Biochemical characterization and subcellular localization of the sterol C-24(28) reductase, Erg4p, from the yeast *Saccharomyces cerevisiae*

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Abstract The yeast *ERG4* gene encodes sterol C-24(28) reductase which catalyzes the final step in the biosynthesis of ergosterol. Deletion of *ERG4* resulted in a complete lack of ergosterol and accumulation of the precursor ergosta-5,7,22,24(28)-tetraen-3β-ol. An *erg4* mutant strain exhibited pleiotropic defects such as hypersensitivity to divalent cations and a number of drugs such as cycloheximide, miconazole, 4-nitroquinoline, fluconazole, and sodium dodecyl sulfate. Similar to *erg6* mutants, *erg4* mutants are sensitive to the Golgi-destabilizing drug brefeldin A. Enzyme activity measurements with isolated subcellular fractions revealed that Erg4p is localized to the endoplasmic reticulum. This view was confirmed in vivo by fluorescence microscopy of a strain expressing a functional fusion of Erg4p to enhanced green fluorescent protein. We conclude that ergosterol biosynthesis is completed in the endoplasmic reticulum, and the final product is supplied from there to its membranous destinations.

Key words: Sterol C-24(28) reductase; Ergosta-5,7,22,24(28)-tetraen-3β-ol; Ergosterol; Endoplasmic reticulum; Green fluorescent protein; Brefeldin A

1. Introduction

Sterols are essential components of eukaryotic cells. They are important membrane constituents, regulating membrane fluidity and permeability [1–4], but also specifically interact with proteins and thus modulate their activity. Sterols of higher eukaryotes are precursors of steroid hormones which are involved in many different cellular processes (for a review see [5]). The major sterol of yeast, ergosterol, is structurally and functionally related to sterols of higher eukaryotes. Ergosterol is synthesized through a complex pathway involving numerous enzymatic steps for all of which the structural genes have been cloned (for a review see [6]). The final step of this biosynthetic sequence is catalyzed by sterol C-24(28) reductase (Fig. 1), encoded by the *ERG4* gene (systematic ORF name YGL022W). Expression of Erg4p was shown to be non-essential for cell viability [7,8], and deletion of the gene does not result in sterol auxotrophy [9] or other auxotrophic requirements on complex glucose containing media.

Ergosterol synthesis is thought to be a process occurring in the endoplasmic reticulum [10], although some enzymes of the pathway have not yet been unambiguously localized. Recently, some Erg proteins in yeast were shown to be localized to both the endoplasmic reticulum and the lipid particle fraction [11]. Lipid particles are a distinct subcellular compartment which consists of a hydrophobic core containing triacylglycerols and steryl esters [12] and a surface phospholipid monolayer harboring a small amount of characteristic proteins. Among these proteins [13] three enzymes involved in sterol metabolism, squalene epoxidase, Erg1p [11], sterol Δ24-methyltransferase, Erg6p [12], and lanosterol synthase, Erg7p (Balliano et al., manuscript in preparation) were identified. Erg6p and Erg7p are abundant in lipid particles, and only minor quantities of these enzymes were detected in the endoplasmic reticulum. In contrast, the major location of Erg1p [11,14] is the endoplasmic reticulum.

The presence of some sterol biosynthetic enzymes in lipid particles suggests an active contribution of this organelle to cellular sterol synthesis and perhaps to the regulation of the flux of intermediates through the pathway. Thus, it was of interest to investigate the subcellular distribution of further enzymes of sterol synthesis that had not previously been localized. In this report, we describe the analysis of sterol C-24(28) reductase, Erg4p, which catalyzes the conversion of ergosta-5,7,22,24(28)-tetraen-3β-ol to ergosterol, the final step of the sterol biosynthetic pathway in yeast. We describe the phenotypic consequences of a lack of Erg4p function and the subcellular distribution of the enzyme, based on activity measurements in subcellular fractions in vitro and microscopic inspection of a functional fusion to green fluorescent protein (GFP) in vivo.

2. Materials and methods

2.1. Strains and culture conditions

The wild-type yeast strain *Saccharomyces cerevisiae* W303D (*MATa/α ura3-1/ura3-1 leu2-3,112/leu2-3,112 trp1-1/trp1-1 his3-11,15/his3-11,15 ade2-1/ade2-1 can1-100/can1-100*) and the mutant strains DZW224 (W303; *MATa/α, erg4Δ::URA3/ERG4 ura3-1/ura3-1*) and DZW244 (W303; *MATa, erg4Δ::URA3*), which was derived

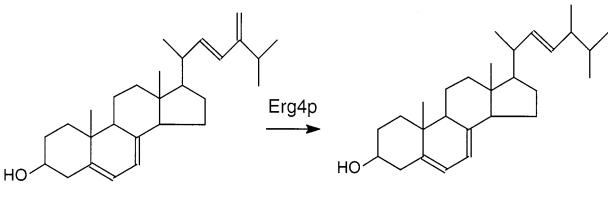

Fig. 1. Enzymatic reaction catalyzed by Erg4p.

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by sporulation and tetrad dissection from DZW224, were used throughout this study. Strain RH4217 (*MAT**a** erg4::URA3 his4 leu2 lys2 ura3 bar1*) was provided by H. Riezmann, Biocenter Basel, Switzerland, and *erg6* mutant FKY212 (*MAT**a** erg6Δ ura3-1 leu2-3,112 his3-11,15 trp1-1 ade2-1 can1-100*) was obtained from F. Kepes, Gif-sur-Yvette, France. For preparation of lipid cells and subcellular fractionation cells were grown aerobically in 2 l Erlenmeyer flasks to the late exponential phase at 30°C in YPD medium containing 1% yeast extract (Oxoid), 2% peptone (Oxoid) and 2% glucose (Merck). 500 ml of culture medium was inoculated with 0.3 ml of a preculture grown aerobically at 30°C for 48 h. Growth was monitored by measuring the optical density at 600 nm. Sporulation media were as described by Sherman et al. [15].

Transformants harboring plasmids encoding Erg4p–EGFP fusions were grown on uracil-free minimal medium (−URA) containing 0.67% yeast nitrogen base and 1% glucose supplemented with all amino acids and bases, except uracil. For derepression of the *MET25*^{p} promoter (see below) cells were harvested from −URA medium and resuspended in minimal medium lacking uracil and methionine (−URA, −MET).

Growth tests were carried out on YPD media plates containing 2% Bacto agar (Difco). Drugs, cations or detergents were added at various concentrations as indicated. Cells were pre-grown to the mid exponential phase in liquid media, diluted with YPD to an OD_{600nm} of 1, 0.1 and 0.01 and spotted onto the plates.

Escherichia coli XL-Blue (Stratagene) served as the host for plasmids used in this study. Transformed XL-Blue strains were grown in LB liquid medium (0.5% yeast extract, 1% tryptone, 0.5% NaCl) containing 100 mg/l ampicillin (United States Biochemicals).

2.2. Isolation of yeast subcellular fractions

Yeast spheroplasts and mitochondria were isolated following published procedures [16]. The 40 000×*g* microsomal fraction was prepared from the post-mitochondrial supernatant [17]. Vacuoles and highly purified yeast lipid particles were prepared as described by Leber et al. [12], and plasma membrane was prepared by the method of Serrano et al. [18].

2.3. Construction of an erg4 deletion strain

The plasmid pCM2 (kindly provided by C. Marcireau, Centre de Recherche de Vitry-Alfortville), an 11 664 bp construct with a 685 bp fragment of *ERG4* replaced by a *URA3* cassette, was used for deletion of the *ERG4* gene. pCM2 originates from vector pA-B6.5 [19] which carries an 8784 bp DNA fragment of chromosome VII including *ERG4*. For construction of pCM2 and deletion of *ERG4* a 685 bp *Sna*BI/*Avr*II fragment was replaced by a 2729 bp *URA3* cassette flanked by direct repeats. A 6937 bp *ERG4* gene disruption cassette was obtained by cleaving pCM2 with *Sac*I and *Hpa*I. The linear DNA was transformed into diploid W303 using the high efficiency lithium acetate transformation protocol [20] and stable uracil prototrophic transformants were isolated. Sporulation and tetrad analysis of the Ura^{+} transformants were carried out by standard techniques [15] and yielded four viable spores and the uracil prototrophic marker segregated $2^{+}:2^{-}$, indicating that *ERG4* is not essential for vegetative growth [7,8]. Deletion of *ERG4* was confirmed by Southern hybridization in diploid transformants and the resulting segregants. The *URA3* gene used for gene disruption/selection was eliminated by intrachromosomal recombination through the flanking direct repeats by replicating yeast on complete medium containing 5-fluoroorotic acid (0.1%) [21].

2.4. Construction of an ERG4–EGFP fusion

1440 bp of the coding region of *ERG4* including 24 bp upstream of the ATG were amplified by PCR using genomic DNA of strain W303D as the template. The PCR primers P1 and P2 (Fig. 2) used for the amplification were generated such to introduce *Xba*I (5′-end) and *Bam*HI (3′-end) restriction sites for subsequent subcloning into GFP fusion vectors, and also to replace the stop codon in order to obtain an in-frame GFP fusion. The PCR fragment was cut with *Xba*I and *Bam*HI and introduced into the respective sites of the yeast *CEN* vector pGFP-C-FUS (kindly provided by J. Hegemann, Heinrich-Heine-Universität Düsseldorf, Germany), which led to the formation of a C-terminal GFP fusion. This vector contains the yeast *MET25*^{p} promoter that allows regulated expression of *ERG4* under conditions of methionine depletion. The correct sequence of the construct was confirmed by DNA sequencing. For enhanced fluorescence of the fusion protein the *MET25*^{p}*–ERG4* fragment was subcloned as *Bam*HI fragment (1863 bp) (primers P2 and P3, see Fig. 2) into the respective sites of plasmid pRS416 harboring the coding region of enhanced GFP (EGFP) in its *Eco*RI site (kindly provided by L. Robinson, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, Shreveport, Louisiana, USA).

	Primer (5' -> 3')
P1	TCATCTAGAGTGTACATAGATTAGCATCGC
P2	TGCGGATCCGAAAACATAAGGAATAAAGAC
P3	TGAGGATCCGAACAAAAGCTGGAGCTCCG

Fig. 2. Primers used for the construction of the *ERG4–EGFP*.

The plasmid encoding Erg4p–EGFP was transformed into an *erg4* deletion strain using the lithium acetate method [22], and transformants were selected for uracil prototrophy on synthetic media lacking uracil. Transformation of *E. coli* XL1-Blue (Stratagene) was performed by the $CaCl_2$ method [23].

2.5. Fluorescence microscopy

Yeast cells were grown overnight in liquid media lacking uracil. For derepression of *MET25*^{p}-controlled gene expression cells were inoculated into minimal media lacking uracil and methionine. After various periods of time 1 ml aliquots were withdrawn and cells were concentrated by centrifugation at 1000 rpm (600×*g*) for 2 min. Preparation of cells for microscopic inspection and confocal microscope set-up were as described previously [24]. GFP fluorescence was observed by laser scanning confocal microscopy on a Leica TCS 4D confocal microscope, using the 488 nm excitation line (Ar/Kr laser), a TK500 beam splitter and a TK525/50 band pass filter for GFP fluorescence detection.

2.6. Lipid analysis

For the analysis of total sterols the wild-type strain W303D and *erg4* mutant cells were grown in YPD for 24 h, harvested and washed with distilled water. Cells (approximate 300 mg wet weight) were taken up in 1.5 ml methanol, 1 ml of 0.5% pyrogallol and 1 ml of 60% KOH, and heated for 2 h at 85°C to hydrolyze steryl esters. Hydrolysates were extracted three times with 3 ml light petroleum each, and the organic phases were combined and taken to dryness under a stream of nitrogen. Lipids were dissolved in a small volume of cyclohexane and subjected to gas liquid chromatography (GLC) or GLC/mass spectrometry (GLC/MS), as described previously [24]. UV absorption spectra of lipid extracts were measured on a U-3210 spectrophotometer (Hitachi).

2.7. Measurement of sterol C-24(28) reductase activity

Enzymatic activity of Erg4p in yeast subcellular fractions of wild-type and *erg4* mutant cells was analyzed by measuring the conversion of ergosta-5,7,22,24(28)-tetraen-3β-ol to ergosterol. The assay is based on the method described by Neal and Parks [25] and was performed with several modifications. The standard assay mixture with a total volume of 1 ml contained 1 μmol NADPH, 20.5 μmol EDTA and 100 nmol ergosta-5,7,22,24(28)-tetraen-3β-ol substrate (from a stock solution in ethanol, see below) in 0.1 M Tris–HCl, pH 7.5. The reaction was started by adding 0.5 mg protein of various subcellular fractions; incubations were carried out at 28°C. Samples of 100 μl were taken at 0, 0.5, 1 and 2 h, and lipids were extracted in 4 ml chloroform/methanol (2:1; v/v) for 1 h. Lipid extracts were washed with 1 ml of 0.034% $MgCl_2$ and 1 ml of methanol/water/chloroform (48:47:3, v/v). The organic phase was dried under a stream of nitrogen and samples were taken up in 0.75 ml methanol, 0.5 ml of 0.5% pyrogallol and 0.5 ml of 60% KOH, and heated for 2 h at 85°C to hydrolyze steryl esters. Hydrolysates were extracted three times with 3 ml light petroleum each, and the organic phases were combined and taken to dryness. Lipids were dissolved in 50 μl cyclohexane and subjected to GLC (see above). Authentic ergosterol (Sigma) was used as a standard. Relative retention times of sterols were as described previously [26–28]. The activity of sterol C-24(28) reductase was estimated based on the increase of the amount of ergosterol.

For preparation of ergosta-5,7,22,24(28)-tetraen-3β-ol the *erg4* deletion strain DZW244 was cultivated in synthetic medium without ergosterol for 24 h, harvested and treated with methanol–KOH as

described above. Lipids extracted with light petroleum were applied to a column (20×1 cm) filled with silica gel 60 (corn diameter 0.063–0.2 mm; Merck, Darmstadt, Germany). The solvent system used for the elution of lipids was a gradient of light petroleum/diethylether starting at a ratio of 70:30 with increasing amounts of diethylether. Fractions of 1 ml were collected, and samples containing ergosta-5,7,22,24(28)-tetraen-3β-ol were combined and dried under nitrogen. The resulting product was dissolved in a small volume of cyclohexane and quantified by GLC with ergosterol (Sigma) as a standard.

2.8. Miscellaneous methods

Protein was quantified by the method of Lowry et al. [29] using bovine serum albumin as a standard. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE) was carried out by the method of Laemmli [30]. Western blot analysis was performed as described by Haid and Suissa [31], and immunoreactive proteins were detected by ELISA using rabbit antisera as the first and goat anti-rabbit IgG linked to peroxidase or phosphatase as the second antibody.

Fluidity of the plasma membrane was determined in vitro by measuring the fluorescence anisotropy of TMA–DPH (trimethylammonium–diphenylhexatriene) [32].

3. Results and discussion

3.1. Phenotypic analysis of an erg4 null mutant

Sterol C-24(28) reductase, Erg4p, catalyzes the final step in the ergosterol biosynthetic pathway in yeast. Deletion of *ERG4* resulted in an accumulation of ergosta-5,7,22,24(28)-tetraen-3β-ol up to 90% of total sterols, confirming previous findings by Lai et al. [8]. Since *erg4* mutants lack detectable levels of ergosterol it can be assumed that Erg4p is the only sterol C-24(28) reductase present in yeast.

Ergosterol is a main constituent of the yeast plasma membrane and is known to affect membrane permeability, fluidity and activity of membrane-bound proteins [1–4,33]. Thus, changes of the sterol composition can be expected to affect certain properties of the plasma membrane. In the plasma membrane of the *erg4* mutant, ergosterol is completely replaced by ergosta-5,7,22,24(28)-tetraen-3β-ol (Table 1). Although this sterol precursor is structurally highly related to ergosterol, distinct phenotypic defects were observed with the *erg4* mutant indicating that ergosta-5,7,22,24(28)-tetraen-3β-ol cannot fully replace ergosterol as a membrane component.

As shown in Table 2 *erg4* mutant cells are hypersensitive to various drugs, such as cycloheximide, miconazole, terbinafine, fluconazole and, to a lesser extent, nystatin and 4-nitroquinoline. Lack of ergosterol in the plasma membrane of *erg4* cells may facilitate the import of certain drugs. This effect, however, cannot be simply explained by an alteration of the membrane fluidity: anisotropy measurements using a TMA–DPH chromophore, which was incorporated into the plasma membrane, revealed no differences in chromophore mobility between *erg4* mutant and wild-type (data not shown). Alternatively, *erg4* mutants may become more sensitive to drugs due to a less efficient efflux of these components mediated by membrane-bound pumps of the multidrug resistance family. Some of these transporters [34] may require ergosterol for optimum activity although such a dependence has not yet been systematically tested.

Table 1
Sterol composition of plasma membranes of an *erg4* deletion strain (DZW244) and the corresponding wild-type (W303)

	Total sterols (%)	
	Wild-type	*erg4*
Ergosterol	89.5	ND
Ergosta-5,7,22,24(28)-tetraen-3β-ol	ND	89.0
Fecosterol	0.8	ND
Ergosta-5,7,24-trien-3β-ol	5.2	6.2
Episterol	1.2	1.0
Squalene	1.3	1.0
Zymosterol	0.9	0.3
Lanosterol	0.7	1.0
Others	0.4	1.5

ND, not detectable. Values were obtained from two independent estimations with a deviation of ±5%.

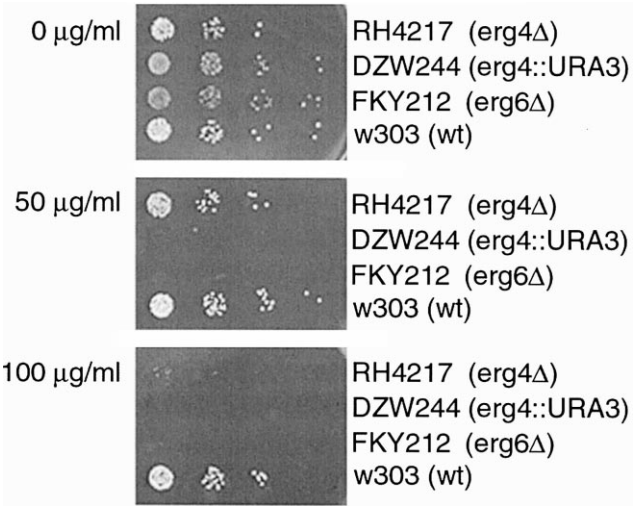

Fig. 3. Growth inhibition of *erg4* and *erg6* deletion strains by brefeldin A. Exponential phase cells were spotted at densities of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} onto YPD media plates without or with brefeldin A (50 μg/ml and 100 μg/ml) and incubated for 3 days at 30°C.

Increased sensitivity of *erg4* mutant strains to the pore forming macrolide antibiotic nystatin (see Table 2) is an interesting phenomenon and suggests that this drug can interact with ergosta-5,7,22,24(28)-tetraen-3β-ol in a similar way as described previously for ergosterol to exert its growth inhibitory activity [35]. In contrast, other mutants with defects in ergosterol biosynthesis, such as *erg6*, *erg3* and *erg5*, which accumulate sterols that are structurally more distant from ergosterol, are nystatin-resistant [36]. Sterols accumulating in these strains obviously fail to form complexes with the drug as proposed for ergosterol.

As shown in Fig. 3, growth of *erg4* mutants in two different genetic backgrounds is drastically reduced in the presence of brefeldin A. This fungal metabolite inhibits the exchange of ADP ribosylation factor-bound GDP for GTP by a Golgi-

Table 2
Sensitivity of the *erg4* mutant to various reagents

Reagent	Minimal inhibitory concentration	
	Wild-type	*erg4*
Cycloheximide	1 μg/ml	0.05 μg/ml
Miconazole	0.05 μg/ml	0.01 μg/ml
Fluconazole	20 μg/ml	2 μg/ml
Terbinafine	100 μg/ml	10 μg/ml
4-Nitroquinoline	0.5 μg/ml	0.25 μg/ml
Nystatin	5 μg/ml	2 μg/ml
SDS	0.02%	0.0025%

associated guanine nucleotide exchange protein [37,38] and thus disrupts the Golgi apparatus and the flux of secretory proteins [39]. Whereas wild-type yeast cells are resistant against more than 100 μg/ml brefeldin A, mutants lacking the *ERG6* gene are highly susceptible to this drug (Fig. 3, [40]). For this reason *erg6* mutants have been widely used for studies involving brefeldin A inhibition. Here we show that mutants lacking *ERG4* are as sensitive to brefeldin A as *erg6* mutant cells and may thus be used as an alternative model system to study brefeldin A action in vivo with the advantage of a less altered membrane sterol composition.

The *erg4* deletion strain is also highly sensitive to SDS (Table 2). Wild-type cells are able to tolerate 8–10-fold higher concentrations of this ionic detergent compared to the *erg4* mutant, whereas non-ionic detergents such as Brij 58 or Triton X-100 had similar effects on mutant and wild-type (data not shown). SDS may penetrate the plasma membrane of the mutant more easily than that of wild-type, which, however, is not due to an increased osmo-sensitivity of the mutant, since *erg4* cells grow like wild-type in the presence of 1 M sorbitol (data not shown).

As shown in Table 3 the *erg4* mutant is also more sensitive to various divalent cations than wild-type. Resistance to divalent cations is often conferred by membrane ATPases which serve as pumps to protect cells from toxic compounds, or by oxidoreductases which are responsible for detoxification [41]. These membrane-associated activities may be altered in the mutant due to lack of ergosterol and/or accumulation of its precursor.

3.2. Localization of Erg4p

At least three enzymes of the ergosterol biosynthetic pathway, namely Erg1p, Erg6p and Erg7p [11–13], are dually located both in the endoplasmic reticulum and in lipid particles. It was suggested that lack or paucity of transmembrane spanning domains is the reason for a preferred occurrence of these proteins in the membrane monolayer which surrounds lipid particles [13]. In contrast to the above mentioned enzymes, Erg4p contains eight transmembrane regions and is thus predicted to be embedded in a bilayer membrane. Indeed, highest activity of Erg4p had previously been found in a $25\,000\times g$ membrane pellet [25].

To identify the subcellular localization of Erg4p in more detail we tested isolated subcellular fractions for sterol C-24(28) reductase activity by analyzing the conversion of ergosta-5,7,22,24(28)-tetraen-3β-ol to ergosterol. The only significant activity (0.32 nmol/min mg) was found in the $40\,000\times g$ microsomal fraction, corresponding largely to endoplasmic reticulum membranes. The enrichment factor of 7 over the homogenate for Erg4p reflects the enrichment of typical components of the endoplasmic reticulum [17]. Other subcellular fractions such as lipid particles, mitochondria, vacuoles, and the plasma membrane were devoid of Erg4p activity.

Table 3
Cation sensitivity of the *erg4* mutant

Cation	Minimal inhibitory concentration (mM)	
	Wild-type	*erg4*
Ca^{2+}	>100	75
Mg^{2+}	>200	75
Zn^{2+}	7.5	2.5
Mn^{2+}	20	10
Cu^{2+}	>5	2.5

Cations were added to solid media at the indicated concentrations as chlorides.

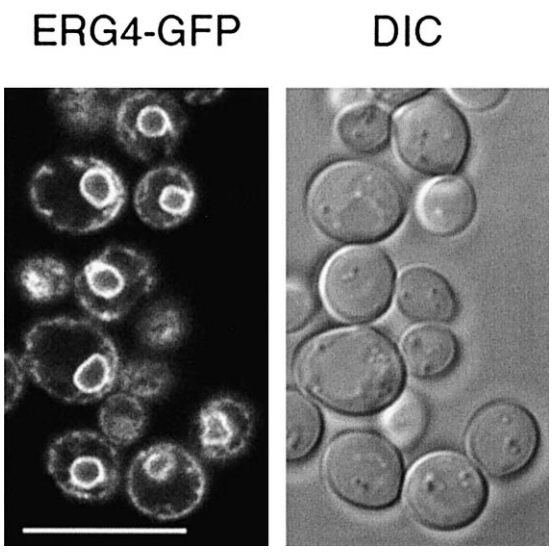

Fig. 4. Visualization of an Erg4p–EGFP fusion protein by fluorescence microscopy in vivo. The *ERG4–EGFP* hybrid was constructed as described in Section 2. Expression of the fusion protein was induced for 4 h in media lacking methionine. Left panel: GFP fluorescence (single confocal section; 512×512 pixel; 4× line averaging mode); right panel: DIC (differential interference contrast) image of the respective cells. Bar represents 10 μm.

Subcellular localization of Erg4p as determined by subcellular fractionation of yeast cells was confirmed in vivo using a C-terminal fusion of Erg4p to EGFP. The Erg4p–EGFP hybrid was expressed under the control of a heterologous $MET25^{p}$ promoter in an *erg4* mutant background, and visualized by confocal laser scanning microscopy. Under derepressing conditions, i.e. in the absence of methionine, the fusion protein fully restored synthesis of ergosterol in the *erg4* mutant strain as determined by GLC analysis of total cellular lipids, suggesting that the fusion protein was indeed functional. Immunoblots of a homogenate that was prepared from transformants expressing Erg4p–EGFP using GFP-specific antibody unveiled a fusion protein of the predicted mass of 86 kDa (56 kDa for Erg4p and 30 kDa for GFP).

Fluorescence microscopy of yeast transformants grown under derepressing conditions demonstrates that Erg4p–EGFP is localized to the endoplasmic reticulum (Fig. 4). GFP fluorescence was observed around the nucleus and in proximity to the cell periphery, which is typical of endoplasmic reticulum-resident proteins. Thus, based on activity measurements in subcellular fractions in vitro and by fluorescence microscopy in vivo, Erg4p is a component of the endoplasmic reticulum in yeast, and is clearly absent from lipid particles.

3.3. Conclusions

As a consequence of the localization of Erg4p, sterol C-24(28) reductase, which catalyzes the final step in the ergosterol biosynthetic pathway in yeast, in the endoplasmic reticulum this compartment must be regarded as the origin of ergosterol, despite the fact that certain intermediate steps of the pathway may also occur in lipid particles. Ergosterol syn-

thesized in the endoplasmic reticulum is transported to its destinations, mainly to the plasma membrane where it occurs in the highest concentrations, presumably via the secretory pathway (H. Pichler, unpublished observations). The contribution to sterol synthesis in vivo of Erg1p, Erg6p and Erg7p located to lipid particles remains to be shown.

Another important consequence of the presence of sterol C-24(28) reductase in the endoplasmic reticulum is that ergosterol, the product formed by Erg4p, can be immediately metabolized to ergosteryl esters in situ without prior translocation to another organelle/membrane system. Both steryl ester synthases of the yeast, Are1p and Are2p [42,43], are also components of the endoplasmic reticulum [24]. Interestingly, steryl esters formed by these two enzymes are barely detectable in the endoplasmic reticulum and are exclusively stored in lipid particles, which suggests rapid and efficient interorganelle translocation of these compounds in vivo. Furthermore, only about 50% of steryl esters in lipid particles are derivatives of ergosterol, whereas the other half contain sterol precursors. Thus, steryl ester synthases may interact with several sterol biosynthetic enzymes in the endoplasmic reticulum and thus remove intermediates from the pathway by esterification and subsequent storage in lipid particles. The presence of sterol intermediates in esterified form in lipid particles as well as some key enzymes of sterol synthesis, such as Erg1p, Erg6p and Erg7p, suggests a depot function for lipid particles in regulating the flux through the ergosterol biosynthetic pathway at the level of both enzymes and intermediate substrates. Indeed, during the early exponential growth phase [44] or upon starvation for sterols in the presence of metabolic inhibitors [45], sterols are mobilized from lipid particles and precursors are converted to ergosterol. Proteins involved in this translocation process, enzymes responsible for steryl ester cleavage and factors governing regulation of the interaction between lipid particles and the endoplasmic reticulum, however, remain to be identified.

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