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4-Methyl Sterols Regulate Fission Yeast SREBP-Scap under Low Oxygen and Cell Stress

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In fission yeast, orthologs of mammalian SREBP and Scap, called Sre1 and Scp1, monitor oxygen-dependent sterol synthesis as a measure of cellular oxygen supply. Under low oxygen conditions, sterol synthesis is inhibited, and Sre1 cleavage is activated. However, the sterol signal for Sre1 activation is unknown. In this study, we characterized the sterol signal for Sre1 activation using a combination of Sre1 cleavage assays and gas chromatography sterol analysis. We find that Sre1 activation is regulated by levels of the 4-methyl sterols 24-methylene lanosterol and 4,4-dimethylfecosterol under conditions of low oxygen and cell stress. Both increases and decreases in the level of these ergosterol pathway intermediates induce Sre1 proteolysis in a Scp1-dependent manner. The SREBP ortholog in the pathogenic fungus Cryptococcus neoformans is also activated by high levels of 4-methyl sterols, suggesting that this signal for SREBP activation is conserved among unicellular eukaryotes. Finally, we provide evidence that the sterol-sensing domain of Scp1 is important for regulating Sre1 proteolysis. The conserved mutations Y247C, L264F, and D392N in Scp1 that render Scap insensitive to sterols cause constitutive Sre1 activation. These findings indicate that unlike Scap, fission yeast Scp1 responds to 4-methyl sterols and thus shares properties with mammalian HMG-CoA reductase, a sterol-sensing domain protein whose degradation is regulated by the 4-methyl sterol lanosterol.

Lipid homeostasis in mammals is regulated at the transcriptional level by sterol regulatory element-binding proteins (SREBPs), a family of ER membrane-bound transcription factors (1, 2). SREBPs control expression of over 30 genes required for low density lipoprotein uptake and synthesis of fatty acids, triglycerides, phospholipids, and cholesterol (3). SREBP contains two transmembrane segments and is inserted into the ER membrane in a hairpin fashion such that the N and C termini are in the cytosol. The N terminus of SREBP is a basic helix-loop-helix leucine zipper (bHLH-zip) transcription factor. The C terminus of SREBP binds SREBP cleavage activating protein (Scap), an ER membrane protein required for the function of SREBP.

SREBP transcriptional activity is controlled by cellular cholesterol concentration through a negative feedback system. In cells with high levels of cholesterol, the SREBP-Scap complex is retained in the ER in an inactive state. ER retention is mediated by an interaction of Scap with the ER resident protein Insig (4). A drop in cholesterol levels disrupts the binding of Scap and Insig, facilitating ER exit of SREBP-Scap and transport to the Golgi apparatus. In the Golgi, SREBP is activated by two proteolytic cleavage events mediated by the Site-1 and Site-2 proteases (5). Upon cleavage, the N-terminal bHLH-zip domain of SREBP is released from the membrane and enters the nucleus to activate transcription of target genes required for lipid homeostasis.

The sterol-sensing ability of the SREBP pathway is mediated by Scap, a polytopic ER membrane protein. Scap consists of 8 transmembrane segments and a C-terminal cytosolic domain that binds to SREBP (1). Transmembrane segments 2–6 of Scap comprise the sterol-sensing domain (SSD) that regulates SREBP activation in response to sterol levels (6). Previous studies identified three mutations in the SSD (Y298C, L315F, and D443N) that each render Scap insensitive to sterol levels and abolish Insig binding, causing constitutive ER exit and proteolytic activation of SREBP (7). Although these residues are important for Scap function, the exact mechanism by which Scap measures cellular sterol content is unclear. Recent studies suggest that cholesterol binds directly to the SSD of Scap and induces a conformational change in the protein that enhances Insig binding and ER retention of SREBP-Scap (8–10).

Recently, our laboratory has characterized orthologs of SREBP and Scap, named Sre1 and Scp1, in the fission yeast Schizosaccharomyces pombe (11). Our studies indicate that Sre1 and Scp1 function in an oxygen sensing pathway that monitors sterol synthesis as an indirect measure of cellular oxygen availability. Under oxygen-replete conditions, oxygen-dependent synthesis of the fungal sterol ergosterol is high, and Sre1 is inactive. When oxygen becomes limiting, ergosterol synthesis is inhibited, leading to rapid proteolytic activation of Sre1 in a Scp1-dependent manner (11). Following proteolysis, the soluble N-terminal bHLH-zip domain of Sre1 enters the nucleus and binds Sre1, facilitating ER exit and proteolytic activation.
and directly activates transcription of genes required for oxygen consumptive pathways including ergosterol, heme, sphingolipid, and ubiquinone biosynthesis (12). Activation of genes in these pathways ensures cellular survival under low oxygen conditions as both sre1+ and scp1+ are required for low oxygen growth. Our previous data suggest that like mammalian Scap, Scp1 is required for sterol sensing in fission yeast (11). Sre1 is unstable in the absence of Scp1, and the activation of Sre1 under low oxygen requires Scp1. Additionally, the three residues required for Scap sterol sensing, Tyr298, Leu315, and Asp443, are all conserved in Scp1 (Tyr247, Leu264, and Asp392). Although Scp1 is likely the sterol sensor in fission yeast, the identity of the molecular signal for Sre1 activation is unknown.

In this study, we characterize the sterol signal for Sre1 activation using a combination of Sre1 cleavage assays and gas chromatography sterol analysis. We find that Scp1-dependent activation of Sre1 is regulated by both an increase and decrease of 4-methyl sterol intermediates in the ergosterol synthetic pathway under conditions of low oxygen and cell stress. C. neoformans SREBP is also activated by increased 4-methyl sterols, suggesting that this signal for SREBP activation is conserved across phyla in fungal eukaryotes. Finally, we provide evidence suggesting that this signal for SREBP activation is conserved across phyla in fungal eukaryotes.

**EXPERIMENTAL PROCEDURES**

**Materials**—We obtained yeast extract from BD Biosciences; Edinburgh minimal medium (EMM) and amino acids from Q-Biogene; oligonucleotides from Integrated DNA Technologies; horseradish peroxidase-conjugated, affinity-purified donkey anti-rabbit, and anti-mouse IgG from Jackson Immunoresearch; cholesterol (C6760) and lanosterol (C3250) from Steraloids; itraconazole, compactin, A23187, lysing enzymes, and H2O2 from Sigma; anti-HA-HRP and alkaline phosphatase from Roche Applied Sciences; anti-Myc 9E10 IgG from Santa Cruz Biotechnology; anti-SpSre1 and anti-CnSre1p IgG have been previously described (11, 13).

**Strains and Cell Culture**—Schizosaccharomyces pombe strains KGY425 (wild type) and sre1Δ culture conditions for Sre1 cleavage assays and sterol analysis, hypoxic growth conditions, and yeast transformation were previously described (11). C. neoformans serotype D strain B3501A and culture conditions for Sre1 cleavage assay, logarithmically growing yeast (7.5 × 107 cells) were harvested by centrifugation, transferred to glass tubes, and subjected to methanolic saponification with 9 ml of methanol and 4.5 ml of 16% (w/v) KOH for 2 h at 75 °C after addition of 5 μl of cholesterol as a recovery standard. Non-saponifiable sterols were extracted with 4 ml of petroleum ether, evaporated to dryness under a stream of nitrogen gas, and resuspended in 250 μl of heptane. 3 μl of each sample was injected in splitless mode using an automatic sampler onto an Agilent 6850 gas chromatograph equipped with an HP-1 column and FID detector. Peak areas for each sterol were normalized to cholesterol peak area to correct for recovery.

**Sterol Analysis**—Sterol extraction and gas chromatography analysis were conducted as previously described (16). Briefly, exponentially growing yeast (5 × 107 cells) were harvested by centrifugation, transferred to glass tubes, and subjected to methanolic saponification with 9 ml of methanol and 4.5 ml of 60% (w/v) KOH for 2 h at 75 °C after addition of 5 μg of cholesterol for the end product ergosterol are shown.

**Immunoblot Analysis**—Protein preparation, alkaline phosphatase treatment, and immunoblot analysis for S. pombe experiments were described previously (11). Protein preparation and immunoblot analysis for C. neoformans experiments were also described previously (13).

**Spheroplasting**—To spheroplast yeast for A23187 Sre1 cleavage assay, logarithmically growing yeast (7.5 × 107 cells) at a...
Concentration of 1 x 10^7 cells/ml were collected by centrifugation at 2,500 x g for 5 min. Cells were resuspended in 1 ml of buffer SP1 (1.2 M sorbitol, 10 mM Tris-HCl, pH 7.6) and then resuspended in 7.5 ml of rich medium (YES) plus 1.2 M sorbitol prior to addition of Me_2SO or A23187 for a Sre1 cleavage assay.

RESULTS

Low Oxygen Increases 4-Methyl Sterols and Activates Sre1—Our previous studies indicated that Sre1 monitors oxygen-dependent sterol synthesis as a measure of cellular oxygen supply (11). The ergosterol synthesis pathway requires 12 molecules of dioxygen for the production of each ergosterol molecule (Fig. 1 and Ref. 17). Under low oxygen conditions, ergosterol synthesis decreases and Sre1 cleavage is activated. However, addition of ergosterol to cells grown under low oxygen does not suppress Sre1 activation (data not shown). Therefore, we hypothesized that low oxygen Sre1 activation may be mediated by changes in ergosterol synthesis intermediates. To investigate this possibility, wild-type and sre1/H9004 cells were grown at 0.5% oxygen for increasing time, and samples were harvested for immunoblot and sterol analysis to determine if Sre1 activation correlated with changes in sterol intermediates. Antibody to the N terminus of Sre1 detected an accumulation of cleaved nuclear Sre1 by 1 h (Fig. 2, A, lane 2). Sre1 activation was maximal 4 h after shifting to low oxygen and then declined at 6 h.

From the same samples, we measured the relative quantities of squalene and downstream sterol species by gas chromatography (Fig. 1). This analysis revealed significant changes in only 3 sterol intermediates. Squalene levels were unchanged at 0.5% oxygen. Ergosterol levels in a wild-type strain decreased to a minimum at 2 h and then returned to normal levels at 6 h (Fig. 2B). Paralleling Sre1 activation, the amount of 24-methylene lanosterol increased up to 4 h after a shift to low oxygen and returned to normal at 6 h, suggesting that the oxygen-dependent enzyme Erg11 is rate-limiting under these conditions (Fig. 2C). Low oxygen also decreased
zymosterol, ergosterol, cholesterol, 25-hydroxycholesterol, and 19-hydroxycholesterol (data not shown). Importantly, sterol analysis revealed that ergosterol levels were unchanged 30 min after lanosterol addition (data not shown). These data suggest that an increase in 4-methyl sterols is sufficient to activate Sre1.

**Cell Stress Increases 4-Methyl Sterols and Activates Sre1**—In addition to being activated by direct sterol depletion, mammalian SREBP is cleaved in response to cell stress-inducing agents such as thapsigargin, hypotonicity, and bacterial pore forming toxins (18, 19). To test if Sre1 is activated in response to cell stress in fission yeast, wild-type cells containing an empty vector were cultured in the presence of 50 μM hydrogen peroxide (H₂O₂) for increasing amounts of time. Immunoblot analysis indicated that Sre1 was rapidly cleaved in response to H₂O₂, with activation occurring 10 min after H₂O₂ addition (Fig. 4A, upper panel, lanes 1–4). Interestingly, sterol analysis under these conditions showed that H₂O₂ treatment caused an accumulation of 24-methylene lanosterol and 4,4-dimethylfecoesterol, substrates for Erg11 and Erg25, respectively, and a minor decrease in ergosterol (Fig. 4, B–D, closed symbols). These data suggest that H₂O₂ treatment may inhibit the sterol synthesis enzymes Erg11 and Erg25, leading to an increase in 4-methyl sterols and Sre1 activation.

To determine if H₂O₂-induced Sre1 activation requires 4-methyl sterol accumulation, we attempted to reduce the levels of these intermediates by overexpressing erg11⁺ and erg25⁺. We examined Sre1 cleavage after H₂O₂ treatment in a wild-type strain overexpressing Myc-tagged erg11⁺ and HA-tagged erg25⁺ from the strong nmt promoter. Immunoblot analysis showed that both enzymes were expressed at a constant level during the experiment (Fig. 4A, lower panels). Overexpression of erg11⁺ and erg25⁺ suppressed Sre1 activation in response to H₂O₂, indicating that Sre1 induction by H₂O₂ is sterol-dependent (Fig. 4A, upper panel, lanes 4–8). Contrary to our expectation, sterol analysis revealed that cells overexpressing erg11⁺ and erg25⁺ had increased levels of ergosterol, 24-methylene lanosterol, and 4,4-dimethylfecoesterol compared with wild-type cells (Fig. 4, B–D, t = 0). However, the 4-methyl sterol to ergosterol ratio in this overexpression strain under untreated conditions was equal to that of the wild-type strain, suggesting that overall sterol synthesis was increased in this strain by an unknown mechanism (Fig. 4E). Upon addition of H₂O₂, the 4-methyl sterol to ergosterol ratio increased 3-fold in wild-type cells and was largely unchanged in the erg11⁺-erg25⁺ overexpressing cells (Fig. 4E). A small increase in this ratio was observed at 30 min in the erg11⁺-erg25⁺ overexpression strain, and this correlated with a slight increase in Sre1 cleavage at this time point (Fig. 4A, lane 8). Collectively, these data show that H₂O₂ activation of Sre1 is sterol-dependent and suggest that Sre1 responds to changes in the ratio of 4-methyl sterol to ergosterol in the cell.

To examine Sre1 activation by other cell stresses, we analyzed Sre1 cleavage in wild-type cells after treatment with the calcium ionophore A23187. In mammalian cells, A23187 depletes ER calcium stores and has been shown to increase HMG-CoA reductase and HMG-CoA synthase transcript levels, known transcriptional targets of mammalian SREBP (20). To facilitate A23187 delivery, we removed the yeast cell wall by
spheroplasting prior to treatment with the ionophore. Sre1 activation in response to inhibition of sterol synthesis was normal in yeast spheroplasts (data not shown). Treatment of wild-type cells for 4 h with 1 μM A23187 caused an accumulation of cleaved nuclear Sre1 compared with untreated and vehicle-treated (Me2SO) cells (Fig. 5A). A23187 addition appeared to inhibit Erg25, causing an accumulation of 4,4-dimethylfecosterol and a decrease in ergosterol (Fig. 5B). No significant change in 24-methylene lanosterol was observed. These data suggest that perturbing ER calcium homeostasis may disrupt the function of Erg25, leading to 4,4-dimethylfecosterol accumulation and activation of Sre1.

4-Methyl Sterol Activation of Sre1 Is Conserved in C. neoformans—Recent studies have identified orthologs of SREBP and Scap in the pathogenic basidiomycete C. neoformans, named Sre1p and Scp1p, respectively (13). As in the ascomycete S. pombe, C. neoformans Sre1p is cleaved and activated by inhibition of sterol synthesis in a Scp1p-dependent manner. To determine if an increase in 4-methyl sterols is sufficient for Sre1p activation in this organism, cells were cultured in the absence or presence of increasing concentrations of lanosterol for 2 h. Immunoblot analysis with an antibody directed toward the N terminus of Sre1p showed that lanosterol induced Sre1p cleavage in a concentration-dependent manner (Fig. 6A). Kinetic analysis of Sre1p activation with 10 μg/ml lanosterol showed Sre1p cleavage as early as 15 min after lanosterol addition with maximal activation by 90 min (Fig. 6B). No changes in ergosterol levels were observed at the time of Sre1 induction (data not shown). These results indicate that the sterol signal is conserved between fungal phyla and suggest that S. pombe Sre1-Scp1 and C. neoformans Sre1p-Scp1p respond to an alteration in 4-methyl sterol levels.

Sre1 Responds to Both Increases and Decreases in 4-Methyl Sterols—Thus far, we have provided evidence that an increase in 4-methyl sterols regulates Sre1 in fission yeast. Yet, in a previous study we demonstrated activation of Sre1 by compactin, an inhibitor of HMG-CoA reductase (11). Treatment of cells with 200 μM
compacting for 8-h induced Sre1 activation (Fig. 7A, lane 2), but did not increase 4-methyl sterol levels (Fig. 7B). As expected, compacting treatment lowered the levels of all sterols, including ergosterol and 4-methyl sterols. Treatment of cells with 100 μM itraconazole, an Erg11 inhibitor, also activated Sre1 and decreased ergosterol levels, but resulted in the accumulation of 24-methylene lanosterol as expected. Taken together, these results suggest that Sre1-Scp1 may respond to (1) either an increase or decrease in 4-methyl sterol levels or (2) a decrease in ergosterol. To differentiate between these possibilities, we treated wild-type cells with a combination of 200 μM compactin and 100 μM itraconazole. This treatment inhibited sterol synthesis at two steps (HMG-CoA reductase and Erg11) and caused a depletion of ergosterol equal to that observed in cells treated with either compactin or itraconazole alone (Fig. 7B). However, because we inhibited HMG-CoA reductase and Erg11 simultaneously, the level of 24-methylene lanosterol in these cells was almost unchanged. Despite a decrease in ergosterol equal to that in cells treated with either compactin or itraconazole alone, Sre1 activation was suppressed by the combination treatment as evidenced by a decrease in the nuclear form of Sre1 and an increase in the Sre1 precursor (Fig. 7A). This result indicates that depletion of ergosterol is not sufficient for full Sre1 activation. Instead, these data suggest that Sre1 responds to either an increase (low oxygen, H2O2, A23187, itraconazole treatments) or a decrease (compactin treatment) in 4-methyl sterols.

Fission Yeast SREBP-Scap Responds to 4-Methyl Sterols

![Sterol Synthesis Inhibitors (8 h)](image)

**FIGURE 7. Sre1 responds to both increased and decreased 4-methyl sterol levels.** Wild-type yeast were grown in rich medium for 8 h in the absence or presence of ergosterol synthesis inhibitors: C, 200 μM compactin; I, 100 μM itraconazole; CI, 200 μM compactin plus 100 μM itraconazole. A, total cell extracts (10 μg) were subjected to immunoblot analysis with anti-Sre1 IgG after treatment with alkaline phosphatase. P and N denote the membrane-bound precursor and cleaved nuclear forms of Sre1, respectively. B, sterols extracted from 5 × 10^7 cells were analyzed by gas chromatography. Cholesterol (5 μg) was added as an internal standard. Data are the average of three independent replicates. Error bars equal one S.D.

**DISCUSSION**

In our previous studies, we characterized fission yeast SREBP and showed that it functions in an oxygen-sensing pathway that monitors sterol synthesis as an indirect measure of cellular oxygen availability (11). Although Sre1 responded to inhibition of sterol synthesis, the nature of the sterol signal for Sre1 activation was unclear. In this study, we show that the Sre1-Scp1 complex responds to levels of 4-methyl sterol intermediates in the ergosterol synthesis pathway. This conclusion is supported by the following evidence: 1) low oxygen, hydrogen peroxide, and depletion of ER calcium increased 4-methyl sterol concentrations and activated Sre1 cleavage; 2) removal of 4-methyl sterol intermediates by overexpression of erg11+ and erg25+ suppressed Sre1 activation in cells grown under low oxygen or treated with H₂O₂ (Figs. 2E and 4A); 3) exogenous 4-methyl sterol activated Sre1 cleavage (Fig. 3); and 4) depletion of ergosterol alone was not sufficient for full Sre1 activation (Fig. 7). Our results suggest that the C-4 methyl group is responsible for Sre1 activation as addition of exogenous sterols lacking a C-4 methyl group, such as zymosterol, did not activate Sre1 (data not shown). It is possible that the C-14 methyl group of 24-methylene lanosterol also contributes to Sre1 activation. However, we consider this unlikely because 4,4-dimethylfeco-osterol lacks the C-14 methyl group and both 24-methylene lanosterol (Fig. 1, low oxygen treatment) and 4,4-dimethylfeco-sterol (Fig. 5, A23187 treatment) activated Sre1. Further evidence that Sre1-Scp1 responds to 4-methyl sterols comes from experiments in the distantly related basidiomycete C. neoformans. Similar to S. pombe, low oxygen and lanosterol addition activated C. neoformans Sre1p (Fig. 6) (13). The conservation of this sterol signal in these organisms and the presence of SREBP homologs in other fungi suggest that 4-methyl sterols may be a conserved signal for SREBP activation among unicellular eukaryotes.

Although our data suggest that 4-methyl sterols are a signal for Sre1 activation, further studies are required to understand the molecular mechanism of this activation. Sre1 activation is Scp1-dependent but we do not yet fully understand how Scp1 senses sterols. In mammals, it is thought that Scap monitors sterol levels through direct binding of cholesterol (10). It is possible that like Scap, Scp1 binds directly to 4-methyl sterols. Alternatively, 4-methyl sterols may regulate the ability of another sterol, perhaps ergosterol, to bind to Scp1. A third possibility is that Scp1 may bind to two different sterols, allowing Scp1 to sense the 4-methyl sterol to ergosterol ratio in the cell. These latter models could help explain the apparent results from Fig. 7 in which both an increase and decrease in 4-methyl sterols activate Sre1. Future studies are required to distinguish among these possibilities.

4-Methyl sterols represent a new class of signal for oxygen-sensing. In mammals, one way oxygen levels are monitored is through the regulation of the hypoxic transcription factor Hif-1α by oxygen-dependent prolyl hydroxylases (21). In S. cerevisiae, heme levels are monitored as an indirect measure of oxygen availability by the transcriptional activator Hap1p and repressor Rox1p (22). While similar systems such as these may exist in fission yeast, our previous studies indicate that Sre1 is a principal regulator of low oxygen transcription and thus, 4-methyl sterols represent a new class of signal for oxygen sensing in eukaryotes (12). Whether these sterols play an unrecognized role in oxygen sensing in other organisms is unknown. Indeed, 4-methyl sterols are ideally positioned to function as a signal for low oxygen. The 4-methyl sterols 24-methylene lanosterol and 4,4-dimethylfeco-sterol are substrates for the oxygen requiring enzymes Erg11 and Erg25 (Fig. 1), and removal of the 4-methyl groups in 24-methylene lanosterol requires 9 molecules of dioxygen. Under low oxygen, these reactions will be inhibited, leading to rapid accumulation of 4-methyl sterols. Erg11 and Erg25 are broadly conserved in eukaryotes, further supporting the idea that 4-methyl sterols may play an unrecognized role in oxygen sensing in other organisms.

In addition to serving as a signal for low oxygen, our data suggest that 4-methyl sterols may function as a signal for cell stress. Treatment of fission yeast cells with H₂O₂ or the calcium ionophore A23187 caused an increase in 4-methyl sterols and Scp1-dependent activation of Sre1 (Figs. 4 and 5). Sre1 up-regulates erg11+ and erg25+ transcription, and Sre1 may serve to reduce the levels of these intermediates during cell stress. However, we cannot rule out additional functions for Sre1 or actions of 4-methyl sterols during cell stress. These results highlight an important link between sterol synthesis and cell stress that has been observed by others. A recent study demonstrated that...
both activities. We examined the function of these three amino acids in Scp1. As in mammalian cells, single mutation of any of these conserved amino acids in Scp1 (Y247C, L264F, or D392N) also activated Sre1 in cells with normal sterol levels (Fig. 8). Interestingly, fission yeast Insig, named Ins1, is not required for sterol regulation of Sre1 (11). Collectively, these data suggest that these three SSD residues play a conserved role in sterol sensing by Scap and are not only required for Insig binding.

Identification of the sterol signal for Scp1 in fission yeast also allows us to draw comparisons between Scp1 and other SSD-containing proteins, such as HMG-CoA reductase, NPC1, and Patched (24). Of these, the function of the SSD in HMG-CoA reductase is the best understood. Recent studies have shown that HMG-CoA reductase is regulated by sterols through a negative feedback system that requires Insig (1). However, unlike Scap, Insig-mediated regulation of HMG-CoA reductase leads to its ubiquitinylation and degradation in the presence of sterols, not its retention. Recent studies have identified the 4-methyl sterol lanosterol as the physiological signal for Insig-mediated HMG-CoA reductase degradation (25). Thus, yeast Scp1 appears to possess properties of both Scap and HMG-CoA reductase. Scp1 binds to Sre1 and regulates Sre1 proteolysis in response to sterol levels. Indeed, conserved amino acids in Scp1 and Scap function to sense sterols in both proteins. However, like HMG-CoA reductase, Scp1 responds to 4-methyl sterols, suggesting that the SSD of mammalian HMG-CoA reductase and fission yeast Scp1 are functionally similar. In our study, we have shown that 4-methyl sterols activate Sre1 in response to low oxygen and cell stress. Therefore, it is interesting to speculate that low oxygen and cell stress may be physiological regulators of mammalian HMG-CoA reductase in vivo. Future studies will focus on a more detailed analysis of fission yeast SSD proteins with the goal of advancing our understanding of sterol sensing and lipid homeostasis.

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