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MINIREVIEW

Sterol Regulatory Element Binding Proteins in Fungi: Hypoxic Transcription Factors Linked to Pathogenesis[▽]

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Sterol regulatory element binding proteins (SREBPs) are membrane-bound transcription factors whose proteolytic activation is controlled by the cellular sterol concentration. Mammalian SREBPs are activated in cholesterol-depleted cells and serve to regulate cellular lipid homeostasis. Recent work demonstrates that SREBP is functionally conserved in fungi. While the ability to respond to sterols is conserved, fungal SREBPs are hypoxic transcription factors required for adaptation to a low-oxygen environment. In the fission yeast *Schizosaccharomyces pombe*, oxygen regulates the SREBP homolog Sre1 by independently controlling both its proteolytic activation and its degradation. SREBP is also required for adaptation to hypoxia in the human pathogens *Cryptococcus neoformans* and *Aspergillus fumigatus*. In these organisms, SREBP is required for virulence and resistance to antifungal drugs, making the SREBP pathway a potential target for antifungal therapy.

Sterol lipid is essential for maintenance of the cell membrane structure and fluidity in eukaryotic organisms. For several decades, researchers have been working to understand the mechanisms by which animal cells maintain proper cholesterol levels. Interest in understanding cholesterol regulation stems from the fact that alterations in cholesterol are linked to human disease, including atherosclerosis and heart disease. Studies primarily from the laboratory of Brown and Goldstein elucidated pathways involved in the complex regulation of cholesterol homeostasis in mammalian cells (5). One such pathway involves a group of mammalian transcription factors called sterol regulatory element binding proteins (SREBPs), which act as principal regulators of both cellular cholesterol uptake and *de novo* synthesis (12). SREBPs are synthesized as inactive membrane-bound proteins, requiring cleavage from the membrane for activation. In addition, SREBPs undergo a unique regulatory mechanism in which sterols directly control SREBP activity and inhibit proteolysis when cellular sterol levels are sufficient (13).

In 2005, the first fungal ortholog of SREBP, called Sre1, was identified in the fission yeast *Schizosaccharomyces pombe* (19). Like SREBP, Sre1 also undergoes sterol-dependent proteolytic activation and regulates genes required for maintaining cellular sterol homeostasis. In addition, Sre1 regulates genes involved in the transcriptional response to hypoxia and is required for growth under low-oxygen conditions (45). Sre1 is also regulated hypoxically by a sterol-independent mechanism through the putative dioxygenase Ofd1 (20). Recently, SREBP-like proteins have been identified in the pathogenic fungal species *Cryptococcus neoformans* and *Aspergillus fumigatus* (7, 9, 48). In addition to their role in hypoxic adaptation, these proteins are important for pathogenesis and resistance to antifungal drugs. Here, we present a review of fungal SREBP pathways and their importance in human disease.

MAMMALIAN SREBP PATHWAY

The human genome contains two genes which code for three SREBPs: SREBP1a, SREBP1c, and SREBP2 (4, 11, 12). These proteins contain a highly conserved basic-helix-loop-helix (bHLH) leucine zipper DNA-binding domain in the N terminus. Compared to other bHLH domain transcription factors, SREBPs contain a unique tyrosine residue instead of a basic residue in the bHLH region. This single-amino-acid difference alters the DNA-binding properties such that SREBPs do not bind exclusively to E-box DNA motifs in target gene promoters but can bind to unique sterol regulatory elements (SREs) (33).

SREBPs are synthesized as inactive precursor proteins on the endoplasmic reticulum (ER) membrane and contain two transmembrane segments such that the N and C termini face the cytosol (Fig. 1) (17). On the membrane, SREBPs bind to the sterol-sensing protein Scap (*S*REBP *c*leavage *a*ctivating *p*rotein), which mediates sterol-dependent regulation of SREBP activity (16, 38). When membrane cholesterol levels are sufficient, Scap binds cholesterol leading to a conformational change (3, 37). In this conformation, Scap interacts with the ER-resident protein INSIG, and SREBP remains inactive on the ER membrane. When cholesterol levels are reduced, Scap-INSIG binding is disrupted and the SREBP-Scap complex is transported to the Golgi apparatus via COPII vesicle-mediated transport (12). In the Golgi apparatus, two proteases release SREBP from the membrane. The site 1 serine protease first cleaves SREBP in its Golgi lumenal loop. Next, the site 2 zinc metalloprotease cleaves SREBP within the first transmembrane segment to release the N-terminal transcription

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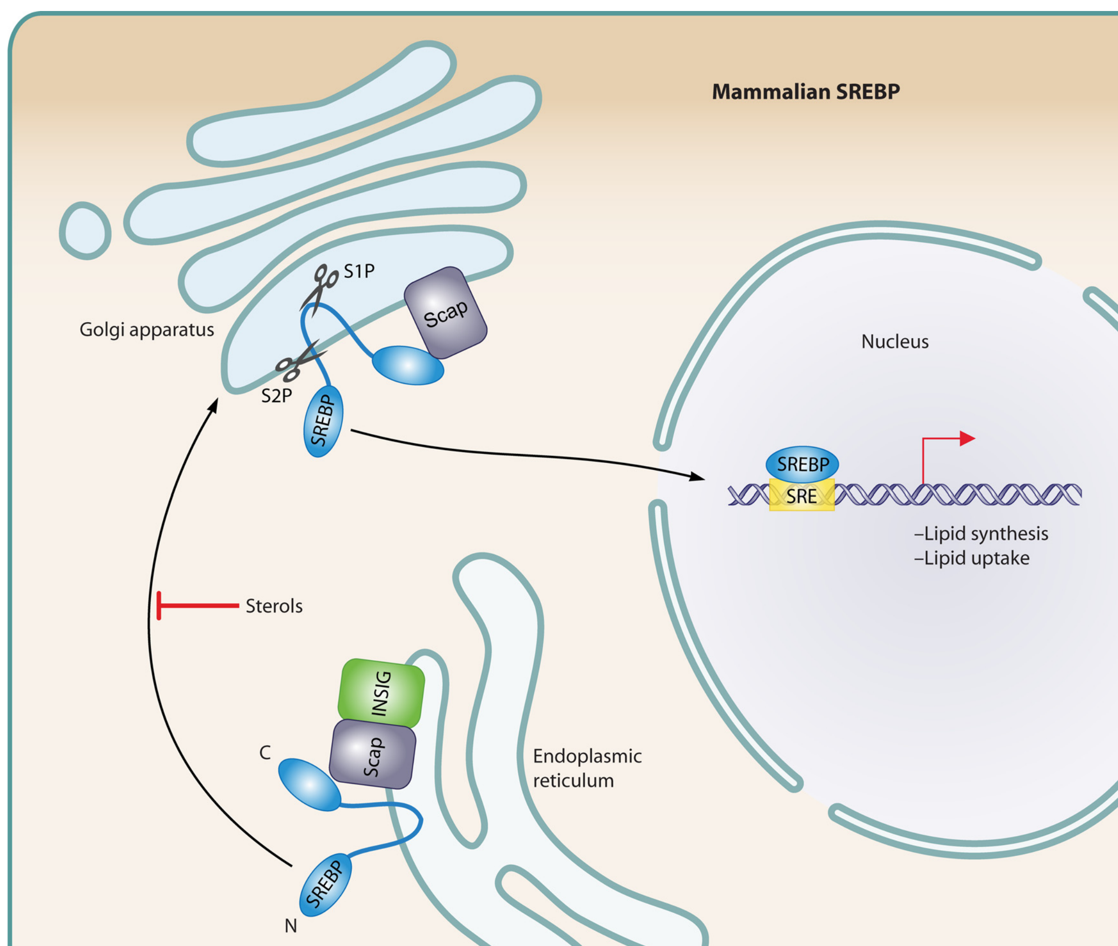

FIG. 1. Mammalian SREBP pathway. Under sterol replete conditions, the SREBP-Scap complex is retained in the ER bound to INSIG. When sterol levels decrease, SREBP-Scap is transported to the Golgi apparatus, where SREBP is cleaved by the site 1 and site 2 proteases. The soluble N terminus of SREBP enters the nucleus and activates genes required for the synthesis and uptake of sterols and other lipids.

factor domain into the cytosol, where it travels to the nucleus to regulate gene expression (12). Target genes of SREBPs include genes involved in *de novo* cholesterol biosynthesis from acetyl-coenzyme A (CoA), cholesterol uptake via the low-density-lipopeptide (LDL) receptor, and other pathways involved in lipid metabolism (15). The subsequent increase in cholesterol supply inhibits Golgi transport of Scap, thereby repressing SREBP activation.

SCHIZOSACCHAROMYCES POMBE SREBP PATHWAY

Proteins with sequence homology to SREBP, Scap, and INSIG, called Sre1, Scp1, and Ins1, respectively, have been identified in the fission yeast *S. pombe* (Table 1) (19). Sre1 contains two predicted transmembrane segments and a bHLH DNA-binding domain with the unique tyrosine residue found in all SREBP orthologs. Initial studies of these proteins re-

TABLE 1. Conserved SREBP pathway components in fungi

Organism	Name(s) (accession no.[a]) of homolog(s) of indicated protein					
	Sre1	Sre1N-like	Scp1	Ins1	Stp1	Ofd1
S. pombe	Sre1 (NP_595694); Sre2 (NP_595229)		Scp1 (NP_596673)	Ins1 (NP_587813)		Ofd1 (NP_596087)
C. neoformans	Sre1 (XP_567526)		Scp1 (XP_569410)		Stp1 (XP_571333)	(XP_566748)
A. fumigatus	SrbA (XP_749262)	(XP_746533)		InsA (XP_752057)		(XP_755813)
C. albicans	Cph2 (XP_712449)	Tye7 (XP_722152); (XP_716760)				(XP_711450)
S. cerevisiae		Hms1p (NP_014675); Tye7p (NP_014989)		Nsg1p (NP_012001); Nsg2p (NP_014243)		Tpa1p (NP_010969)
M. grisea	(XP_001410973)	(XP_001522006)	(XP_362552)			(XP_362298)

[a] NCBI reference sequence accession number(s).

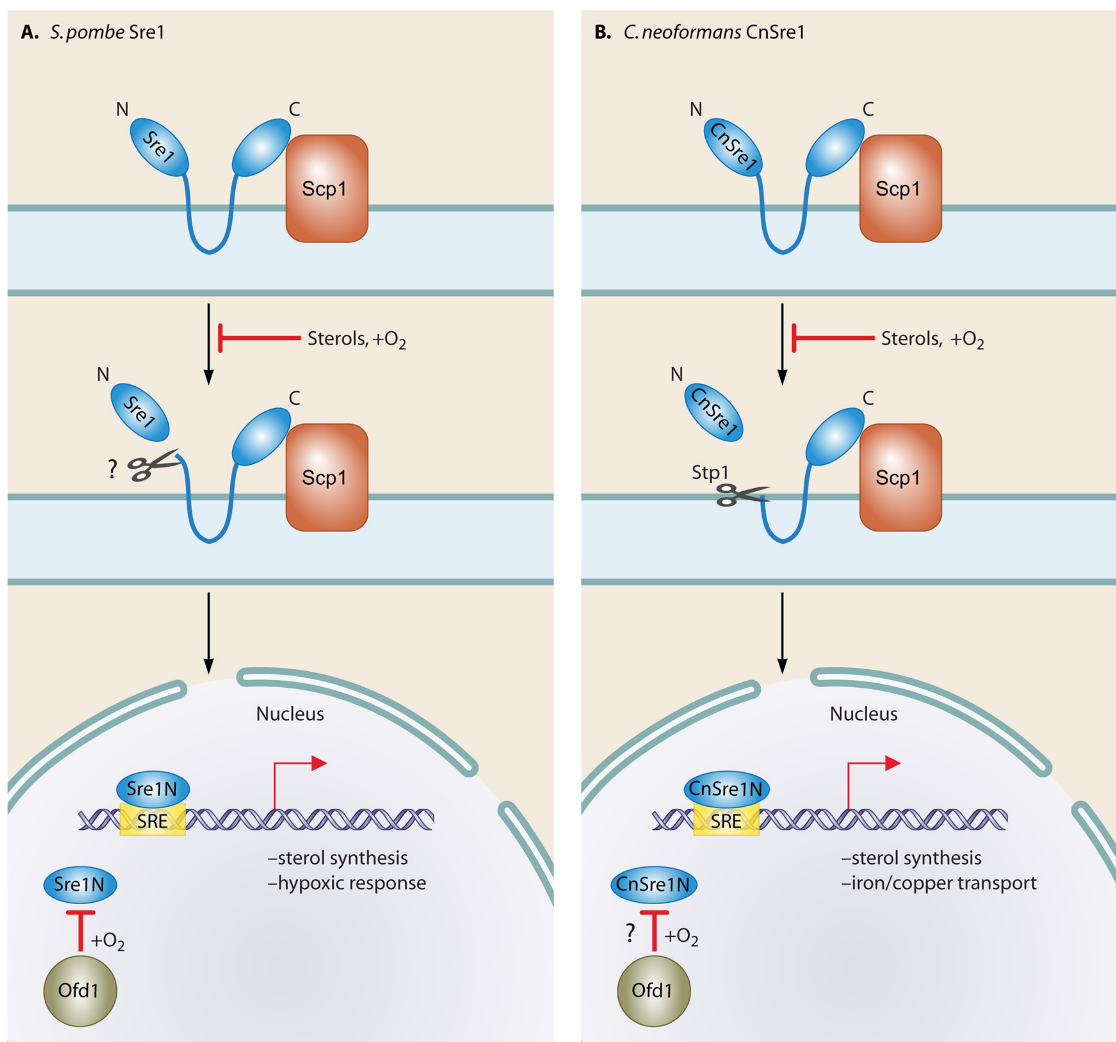

FIG. 2. SREBP pathway in fungi. (A) *S. pombe*. In the presence of oxygen, sterols inhibit proteolysis of Sre1 and Ofd1 accelerates Sre1N degradation. In the absence of oxygen, both Sre1 proteolysis and stability increase, leading to upregulation of genes required for sterol synthesis and adaptation to low oxygen levels. How Sre1 is released from the membrane is unknown. (B) *C. neoformans*. Under low-oxygen conditions, CnSre1 is released from the membrane by the site 2 protease homolog Stp1 and CnSre1N activates genes required for sterol synthesis and metal homeostasis. Whether CnSre1N is regulated by Ofd1 is not known.

vealed many similarities to the mammalian SREBP system (Fig. 2A). First, Sre1 is synthesized as a membrane-bound precursor and is cleaved in response to a decrease in ergosterol, the fungal equivalent of cholesterol. This has been demonstrated using specific pharmacological inhibitors of sterol biosynthesis, including statin and azole drugs (18, 19). Second, Sre1 and Scp1 physically interact, and Scp1 is required for Sre1 cleavage and activation (19). Third, *in vitro* and *in vivo* experiments determined that Sre1 binds directly to conserved SRE sequences in target gene promoters (45). Finally, Sre1 activates genes required for ergosterol biosynthesis, and Sre1 mutants are defective for maintaining proper sterol levels (18, 45). On the basis of these findings, it is clear that the SREBP pathway is conserved in fungi as an important regulator of sterol homeostasis.

Despite these similarities, the *S. pombe* Sre1 pathway displays interesting differences from the mammalian SREBP pathway. Most importantly *S. pombe* Sre1 is a major regulator of the fission yeast hypoxic response (19). Sre1 activity is dramatically upregulated in the absence of oxygen. One reason for this is that, unlike mammals, fission yeast is unable to transport sterols from the extracellular medium and cells rely solely on the biosynthesis of ergosterol, which is heavily oxygen-requiring. Consistent with this, under low-oxygen conditions, ergosterol synthesis decreases and Sre1 cleavage is induced. In this way, environmental oxygen concentrations directly affect cellular sterol levels and indirectly regulate Sre1 activity. In addition to regulating sterol biosynthetic enzymes, Sre1 activates genes coding for enzymes in other oxygen-dependent metabolic pathways, including heme, sphingolipid, and ubiquinone biosynthesis. Thus, Sre1 regulates the transcriptional responses under both low-sterol and low-oxygen conditions (45). Sre1 does not regulate pathways involved in respiration or fermentation, suggesting that other transcription factors exist to regulate changes in energy metabolism under low-oxygen conditions. Transcriptional profiling experiments revealed that Sre1

is a central regulator of hypoxic gene expression and that consequently, Sre1 is essential for growth under anaerobic conditions.

Further investigation of Sre1 in the hypoxic transcriptional response revealed a sterol-independent mechanism for the regulation of Sre1 activity. The cleaved soluble N terminus of Sre1 (Sre1N) undergoes oxygen-mediated regulation by the 2-*o*xoglutarate *F*e(II) *d*ioxygenase domain-containing protein Ofd1 (20, 25). Ofd1 regulates Sre1N through a mechanism reminiscent of the mammalian hypoxia-inducible factor (HIF) transcription factor pathway (22, 42). In that system, the alpha subunit of HIF is negatively regulated under normoxic conditions by the members of the 2-oxoglutarate Fe(II) dioxygenase prolyl hydroxylase (PHD) family of enzymes. PHDs bind directly to oxygen and catalyze the hydroxylation of HIF alpha, resulting in its ubiquitinylation and proteasomal degradation. When oxygen levels are low, PHDs can no longer catalyze this reaction and HIF is stabilized and activates the transcriptional response under low-oxygen conditions. Similarly, *S. pombe* Ofd1 negatively regulates Sre1N stability in an oxygen-dependent manner (Fig. 2A) (20). Under normoxia, Ofd1 accelerates the proteasomal degradation of Sre1N. Under anaerobic conditions, Ofd1 is bound to the nuclear protein Nro1 (*n*egative *r*egulator of *O*fd1), which prevents Sre1N degradation, leading to increased activation of Sre1 target gene expression (25). In this model, oxygen disrupts the binding of Ofd1 to Nro1, thereby allowing Ofd1 to promote Sre1N degradation. Important questions remain regarding the mechanism by which Ofd1 regulates Sre1N stability and whether Ofd1 acts as a prolyl hydroxylase similar to the mammalian PHD enzymes. The dual regulation by oxygen of Sre1 cleavage and protein turnover confers robust amplification of Sre1N levels under low-oxygen conditions. Using these two modules, cells are able to fine-tune levels of Sre1 activity in response to the oxygen and lipid environment.

While *S. pombe* has an INSIG homolog called Ins1, it does not function directly in the Sre1 pathway (6, 19). Mammalian INSIG regulates sterol homeostasis by two mechanisms: (i) INSIG regulates the sterol-dependent retention of Scap-SREBP on the ER membrane (Fig. 1), and (ii) INSIG promotes the degradation of the sterol biosynthetic enzyme HMG-CoA reductase (HMGR) under high-sterol conditions (13). *S. pombe* Ins1 functions to regulate sterol homeostasis similarly to this second mechanism (6). However, instead of regulating HMGR protein stability, Ins1 is a negative regulator of HMGR enzymatic activity.

In addition to Sre1, fission yeast carries another SREBP-like protein, called Sre2 (19). Sre2 contains the conserved tyrosine in its DNA-binding domain and has two predicted transmembrane segments. However, unlike Sre1 or SREBP, Sre2 has a short C-terminal cytoplasmic tail (23 amino acids) that does not bind Scp1. Therefore, decreased sterol levels do not activate Sre2, and Sre2 is cleaved constitutively. This sterol-independent cleavage raises questions as to why Sre2 is associated with the membrane when its release from the membrane does not appear to be regulated by sterol levels. Further studies will be required for identification of the transcriptional targets of Sre2 and the factors that regulate its activity.

Another difference between the fission yeast and mammalian SREBP systems is the lack of site 1 and site 2 protease homologs in the fission yeast genome, necessitating an alternate mechanism for Sre1 and Sre2 cleavage. Further studies of the mechanism of Sre1 and Sre2 proteolysis in *S. pombe* may reveal interesting insights into the evolution of SREBPs as well as potentially novel factors involved in mammalian SREBP processing.

SREBP PATHWAY IN OTHER FUNGAL SPECIES: PERMUTATIONS OF *S. POMBE*?

Recently, functionally conserved SREBP pathways have been identified in other fungal species (Table 1). These species include the human fungal pathogens *Cryptococcus neoformans* and *Aspergillus fumigatus* (2, 7, 9, 48). *C. neoformans* belongs to the fungal phylum Basidiomycota, which is distantly related to the phylum Ascomycota, which includes *S. pombe*, *Saccharomyces cerevisiae*, *A. fumigatus*, and most other well-studied fungal species. The presence of SREBPs in these two divergent fungal phyla suggests widespread conservation of the SREBP pathway in the fungal kingdom. Studies characterizing these different fungal systems have led to interesting findings about the evolution of SREBP pathway regulation.

CRYPTOCOCCUS NEOFORMANS SREBP PATHWAY

Parallel studies performed by two groups of investigators identified the *C. neoformans* SREBP pathway as important for sterol homeostasis, oxygen sensing, and virulence in mice (7, 9). *C. neoformans* contains single SREBP and Scap homologs, named Sre1 and Scp1, respectively (Table 1). *C. neoformans* Sre1 (referred to here as CnSre1) is cleaved and activated in response to both low-sterol and low-oxygen conditions, and the activation of CnSre1 requires Scp1 (Fig. 2B) (7, 9). However, *C. neoformans* lacks INSIG. *C. neoformans* also contains a homolog of fission yeast, Ofd1. It is unknown whether Ofd1 plays a role in the hypoxic regulation of the N terminus of CnSre1.

Interestingly, *C. neoformans* carries a conserved site 2 protease (Stp1) that is required for the processing of CnSre1 (2, 9). DNA database searches identify homologs of the mammalian site 2 protease in many members of the basidiomycete phylum, including *Postia placenta*, *Malassezia globosa*, *Moniliophtora perniciosa*, *Laccaria bicolor*, and *Coprinopsis cinerea*. In contrast, site 2 proteases have not been found in ascomycetes, suggesting that this protein was lost after the divergence of the two fungal phyla. *C. neoformans* Stp1 contains conserved catalytic residues common to all site 2 protease homologs, and Stp1 cleaves within the first transmembrane segment of CnSre1 (2). However, no clear homolog of the site 1 protease exists in *C. neoformans*. Consistent with this, cells lacking *STP1* do not accumulate an intermediate form of CnSre1 protein, suggesting that perhaps Stp1 is the sole CnSre1 protease.

The transcriptional program controlled by CnSre1 resembles both the mammalian and the fission yeast systems (2, 7, 9, 26). Similar to mammalian SREBP, CnSre1 regulates most enzymes in the sterol biosynthetic pathway, and CnSre1 is required for normal sterol synthesis under both normoxic and hypoxic conditions (7). This is in contrast to *S. pombe* Sre1, which primarily regulates the oxygen-requiring enzymes in the ergosterol biosynthetic pathway (45). Consequently, *S. pombe*

Sre1 mutants are not significantly defective for sterol synthesis under normoxic conditions (18).

Similar to *S. pombe* Sre1, CnSre1 regulates other pathways involved in the adaptation to hypoxia. For example, CnSre1 induces the low-oxygen activation of genes involved in high-affinity uptake of iron and copper, including genes in both siderophore-mediated and reductive iron transport (7). Since most oxygen-dependent enzymes are also iron-containing, the iron starvation response is often coordinately regulated with the response to hypoxia (35, 40). In addition, the reductive pathway for high-affinity iron transport involves multicopper oxidase enzymes that require oxygen and copper for oxidization of iron at the plasma membrane (23). Therefore, the cellular needs for oxygen, copper, and iron are tightly linked. In *C. neoformans*, CnSre1 appears to coregulate the hypoxic activation of these three nutrient starvation pathways.

Recently, a genetic screen used to find factors required for growth under hypoxic conditions in *C. neoformans* revealed several putative regulators of CnSre1 activity (8). Interestingly, many of these regulators are homologous to factors involved in regulation of the mammalian SREBP pathway, including a protein involved in exit of SREBP from the ER (Sec24), a protein required for SREBP import into the nucleus (importin β), and a kinase involved in the regulation of SREBP transcriptional activity in the nucleus (Gsk3β). Mutations in these genes alter Sre1 protein levels, either through synthesis or through degradation.

ASPERGILLUS FUMIGATUS SREBP PATHWAY

The filamentous mold pathogen *Aspergillus fumigatus* also contains a SREBP homolog important for hypoxic adaptation and pathogenesis (48). Mutants lacking the SREBP homolog SrbA grow normally in atmospheric oxygen at 21% but fail to grow at 1% oxygen. In addition, SrbA is required for the hypoxic induction of genes involved in ergosterol biosynthesis, and SrbA mutants are defective for sterol synthesis. Despite the role of SrbA in regulating sterol homeostasis, *A. fumigatus* does not contain an obvious homolog of Scap, the protein responsible for sterol-dependent cleavage of SREBPs in other organisms (Table 1). Thus, SrbA may regulate sterol synthesis without being directly regulated by sterols, although a Scap homolog that cannot be identified by sequence database searching may exist. Assuming that SrbA is synthesized as a membrane-bound inactive precursor, other sterol sensing factors may exist to regulate SrbA proteolysis. One candidate is the *A. fumigatus* INSIG homolog InsA, as mammalian INSIGs also have sterol binding properties (36). Finally, *A. fumigatus* contains an Ofd1 homolog, which could regulate the N terminus of SrbA in response to changes in oxygen levels. Overall, uncovering the mechanisms behind SrbA regulation will allow for interesting comparisons of SREBP pathways across organisms.

Unlike other fungal SREBPs, SrbA mutants are defective for the expression of genes involved in cell wall maintenance (48). SrbA mutants show abnormal hyphal branching, indicating a defect in the establishment of cell polarity, a process which requires cell wall synthesis and restructuring. In contrast to fungal species that exist mainly as yeast, cell polarity maintenance is an essential process in filamentous fungal species such as *A. fumigatus*. Electron microscopy experiments revealed that SrbA mutants have abnormal cell wall-plasma membrane interfaces (48). However, the specific SrbA transcriptional targets required for this process remain unknown, and further experiments are required for determination of whether SrbA directly regulates cell wall biosynthesis. It is possible that defects in ergosterol biosynthesis in SrbA mutants could lead to problems in the maintenance of cell wall architecture as well as defects in filamentous growth.

OTHER FUNGAL SPECIES

To date, in-depth characterizations of fungal SREBPs have been performed for *S. pombe*, *C. neoformans*, and *A. fumigatus*. However, many fungal species contain putative SREBPs and components of the SREBP pathway, including human and plant pathogens (Table 1). For example, the common human fungal pathogen *Candida albicans* contains a predicted membrane-spanning, bHLH domain-containing protein with the unique tyrosine residue in the DNA-binding domain (Table 1). This protein, called Cph2, was identified in a genetic screen as a regulator of the yeast-to-hypha growth transition, a critical process for the virulence of *C. albicans* (24). Cph2 was found to bind directly to SRE-like elements upstream of the *TEC1* gene, which encodes a transcription factor involved in hyphal development. Consequently, *cph2/cph2* homozygous mutants are defective for filamentous growth. In its initial characterization, Cph2 was predicted to code for a soluble protein 290 amino acids in length (24). However, Cph2 is now annotated as 853 amino acids, with two predicted transmembrane segments. Therefore, Cph2 may code for a functional SREBP ortholog. If so, Cph2 is another example of a fungal SREBP regulating hyphal development. Like *A. fumigatus*, *C. albicans* does not possess a Scap homolog, indicating that sterols may not regulate Cph2. This is consistent with the fact that *C. albicans* contains another transcriptional activator of sterol synthesis, called Upc2. Upc2 has been described for both *C. albicans* and the more commonly studied yeast *S. cerevisiae* as being important for the hypoxic induction of ergosterol biosynthetic enzymes (14, 30, 47).

Interestingly, several studies suggest a link between environmental oxygen concentrations and the yeast-to-hypha transition in *C. albicans* (31, 43). Furthermore, it has been shown that hypha-specific genes are induced under low-oxygen conditions (31, 43). *C. albicans* contains an Ofd1 homolog, and it is tempting to speculate that it may act to hypoxically regulate Cph2-dependent regulation of the hyphal response.

DNA database searches for SREBP homologs in fungi reveal an interesting class of proteins that lack predicted transmembrane segments but contain SREBP-like DNA-binding domains, including the unique tyrosine residue. Many fungal species contain these truncated SREBPs, including *S. cerevisiae* and *C. albicans* (Table 1). *S. cerevisiae* and *C. albicans* each contain two such proteins, Tye7p and Hms1p in *S. cerevisiae* and Tye7 and XP_716760 in *C. albicans*. *S. cerevisiae* Hms1p was identified as a high-copy-number suppressor of the hyphal growth phenotype of ammonium permease mutant strains (29). Tye7p (also called Sgc1p) activates genes encoding glycolytic enzymes and Ty1 transposons in *S. cerevisiae* (28, 41). Interestingly, *C. albicans* Tye7 was recently found to be in-

volved in the induction of glycolytic genes under low-oxygen conditions, suggesting a role for Tye7 in the hypoxic response (1). However, little is known about the metabolic signals that control the activity of Tye7p and Hms1p. *S. cerevisiae* also contains an Ofd1 homolog called Tpa1p, which has been described to occur in regulation of translation (21). On the basis of current knowledge of the regulation of fission yeast Sre1N by Ofd1, it would be interesting to investigate the role of Tpa1p in regulating hypoxic gene expression through Hms1p or Tye7p. In addition, although *S. cerevisiae* lacks a full-length SREBP homolog, data suggest a link between Tpa1 and sterol homeostasis. Mutation of Tpa1 was found to suppress the lethal phenotype of other regulators of ergosterol metabolism (46).

In general, fungi contain different assortments of SREBP pathway components (Table 1). Future studies will reveal whether the two oxygen-dependent regulatory mechanisms in fission yeast are selectively conserved as functional modules in other fungal species to control transcription in response to hypoxia.

SREBP PATHWAY IN FUNGAL DISEASE

Recent increases in immunocompromised populations due to HIV/AIDS and immunosuppressive therapy have led to a rise in the prevalence of systemic fungal diseases, including invasive cryptococcosis, aspergillosis, and candidiasis (10). While effective antifungal therapies exist, drug toxicity and increasing instances of antifungal resistant strains underscore the need for novel therapeutics. Additionally, fungistatic drugs such as the commonly used azole class of antifungal can be less effective in immunocompromised patients who cannot rapidly clear nondividing yeast. Consequently, long periods of drug treatment are required for prevention of relapse in these patients (27).

SREBPs are important for virulence of the human opportunistic pathogens *C. neoformans* and *A. fumigatus*. Mouse intravenous and intranasal infection experiments revealed that *C. neoformans* cells lacking components of the CnSre1 pathway are either completely avirulent or severely attenuated compared to wild-type strains, depending on the strain background tested (2, 7, 9). Histological analysis showed that CnSre1 mutants accumulated in the meningeal layer of the brain but were unable to develop cystic lesions throughout the brain matter, as seen in BALB/c mice infected with wild-type *C. neoformans* cells, suggesting that CnSre1 is important for adaptation and growth in the brain tissue but is not required for reaching the brain (7). Similar studies using A/J mice examined the fungal burden of wild-type and *cnsre1*Δ cells in the brain, lung, and spleen (9). While *cnsre1*Δ cells were defective for colonization in all tissues, they displayed a most severe defect in lung colonization (9).

Similarly, *A. fumigatus* cells lacking SrbA were dramatically defective for virulence in two independent mouse models of invasive pulmonary aspergillosis (IPA) (48). The mouse models tested were CD-1 neutropenic mice and mice with X-linked chronic granulomatous disease (X-CGD), which are hypersusceptible to IPA (34). SrbA mutants failed to cause IPA-like symptoms in CD-1 mice and were completely avirulent, while the SrbA mutant-infected X-CDG mice displayed symptoms at early time points postinfection but were severely attenuated for virulence. The authors suggested that the difference between the mouse models could be attributed to the large inflammatory response against fungal antigens in the X-CDG mice that were not possible in the neutropenic mice. *A. fumigatus* SrbA cells were able to germinate at the site of infection, indicating that viable cells were able reach target host tissue. Interestingly, although unable to proliferate in mouse lung tissue, viable SrbA mutant cells could be recovered from lung homogenates at later time points. This resembles CnSre1 mutants in infected mouse brain tissue, which are able to persist but unable to grow and develop fungal lesions (7).

Taken together, these studies suggest a requirement for SREBPs in fungal pathogens to adapt to the host environment. One reason for this could be a decrease in oxygen availability in host tissue, particularly near sites of infection. It has become clear that inflammatory sites create hypoxic microenvironments (32). Indeed, phagocytic cells require low oxygen levels for HIF-mediated activation of microbicidal pathways (49). It has also been shown that hypoxic environments are generated in lung granulomas generated during *Mycobacterium tuberculosis* infection. This bacterium induces a hypoxic transcriptional response to adapt to the limiting oxygen levels and establish latent infection (39). Studies suggest that the brain is more hypoxic than other tissues, and some estimates place brain oxygen concentrations around 1 to 5%, which activate CnSre1 *in vitro* (7, 9, 44). Despite this evidence, it is premature to conclude that the virulence defect in CnSre1 and SrbA mutants is due to low oxygen concentrations in infected host tissues. Other fungal SREBP-mediated pathways may be important for virulence, such as response to low iron availability, maintenance of cell polarity, or other unidentified SREBP-regulated pathways.

IMPLICATIONS FOR ANTIFUNGAL THERAPY

Studies of *C. neoformans* and *A. fumigatus* establish SREBP as an important regulator of virulence. The function of these transcription factors in regulating sterol homeostasis makes the SREBP pathway an attractive target for antifungal therapy. Cells lacking SREBP in both pathogenic fungal species have reduced levels of mRNA for sterol biosynthetic enzymes and are consequently hypersensitive to inhibitors of sterol biosynthesis, including the commonly prescribed azole class of antifungals (7, 9, 48). These drugs cause fungal cell growth arrest by blocking ergosterol biosynthesis. However, azole drugs are fungistatic and not fungicidal agents, in that they do not directly kill fungal cells. This distinction is thought to contribute to the rising incidence of azole resistant fungal strains, particularly in immunocompromised patients who are unable to rapidly clear nondividing fungal cells (27). Excitingly, disruption of the CnSre1 pathway in *C. neoformans* converts azoles to fungicidal drugs. Cell culture experiments demonstrated effective killing of CnSre1 mutants at subnanomolar concentrations of itraconazole (2). Thus, inhibitors of fungal SREBPs may act synergistically with current antifungal drugs to more effectively and rapidly clear fungal infections.

CONCLUSIONS

Here, we summarized the current knowledge of the SREBP pathway in fungi. Like mammalian SREBP, characterized fungal SREBPs are transcriptional activators of sterol biosynthesis and are required for maintenance of proper sterol homeostasis (7, 19, 48). Interestingly, fungal SREBPs are important regulators of the transcriptional response to hypoxia and are required for growth under low-oxygen conditions (7, 9, 19, 48). Studies of the *S. pombe* Sre1 pathway revealed two oxygen-dependent mechanisms that regulate Sre1 activity. By sensing oxygen-dependent sterol synthesis, Sre1 responds to changes in environmental oxygen levels through Scp1 (19). Sre1 is also regulated by the putative hydroxylase Ofd1, which negatively regulates Sre1N levels under normoxic conditions (20, 25). In the basidiomycete *C. neoformans*, CnSre1 proteolysis is regulated by sterols and low levels of oxygen via Scp1 (7), but the role of Ofd1 in the hypoxic regulation of CnSre1 is still unknown. *A. fumigatus* does not contain a Scap homolog, indicating that perhaps the sterol-dependent regulation of SREBP was lost in this organism. An Ofd1 homolog in the filamentous fungus *A. fumigatus* may play a role in regulating SrbA. Further investigation of SREBP pathways in other fungal species will be important for understanding the role of SREBPs in oxygen sensing and sterol metabolism as well as providing potential insights into the mammalian SREBP pathway.

The SREBP pathway is an important virulence determinant in the pathogenic fungal species *C. neoformans* and *A. fumigatus* (7, 9, 48). While SREBP in these two fungal species is required for normal disease progression, mutant cells are able to reach sites of infection, indicating an inability to adapt to the host tissue environment. Important questions remain as to which SREBP transcriptional targets are required for growth in the host.

Finally, the SREBP pathway in fungal pathogens represents a promising target for antifungal therapy development. In the human pathogens *C. neoformans* and *A. fumigatus*, SREBPs are required for the transcriptional activation of ergosterol biosynthetic enzymes (7, 9, 48). Thus, defects in the SREBP pathway render cells hypersensitive to low levels of sterol biosynthesis inhibitors, including the azole class of antifungal drugs. Indeed, studies of *C. neoformans* demonstrate that azoles are fungicidal to CnSre1 pathway mutants (2). Inhibitors specific for the fungal SREBP pathway may prove to be potent and effective antifungal agents, particularly when used in combination with current therapeutics.

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