red activity was based on the expectation that reduction of the \( \Delta 5,7 \)-endogenous yeast sterols would decrease nystatin toxicity. Nevertheless, the possibility to substitute ergosterol by its reduction product was a major concern since disruption of ergosterol biosynthesis is the classical mode of action of antifungal drugs like ketoconazole. In fact, the bulk (structural) function of the sterols can be fulfilled in yeast by some different sterols such as cholestanol, cholesterol, lanosterol, or intermediates of sterol biosynthesis pathway, provided that some residual level of ergosterol be present (18, 19). This is known as the "sparking effect," which is probably related to a cell cycle control mechanism in wild type strains (20). This requirement is abolished in cells harboring fen1 and/or fen2 gene mutations (21, 22) suggesting to use strain FY1679-28C, a naturally occurring fen1 mutant, as a host for the \( \Delta 7 \)-red screening. We also chose a plant, A. thaliana as cDNA source because of the high \( \Delta 7 \)-red activity present in plant microsomes and its low relative genome complexity (23, 24).

**EXPERIMENTAL PROCEDURES**

**Yeast Strains—** FY1679-28C yeast strain (MAT a, ura3–52, trp1Δ63, leu2Δ13, his3Δ200, GAL2) is a derivative of S288C constructed by Thierry Thierry (25). The pl05 strain was obtained from Dr. F. Karst, which is deficient in \( \Delta 7 \)-desaturase, and was isolated by Molyneux and Woods (26) as a polyene-resistant mutant. For construction of PLC1051 (MAT \( a \), erg5, ura3–52, his3Δ200, trp1Δ63, GAL2), the po5 strain was crossed with FY1679 (MAT a) and the resulting diploid cells were sporulated. Spore purification by hydrophobic binding to polypropylene tubing was performed as described (27). The PLC1051 haploid strain was selected after germination as an ura + , his + , trp + , leu + , erg5 clone (accumulation of ergosta-5,7-dien-4-one detected by gas chromatography on a SE 30 capillary column) able to grow on galactose (GAL2 phenotype) and to use glycerol as carbon source (respiration competency). The PLC1051 (MAT \( a \), erg5, ura3–52, his3Δ200, trp1Δ63, GAL2, rhr + ), a double sterol mutant carrying deficiencies in the sterol \( \Delta 22 \)-desaturase and in the sterol \( \Delta 7 \)(28)-reductase genes, was constructed as follows: a spontaneous nystatin-resistant mutant (5 \( \mu \)g/ml nystatin) of the po5 strain which accumulates ergosta-5,7,24(28)-trieneol instead of ergosta-5,7-dien-4-one was isolated by direct screening for alteration in the sterol composition among nystatin-resistant clones. This latter strain was crossed with FY1679-28C, and the diploid was sporulated as described previously. After spore purification and germination, the haploid strain PLC1051 was isolated. This strain accumulates ergosta-5,7,24(28)-trieneol, is auxotrophic for uracil, tryptophan and leucine and can grow in presence of galactose or glycerol as carbon source. PLC1061 (MAT \( a \), erg6, ura3–52, his3Δ200, GAL2) was isolated by sporulation of the diploid formed after crossing po5 with FY1679-28C.

**Media—** Synthetic media are SGI (7 g/liter yeast nitrogen base (YNB)) supplemented with 2% glucose (Difco). The complete medium without carbon source is named YP (2% Bacto-agar (Difco)). Transformation yielded 10⁵ primary transformants per \( \mu \)g of DNA and 5 × 10⁶ cells per plate containing SGI solid medium and both or 2 or 5 \( \mu \)g/ml nystatin. After 3 days of incubation at 28°C, a hundred clones were growing in the presence of 2 \( \mu \)g/ml nystatin. Individual clones were finally analyzed for their sterol composition by HPLC. Among the mutant strain F22 was resistant to 5 \( \mu \)g/ml galactose and exhibited a sterol composition with a lowered \( \Delta 5,7 \) content based on the 280 nm HPLC traces. E. coli was transformed by the plasmidic DNA extracted from clone F22 as described previously (31). The plasmidic DNA from individual E. coli transformants was digested by NotI. Two different classes of cDNA inserts were identified which correspond to 600-bp and 1.6-kb NotI inserts respectively. The plasmid carrying the 1.6-kb DNA insert was found to be also rearranged at the pFL61 level as judged by the altered restriction pattern. The FY1679-28C yeast strain was retransformed with this plasmid and the sterol composition of transformants analyzed. All transformants exhibit the same anomalous sterol pattern as compared with the void pFL61 transformed strain.

**Nucleotide Sequence Determination—** The NotI cDNA insert in pFL61 was extracted and subcloned into the unique NotI site of pUC9-N. The nucleotide sequence was determined using the Sequenase kit (U. S. Biochemical Corp.), the direct and reverse primers of pUC9 and of plBlueScript (T3 and T7 primers) and specific oligonucleotide sequences corresponding to the sequenced gene. After completing the full sequence of one strand, the complementary strand was sequenced using a series of specific oligonucleotides as primers.

**Reformatting and Cloning \( \Delta 7 \)-red cDNA into Expression Vector** pYEpD1/8-2—Deletion of the 5′- and 3′-non-coding regions of the \( \Delta 7 \)-Red cDNA was performed by PCR amplification using specific primers designed to introduce a BamH I restriction site immediately upstream of the initiation codon and a Kpn I site immediately downstream of the stop codon.

**Direct primer:** 5′-ccggcagatccATGGCGGAGACTGTACATTC-3′

**Reverse primer:** 5′-cagggtaccTCAATAAATTTCCCGGAAATG-3′

Sequences identical or complementary to the cDNA are shown as upper-case, and restriction sites are underlined. The \( \Delta 7 \)-red cDNA was amplified using 33 thermal cycles with 2 units of DNA polymerase (Stratagene) in the presence of 10 pmol of each primer and 0.2 μl of each dNTP, in the recommended buffer. The temperature cycles were 10 s at 94°C, 50 s at 52°C, followed by 1 min 30 s at 74°C. The 1300-bp PCR product was BamH I and/or Kpn I digested and inserted between the BamH I and Kpn I sites of pYEpD1/8-2, resulting in plasmid \( \Delta 7 \)-red/V8. The integrity of the PCR-amplified fragment was confirmed by sequencing.

**Integration of Reformatted \( \Delta 7 \)-Red cDNA into the ADE2 Locus of FY1679-28C Strain—** The \( \Delta 7 \)-red cDNA fragment of the yeast ADE2 gene was cloned in the BamH I site of pBS-ADE2. Primers: 5′-agaatt- TGAGGAGATCCGGCCAGAAAAAC-3′ (hybridizing the 3′-ends of URA3) 5′-GATACCGCCAAGCTTTTCCGAAC-3′ (hybridizing the 3′-end of the PGK terminator) were designed to amplify the full \( \Delta 7 \)-Red/V8 expression cassette including the GAL10-5′ region and the PGK transcription terminator: 80 ng of \( \Delta 7 \)-red/V8 template, 0.5 μM phosphorylated primers, 0.2 μl of each dNTP diluted in the commercial buffer were first denatured 1 min at 95°C, after which 1 unit of native Pfu DNA polymerase was added and the reaction mixture cycled for 35-fold using 5 s at 95°C, 30 s at 56°C, and 4 min 30 s at 70°C. The amplified 2440-bp fragment was then purified and subcloned into the unique HindIII site of pBS- ADE2, giving pADJ7. The 4720-bp NotI-PstI fragment of pADJ7 containing the disrupted ADE2 sequence was isolated and used to transform strain FY1679-28C according to previously described methods (30). The resulting yeast strain was called ELR01.

**ERL01**—**Biochemical Analysis—** Genomic DNAs from A. thaliana, yeast, and different transgenic strains were isolated in our laboratory using standard methods. DNA fragments were separated by electrophoresis on a 0.8% agarose gel. DNA was transferred onto nitrocellulose filters (BA85; Schleicher & Schuell). The open reading frame of the \( \Delta 7 \)-red cDNA was isolated from \( \Delta 7 \)-red/V8 on agarose gel and purified with Jetsorb extraction kit (Bio- probe). The hybridization of the 32P labeled probe was performed at 42°C for 3 days using standard procedures except that formamide concentration was reduced from 50 to 30% (by volume) to reduce stringency. Filters were washed using as final conditions 0.1 × SSC, 0.1% SDS, and 37°C before exposition in a PhosphorImager.
Sterol Δ7-Reductase Cloning and Properties

RESULTS

Isolation of the cDNA Encoding Δ7-Red Activity—Effects of polycy an antifungal like nystatin, amphotericin B, or filipin are well separated on the column, and their retention times are quite different from the endogenous yeast sterols.

Nucleotide Sequence of the A. thaliana Δ7-Red—The cDNA in pf22 was extracted and subcloned before sequencing on both strands. The deduced cDNA (Fig. 1) without its poly(A) tail is 1488 bp long and contains an open reading frame of 1290 bp, which is coding for a protein of 430 amino acids with a calculated molecular mass of 49,458 Da. The S'- and the 3'-untranslated regions are 76 and 121 bp long, respectively. A possible consensus polyadenylation signal is located 34 bp upstream of the poly(A) tail. A computer search on a sequence data base reveals that the deduced amino acid sequence of the Δ7-red exhibits a significant similarity with the ones of Δ14- and Δ24(28)-sterol reductases (Fig. 2), suggesting that all known sterol reductases belong to a single sequence family. These reductases are the S. cerevisiae sterol C-14-reductase (34), the Neurospora crassa sterol C-14-reductase (accession no. X77955 in EMBL data base), the YGL022 open reading frame later identified as the S. cerevisiae sterol Δ24(28)-reductase (35), and the Schizosaccharomyces pombe SST1 gene product (27). The latter protein is likely to be a sterol Δ24(28)-reductase too (36). In addition, the Δ7-red shows a striking similarity with the 400 C-terminal amino acids of the lamin B receptors from chicken and human as already evidenced for other sterol reductases (37-39). The N-terminal end of these two proteins contains a typical DNA binding domain (40), absent in all identified sterol reductases including the Δ7-red one.

Consensus sequences involved in the binding sites of NADPH or NADH and/or flavin have been described in different reductases family like P450 reductases or nitrate reductases (41). However, it was not possible to identify similar motif in the sterol reductase protein family, even with the newly included sequence. In addition detailed sequence comparison between Δ7-, Δ14-, and Δ24(28)/sterol reductases (eight sequences) did not allow identification of a clear sequence signature corresponding to the different regio-specificities for sterol reduction (Fig. 2). Globally, sequence conservation within the
family is high in the C-terminal half of the enzymes, with a
dear LLXSGWWGXXR signature almost perfect in all mem-
bers. In contrast, a more limited sequence similarity is present
on the N-terminal half. Particularly the EFGGXX common to
D24(28), D14, and lamin receptor is not present in
D7-red. Interestingly enough, the hydrophobic profiles remain
very similar among all family members even within the N-
terminal half (starting residue 440 for lamin B receptors). The
lamin B receptor sequence cannot be distinguished from that of
other family members either on sequence or on hydrophobic
profile criteria.

Southern Blot Analysis of D7-Red Locus—A. thaliana
genomic DNA was probed with the open reading frame of
D7-red cDNA (Fig. 3). Based on PstI digestion (absent site from
the cDNA) yielding a single hybridizing band in low stringency
conditions, the presence of a single D7-red gene can be deduced.
Absence of overlapping bands was confirmed by double digestion
with BamHI. Cleavage by BamHI alone (two bands: c and
c1) or in combination with PstI (three fragments: a, a1, and a2)
are indicative of the presence of at least one intron in the gene.

PCR applied to genomic DNA using a primer situated at both
extremities of the open reading frame led to amplification of a
single 3.6-kb fragment confirming the presence of a total of 2.5
kb of intronic sequences within the open reading frame of a
unique gene. Digestion with PvuII, which cuts once within the
cDNA, gave rise as expected to two hybridizing bands (e1 and
e). In a second experiment, genomic DNAs of different origins
(human, quail, Drosophila melanogaster, Xenopus laevis,
maize, and yeast) were tested (Fig. 3B). EcoRI-restricted DNA
from parental yeast FY1679-28C exhibits three weak bands at
4.2, 2.5, and 2.3 kb upon low stringency hybridization, which
could correspond to endogenous D14 and D24(28)-reductase
genes. As a control, strain ELR01 (see later), which contains an
expression cassette for A. thaliana D7-red integrated within
the yeast genome, was tested. The strong hybridization signals
corresponding to the two expected EcoRI fragments were ob-
served in addition to two weak signals also found with the
parental strain. Interestingly enough, a well defined hybridiza-
tion signal was found with quail DNA. This hybridization signal
was absent under high stringency conditions, suggesting de-
tectable but limited interspecies sequence conservation. Weak but defined signals were also found with maize, but not with human, X.laevis, and D.melanogaster DNAs, thus illustrating the limits of interspecies cross-hybridization approaches.

Overexpression of D7-Red and Time Course of in Vivo Sterol Conversion—To optimize expression, the cDNA encoding the D7-red was reformatted and cloned into pYeDP1/8-2, placing the flanking sequence-free open reading frame under the transcriptional control of a galactose-inducible GAL10-CYC1 promoter. FY1679-28C cells were transformed by the resulting pV8/D7red. Cells were first grown on glucose-repressed conditions in which the plasmid-borne D7-red composite gene is silent. Following the transfer in inducing culture conditions, the time course of the changes in the yeast cell sterol composition was followed (Fig. 4). As expected, during growth in glucose or in ethanol, the main sterol is ergosterol and ergosta-5,22-dieneol and 5-eneol are hardly (ethanol) or not at all (glucose) detectable. Following the addition of galactose, ergosterol content rapidly decreases with a concomitant increase in ergosta-5,22-dieneol content. A very limited formation of ergosta-5-eneol occurs during the first 2 h following induction. This compound nevertheless slowly accumulates with increasing induction times (up to 9 h), while ergosta-5,22-dieneol content remains constant and then slowly decreases. At the end of the culture, ergosterol accounts for 5% (w/w) of the total sterols, ergosta-5-eneol for 45% (w/w), and ergosta-5,22-dieneol for 50% (w/w). The proportion of the other sterol intermediates could be estimated to less than 10% of the total sterol based on GC analysis. This indicates that in vivo accumulation of ergosta-5,22-dieneol results from the direct reduction of previously accumulated ergosterol. In contrast, accumulation of ergosta-5-eneol requires de novo sterol biosynthesis, and likely results from reduction of the biosynthetic intermediate ergosta-5,7-dieneol (Fig. 5). The decrease in ergosta-5,22-dieneol content during the late induction phase suggests that ergosta-5-eneol is no longer a good substrate for the yeast D22 desaturase enzyme.

In Vivo Analysis of D7-Red Substrate Specificity—To investigate in more detail substrate specificity of the D7-red, mutant strains PLC 1051, 1451, and 1061, which accumulate sterol biosynthesis intermediates were transformed with pV8/D7red. Main sterols accumulated by yeast mutants expressing or not the D7-red activity are listed in Table I. In the D22-desaturase-deficient strain PLC1051, D7-red expression results mainly in...
accumulation of ergosta-5-eneol confirming that ergosta-5,7-dieneol is a good substrate for the enzyme in vivo as predicted from the late accumulation of ergosta-5-eneol in a similar experiment with the wild-type parental strain. Similar experiment with mutant PLC1451, which also lacks the sterol \( \Delta 7 \)-reductase, led to accumulation of ergosta-5,24(28)-dieneol (ostreasterol), the expected reduction product. In PLC1051 and 1451, the expression of the \( \Delta 7 \)-red thus changes the end product synthesized but does not cause accumulation of biosynthesis intermediates. In the erg6 mutant PLC1061, the sterol C-24 5-adenosyl methyl transferase, which converts cholesterol-8,24-dieneol (zymosterol) into ergosta-8,24(28)-dieneol (fucosterol), is deficient, thus leading to zymosterol accumulation and to a lesser extent to cholesterol-5,7,24-trieneol and cholesterol-5,7,22,24-tetraeneol accumulations. Expression of the \( \Delta 7 \)-red in this strain caused the reduction of the \( \Delta 7 \) double bond of cholesterol-derivatives, yielding cholesterol-5,22,24-trieneol and cholesterol-5,24,24-dieneol but zymosterol, which bears a double bond in C-8, remained unaffected, suggesting a high specificity of \( \Delta 7 \)-red for the C-7 position. \( \Delta 7 \)-Red can thus accept in vivo a very large range of ergosta- and cholesta-compounds carrying a \( \Delta 5,7 \)-dieneol structure.

In Vitro Analysis of \( \Delta 7 \)-Red Enzymatic Properties—The subcellular location of the \( \Delta 7 \)-red was analyzed using the cholesterol-5,7-dieneol as substrate since cholesterol, the expected reduction product, is absent from yeast and well resolved from endogenous sterols. GC profiles of sterols were examined after incubation of cholesterol-5,7-dieneol and NADP with microsomal fractions from the FY1679-28C strain expressing \( \Delta 7 \)-red. Two peaks corresponding to the residual substrate (cholesta-5,7-dieneol) and to the cholesterol formed are present and well separated from endogenous sterols. The cholesterol peak was found to be absent when either cholesterol-5,7-dieneol or NADPH were omitted or when microsomes from a yeast transformed with a void plasmid were used. In addition, a negative result was also obtained when NADH was substituted to NADPH. In our hands, the microsomal fractions exhibits the highest specific activity (versus protein content) but some activity was also found in lipid droplets and cytosol, suggesting a rather diffuse subcellular location of the enzyme. The activity of \( \Delta 7 \)-red toward sterol esters was tested using sterol acetate as a model. 7-Dehydrocholesterol acetate and ergosterol acetate (200 \( \mu \)M) were incubated with cytosolic or microsomal fractions from
PLC1051 strain transformed with V8/Δ7-red. Both esters were found to be efficiently reduced at C-7 upon incubation with the cytosolic fractions. 7-Dehydrocholesterol ester was clearly a better substrate than the ergosterol ester (45% versus 15% of conversion). Similar experiments with microsomal fractions led surprisingly to the fast hydrolysis of steryl acetate by some endogenous esterase, a reaction that was absent when cytosolic fractions were used. This indicates that a free hydroxyl group at the C-3 position is not required for activity of Δ7-red and that fatty acid steryl esters might be physiological substrates of this enzyme.

Genome Integration of the Δ7-Red Expression Cassette and Effects on Cell Viability—Genomic integration of the Δ7-red expression cassette opened the way to add additional heterologous activities in engineered cells and allowed us a better analysis of the physiological effects of Δ7 reduction of sterols. The cassette containing the GAL10-CYC1 promoter, the Δ7-red open reading frame, and the PGK terminator was extracted from pV8/Δ7-red by PCR, and the resulting fragment was inserted within a plasmid containing the yeast ADE2 gene (see “Experimental Procedures”). FY1679-28C cells were transformed by the interrupted ADE2 sequence, and homologous recombination events were selected on the basis of the generation of ade2 clones. PCR and Southern blotting analysis (Fig. 3B) of clone ELR01 confirmed that the full Δ7-red expression cassette had been integrated within the ADE2 genomic locus. Galactose induction of the Δ7-red expression induced a dramatic change in sterol composition as was observed with the pV8/Δ7-red transformed strain, except for an even lower level of residual ergosterol. A single integrated copy of the expression cassette is thus sufficient to completely reduce in vivo Δ7-sterols. The ELR01 doubling time and the final cell density at saturation were found to be similar to those of the parental strain under similar culture conditions. Cell viability was analyzed and indicated in both cases that more than 90% of cells in exponential growth phase were viable. Together, these results indicate that Δ7-reduced sterols support yeast growth and cell viability as well as unsaturated sterols under tested conditions.

**DISCUSSION**

The quantitative and qualitative sterol compositions and the presence of double bonds or branched groups are known to depend on others factors, including phospholipid composition and presence of a suitable membrane potential (46). The particularly high level of resistance of the erg2 mutant (deficient in sterol Δ8-Δ7 isomerase) confirms that the presence of Δ5,7-conjugated double bond is determining. Consistently, FY1679-28C expressing Δ7-red is highly nystatin-resistant (up to 80 μg/ml compared with 2 μg/ml for parental FY1679). This resistance correlates with accumulation of almost 95% of C-7-saturated sterols. Upon induction, Δ7-red rapidly converts the whole ergosterol pool, including steryl esters to the corresponding reduction products. This observation supports the finding that esterified sterols could be in vivo substrates for the enzyme. The low content of free sterols within the microsomal membranes in comparison to the value into the plasma membrane is generally explained by a vectorial transport process involving the synthesis of steryl esters in microsomes and their hydrolysis by a specific lipase at the plasma membrane (47). Steryl esters stored into the lipid droplets and the free sterols within the plasma membrane might rapidly interconvert thus explaining reduction of the whole ergosterol pool by Δ7-red.

Ergosta-5,22-dienoic and ergosta-5,7-dienoic, which are, respectively, the reduction products of ergosterol and ergosta-5,7-dienoic, accumulate in the presence of the Δ7-red in transformed FY1679-28C. Ergosta-5-, ergosta-5,24(28)-, or ergosta-5,22,24(28)-enols and cholesta-5,24- or cholesta-5,22,24-enols can be formed in vivo by reduction of the corresponding Δ7-stereols in the different mutants tested. This indicates that Δ7-red can tolerate a large variety of sterol side chains while being highly regio-specific for the Δ7-bond reduction in a Δ5,7-structure. In particular, this enzyme lacks any Δ8-, Δ14-, Δ24(25)-, or Δ24(28)-reductase activity. This raises the question of the basis of the regio-specificity within the sterol reductase family and suggests that the N-terminal part, which is the more divergent sequence region within the family, might be involved in this role. The transformed wild type strain behaves like a leaky erg5 Δ22-desaturase mutant strain since ergosta-5-enol but not ergosta-5,22-dienoic preferentially accumulates as an end product of the de novo sterol synthesis. This suggests that Δ7-reduced sterols are bad substrates for the Δ22-desaturase and that reduction of ergosta-5,7-dienoic by the Δ7-red is highly competitive with the C-22 desaturation.

The deduced amino acid sequence of the A. thaliana Δ7-red demonstrates that the enzyme belongs to the same sequence and consequently structural family as the Δ14- and Δ24(28)-reductases. Of particular interest is the confirmation than lamin B receptor, a nuclear membrane protein featuring a N-terminal DNA binding domain, is a member of the sterol reductase family. A close examination of the sequence alignment demonstrates that all highly conserved regions among Δ7-, Δ14-, and Δ24(28)-reductases are also conserved in lamin B receptors. This puzzling observation strongly suggests that lamin B receptor may actually have a sterol reductase activity in addition to its DNA binding role. Chicken lamin B receptor was expressed in a sterol C-14-reductase-deficient yeast and did not complement the mutation. In addition, no equivalent of lamin B receptor has been found in yeast to date on the basis of the systematic sequence analysis. Yeast has no need for Δ24-reductase activity, a step specifically required for cholesterol biosynthesis. We thus propose that lamin B receptor might be the cholesta-5,24-dienoic Δ24-reductase, the last missing member of the sterol reductase family.

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