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Defect of synthesis of very long-chain fatty acids confers resistance to growth inhibition by inositol phosphorylceramide synthase repression in yeast *Saccharomyces cerevisiae*

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Aureobasidin A (AbA) inhibits Aur1p, an enzyme catalysing the formation of inositol phosphorylceramide in the yeast *Saccharomyces cerevisiae*. AbA treatment results not only in reductions in complex sphingolipid levels but also in accumulation of ceramides, both of which are believed to lead to the growth defect caused by this inhibitor. We screened for mutants showing resistance to this drug, and found that a lack of *ELO3*, the gene involved in synthesis of very long-chain fatty acids, confers resistance to the inhibitor. The resistance as to growth inhibition by reduction in Aur1p activity was also confirmed by repression of *AUR1* expression under the control of a tetracycline-regulatable promoter. Under the *AUR1*-repressive conditions, the *ELO3* mutant showed reduction in the complex sphingolipid levels and the accumulation of ceramide, like wild-type cells. However, with repression of *LCB1* encoding serine palmitoyltransferase or *LIP1* encoding the ceramide synthase subunit, the *ELO3* mutation did not confer resistance to growth inhibition induced by the impaired sphingolipid biosynthesis. Therefore, it is suggested that the *ELO3* mutant shows resistance as to accumulation of ceramides, implying that the chain lengths of fatty acids in ceramide are a critical factor for the ceramide-induced growth defect under *AUR1*-repressive conditions.

Keywords: aureobasidin A/ceramide/fatty acid/*Saccharomyces cerevisiae*/sphingolipid.

Abbreviations: AbA, Aureobasidin A; Cer, ceramide; DHS, dihydrosphingosine; Dox, doxycycline; IPC, inositol phosphorylceramide; LCB, long-chain base; LCBP, long-chain base 1-phosphate; MIPC, mannosylinositol phosphorylceramide; $M(IP)_2C$, mannosyldiinositol phosphorylceramide; MMA, monomethylamine; MSG, L-glutamic acid sodium salt hydrate; PHS, phytosphingosine; VLCFA, very long-chain fatty acid.

Complex sphingolipids are a major component of the eukaryotic plasma membrane (*1*). They consist of a ceramide (Cer) backbone with a polar head group. The Cer moiety comprises a long-chain base (LCB) attached to a fatty acid via an amide bond. Complex sphingolipids are mainly distributed in the external leaflet of the plasma membrane, and dynamically cluster with sterols to form lipid microdomains (*2*). Numerous studies have also demonstrated that the degradation products of membrane sphingolipids, such as Cers, LCBs and LCB 1-phosphates (LCBPs), function as lipid mediators that regulate various signal transduction systems (*3*).

The chain lengths of fatty acids in sphingolipids mainly range from C14 to C26 in mammalian tissues (*4*). In most tissues, C16 fatty acids are predominant, followed by the C24 species. In contrast, in the yeast *Saccharomyces cerevisiae*, the chain length of fatty acids in Cers is primarily C26 (*5*). In yeast, the chain lengths of fatty acids in complex sphingolipids are important for numerous cellular functions, including the formation of GPI lipid anchors and the trafficking of proteins through the secretory pathway (*6*, *7*). Sphingolipids containing C22 and C24 fatty acids are partly able to complement the functions of that containing C26 fatty acids; however, complete loss of very long-chain fatty acids (longer than 20 carbons; VLCFAs) results in a lethal phenotype (*8*). VLCFAs are synthesized from medium-chain fatty acids (C16 and C18) via the VLCFA elongation cycle composed of four steps (Fig. 1A). Both Elo2p and Elo3p catalyse the first step of this cycle. Elo2p is involved in the elongation of fatty acids up to C22 or C24, whereas Elo3p exhibits a broader substrate specificity, and is essential for the conversion of C24 to C26. Thus, the *ELO3* mutant shows numerous phenotypes, which are related to the specific functions of C26 fatty acids (*6*, *7*).

In yeast, there are three classes of complex sphingolipids that contain *myo*-inositol, namely inositol phosphorylceramide (IPC), mannosylinositol phosphorylceramide (MIPC) and mannosyldiinositol phosphorylceramide [$M(IP)_2C$] (*5*). *AUR1* is an essential gene that encodes IPC synthase, an enzyme catalysing the transfer of the head group of phosphatidylinositol to Cers (*9*) (Fig. 1A). Aureobasidin A (AbA) is a cyclic depsipeptide anti-fungal antibiotic, which inhibits Aur1p (*9*, *10*). AbA treatment results not only in reductions in all the complex sphingolipid levels but also

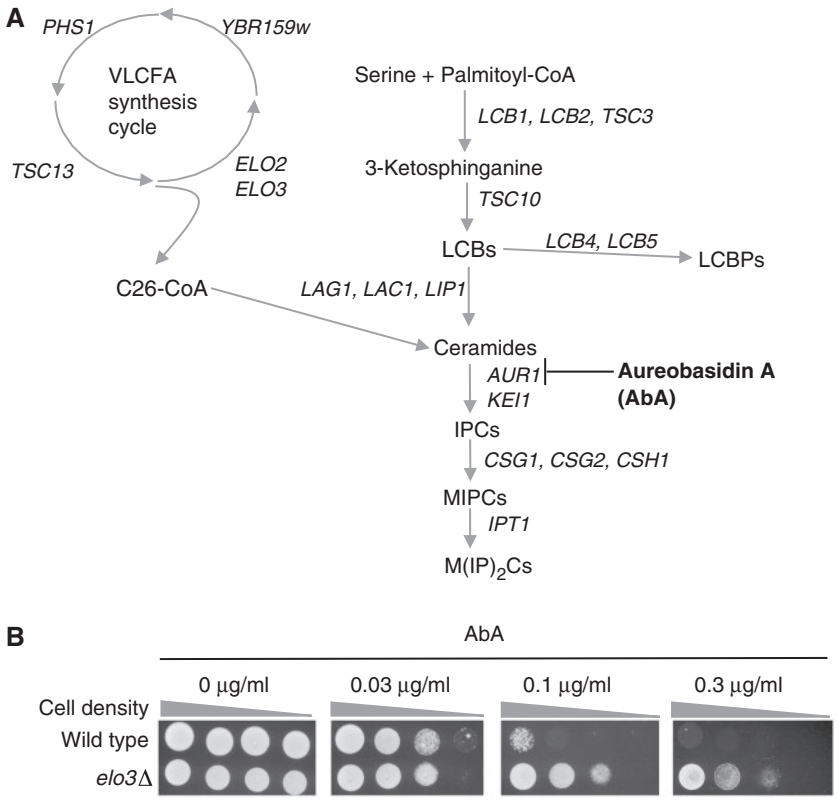

Fig. 1 AbA resistance of *elo3*Δ cells. (A) Sphingolipid synthesis pathway in yeast *Saccharomyces cerevisiae*. The pathway and responsible enzyme genes are shown for the synthesis of yeast sphingolipids. (B) Effect of AbA on growth of wild-type (MTY174) and *elo3*Δ cells. Cells were cultured overnight in YPD medium, and then spotted onto YPD plates with or without the indicated concentrations of AbA in 3.2-fold serial dilutions starting with a density of 7 A_{600} U/ml. The plates were incubated at 30°C and photographed after 2 days.

in the accumulation of Cers, both of which are believed to lead to the growth defect caused by this inhibitor (*9*, *11*, *12*); however, the molecular mechanism underlying the growth defect is largely unknown. To address the mechanisms of the AbA-induced growth defect, we screened a collection of ~4,800 yeast mutant strains each lacking a nonessential gene for mutants showing resistance to this drug, and found that *elo3*Δ cells show resistance to the inhibitor. This indicated that a defect of the synthesis of VLCFAs confers resistance to growth inhibition by *AUR1* repression.

Materials and Methods

Yeast strains and media

The *S. cerevisiae* strains used are listed in Table I. To generate a yeast in which the expression of *AUR1, LIP1* or *LCB1* is regulated by doxycycline (Dox) (*tet-AUR1*::*kanMX4*, *tet-LIP1*::*kanMX4* or *tet-LCB1*::*kanMX4* cells), the *AUR1* upstream region from −1 to −60 bp, the *LIP1* upstream region from −1 to −60 bp, or the *LCB1* upstream region from −1 to −70 bp was replaced with a tetracycline operator cassette containing a repressor binding site and a TetR-VP16 tTA transactivator ($tetO_7$), as described earlier (*13*). Disruption of *ELO3* and *LCB4* was performed by replacing their open reading frames with the *URA3* marker from pRS406 (*14*). Disruption of *ELO3* was also performed by replacing its open reading frame with the *natMX4* marker from p4339 (pCRII-TOPO::natMX4) (*15*). To provide uniform auxotrophic conditions, *ura3* cells were transfected with the *URA3* fragment, thereby generating *URA3* cells. The cells were grown in either YPD medium (1% yeast extract, 2% peptone and 2% glucose) or synthetic complete (SC/MSG) medium [0.17% yeast nitrogen base w/o amino acids and ammonium sulphate (BD Difco, Heidelberg, Germany), 0.1% L-glutamic acid sodium salt hydrate (MSG; Sigma) and 2% glucose] containing nutritional supplements (*15*).

[^{3}H]myo-inositol labelling

Yeast cells grown in YPD medium were collected by centrifugation and then washed with SC/MSG medium with minimum inositol (2 μg/ml). Then the cells were resuspended in 500 μl of SC/MSG medium with minimum inositol to 2 A_{600} U/ml and labelled with [^{3}H]*myo*-inositol (1 μCi/1 A_{600} U of cells; PerkinElmer Life Sciences, Norwalk, CT, USA) for 1 h at 30°C. The cells were chilled on ice, collected by centrifugation, washed with distilled water, and then suspended in 150 μl of ethanol/ water/diethyl ether/pyridine/ 15 N ammonia (15 : 15 : 5 : 1 : 0.018, v/v). Radioactivity was measured using a liquid scintillation system, and samples exhibiting equal radioactivity were used for further experiments. After 15-min incubation at 60°C, the samples were centrifuged at 10,000*g* for 1 min and the supernatants were withdrawn. Lipids were extracted from the residues once more in the same manner. The resulting supernatants were dried. Occasionally, the lipids were subjected to mild alkaline treatment using monomethylamine (MMA). For this, the lipid extracts were dissolved in 150 μl MMA (40% methanol solution)/water (10 : 3, v/v) or methanol/water (10 : 3, v/v), incubated for 1 h at 53°C, and then dried. The lipids were suspended in 20 μl of chloroform/methanol/water (5 : 4 : 1, v/v) and then separated on Silica Gel 60 TLC plates (Merck, Whitehouse Station, NJ, USA) with chloroform/methanol/4.2 N ammonia (9 : 7 : 2, v/v) as the solvent system.

[^{3}H]dihydrosphingosine labelling

Yeast cells grown in YPD medium were collected by centrifugation and then resuspended in 600 μl of fresh YPD medium to 1.7 A_{600} U/ml. 0.5 μCi [4,5-^{3}H]dihydrosphingosine (DHS) (American Radiolabelled Chemical Inc., St. Louis, MO, USA) in 166.5 μl of YPD medium containing 4 mg/ml BSA was added to the cultures, followed by incubation for 1 h at 30°C. The cells were chilled on ice, collected by centrifugation, and then washed with YPD medium containing 1 mg/ml BSA. The lipids were

Table I. Strains used in this study.

Strain	Genotype	Source
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	(*16*)
MTY174	*MATa his3Δ1 leu2Δ0 met15Δ0 URA3*	This study
MTY51	BY4741, *elo3Δ::URA3*	This study
MTY175	BY4741, *tetO$_7$-AUR1::kanMX4 URA3*	This study
MTY47	BY4741, *tetO$_7$-AUR1::kanMX4 elo3Δ::URA3*	This study
MTY176	BY4741, *tetO$_7$-LIP1::kanMX4 URA3*	This study
MTY552	BY4741, *tetO$_7$-LIP1::kanMX4 elo3Δ::URA3*	This study
MTY615	BY4741, *tetO$_7$-LCB1::kanMX4 URA3*	This study
MTY550	BY4741, *tetO$_7$-LCB1::kanMX4 elo3Δ::URA3*	This study
MTY542	BY4741, *tetO$_7$-AUR1::kanMX4 lcb4Δ::URA3*	This study
MTY543	BY4741, *tetO$_7$-AUR1::kanMX4 lcb4Δ::URA3 elo3Δ::natMX4*	This study

extracted, subjected to mild alkaline treatment, and then separated by TLC as described under '*[^{3}H]myo-inositol labelling*'.

[^{14}C]serine labelling

Yeast cells were cultured overnight in YPD medium, diluted (0.2 A_{600} U/ml) in fresh YPD medium with or without 10 μg/ml Dox, and then incubated for 4 h. Cells were collected by centrifugation, washed with SC/MSG medium lacking serine, resuspended in SC/MSG medium lacking serine with or without 10 μg/ml Dox, and then incubated at 30°C for 1 h. Cells were collected by centrifugation and resuspended in 700 μl of fresh SC/MSG medium lacking serine with or without 10 μg/ml Dox to 1 A_{600} U/ml. Then the cells were labelled with [^{14}C]serine (0.7 μCi/1 A_{600} U of cells; PerkinElmer Life Sciences, Norwalk, CT, USA) for 5 h at 30°C. The lipids were extracted, subjected to mild alkaline treatment as described under '*[^{3}H]myo-inositol labelling*'. The extracted lipids were separated on Silica Gel 60 TLC plates with chloroform/methanol/acetic acid (100 : 5.2 : 0.58, v/v) or chloroform/methanol/acetic acid (90 : 10 : 1, v/v) as the solvent system, and then analysed with a Bio Imaging analyser FLA-2000 (Fuji Photo Film, Kanagawa, Japan).

Detection of Cer by a copper sulphate and orthophosphoric acid reagent

Yeast cells were cultured overnight in YPD medium, diluted (0.1 A_{600} U/ml) in fresh YPD medium with or without 10 μg/ml Dox, and then incubated for 9 h at 30°C. Lipids (3.5 A_{600} U) were extracted, treated with MMA, and then separated on TLC plates as as described under '*[^{14}C]serine labelling*'. The TLC plates were sprayed with 10% copper sulphate in 8% orthophosphoric acid and then heated at 180°C to visualize Cers.

Results

elo3*Δ* cells exhibit AbA resistance

To identify mutant strains showing resistance to AbA, we used a yeast knockout library (*16*). All the library strains were subjected to cultivation on YPD plates containing 0.1 μg/ml AbA, and then cell growth was evaluated. Although almost all the strains ceased growing in the presence of 0.1 μg/ml AbA, *elo3*Δ cells showed siginificant resistance to this drug. Figure 1B shows the growth of wild-type and *elo3*Δ cells with various concentrations of AbA. In the presence of 0.3 μg/ml AbA, the growth of wild-type cells was almost completely inhibited, whereas that of *elo3*Δ cells was only partly inhibited.

Sphingolipid metabolism of elo3*Δ cells under* AUR1*-repressive conditions*

To eliminate the possibility that the AbA resistance of *elo3*Δ cells is caused by a non-specific effect of the inhibitor, we used a mutant strain that carries the *AUR1* gene under the control of a tetracycline-regulatable (Tet) promoter (*tet-AUR1*) for further analysis. Supplementary Figure S1 shows the time course of growth of *tet-AUR1* cells in the presence or absence of doxycycline (Dox), which represses expression of the gene under the Tet promoter. The growth rate of *tet-AUR1* cells, but not that of wild-type cells, began to slow down at 7–9 h after the addition of 10 μg/ml Dox. Figure 2A shows the growth of *tet-AUR1* and *tet-AUR1 elo3*Δ cells in the presence of various concentrations of Dox. *tet-AUR1* showed slow growth on a YPD plate containing 0.7 μg/ml Dox, but strong growth inhibition was observed in the presence of 10 μg/ml Dox. In contrast, *tet-AUR1 elo3*Δ cells did not show a growth defect even in the presence of 10 μg/ml Dox (Fig. 2A). This together with the effect of AbA (Fig. 1B) indicated that *elo3*Δ cells are resistant to the growth inhibition induced by *AUR1* repression. To examine the complex sphingolipid levels under *AUR1*-repressive conditions, *tet-AUR1* and *tet-AUR1 elo3*Δ cells were radiolabelled with [^{3}H]*myo*-inositol (Fig. 2B). Reductions in complex sphingolipids in Dox-treated *tet-AUR1* and *tet-AUR1 elo3*Δ cells were observed at 9 h after the addition of 10 μg/ml Dox. In Dox-treated *tet-AUR1* cells, accumulation of Cer-C, which contains phytosphingosine (PHS) and α-OH-C26 fatty acid, was observed in the presence of 10 μg/ml Dox when cells were radiolabelled with [^{14}C]serine (Fig. 2C, left panel). Detection of Cers by a copper sulphate and orthophosphoric acid reagent also showed the accumulation of Cer-C by treatment with Dox (Fig. 2C, right panel). The accumulation was similarly observed in Dox-treated *tet-AUR1 elo3*Δ cells; however, the accumulated Cer-C in Dox-treated *tet-AUR1 elo3*Δ cells exhibited a somewhat lower R_f value than that in Dox-treated *tet-AUR1* cells (Fig. 2C and D), because of the shorter chain lengths of the fatty acids (24 carbons or shorter) (*17*). These results indicated that under *AUR1*-repressive conditions the reductions in complex sphingolipid levels and the accumulation of Cers occur in *elo3*Δ cells, like in wild-type cells.

Growth of elo3*Δ cells under sphingolipid synthesis-defective conditions*

Both the reductions in the complex sphingolipid levels and the accumulation of Cers are responsible for the growth inhibition by *AUR1* repression (*9*, *11*, *12*).

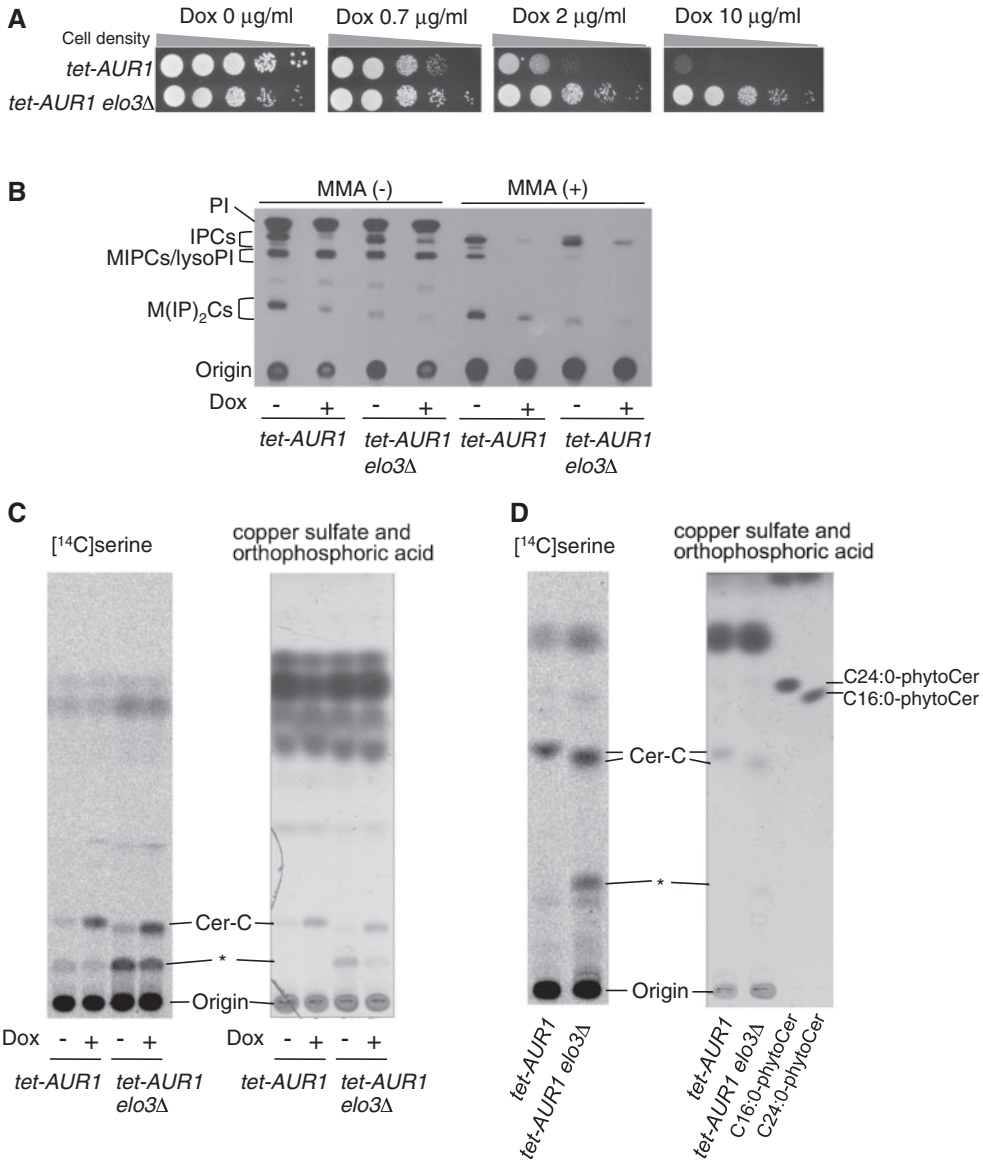

Fig. 2 Growth and sphingolipid metabolism of *elo3*Δ cells under *AUR1*-repressive conditions induced by the Tet promoter. (A) *tet-AUR1* and *tet-AUR1 elo3*Δ cells were cultured overnight in YPD medium, and then spotted onto YPD plates with or without the indicated concentrations of Dox in 10-fold serial dilutions starting with a density of 0.7 A_{600} U/ml. (B) [^{3}H]*myo*-inositol labelling of *tet-AUR1* and *tet-AUR1 elo3*Δ cells. Cells were cultured overnight in YPD medium, diluted (0.1 A_{600} U/ml) in fresh YPD medium with or without 10 μg/ml Dox, incubated for 8 h, and then labelled with [^{3}H]*myo*-inositol for 1 h. Radiolabelled lipids were extracted, treated with MMA, and then separated by TLC. (C and D) *tet-AUR1* and *tet-AUR1 elo3*Δ cells were cultured with or without 10 μg/ml Dox, and then labelled with [^{14}C]serine. Radiolabelled lipids were extracted, treated with MMA, and then separated by TLC with chloroform/methanol/acetic acid (100 : 5.2 : 0.58, v/v) (C) or chloroform/methanol/acetic acid (90 : 10 : 1, v/v) (D; all samples were treated with Dox) as the solvent system. Alternativly, lipids were visualized with a copper sulphate and orthophosphoric acid reagent. C16:0-phytoCer and C24:0-phytoCer (10 nmol) (Avanti polar lipids, Alabaster, Alabama) were spotted as standards. The asterisks indicate unidentified band. The Cer-C band was identified by [^{14}C]serine labelling of wild-type, *scs7*Δ, and *sur2*Δ cells (Supplementary Fig. S2). The details are given under 'Materials and Methods' section.

To investigate why *elo3*Δ cells acquire resistance to the growth inhibition by *AUR1* repression, upstream of *AUR1* in the *de novo* sphingolipid biosynthetic pathway was repressed in the *elo3*Δ cells. *LCB1* encodes serine palmitoyltransferasae, the first step enzyme in sphingolipid biosynthesis, and repression causes reductions in all sphingolipid levels (Fig. 1A) (*5*). *LIP1* encodes the subunit of ceramide synthase, and repression causes reductions in both the Cer and complex sphingolipid levels (Fig. 1A) (*5*). We used a mutant strain that carries the Tet promoter-regulated *LCB1* gene (*tet-LCB1*) or *LIP1* gene (*tet-LIP1*). Figure 3A shows the [^{3}H]*myo*-inositol labelling of *tet-LCB1* and *tet-LIP1* cells in the presence or absence of Dox. When *tet-LCB1* cells were pulse-radiolabelled after 8-h treatment with Dox, the complex sphingolipid levels were dramaticaly decreased. In contrast, *tet-LIP1* cells showed only slight decreases in the complex sphingolipid levels after 8-h treatment with Dox; however, significant decreases in the complex sphingolipid levels were observed when *tet-LIP1* cells were treated with Dox overnight (Fig. 3A). Both Dox-treated *tet-LIP1* and *tet-LIP1 elo3*Δ cells can grow in the presence of Dox, but the growth of Dox-treated *tet-LIP1 elo3*Δ

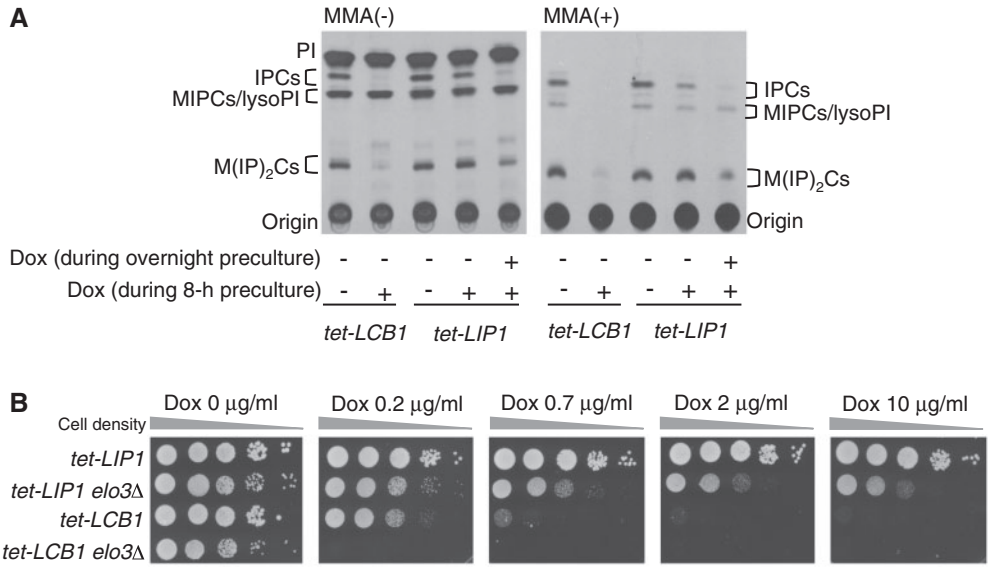

Fig. 3 Cell growth under *LIP1*- or *LCB1*-repressive conditions. (A) [^{3}H]*myo*-inositol labelling of *tet-LIP1* and *tet-LCB1* cells. Cells were cultured overnight in YPD medium with or without 10 μg/ml Dox, diluted (0.1 A_{600} U/ml) in fresh YPD medium with or without 10 μg/ml Dox, incubated for 8 h, and then labelled with [^{3}H]*myo*-inositol for 1 h. Radiolabelled lipids were extracted, treated with MMA, and then separated by TLC. The details are given under 'Materials and Methods' section. (B) Cells were cultured overnight in YPD medium, and then spotted onto YPD plates with or without the indicated concentrations of Dox in 10-fold serial dilutions starting with a density of 0.7 A_{600} U/ml.

cells was significantly inhibited (Fig. 3B). It was previously reported that *elo3*Δ cells show high sensitivity to Australifungin, an inhibitor of Cer synthase (*18*), which well coincides with the results of the present study. *tet-LCB1* cells showed a strong growth defect in the presence of Dox, and the deletion of *ELO3* caused a more severe growth defect (Fig. 3B). Taken together, these results suggested that the reductions in sphingolipid levels including those of complex sphingolipids cause the growth defect of *elo3*Δ cells.

Accumulation of LCB and LCBP is not involved in resistance of elo3*Δ cells to* AUR1 *repression*

In *elo3*Δ cells, LCB and LCBP are highly accumulated (*18*). Since both lysosphingolipids exhibit various bioactivities, such as regulation of the cell cycle and stress responses (*3*, *5*), we next investigated whether or not the accumulation of LCBs or LCBP affects the growth of Dox-treated *tet-AUR1* and *tet-AUR1 elo3*Δ cells. To investigate the effect of accumulation of LCB under *AUR1*-repressive conditions, Dox-treated *tet-AUR1* and *tet-AUR1 elo3*Δ cells were cultured in the presence of PHS, a major LCB in yeast; however, exogenously added PHS did not complement the growth defect of Dox-treated *tet-AUR1* cells (Fig. 4A). To investigate the effect of accumulation of LCBP, *LCB4*, encoding major LCB kinase (*5*), was deleted. As shown in Fig. 4B, the deletion of *LCB4* resulted in dramatic reductions in the LCBP levels in both *tet-AUR1* and *tet-AUR1 elo3*Δ cells, but did not affect the resistance of *elo3*Δ cells to *AUR1* repression (Fig. 4C). These results indicated that the accumulation of LCB and LCBP in *ELO3*-deleted cells is not related to the resistance to growth inhibition caused by *AUR1* repression.

Discussion

In the present study, it was found that the *ELO3* mutation confers resistance to IPC synthase inhibitor AbA. This resistance was also confirmed by repression of the expression of *AUR1* by the Tet promoter. Under *AUR1*-repressive conditions, the *ELO3* mutant showed reductions in complex sphingolipid levels and accumulation of Cer-C, like the wild-type cells. In contrast, with repression of either *LCB1* or *LIP1*, both of which cause reduction in complex sphingolipid levels, the *ELO3* mutation did not confer resistance to growth inhibition induced by impaired sphingolipid biosynthesis. Therefore, it was suggested that the *ELO3* mutant shows resistance as to Cer accumulation. The *ELO3* mutation causes a defect of the synthesis of VFCFAs, and, in particular, complete loss of the synthesis of C26 fatty acids, which are the primary constituent of yeast sphingolipids (*8*). Thus, it is implied that Cers with C26 fatty acids are more toxic than those with C24 or shorter fatty acids under *AUR1*-repressive conditions.

Over the past few decades, Cers have been suggested to be intracellular lipid mediators that regulate various signal transduction systems involved in cell differentiation, proliferation and apoptosis in eukaryotes ranging from yeast to mammals (*3*, *19*). To investigate the function of Cers, one of the most popular approaches is the addition of exogenous Cers to cells (*20*). Since natural Cers, which mainly contain fatty acids with carbon chain lengths of more than 14, are not water-soluble and are incorporated into cells with difficulty, synthetic short-chain Cers, particularly C2- and C6-Cers, which are water-soluble and membrane-permeable, are used widely as experimental tools. These short-chain Cer analogues are believed to be able to mimic endogenous Cers, but they often cause

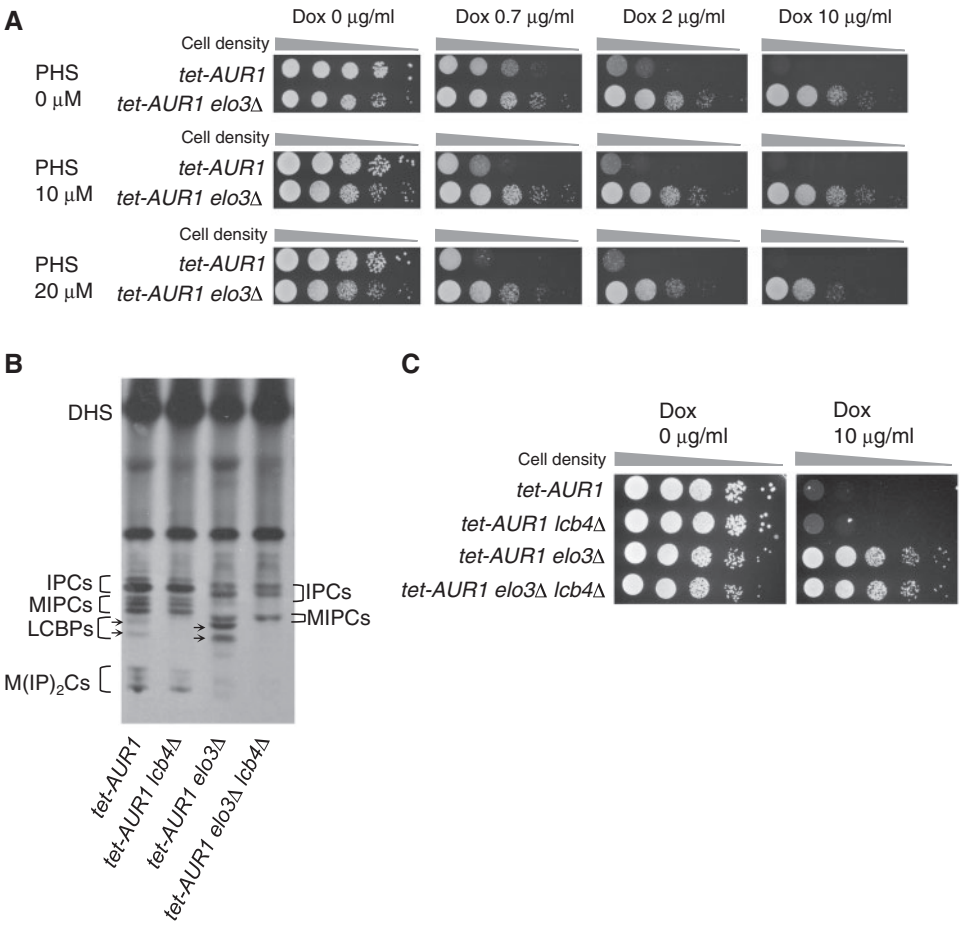

Fig. 4 Effects of exogenously added PHS and depletion of LCBP on the growth of Dox-treated *tet-AUR1* and *tet-AUR1 elo3*Δ cells. (A) Effects of exogenously added PHS. Cells were cultured overnight in YPD medium, and then spotted onto YPD plates with or without 10 or 20 μM PHS and the indicated concentrations of Dox in 10-fold serial dilutions starting with a density of 0.7 A_{600} U/ml. Nonidet P-40 (0.0015%) was also included in the plates as a dispersant. The plates were incubated at 30°C and photographed after 2 days. (B) [^{3}H]DHS labelling of *LCB4*-deleted cells. Cells were cultured overnight in YPD medium, diluted (0.1 A_{600} U/ml) in fresh YPD medium, incubated for 5 h, and then labelled with [^{3}H]DHS for 1 h. Radiolabelled lipids were extracted, treated with MMA, and then separated by TLC. (C) Effects of the deletion of *LCB4* on Dox-treated *tet-AUR1* and *tet-AUR1 elo3*Δ cells. Cells were cultured overnight in YPD medium, and then spotted onto YPD plates with or without 10 μg/ml Dox in 10-fold serial dilutions starting with a density of 0.7 A_{600} U/ml. The details are given under 'Materials and Methods' section.

artificial effects due to the large differences in fatty acid chain length (*20*). In contrast, analysis of Cer-metabolizing enzymes have revealed that endogenous Cers with different fatty acid chain lengths might play distinct functions. For instance, overexpression and siRNA experiments on neutral sphingomyelinase 2, one of the key enzymes for Cer generation, suggested that C24 and C24:1-Cers, but not C16-Cer, are putative key regulators of confluence-induced growth arrest of MCF7 cells (*21*). Mammalian cells have six Cer synthases with different substrate specificities, which determines the structural diversity of the fatty acid portion of Cers (*3*). In head and neck squamous cell carcinomas, generation of C18-Cer by Cer synthase 1 inhibits the tumour progression, whereas that of C16-Cer by Cer synthase 6 prevents apoptosis of the tumour cells (*22*). The question arises as to why Cers with different fatty acid chain lengths have different effects on the cellular functions. It has been proposed that Cers directly regulate the activities of proteins involved in signal transduction systems, such as protein phosphatase 2A, protein phosphatase 1, cathepsin D and protein kinase Cζ, etc. (*3*). It is possible that the structural specificity of Cers affects the protein–lipid interaction.

In summary, the results of this study revealed that a defect of the synthesis of VLCFAs confers resistance to growth inhibition by *AUR1* repression in yeast. Further study involving mechanistic analysis as to this observation will provide a new insight into Cer-mediated cellular functions.

Supplementary Data

Supplementary Data are available at *JB* Online.

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Conflict of interest
None declared.

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