In fission yeast, the endoplasmic reticulum membrane-bound proteins Sre1 and Scp1, orthologs of mammalian sterol regulatory element binding protein (SREBP) and Scap, monitor sterol synthesis as an indirect measure of oxygen supply. When cellular oxygen levels are low, sterol synthesis is inhibited, and the Sre1-Scp1 complex responds by increasing transcription of genes required for adaptation to hypoxia. Scap senses the condition is clear. Here, we demonstrate that Sre1-Scp1 senses ergosterol. Processing experimental data with a mathematical model of Sre1 and Scp1 function reveals a clear quantitative relationship between ergosterol concentration in the endoplasmic reticulum and Sre1 activation. Based on this relationship, we predict that the Sre1-Scp1 complex exists under “active” and “inactive” states and that the transition between these states is cooperatively mediated by ergosterol.

Mammalian cholesterol synthesis is a tightly controlled process regulated by negative feedback inhibition (1). The central components of this process are two endoplasmic reticulum (ER)-resident integral membrane proteins, SREBP and its binding partner Scap. SREBP is a membrane-bound transcription factor that, when proteolytically activated, enters the nucleus and induces transcription of genes required to increase intracellular cholesterol levels (2). Regulated SREBP activation requires both Scap and a third ER membrane protein, Insig. Scap senses ER cholesterol concentration by directly binding cholesterol in the membrane (3), and cholesterol binding induces a conformational change in Scap that promotes binding to Insig, retaining the SREBP-Scap complex in the ER (4, 5). When cholesterol is depleted, SREBP-Scap dissociates from Insig and can then be transported to the Golgi apparatus (4, 6). The N-terminal transcription factor domain of SREBP is liberated from the membrane by two Golgi-resident proteases, allowing SREBP to enter the nucleus and activate transcription of its target genes (2). Activation of SREBP target genes ultimately restores ER cholesterol, which in turn promotes Scap-Insig binding, thus blocking further ER-to-Golgi transport of SREBP-Scap and completing a negative feedback loop.

Studies of SREBP in the genetically tractable fission yeast Schizosaccharomyces pombe revealed that this mammalian sterol-sensing mechanism is conserved and that SREBP functions as a principal hypoxic transcription factor in fungi (7). Importantly, in the pathogenic fungi Cryptococcus neoformans and Aspergillus fumigatus, SREBP is required for virulence in fungal disease models (8–10). Fission yeast has homologs of SREBP and Scap called Sre1 and Scp1, respectively (11). The Sre1-Scp1 complex monitors synthesis of ergosterol, the fungal equivalent of cholesterol, as an indirect measure of oxygen supply. When cellular oxygen levels are low, oxygen-dependent sterol synthesis is inhibited, and Sre1-Scp1 responds by increasing transcription of genes required for adaptation to hypoxia, including enzymes involved in ergosterol biosynthesis.

Given the broad conservation, importance for fungal disease, and detailed knowledge of SREBP pathway components and function, we seek to create a mathematical model of this sterol regulatory system. As a first step, we have focused on the fission yeast Sre1 pathway. A comprehensive mathematical model would enable a thorough systems-level analysis of this pathway, including quantifying the contributions of its different parts to overall performance and evaluating the system’s robustness to mutations (12, 13). Because of the conserved structure and function between the components of the mammalian and yeast SREBP pathways, such a model will likely also shed light on the process of cholesterol regulation in mammals.

The Sre1 pathway can be divided conceptually into three regulatory steps as follows: Sre1 transport and cleavage, Sre1 activity in the nucleus, and ergosterol biosynthesis. As an initial step in modeling this pathway, we chose to investigate quantitatively the mechanism by which sterols control the rate of ER-Golgi transport and cleavage of Sre1-Scp1. Although mammalian cells respond to the biosynthetic end...
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### Table 1

Processes identified in Sre1 transport-cleavage mechanism in sre1-MP ubr1Δ hrd1Δ cells

| Process                     | Rate coefficient | Value in normalized model |
|-----------------------------|------------------|----------------------------|
| Sre1 transcription/translation | $k_p^X$          | $k_p^X/X_p = 0.23 \, h^{-1}$ |
| Scp1 transcription/translation | $k_p^Y$          | $k_p^Y/X_p = 0.23 \, h^{-1}$ |
| Sre1-Scp1 binding           | $k_1$            | $k_1 = 850 \, h^{-1}$      |
| Sre1-Scp1 unbinding         | $k_2$            | $k_2 = 0.2 \, h^{-1}$      |
| Sre1-Scp1 transport/cleavage| $f_{TC}$         | Varies                     |
| Dilution                    | $\beta$          | $\beta = 0.23 \, h^{-1}$   |

In this study, we investigated the sterol-dependent transport and cleavage of Sre1-Scp1 using a combination of experimental and mathematical techniques. We specifically sought to understand what sterol the Sre1-Scp1 complex senses, how this sensing could be described quantitatively, and what molecular mechanism could perform it. To facilitate this, we created a genetically modified strain of *S. pombe* that isolated the transport-cleavage process from several other factors known to affect it. Based on prior knowledge of the system, we constructed a mathematical model of this modified strain consisting of nonlinear differential equations describing the known interactions between the components in the pathway. We then performed experiments on the modified strain of yeast, interpreted the results with the aid of our model, and used the results to expand the model to include a quantitative empirical description of how the process of Sre1-Scp1 transport and cleavage responds to changes in cellular sterols. Our results suggest that ergosterol is the signal that regulates Sre1-Scp1 transport and cleavage and that the mechanism for this activation is similar to that used by mammalian Scap.

### Experimental Procedures

Yeast cell culture and immunoblot analysis were performed as described previously (11). Compactin, terbinafine, lanosterol (L5768), and itraconazole were obtained from Sigma; 25-thialanosterol was a gift of W. David Nes (Texas Tech University). Lanosterol (lanosterol/24,25-dihydrolanosterol mixture) and 24,25-dihydrolanosterol were from Steraloids (C3250 and C8600, respectively).

**Strains and Media**—Haploid *S. pombe* strains were grown to log phase at 30 °C in YES medium (0.5% (w/v) yeast extract plus 3% (w/v) glucose and supplements, 225 mg/liter each of uracil, adenine, leucine, histidine, and lysine). The yeast strains sre1-MP ubr1Δ hrd1Δ (JBY517; h− sre1-MP scp1−13nyc::kanMX6 Δubr1::natMX6 Δhrd1::kanMX6 his3-D1 leu1-32 ura4-D18 ade6-m210), are1Δ (JBY529; h− Δare1::natMX6 his3-D1 leu1-32 ura4-D18 ade6-m210), and are1Δ are2Δ (JBY547; h− Δare2::kanMX6 his3-D1 leu1-32 ura4-D18 ade6-m210) were derived from wild-type KGY425 (h− his3-D1 leu1-32 ura4-D18 ade6-m210) by standard genetic techniques (16, 17).

**Antibodies**—Polyclonal antiserum and affinity purified IgG recognizing Sre1 (amino acids 1–260) were generated as described previously (11).

**Sre1 Cleavage Assay**—Sre1 cleavage was induced by addition of drugs or depletion of oxygen (as indicated in figure legends) at time = 0. Separate cultures were started for each time point such that cells reached a density of $1 \times 10^7$ cells/ml at the time of harvest. Samples for gas chromatography (5 × 10^7 cells) and immunoblotting (2 × 10^7 cells) were removed at different times and frozen for later analysis. Prior to SDS-PAGE, protein samples were treated with alkaline phosphatase to collapse the heavily phosphorylated Sre1N into a single band for quantitation (11). Western blots were quantified using the Versadoc Imaging System with Quantity One software (Bio-Rad). The percent cleavage of Sre1 (100 × Sre1N/[Sre1N + Sre1 precursor]) was determined using signals from each individual lane.

**Determination of Yeast Sterols**—Gas chromatography of total sterols was performed as described previously (14). For discrimination of free and total sterols, $1 \times 10^8$ yeast cells were grown as indicated, lysed at 4 °C by vortexing for 5 min with glass beads in 100 mM potassium phosphate, pH 7.2, and adjusted to a volume of 0.5 ml. Cholesterol recovery standard (10 μg) was added to the lysate, which was then divided in half and either saponified in 13.5 ml of 2:1 methanol, 60% KOH at 75 °C for 2 h or mixed with 13.5 ml of 2:1 methanol, 100 mM potassium phosphate, pH 7.2, at room temperature. Both saponified and unsaponified fractions were extracted with 4 ml of petroleum ether and analyzed by gas chromatography as described previously (14). Percent esterification was calculated for each sterol species by dividing the difference between quantities of total and free sterol by the quantity of total sterol.

**Modeling Sre1 Dynamics**—To understand the relationship between the rate of Sre1-Scp1 transport and cleavage and the fraction of Sre1 cleavage observed over time, we developed a mathematical model of Sre1 dynamics in sre1-MP ubr1Δ hrd1Δ yeast (Fig. 3A). This model describes a population of cells growing exponentially and asynchronously, thus avoiding the need to model cell cycle-dependent effects and enabling the model to be used with data gathered from growing populations of yeast.

Table 1 lists the processes included in this model, described under “Results,” along with their rate coefficients. The symbols $W$, $X$, $Y$, and $X_p$ represent Scp1, Sre1, Sre1-Scp1 com-
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\[ \frac{dx}{dt} = f_{tc}y - \beta x \]  
\[ \frac{dw}{dt} = \frac{k_{pw}}{X_T} - k_s x w + k_s y + f_{tc}y - \beta w \]

Similarly, if we denote the total concentration of Scp1 in the normalized model as \( w_T = w + y \), then adding Equations 7 and 9 gives us Equation 10:

\[ \frac{dw_T}{dt} = \frac{k_{pw}}{X_T} - \beta w_T \]

If the rate of Scp1 production \( (k_{pw}) \) remains constant, then \( w_T \) will approach the steady-state value \( \frac{k_{pw}}{\beta X_T} \). Assuming that each experiment begins at steady state and that the experimental treatments do not significantly affect Scp1 production, then \( w_T \) is also constant over the course of each experiment. This simplifies the solution of Equations 6–9.

Having normalized the model, we now estimate its rate constants. Untreated \( sre1{-}MP \) \( ubr1{-}\Delta hrd1{-}\Delta \) cells have a doubling time of \( \approx 3 \) h, which gives \( \beta = \ln(2)/3 = 0.23 \) h\(^{-1}\) (supplemental material). Because \( X_T \) is constant, we can set the left side of Equation 5 to zero, yielding \( k_{px}/X_T = \beta = 0.23 \) h\(^{-1}\). In untreated \( sre1{-}MP \) \( ubr1{-}\Delta hrd1{-}\Delta \) cells, the concentration of Scp1 is approximately equal to that of Sre1 precursor (supplemental material), and on average, Sre1 precursor levels in untreated cells were about 20% of total Sre1 levels (see under “Results”). Thus, \( w_T = (W + Y)/X_T = 0.2 \), and because \( w_T \) is constant, we can set the left side of Equation 10 to zero to yield \( k_{pw}/X_T = \beta w_T = 0.046 \) h\(^{-1}\). We also compute \( k_s X_T = 850 \) h\(^{-1}\) and estimate \( k_s = 0.2 \) h\(^{-1}\) (supplemental material).

Calculating Transport-Cleavage Rate Coefficient—Given a time-varying rate coefficient for Sre1-Scp1 transport and cleavage, \( f_{tc}(t) \), we can solve Equations 6–9 to compute the resulting time-varying fraction of cleaved Sre1, \( x_s(t) \). However, in our case, we have the following inverse problem: given \( x_s(t) \) for the duration of an experiment, we want to compute the time-varying transport-cleavage rate coefficient \( f_{tc}(t) \) that led to that fraction of cleaved Sre1. To do this, we rewrite Equation 7, making the substitutions \( x = 1 - x_s - y \) and \( w = w_T - y \) as well as a substitution for \( f_{tc} \) from Equation 8 to give Equation 11,

\[ \frac{dy}{dt} = k_s x (1 - x_s - y)(w_{T} - y) - k_s y - \left( \frac{dx_s}{dt} + \beta x_s \right) - \beta y \]

For each experiment, at each sampling time \( t_s \), we measured Sre1 precursor \( (X(t_s) + Y(t_s)) \) and Sre1N \( (X_N(t_s)) \) by quantifying Western blots as described above and calculating \( x_s(t_s) = X_s(t_s)/(X(t_s) + Y(t_s) + X_N(t_s)) \). We then estimated \( x_s(t) \) for the duration of the experiment by interpolating the \( x_s(t_s) \) points with a piecewise cubic Hermite interpolating polynomial in Matlab (Mathworks, Natick, MA) (19). For numerical stability in solving the model equations, we capped the value of \( x_s(t) \) at 0.995. To find an initial condition \( y(0) \) for the experiment, we used the starting point \( x_s(0) \), assumed to be the steady-

plex, and Sre1N, respectively. Because the Sre1 positive feedback loop is broken in this strain (see under “Results”), we can assume that Sre1 and Scp1 are produced by transcription and translation at constant concentration rates \( k_{px} \) and \( k_{pw} \) respectively. We assume a one-to-one stoichiometry between Sre1 and Scp1; these proteins bind with a rate constant \( k_1 \) and unbind with a rate constant \( k_2 \). Furthermore, we treat transport and cleavage of Sre1-Scp1 complex as a single step and assume that the rate coefficient for this step, \( f_{tc} \), is not constant but changes in time. This accounts for the capability of the Sre1-Scp1 complex to increase its rate of transport and cleavage upon detecting some signal, likely a change in the rate of sterol synthesis. We also assume that, as part of the transport and cleavage step, Scp1 is liberated from the Sre1-Scp1 complex and recycled to the ER to form new Sre1-Scp1 complexes (18). Each chemical species is diluted due to the exponential growth of the population of cells; this happens at a rate proportional to the concentration of the species with a rate coefficient \( \beta \). A mathematical justification for this step and a procedure for calculating \( \beta \) are presented in the supplemental material. Denoting concentrations of chemical species in italics, we can write rate equations to describe the dynamics of this system as shown in Equations 1–4.

\[ \frac{dX}{dt} = k_{px} - k_s x W + k_s y - \beta X \]  
\[ \frac{dY}{dt} = k_s x W - k_s y - f_{tc} y - \beta Y \]  
\[ \frac{dx_N}{dt} = f_{tc} y - \beta x_N \]  
\[ \frac{dw}{dt} = k_{pw} - k_s x w + k_s y + f_{tc} y - \beta w \]

The total concentration of Sre1 in the system is \( X_T = X + Y + X_N \). Adding Equations 1–3 gives us Equation 5,

\[ \frac{dX_T}{dt} = k_{px} - \beta X_T \]

One can see from Equation 5 that if the rates of Sre1 production \( (k_{px}) \) and dilution due to cell growth \( (\beta) \) remain constant, \( X_T \) will approach the steady-state value \( \frac{k_{px}}{\beta} \). It follows that if each experiment begins at steady state and the experimental treatments do not significantly change the rates of Sre1 production or cell growth, then \( X_T \) is constant over the course of each experiment. Thus, we can normalize the model by dividing Equations 1–4 by \( X_T \), making the substitutions \( w = W/X_T, x = X/X_T, y = Y/X_T \), and \( x_N = X_N/X_T \) as shown in Equations 6–9,

\[ \frac{dx}{dt} = \frac{k_{px}}{X_T} - k_s x w + k_s y - \beta x \]  
\[ \frac{dy}{dt} = k_s x w - k_s y - f_{tc} y - \beta y \]  

\[ \frac{dx_N}{dt} = f_{tc} y - \beta x_N \]  

\[ \frac{dw}{dt} = \frac{k_{pw}}{X_T} - k_s x w + k_s y + f_{tc} y - \beta w \]
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state value of $x_N$ before treatment, to solve Equations 6–9 at steady state (supplemental material). With the continuous input $x_N(t)$ and initial condition $y(0)$, we solved Equation 11 with Matlab’s ode15s routine to compute $y(t)$ for the duration of the experiment. Finally, we solved for $f_{TC}(t)$ by Equation 8.

Estimating Ergosterol in the ER—As described under “Results,” experimental evidence points to ergosterol as the signal that regulates the transport and cleavage of the Sre1-Scp1 complex. However, most of the ergosterol in a yeast cell is not located in the ER (20) and thus is not detectable by Sre1 and Scp1, which are embedded in the ER membrane. We used a mathematical model (supplemental material) to estimate $[S_{ER}]$, the concentration of detectable ergosterol in the ER, from $[S_T]$, the measured total amount of ergosterol per cell. $[S_T]$ includes both nonesterified and esterified ergosterol.

Curve Fitting and Error Measurements—Our method for fitting a curve to calculated points of $f_{TC}$ and $[S_{ER}]$ (Fig. 6A) is informed by the structure of the model of Sre1 dynamics. Solving Equations 6–10 for the value of $f_{TC}$ required to obtain a given value of $x_N$ at steady state (supplemental material) reveals that $f_{TC}$ scales exponentially with $x_N$ for $x_N < 0.8$ and superexponentially for higher values of $x_N$ (supplemental Fig. 2). Because this relationship describes the way that error in measuring Sre1 levels leads to error in the calculated value of $f_{TC}$, it is most appropriate to consider the logarithm of $f_{TC}$ when fitting a curve to it. Thus, when computing the best fit curve to relate calculated values of $f_{TC}$ and $[S_{ER}]$ (Fig. 6A), we minimized the sum of squared differences between the logarithm of the calculated $f_{TC}$ values and the logarithm of the $f_{TC}$ values predicted by the curve and computed $R^2$ accordingly. Calculation of the best fit curve and 95% confidence intervals for the parameters of the curve was performed by nonlinear regression in Mathematica (Wolfram Research, Champaign, IL).

To compare the percentages of Sre1 cleavage predicted by model simulations with those calculated from experimental measurements (Fig. 7), we defined an error metric that takes into account how normalizing distorts errors in the original data. For example, if the actual percentage of Sre1 cleavage ($x_N$) is 95%, a 10% overestimate of $X_N$ leads to a 0.45% mismatch in $x_N$, but if the actual $x_N$ is 75%, the same 10% overestimate of $X_N$ leads to a 2.3% mismatch in $x_N$. Because of this, our error metric, described in detail in the supplemental material, considers how much error in the measurements ($X + Y$) and $X_N$ would be required to produce a given error in $x_N$.

RESULTS

Sre1 Pathway—We developed a model (Fig. 1) depicting our current understanding of the fission yeast Sre1 pathway (11, 14, 16, 21–24). Sre1 ($X$) is an ER membrane-bound transcription factor that binds to Scp1 ($W$) to form a complex ($Y$) (11). When production of ergosterol is disrupted by low oxygen or chemical inhibitors, the Sre1-Scp1 complex is transported to the Golgi apparatus and proteolytically cleaved. It is believed that this transport is triggered by the change in ER membrane sterol composition, as Scp1 has a sterol-sensing domain (SSD) that shares essential residues with the SSD in mammalian Scap (11). The resulting N-terminal cleavage product Sre1N ($X_N$) is an active transcription factor that is released from the membrane and enters the nucleus to activate hypoxic gene expression, including that of ergosterol biosynthetic enzymes (21). Additionally, Sre1N promotes the transcription of its own precursor (Sre1), forming a positive feedback loop (11, 21). Following Sre1-Scp1 cleavage, Scp1 is believed to recycle back to the ER to form new Sre1-Scp1 complexes (18). Sre1 that is not bound to Scp1 can be degraded through the ER-associated degradation pathway by the E2 ubiquitin-conjugating enzyme Ubc7 and the E3 ubiquitin ligase Hrd1 (16). Degradation of Sre1N requires the N-end rule E3 ligase Ubr1, and this degradation is accelerated in the presence of oxygen by the prolyl hydroxylase Ofd1 (22). One notable difference between the S. pombe homolog of Insig, Ins1, does not regulate Sre1-Scp1 (11, 25).

Exogenous Lanosterol Does Not Stimulate Sre1 Cleavage—Previous studies indicated that the ER-Golgi transport and cleavage of the Sre1-Scp1 complex is regulated by 4-methylated sterol intermediates such as lanosterol (14). As the mechanism by which this worked was unclear, we sought to further investigate the relationship between cellular sterol composition and Sre1 cleavage. To determine whether Sre1 responded to increased lanosterol, we added exogenous lanosterol (Steraloids C3250) to exponentially growing wild-type cells. Lanosterol treatment activated Sre1 cleavage (Fig. 2A, lanes 1–3) as reported previously (14). However, gas chromatography revealed that addition of this compound inhibited ergosterol synthesis (Fig. 2B). This finding, together with recent data regarding this specific commercial preparation of lanosterol, suggested that this inhibition of sterol synthesis is not caused by lanosterol itself, but rather is caused by 24,25-dihydrolanosterol, a prominent contaminant of the commercial preparation (15). Consistent

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with this hypothesis, addition of pure lanosterol (Sigma L5768) did not induce Sre1 cleavage (Fig. 2A, lanes 4–6) or inhibit ergosterol production (Fig. 2B), whereas addition of 24,25-dihydrolanosterol both blocked ergosterol synthesis (Fig. 2B) and induced Sre1 cleavage (Fig. 2A, lanes 7–9). From these results, we conclude that exogenous lanosterol either does not induce Sre1 cleavage or cannot enter the cells and that 24,25-dihydrolanosterol inhibits ergosterol synthesis.

Simplifying the System—In light of this finding, we sought to investigate more broadly and quantitatively which elements of the sterol biosynthetic pathway could be consistently connected with Sre1 cleavage. To make the Sre1-Scp1 transport-cleavage system more amenable to quantitative experimental analysis, we created a strain of S. pombe with several genetic modifications to simplify the system (Fig. 3A). We mutated the Sre1N-binding sites on the sre1 promoter (called sre1-MP = mutant promoter) to keep Sre1N from up-regulating sre1 transcription (22). To prevent Sre1N from being degraded via Ubr1, we deleted the ubr1 gene. Similarly, we deleted the hrd1 gene to keep Hrd1 from degrading the Sre1 precursor. Consistent with the inability to degrade Sre1N due to the ubr1 deletion, sre1-MP ubr1Δ hrd1Δ cells have much higher levels of Sre1N than wild-type (Fig. 3B) or sre1-MP hrd1Δ cells (16). However, the level of Sre1 precursor is similar between wild-type and sre1-MP ubr1Δ hrd1Δ cells, indicating that the genetic modifications do not affect the pool of Sre1 available for transport and cleavage. This modified strain allowed us to examine the transport-cleavage process independent from other known regulation.

The Sre1 transport-cleavage mechanism in sre1-MP ubr1Δ hrd1Δ cells can be described mathematically by a system of four differential equations ("Experimental Procedures," Equa-
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...tions 1–4). Briefly, in this strain we can assume constant rates of protein production and dilution of each chemical species due to exponential cell growth. Sre1 precursor (X) is the only component consumed, as it can be converted to its active nuclear form (Xₙ) through proteolytic cleavage (Fig. 3A). Furthermore, we treat transport and cleavage of the Sre1-Scp1 complex as a single step and assume that the rate coefficient for this step, \( f_{TC} \), is not constant but changes in time. This accounts for the capability of the Sre1-Scp1 complex to detect changes in some signal (likely ER membrane sterol composition) and respond by increasing the rate of transport and cleavage. Notably, the mathematical model makes no assumptions regarding regulation by sterols, only that the regulatory signal affects the transport-cleavage step.

**Sre1 Cleavage Is Consistent with Ergosterol as the Sterol Signal**—To examine the link between cellular sterol levels and Sre1 cleavage, we subjected sre1-MP ubr1Δ hrd1Δ cells to different treatments known to induce cleavage, including chemical inhibitors and low levels of ambient oxygen. The steps in ergosterol biosynthesis blocked by each of these treatments are shown in supplemental Fig. 1. In each experiment we measured Sre1 precursor, Sre1N nuclear form, and major intermediates in the ergosterol production pathway for 6 h. Fig. 3C shows a sample set of Sre1 Western blots obtained over the duration of an experiment in which cells were treated with the sterol synthesis inhibitor itraconazole. Full quantitative results from six representative experiments are shown in Fig. 4.

As expected, applying inhibitors known to block the pathway at particular steps led to lower concentrations of all intermediates following those steps and accumulation of the intermediates preceding those steps (Fig. 4, A–E, left column). Thus, squalene, lanosterol, and 24-methylene lanosterol increased and decreased depending on the inhibitor applied; ergosterol decreased over time in every experiment employing a chemical inhibitor of sterol synthesis. We also observed increased Sre1 cleavage in every chemical inhibitor experiment (Fig. 4, A–E, right column). Shifting the cells to low oxygen caused transient changes in sterol levels and Sre1 cleavage but no long term changes (Fig. 4F), likely because of the incomplete nature of hypoxic inhibition and to negative feedback from increased ergosterol synthesis. These results show that ergosterol is the only major element of the sterol biosynthetic pathway that has a consistent correlation with Sre1 cleavage, suggesting that it is the sterol signal sensed by Scp1.

**Elevated Free Ergosterol Decreases Sre1 Transport and Cleavage**—As an independent test of the hypothesis that ergosterol regulates Sre1 transport and cleavage, we sought to assay Sre1 cleavage in cells with elevated levels of ergosterol. Previous studies indicated that *S. pombe* cells do not take up exogenous ergosterol (11). Thus, we investigated whether we could alter cellular sterol composition by deletion of acyl-CoA:sterol acyltransferase enzymes that convert free sterols into sterol esters. *S. pombe* codes for two acyl-CoA:sterol acyltransferases, *are1* (SPAC13G7.05) and *are2* (SPCP1E11.05c). Gas chromatography analysis of *are1Δ, are2Δ*, and *are1Δ are2Δ* cells grown to saturation revealed that Are1 and Are2 display different substrate preferences and account for all sterol acyltransferase activity *in vivo* (Fig. 5A). Are1 esterifies sterol pathway intermediates, mainly lanosterol and 24-methylene lanosterol, and Are2 esterifies ergosterol. These results are similar to those found for the *Are1* and *Are2* homologs in *Saccharomyces cerevisiae* (26).

Exponentially growing wild-type, *are1Δ*, and *are2Δ* cells contained equal amounts of total ergosterol and sterol intermediates, and in wild-type cells ergosteryl esters made up ~90% of total sterol ester (Fig. 5B). *are2Δ* cells lacked ergosteryl esters and contained increased free ergosterol (~2-fold) compared with wild-type and *are1Δ* cells. We assayed Sre1 cleavage in response to compactin treatment in wild-type, *are1Δ*, and *are2Δ* cells. Consistent with ergosterol being a repressive signal for Sre1 transport and cleavage, *are2Δ* cells showed reduced cleavage compared with wild-type or *are1Δ* cells (Fig. 5C).

**Transport-Cleavage Rate Is a Function of ER Ergosterol**—Our data from two different types of experiments suggest that ergosterol is the signal that regulates Sre1 transport and

![FIGURE 4. Sre1 cleavage is consistent with ergosterol as the sterol signal. sre1-MP ubr1Δ hrd1Δ cells were grown to exponential phase in YES and treated with compactin (200 μM), terbinaine (122 μM), 25-thialanosterol (1 μM), itraconazole (2 μM), or low oxygen as indicated. Protein extracts were prepared, phosphatase-treated, and immunoblotted with anti-Sre1 IgG. Sre1 cleavage was quantified and normalized as described under “Experimental Procedures.” Nonsaponifiable lipids were extracted and analyzed by gas chromatography. Quantities of sterol intermediates and ergosterol are normalized to total sterol intermediates plus ergosterol in B–F because terbinaine, 25-thialanosterol, itraconazole, and low oxygen block steps in sterol production that occur after squalene. A and E, sterol intermediates are normalized to (total sterol intermediates + ergosterol) × e⁻⁷ᵗ because compactin blocks a step in sterol production prior to squalene, causing the concentration of subsequent sterol intermediates to be diluted with exponential growth of the cell population.](image-url)
cleavage (Fig. 3A). The Sre1-Scp1 complex likely senses the ER pool of ergosterol due to its ER localization and similarities to the mammalian system (27, 28). To evaluate ER ergosterol as the regulatory signal, we asked whether the rate coefficient for Sre1-Scp1 transport and cleavage could be expressed as a function of ER ergosterol levels. For this, we selected six experiments in which ergosterol production in sre1-MP ubr1Δ hrd1Δ cells had been blocked by one or more chemical inhibitors. For each of these experiments, we estimated the transport-cleavage rate coefficient \( f_{TC}(t) \) from the measurements of percentage Sre1 cleavage \( x_N(t) \) over the duration of the experiment using the methods described under “Experimental Procedures.” This calculation did not involve ergosterol or sterol intermediates. We also estimated the ER ergosterol concentration \( [S_{ER}](t) \) from the measurements of total ergosterol per cell \( S_{ER}(t) \) as described under “Experimental Procedures.” We then sampled the resulting \( f_{TC}(t) \) and \( [S_{ER}](t) \) signals at the original sampling times from each experiment and plotted these samples on a graph of \( f_{TC} \) versus \( [S_{ER}] \) (Fig. 6A).

To quantitatively assess the relationship between \( f_{TC} \) and \( [S_{ER}] \), we calculated Spearman’s rank correlation coefficient (\( \rho \)) for the samples. The calculated value of \( \rho = -0.884 \) indicates a strong negative correlation between \( f_{TC} \) and \( [S_{ER}] \). To model this relationship, we fit a curve of the form \( f_{TC}([S_{ER}]) = k_{TC}/(1 + k[S_{ER}]^n) \) to the points on the graph as described under “Experimental Procedures” (Fig. 6A). As explained under “Discussion,” this form of curve corresponds to a steady-state reduction in available Sre1-Scp1 complex as a result of cooperative inactivation by ER ergosterol. \( k_{TC} \) corresponds to the maximum rate of Sre1-Scp1 transport and cleavage, \( n \) is the Hill coefficient for cooperative inactivation, and \( k \) is such that \( k^{-1/n} \) is the level of ER ergosterol at which Sre1-Scp1 is transported and cleaved at half the maximum rate. This regulatory structure is motivated by the regulation of Scap in mammalian cells (18). With parameters and 95% confidence intervals \( k_{TC} = 57 \pm 49 \) h\(^{-1} \), \( k = 1100 \pm 1050 \), and \( n = 1.8 \pm 0.46 \), the curve fit the samples with \( R^2 = 0.77 \).

Next, we tested whether the transport-cleavage rate coefficient can be modeled using other sterol pathway intermediates. For each of these experiments, we estimated the transport-cleavage rate coefficient \( f_{TC}(t) \) from the measurements of percentage Sre1 cleavage \( x_N(t) \) over the duration of the experiment using the methods described under “Experimental Procedures.” This calculation did not involve ergosterol or sterol intermediates. We also estimated the ER ergosterol concentration \( [S_{ER}](t) \) from the measurements of total ergosterol per cell \( S_{ER}(t) \) as described under “Experimental Procedures.” We then sampled the resulting \( f_{TC}(t) \) and \( [S_{ER}](t) \) signals at the original sampling times from each experiment and plotted these samples on a graph of \( f_{TC} \) versus \( [S_{ER}] \) (Fig. 6A).
ates as the regulatory signal. We performed analyses similar to those above for ergosterol to generate plots of \( f_{TC} \) versus squalene, lanosterol, and 24-methylene lanosterol (Fig. 6, B–D) and calculate values for these data. These pathway intermediates showed much less correlation with \( f_{TC} \). The ability to model the Sre1-Scp1 transport-cleavage rate coefficient as a function of ER ergosterol, and not as a function of other sterol pathway intermediates, further supports the case that ER ergosterol is the regulator of Sre1-Scp1 transport and cleavage.

**Model Validation**—To validate the \( f_{TC}([SER]) \) model described above, we performed simulations to compare the predictions of the model with data from our experiments with \( sre1-MP\, ubr1\Delta\, hrd1\Delta \) cells. Using data for total ergosterol concentration \([S_T](t)\) over the duration of each experiment, we estimated the concentration of ergosterol in the ER \([SER](t)\) with the model of ergosterol transport described under “Experimental Procedures.” We then used the \( f_{TC}([SER]) \) curve (Fig. 6A) to calculate the Sre1 transport-cleavage rate coefficient \( f_{TC}(t) \) over the duration of each experiment. Given \( f_{TC}(t) \), we were able to use the model of Sre1 dynamics described under “Experimental Procedures” to predict \( x_N(t) \), the percentage of Sre1 cleaved, over the course of each experiment.

Fig. 7, A–F, shows how the model simulations compare with the Sre1 cleavage data in the six experiments used to generate the \( f_{TC}([SER]) \) model. Much of the discrepancy between the predicted and measured levels of Sre1 cleavage is likely due to measurement error, as Sre1 cleavage differed in identical experiments even before treatment (compare Fig. 7, B and C). To compare the predicted percentages of Sre1 cleavage with their experimentally measured values, we evaluated the error between them as described under “Experimental Procedures.” This error value ranges from 13 to 45%, which points to the lack of precision in the quantitative data obtained through Western blots.

To validate the model further, we performed the same simulations for additional experiments whose data were not used to generate the model (Fig. 7, G–L). Importantly, the model predicts the response of the system to 25-thialanosterol, a chemical inhibitor of ergosterol biosynthesis that was not used in model identification (Fig. 7, G and H). Furthermore, the model predicts much of the system’s response to low oxygen (Fig. 7, I–L). Overall, these two sets of simulations suggest that our model accurately captures the qualitative behavior of Sre1-Scp1 transport and cleavage in the \( sre1-MP\, ubr1\Delta\, hrd1\Delta \) strain.

**DISCUSSION**

In this study, we show that ergosterol regulates the rate of transport and cleavage of the Sre1-Scp1 complex in fission yeast. Previous experiments in which addition of exogenous lanosterol activated Sre1 cleavage were found to be misleading, as the lanosterol mixture used contained a contaminant that inhibits ergosterol production and causes the concentration of cellular ergosterol to decrease (Fig. 2). This motivated
further investigation, which concluded that ergosterol is the signal regulating Sre1-Scp1 transport and cleavage. Multiple lines of evidence support this conclusion. First, different chemical inhibitors of ergosterol synthesis, which inhibit enzymatic steps both early and late in the ergosterol biosynthetic pathway, induce Sre1 cleavage (Fig. 4, A–E). This cleavage is consistent with a drop in ergosterol concentration but inconsistent with changes in the concentration of any other sterol pathway intermediates measured. Second, elevated levels of free ergosterol achieved by blocking ergosterol esterification suppress Sre1 cleavage (Fig. 5). Third, the transport-cleavage rate coefficient ($f_{TC}$) is a function of ergosterol but not other sterol pathway intermediates (Fig. 6). Importantly, although the mathematical model used to compute $f_{TC}$ from experimental data made no assumptions as to the nature of the signal regulating $f_{TC}$, the model using ER ergosterol as the signal captures the behavior of the system (Fig. 7). Because ergosterol is the end product of the sterol biosynthetic pathway in yeast and the primary sterol component of the yeast plasma membrane and subcellular membranes (20), it is fitting that the Sre1-Scp1 complex monitors ergosterol to assess the state of sterol production.

The sensing of ergosterol by Sre1-Scp1 in fission yeast and Scap sensing cholesterol in mammals suggests a possible molecular mechanism for how ergosterol regulates ER-Golgi transport of Sre1-Scp1 and subsequent cleavage of Sre1. Mammalian Scap measures cholesterol levels by binding directly to cholesterol (3), and cholesterol changes the conformation of Scap (30, 31). Cholesterol promotes Scap binding to Insig, which retains the SREBP-Scap complex in the ER, thereby preventing SREBP cleavage in the Golgi (4, 5). However, cholesterol has been shown to block SREBP-Scap cleavage even in the absence of Insig (5). Furthermore, it has been shown that cholesterol regulates SREBP-Scap transport in a cooperative manner (28). These data suggest a model in which Scap exists in two conformations as follows: one “active” conformation that is available for transport and proteolytic cleavage, and one “inactive” conformation that is retained in the ER and available to bind Insig (18). In this model, cholesterol cooperatively promotes the transition from the active to the inactive state.
Ergosterol Regulates Sre1 Cleavage

FIGURE 8. Sre1 pathway in wild-type yeast with a possible mechanism for regulation of Sre1-Scp1 transport and cleavage by ergosterol. Scp1 bound to Sre1 has two conformations, an active state Y and an inactive state Z. The equilibrium between these conformations is rapid and cooperatively dependent on levels of ergosterol in the ER. The active state Y is transported from the ER to the Golgi and cleaved to form Sre1N with a constant rate coefficient \( k_{TC} \).

Based on our results here, we propose a similar model for regulation of Sre1-Scp1 cleavage by ergosterol, noting that Ins1, the fission yeast homolog of Insig, does not play a role in this regulation (11, 25). Suppose that Scp1, like Scap, has two conformations, an active state Y and an inactive state Z in rapid equilibrium, and the transition between these conformations is cooperatively dependent on the concentration of ergosterol in the ER \( [S_{ER}] \) with a Hill coefficient \( n \) (Fig. 8). Then for some constants \( \alpha \) and \( K \), we have Equation 12,

\[
Z = \frac{\alpha[S_{ER}]^n}{K^n + [S_{ER}]^n} Y
\]

(Eq. 12)

In our model of Sre1-Scp1 transport described above, \( f_{TC}(S_{ER}) \) is the rate coefficient for Sre1-Scp1 transport and cleavage, i.e. the rate of transport and cleavage per unit of concentration of complex. Suppose that the transport-cleavage rate coefficient is actually a constant \( k_{TC} \), but only the active state Y is available for this. Then the rate of transport and cleavage is \( k_{TC} Y \) and the concentration of Sre1-Scp1 complex is \( Y + Z \). By the above definition of \( f_{TC}(S_{ER}) \), we now have Equation 13,

\[
f_{TC}(S_{ER}) = k_{TC} Y = \frac{k_{TC} Y}{Y + Z} = \frac{k_{TC} Y}{Y + \frac{\alpha[S_{ER}]^n}{K^n + [S_{ER}]^n} Y} = 1 + \frac{\alpha[S_{ER}]^n}{K^n + [S_{ER}]^n}
\]

(Eq. 13)

if \( K \gg [S_{ER}] \).

As shown under “Results” (Fig. 6A), this function describes our findings, demonstrating that a mechanism whereby ergosterol in the ER cooperatively mediates a transition between active and inactive forms of Sre1-Scp1 complex is a plausible explanation for our observations. Further studies will be required to confirm the existence of multiple conformations of Scp1 and the role of ergosterol in determining those conformations.

Overall, the model developed here describes Sre1-Scp1 transport and cleavage with a reasonable degree of quantitative accuracy given the limitations of our measurements. Western blots of Sre1 are subject to significant experimental variability, and the nature of our procedure for calculating \( f_{TC} \) tends to amplify this noise. Also, this model does not include the process of ergosterol esterification, which affects the amount of ergosterol available to be detected by the Sre1-Scp1 complex. Even with these limitations, the model describes most of the variability in the data \( (R^2 = 0.77) \), suggesting that it captures the essence of the Sre1-Scp1 transport-cleavage dynamics. Furthermore, the process of curve fitting estimated the Hill coefficient for ergosterol sensing by Sre1-Scp1 within a fairly narrow range \( (1.8 \pm 0.46) \). Although this model was developed in a genetically modified strain of S. pombe, it should be applicable to wild-type yeast, as we have no reason to believe that any mutation in this strain directly affects the machinery that performs Sre1-Scp1 transport and cleavage.

In this study, we present evidence that ergosterol regulates the ER-Golgi transport and cleavage of Sre1-Scp1, the fission yeast homologs of mammalian SREBP-Scap. Additionally, we present a quantitative empirical model that describes the nature of this regulation and suggests a possible molecular mechanism by which it could work. The experiments used to generate and test this model were conducted in a modified yeast strain that lacks some of the complexity found in the Sre1-Scp1 system of wild-type yeast. However, we expect this model to serve as a discrete module that will be integrated into the future with models of the other subsystems (Fig. 1), ultimately allowing construction of a complete model for the fission yeast SREBP pathway.

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