In mammalian cells, intracellular sphingosine 1-phosphate (SIP) can stimulate calcium release from intracellular organelles, resulting in the activation of downstream signaling pathways. The budding yeast Saccharomyces cerevisiae expresses enzymes that can synthesize and degrade SIP and related molecules, but their possible role in calcium signaling has not yet been tested. Here we examine the effects of SIP accumulation on calcium signaling using a variety of yeast mutants. Treatment of yeast cells with exogenous sphingosine stimulated Ca\(^{2+}\) accumulation through two distinct pathways. The first pathway required the Cch1p and Mid1p subunits of a Ca\(^{2+}\) influx channel, dependent upon the function of sphingosine kinases (Lcb4p and Lcb5p), and was inhibited by the functions of SIP lyase (Dpl1p) and the SIP phosphatase (Lcb3p). The biologically inactive stereoisomer of sphingosine did not activate this Ca\(^{2+}\) influx pathway, suggesting that the active SIP isomer specifically stimulates a calcium-signaling mechanism in yeast. The second Ca\(^{2+}\) influx pathway stimulated by the addition of sphingosine was not stereospecific, was not dependent on the sphingosine kinases, occurred only at higher doses of added sphingosine, and therefore was likely to be nonspecific. Mutants lacking both SIP lyase and phosphatase (dpl1 lcb3 double mutants) exhibited constitutively high Ca\(^{2+}\) accumulation and signaling in the absence of added sphingosine, and these effects were dependent on the sphingosine kinases. These results show that endogenous SIP-related molecules can also trigger Ca\(^{2+}\) accumulation and signaling. Several stimuli previously shown to evoke calcium signaling in wild-type cells were examined in lcb4 lcb5 double mutants. All of the stimuli produced calcium signals independent of sphingosine kinase activity, suggesting that phosphorylated sphingoid bases might serve as messengers of calcium signaling in yeast during an unknown cellular response.

Sphingolipid metabolites, such as ceramide, sphingosine, and SIP,\(^1\) function as important second messengers in mammalian cells mediating processes such as cell proliferation and motility, differentiation, senescence, stress responses, and apoptosis (1). SIP accumulates in response to various physiological stimuli in mammals. In RBL-2H3 cells, for example, the clustering of the IgE receptor FcεRI activates sphingosine kinase, resulting in SIP production. Inhibitors of sphingosine kinases block the agonist-stimulated accumulation of SIP and also suppress the normal mobilization of Ca\(^{2+}\) stored in the endoplasmic reticulum (2). In permeabilized cells, SIP also triggers the release of Ca\(^{2+}\) through a mechanism independent of the known Ca\(^{2+}\) release pathways, suggesting that SIP activates a novel type of a Ca\(^{2+}\) release channel (3–5) capable of elevating cytosolic-free Ca\(^{2+}\) concentrations ([Ca\(^{2+}\)]\(_i\)) and stimulating capacitative Ca\(^{2+}\) entry (CCE) mechanisms. The hypothetical intracellular SIP receptor and/or Ca\(^{2+}\) release channel has not yet been identified.

Sphingolipids are abundant components of the plasma membrane in yeast, comprising 30% of total membrane phospholipids (6). They differ slightly from mammalian sphingolipids, using a derivative of sphingosine known as phytosphingosine. The enzymes in yeast responsible for phosphorylation of endogenous phytosphingosine and exogenous long chain bases such as sphingosine have recently been identified. Two related sphingosine kinases were identified in yeast as the products of the LCB4 and LCB5 genes (7, 8). Mutants lacking both sphingosine kinases accumulate no detectable SIP-related molecules but, nevertheless, are viable and exhibit no obvious phenotypes (7, 9). Yeast also expresses two related SIP phosphatases encoded by the LCB3/YSR2 and YSR3 genes, the former being the major enzyme (10–12). Additionally, yeast cells express SIP lyase encoded by DPL1 (formerly BST1), which cleaves SIP to yield ethanolamine-1-phosphate and hexadecanal (13). Mutants lacking SIP lyase (dpl1/bst1 mutants) accumulate phytos-SIP and dihydro-SIP at slightly elevated levels (9, 14) and reach maximal SIP accumulation levels within 60 min of the addition of sphingosine to the culture medium (13). These effects are further exacerbated in dpl1 lcb3 double mutants lacking both SIP lyase and the major SIP phosphatase with 500 times greater levels of phyto-SIP and dihydro-SIP relative to wild type (7, 14). Although the functions of SIPs in yeast are largely unknown, recent evidence suggests a role for these lipids in resistance to heat stress in the regulation of cell proliferation and in the shift from fermentative to respiratory growth (8, 14–16). The possibility that SIPs regulate calcium signaling in yeast cells has not been examined.

In yeast, calcium signals are generated in response to a cytosolic-free Ca\(^{2+}\) concentration; CCE, capacitative calcium entry; SC, synthetic complete; IP\(_3\), inositol 1,4,5-trisphosphate; YPD, yeast extract/peptone/dextrose; BAPTA, 1,2-bis(2-aminophenoxy)ethane-N, N', N'-tetraacetic acid.
variety of external stimuli including mating pheromones, salt stress, glucose-1-phosphate accumulation, and depletion of Ca\(^{2+}\) from secretory organelles (17–21). Yeast expresses a Ca\(^{2+}\) influx channel related to voltage-gated Ca\(^{2+}\) channels of animals (22–24) in addition to various intracellular Ca\(^{2+}\) pumps and exchangers related to animal enzymes (25–29). However, homologs of the sarcoendoplasmic reticulum calcium ATPase family (21, 29) are not evident in the yeast genome. Nevertheless, the yeast endoplasmic reticulum accumulates sufficient Ca\(^{2+}\) to facilitate protein folding and secretion, in part through Pmr1p, a member of the secretory pathway calcium ATPase family (21, 29). Sequences of the mammalian S1P-receptor involved in Ca\(^{2+}\) release from microsomes have not been reported.

Here we show that conversion of exogenously added sphingosine to sphingosine 1-phosphate stimulates Ca\(^{2+}\) influx, accumulation, and signaling in yeast. Similar to the CCE-like mechanism of yeast (15), the calcium channel subunit Cch1p was required for the majority of S1P-stimulated Ca\(^{2+}\) accumulation. Therefore, yeast may retain S1P-regulated calcium-signaling mechanisms analogous to those of mammalian cells.

**MATERIALS AND METHODS**

**Yeast Strains and Growth Media**—All yeast strains (Table I) were maintained on either YPD medium or synthetic complete medium (SC) lacking leucine or uracil. Strains of the JK9-3d background CBY31, CBY32, CBY33, and CBY34 were constructed from MSS200, MSS204, MSS205, and MSS207 (14), respectively, by curing the leucine or uracil-lysing 45Ca\(^{2+}\) of 1–2, and then diluted 2-fold into medium supplemented with 12.5 \(\mu\)M sphingosine, 10 mM BAPTA, and/or 5 \(\mu\)g/ml cycloheximide. The resulting luminescence was measured at intervals for 2–3 h. Cells were subsequently permeabilized with 250 \(\mu\)M digitonin, and the luminescence was recorded to standardize for aequorin loading between strains.

**β-Galactosidase Assays**—Cells expressing pCK190 or pDM5 (26, 33) were grown to log phase overnight at 30 °C in SC medium lacking uracil. Cultures were harvested and resuspended to a final A\(_{600}\) of 1 in 2 ml of fresh SC lacking uracil or YPD medium supplemented with sphingosine, NaCl, o-mating factor, and/or FK506 as noted in the text. Cells were incubated with shakting at 30 °C for 3–4 h before assaying for β-galactosidase activity as described previously (34).

**RESULTS**

Sphingosine 1-Phosphate Stimulates Calcium Influx and Signaling in Yeast—Exogenous sphingosine added to culture medium can be taken up by yeast cells and phosphorylated to S1P by sphingosine kinases (13). S1P can be dephosphorylated by the phosphatase Leb\(_3\)p or degraded by the lyase Dpl1p (11–13). To determine whether S1P accumulation can evoke calcium signaling in yeast, we first monitored the accumulation of 45Ca\(^{2+}\) from the medium into growing yeast cells treated with a wide range of exogenous sphingosine. The addition of sphingosine to the culture medium at concentrations >25 \(\mu\)M stimulated 45Ca\(^{2+}\) accumulation in wild-type yeast strains up to 2× the basal level (Fig. 1A). Mutants lacking the S1P phosphatase (leb\(_3\)p mutants) were indistinguishable from wild type in this assay. In contrast, dpl1 mutants lacking S1P lyase accumulated 5-fold higher amounts of 45Ca\(^{2+}\) after treatment with only 10–15 \(\mu\)M sphingosine. These results show that exogenous sphingosine can stimulate Ca\(^{2+}\) accumulation and that this response can be inhibited by the S1P lyase Dpl1p.

If phosphorylation of exogenous sphingosine was required to promote Ca\(^{2+}\) influx, mutants lacking the sphingosine kinases would exhibit less Ca\(^{2+}\) accumulation after treatment with sphingosine. Indeed, the 5-fold increase in 45Ca\(^{2+}\) accumulation seen in a dpl1 mutant at 12.5 \(\mu\)M sphingosine was abolished in a dpl1 leb\(_3\)p leb\(_5\) triple mutant (Fig. 1B, left), indicating that this dramatic increase in Ca\(^{2+}\) accumulation was dependent on the S1P produced by Leb\(_4\)p and Leb\(_5\)p. However, the leb\(_4\) leb\(_5\) double mutants exhibited wild-type sensitivity to sphingosine (Fig. 1B, right). Thus, exogenous sphingosine pro-

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**Table I**

List of yeast strains used in this study

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<th>Strain</th>
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<th>Source</th>
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<td>MAT_a leu2-3,112 ura3-52 his4 trpl1 me1::URA3</td>
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duced two separable calcium responses in yeast, one that was relatively small and independent of sphingosine kinases and another larger response that was dependent on sphingosine kinases and sensitive to S1P lyase and phosphatase. All future experiments will examine the properties of the latter S1P-specific response, which is prominent in dpl1 mutants.

Sphingosine kinases typically phosphorylate the natural D-isomer of sphingosine and are unable to act on the L-isomer (8). The addition of L-sphingosine to yeast cultures stimulated 45Ca2+ accumulation in dpl1 mutants only at very high concentrations similar to those effective in dpl1 lcb4 lcb5 triple mutants (Fig. 1C). Thus, L-sphingosine failed to stimulate Ca2+ accumulation through the pathway involving sphingosine kinases even in the supersensitive dpl1 strain. The results confirm the existence of two separable responses to added sphingosine, one that is not stereospecific or dependent on sphingosine kinases and one that is specific for the biologically active D-isomer of sphingosine, dependent on sphingosine kinases, and sensitive to S1P lyase and phosphatase.

Yeast and other fungi synthesize phytosphingosine rather than sphingosine, as well as its precursor dihydrosphingosine. These molecules differ in the level of saturation and hydroxylation at C-4 (35). Exogenous phytosphingosine and dihydrosphingosine stimulated the nonspecific Ca2+ response much like sphingosine but only weakly stimulated the specific Ca2+ response in dpl1 mutants (Fig. 2A). The weaker effects of exogenous phytosphingosine and dihydrosphingosine might be explained if they are poorer substrates than sphingosine for the kinases (7, 8) or if their phosphorylated products are better substrates than S1P for Lcb3p.

The lcb3 single mutants accumulate approximately 10-fold higher levels of phyto-S1P and dihydro-S1P than wild type during vegetative growth (9, 14), but they do not accumulate more 45Ca2+ than wild type with or without added sphingosine (Fig. 1A). Therefore, a higher threshold level of the native S1Ps may be necessary to stimulate Ca2+ influx. To evaluate the possible roles of phyto-S1P and dihydro-S1P more carefully, we

FIG. 1. Sphingosine 1-phosphate accumulation specifically stimulates calcium accumulation. A, wild-type strains and strains lacking either S1P lyase (dpl1), S1P phosphatase (lcb3) or both were treated with sphingosine concentrations ranging from 0 to 30 μM. Total cell-associated 45Ca2+ was quantitated after a 3-h incubation period. B, strains lacking S1P lyase (dpl1), sphingosine kinases (lcb4 lcb5), or both (dpl1 lcb4 lcb5) were treated with sphingosine and assayed for 45Ca2+ accumulation as described in A. Results are depicted on separate graphs for clarity. C, the responses of dpl1 mutants and dpl1 lcb4 lcb5 mutants to D-sphingosine and L-sphingosine were compared using the 45Ca2+ accumulation assay. Cells were treated with D-sphingosine (D-Sph), L-sphingosine (L-Sph), or ethanol (control) and processed as described above.

FIG. 2. Endogenous S1P-related molecules stimulate Ca2+ accumulation. A, strains lacking S1P lyase (dpl1), S1P kinases (lcb4 lcb5), or both were treated with sphingosine, dihydrosphingosine, or phytosphingosine and assayed for 45Ca2+ accumulation as described earlier. Calcium accumulation in kinase-deficient strains was subtracted from those in which the kinases were present to normalize for kinase-dependent calcium accumulation. B, wild type (WT) and dpl1 lcb3 double mutant strains and the corresponding sphingosine kinase-deficient strains were placed in SC medium containing 45Ca2+, and samples of each were harvested at 6-min intervals for 30 min. Total cell-associated calcium was determined, and the slopes of each estimated the line used to determine the rates of calcium accumulation for each strain.
greater sensitivity to added sphingosine than the absence of added sphingolipids and also showed even higher levels of phyto-S1P and dihydro-S1P than wild type (14). These results confirm that accumulation of S1P-related molecules native to yeast can stimulate \(\text{Ca}^{2+}\) influx and accumulation and suggest that Dpl1p inhibits the response more potently than Lcb3p.

**SIP-stimulated \(\text{Ca}^{2+}\) Accumulation Involves the Cch1p-dependent Channel**—Cch1p was identified previously as the probable pore-forming subunit of a plasma membrane \(\text{Ca}^{2+}\) influx channel that is activated by a variety of stimuli (22, 23). To determine whether SIP stimulates Cch1p activity, we monitored \(\text{Ca}^{2+}\) accumulation in a dpl1 mutant and a cch1 dpl1 double mutant after the addition of sphingosine. The dpl1 single mutant displayed sensitivity to sphingosine, which was similar to that of the dpl1 single mutant, but the maximal level of \(\text{45Ca}^{2+}\) accumulation was lower in the cch1 dpl1 double mutant than in the dpl1 single mutant (Fig. 3, A and B). The residual effect of sphingosine in the cch1 dpl1 double mutant was not observed in cch1 dpl1 lc44 lc65 quadruple mutants. Therefore, the Cch1p channel was required for the major component of the SIP-stimulated \(\text{45Ca}^{2+}\) accumulation under these conditions.

**SIP Accumulation Elevates [\(\text{Ca}^{2+}\)]c and Activates Calcineurin-signaling Pathways**—The kinetics of the SIP-specific response were monitored using cells expressing the calcium-sensitive photoprotein aequorin in the cytoplasm. Treatment of a dpl1 mutant with 12.5 \(\mu\)M sphingosine produced a detectable increase in aequorin luminescence within 60–80 min of treatment that rose sharply and reached a plateau for at least 1 h (Fig. 4, A and B) (data not shown). Treatment of a dpl1 lc44 lc65 triple mutant in a parallel experiment resulted in little aequorin luminescence over this time frame, indicating that SIP accumulation elevates cytosolic-free \(\text{Ca}^{2+}\) ([\(\text{Ca}^{2+}\)]c) primarily via the specific pathway. The protein synthesis inhibitor cycloheximide completely abolished the response of dpl1 mutants to sphingosine (Fig. 4B), suggesting that protein synthesis was necessary for \(\text{Ca}^{2+}\) influx and [\(\text{Ca}^{2+}\)]c elevation in response to sphingosine. Aequorin luminescence in dpl1 mutants was also abolished by the addition of the \(\text{Ca}^{2+}\) ion chelator BAPTA to the culture medium, suggesting that the [\(\text{Ca}^{2+}\)]c elevation observed was the result of extracellular \(\text{Ca}^{2+}\) influx and not intracellular \(\text{Ca}^{2+}\) release (Fig. 4A). In summary, the specific response to SIP-stimulated \(\text{Ca}^{2+}\) influx through Cch1p and elevated [\(\text{Ca}^{2+}\)]c.

To determine whether [\(\text{Ca}^{2+}\)]c elevation in response to SIP could activate downstream signaling pathways, the expression of a calcineurin-dependent reporter gene PMC1-lacZ was quantified.

![Fig. 3. SIP stimulates \(\text{Ca}^{2+}\) accumulation via Cch1p-dependent and Cch1p-independent pathways.](image)

![Fig. 4. SIP accumulation elevates [\(\text{Ca}^{2+}\)]c.](image)
Calcium Influx and Signaling by Intracellular S1P Accumulation

Do S1Ps Serve as Second Messengers for Calcium Signaling?—Several external stimuli lead to the generation of calcium signals in yeast. Although none of these stimuli has been shown to affect metabolism of S1P-related molecules, it is conceivable that one or more of these stimuli causes increased accumulation of S1Ps, which then triggers calcium signaling. To test whether endogenous S1P-related molecules are required for generating calcium signals in response to known physiological stimuli, we compared the calcium responses of wild type or dpl1 mutant cells to those of lcb4 lcb5 or dpl1 lcb4 lcb5 mutant cells lacking sphingosine kinases over a wide range of conditions. The first stimulus tested, α-mating factor, triggers Ca²⁺ influx and signaling after a time lag similar to that of sphingosine (18). We found that α-mating factor stimulated Ca²⁺ influx and calcineurin-dependent induction of PMC1-lacZ in lcb4 lcb5 double mutants to the same degree as in wild type (Fig. 6A) (data not shown). Thus, the sphingosine kinases (and presumably their products) were not required for Ca²⁺ signaling invoked in response to α-mating factor. The second stimulus tested, high salt, also induced the calcineurin-dependent expression of PMC1-lacZ to an equal extent in wild type and in lcb4 lcb5 double mutants (Fig. 6B). Next, the acute heat shock produced by shifting cells grown at 25–39 °C stimulated a transient elevation of [Ca²⁺], in both dpl1 and dpl1 lcb4 lcb5 mutants as detected by aequorin luminescence (Fig. 6C). Hypotonic shock produced by diluting cells grown in standard medium with hypotonic medium (36) also stimulated aequorin luminescence in both wild type and lcb4 lcb5 double mutants (data not shown). Depletion of Ca²⁺ from secretory organelles using pmr1 mutants was shown to stimulate Ca²⁺ accumulation in yeast (20, 21, 24). However, 45Ca²⁺ accumulation in pmr1 lcb4 lcb5 triple mutants was similar to that of pmr1 mutants (Fig. 6D). Chlorpromazine treatment stimulates Ca²⁺ influx and accumulation in wild-type cells (37) and to an equal degree in lcb4 lcb5 double mutants (Fig. 6E). Finally, Ca²⁺ accumulation in pgm2 mutants stimulated by growth in galactose medium (17) also occurred in pgm2 lcb4 lcb5 triple mutants (Fig. 6F). In summary, the sphingosine kinases Lcb4p and Lcb5p were not required for calcium-signaling events triggered by any of the seven known stimuli. Therefore, it appears that the upstream stimulus for S1P signaling in yeast defines a novel event in yeast calcium signaling.

**DISCUSSION**

The results reported here suggest that accumulation of S1P or related molecules in yeast can stimulate Ca²⁺ influx via Cch1p and other factors, resulting in the elevation of [Ca²⁺]c and activation of calcineurin signaling. Several conditions shown previously to accumulate S1P or the native derivatives phyto-S1P and dihydro-S1P were found to stimulate Ca²⁺ influx and signaling in a manner requiring the homologous sphingosine kinases Lcb4p and Lcb5p. Dpl1p, the S1P lyase, potently blocked the Ca²⁺ influx responses with or without Lcb5p, the major S1P phosphatase. Lcb5p was less effective in this regard and only significant in dpl1 mutants, possibly because of the activity of Ysr3p, a minor S1P phosphatase homologous to Lcb3p (11). The direct targets or derivatives of S1P that lead to Ca²⁺ influx and signaling have not been determined. However, the 1-h lag time after the addition of sphingosine observed before the onset of Ca²⁺ influx and the sensitivity to cycloheximide suggests that the response might reflect the time necessary for expressing new proteins involved in

![Fig. 5. S1P accumulation causes calcineurin-dependent induction of PMC1-lacZ expression.](image-url)

A. Various strains carrying the calcineurin-dependent reporter gene PMC1-lacZ were grown in SC lacking uracil medium at 30 °C and treated with varying concentrations of sphingosine (A) or 12.5 μM sphingosine (B) in the presence or absence of FK506 or BAPTA as indicated. After a 3-h incubation at 30 °C, expression of the PMC1-lacZ reporter gene was determined as described above, except cultures were grown in YPD medium lacking sphingosine but supplemented with 10 mM CaCl₂ and FK506 as indicated.

B. PMC1-lacZ expression induced by wild type and dpl1 lcb4 lcb5 double mutants (Fig. 6A) (data not shown). Thus, the sphingosine kinases (and presumably their products) were not required for Ca²⁺ signaling invoked in response to α-mating factor. The second stimulus tested, high salt, also induced the calcineurin-dependent expression of PMC1-lacZ to an equal extent in wild type and in lcb4 lcb5 double mutants (Fig. 6B). Next, the acute heat shock produced by shifting cells grown at 25–39 °C stimulated a transient elevation of [Ca²⁺]c, in both dpl1 and dpl1 lcb4 lcb5 mutants as detected by aequorin luminescence (Fig. 6C). Hypotonic shock produced by diluting cells grown in standard medium with hypotonic medium (36) also stimulated aequorin luminescence in both wild type and lcb4 lcb5 double mutants (data not shown). Depletion of Ca²⁺ from secretory organelles using pmr1 mutants was shown to stimulate Ca²⁺ accumulation in yeast (20, 21, 24). However, 45Ca²⁺ accumulation in pmr1 lcb4 lcb5 triple mutants was similar to that of pmr1 mutants (Fig. 6D). Chlorpromazine treatment stimulates Ca²⁺ influx and accumulation in wild-type cells (37) and to an equal degree in lcb4 lcb5 double mutants (Fig. 6E). Finally, Ca²⁺ accumulation in pgm2 mutants stimulated by growth in galactose medium (17) also occurred in pgm2 lcb4 lcb5 triple mutants (Fig. 6F). In summary, the sphingosine kinases Lcb4p and Lcb5p were not required for calcium-signaling events triggered by any of the seven known stimuli. Therefore, it appears that the upstream stimulus for S1P signaling in yeast defines a novel event in yeast calcium signaling.
Ca\(^{2+}\) influx and sufficient buildup of S1P. In the presence of extracellular Ca\(^{2+}\) chelators such as BAPTA, sphingosine was completely unable to elevate \([\text{Ca}^{2+}]_c\) (Fig. 4A). Therefore, no evidence for S1P-triggered Ca\(^{2+}\) release was obtained in yeast thus far.

Exogenous D-sphingosine also stimulated Ca\(^{2+}\) accumulation to a smaller degree independent of the sphingosine kinases Lcb4p and Lcb5p. This kinase-independent response may be nonphysiological because a similar effect was detected using the biologically inactive isomerL-sphingosine and because of the very high doses necessary to achieve the response. We have noticed similar responses of yeast cells to low levels of other membrane active compounds such as lyso-phosphatidic acid and detergents.\(^3\)

Unlike these nonspecific effects, the involvement of the Ca\(^{2+}\) channel protein Chh1p and the requirement for S1P biosynthesis and accumulation underscore the existence of specific mechanism for S1P-dependent calcium signaling in yeast. Chh1p activity was stimulated in \(\text{pmr1}\) mutants (lacking the secretory pathway Ca\(^{2+}\) ATPase) upon depletion of Ca\(^{2+}\) from secretory organelles (24). Treatment of mammalian cells with thapsigargin, a specific inhibitor of sarcoendoplasmic reticulum calcium ATPase that rapidly depletes Ca\(^{2+}\) from the endoplasmic reticulum and stimulates CCE mechanisms, was shown to stimulate sphingosine kinase activity (38). However, it seems improbable that accumulation of S1Ps serves as a messenger of CCE in yeast cells, because \(\text{pmr1 lcb4 lcb5}\) triple mutants lacking the sphingosine kinases exhibited as much Ca\(^{2+}\) accumulation as \(\text{pmr1}\) mutants (Fig. 6D). Additionally, the injection of S1P in mammalian RBL cells failed to stimulate the CCE channel known as I\(_{\text{CRAC}}\), and inhibitors of sphingosine kinase failed to prevent I\(_{\text{CRAC}}\) activation by thapsigargin (39). A more reasonable explanation is that accumulation of S1Ps stimu-

\(^3\) C. J. Birchwood, J. D. Saba, and K. W. Cunningham, unpublished observations.
lates CCE in animal and fungal cells by first triggering Ca\textsuperscript{2+} release from secretory organelles.

SIP rapidly stimulates Ca\textsuperscript{2+} release from the endoplasmic reticulum of mammalian cells followed by stimulation of Ca\textsuperscript{2+} influx at the plasma membrane and signaling (3, 5). The receptor for SIP has not been identified, but evidence suggests the Ca\textsuperscript{2+} release channel in the endoplasmic reticulum is distinct from the well characterized IP\textsubscript{3} receptor and ryanodine receptor (25). All these routes of Ca\textsuperscript{2+} release can rapidly deplete the endoplasmic reticulum of Ca\textsuperscript{2+} and activate CCE pathways. The yeast genome lacks sequences orthologous to the IP\textsubscript{3} and ryanodine receptors, but nevertheless, yeast cells may utilize a mechanism resembling CCE to supply Ca\textsuperscript{2+} to secretory organelles (24). Our data do not rule out the possibility that SIP activates a new class of Ca\textsuperscript{2+} release channels in yeast that is potentially related to the unidentified SIP-receptor in animal cells. Support for this hypothesis might be obtained through the identification of new factors required for SIP-stimulated Ca\textsuperscript{2+} signaling in yeast.

What is the purpose of SIP-stimulated Ca\textsuperscript{2+} influx and signaling in yeast? That yeast would encounter either high sphingosine environments or conditions inactivating both SIP lyase and phosphatase seems improbable. Therefore, we examined a number of previously described stimuli that lead to Ca\textsuperscript{2+} signaling in wild type and in \textit{lcb4 lcb5} double mutants and found no evidence for the involvement of SIP or its derivatives in any of the processes. It is possible that a significant contribution of S1P to Ca\textsuperscript{2+} signaling was masked by the action of functionally redundant pathways, similar to what has been previously proposed for the sphingosine kinases in the heat stress response (35). Alternatively, an untested stimulus may be found responsible for the stimulation of calcium influx and signaling by SIP (23). S1Ps to Ca\textsuperscript{2+} signaling is a novel calcium-signaling pathway in yeast that is, thus far, potentially activated by undiscovered stimuli.

Acknowledgments—We thank David Bedwell for yeast strains and plasmids and Fujisawa USA, Inc., for the gift of FK506.

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