Brief Definitive Report

Siderophore Biosynthesis But Not Reductive Iron Assimilation Is Essential for *Aspergillus fumigatus* Virulence

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Abstract

The ability to acquire iron in vivo is essential for most microbial pathogens. Here we show that *Aspergillus fumigatus* does not have specific mechanisms for the utilization of host iron sources. However, it does have functional siderophore-assisted iron mobilization and reductive iron assimilation systems, both of which are induced upon iron deprivation. Abrogation of reductive iron assimilation, by inactivation of the high affinity iron permease (FtrA), has no effect on virulence in a murine model of invasive aspergillosis. In striking contrast, *A. fumigatus* L-ornithine-N^5-monooxygenase (SidA), which catalyses the first committed step of hydroxamate-type siderophore biosynthesis, is absolutely essential for virulence. Thus, *A. fumigatus* SidA is an essential virulence attribute. Combined with the absence of a *sidA* ortholog—and the fungal siderophore system in general—in mammals, these data demonstrate that the siderophore biosynthetic pathway represents a promising new target for the development of antifungal therapies.

Key words: fungal pathogenicity • aspergillosis • iron uptake • virulence factor • ornithine monooxygenase

Introduction

Aspergillus fumigatus is the most common airborne fungal pathogen of humans, causing life-threatening invasive disease in immunocompromised patients. The limitations of therapeutic intervention are reflected in mortality rates, dependent primarily on the immune status of the host, of up to 90% (1, 2). The importance of the host immune status has been underlined by the lack of identified specific virulence attributes in *A. fumigatus* (1, 3–6). Limiting access to essential nutrients is an often overlooked aspect of innate immunity (7). Iron is essential for most organisms, suggesting that its acquisition in vivo may be required for *A. fumigatus* to cause disease (8–10). Iron uptake systems are often required for bacterial and yeast virulence (11, 12).

Fungi have evolved various strategies, often used in parallel, to acquire iron. These include two high affinity uptake mechanisms, reductive iron assimilation, and siderophore-assisted iron mobilization (8–10). Siderophores are low molecular mass, organic, ferric iron–specific chelators, which are excreted during iron starvation (8, 9). Siderophore-bound iron is recovered by cells either by the reductive system or by specific transporters able to internalize the siderophore–iron complex. Furthermore, most fungi utilize intracellular siderophores as an iron storage compound. In *Candida albicans,* the siderophore transporter Arn1p/Sit1p is required for epithelial invasion and penetration but not for systemic infection (13). In systemic virulence of this fungus, the high affinity iron permease Ftr1, a component of the reductive iron assimilation system, has been shown to be essential (11), and high affinity heme uptake may play a role in virulence (14, 15). In contrast to bacteria (12), direct proof of the involvement of siderophore production in systemic fungal virulence is lacking. Indeed specific siderophore production was not found to be critical for phytopathogenesis of the basidiomycete *Ustilago maydis* (16). However, recent data indicate that *A. fumigatus* survival in human serum in vitro involves siderophore-mediated removal of iron from transferrin (17).

We have shown that L-ornithine-N^5-monooxygenase catalyzes the first committed step in biosynthesis of both triacetylfusarinine C (TAFC) and desferriferricrocin (DFFC), the two major siderophores of *Aspergillus nidulans* (18). TAFC is a cyclic tripeptide consisting of three N^2-acetyl-N^5-cis-anhydromevalonyl-N^5-hydroxyornithine residues linked by ester bonds, and DFFC is a cyclic hexapeptide with the

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structure Gly-Ser-Gly-(N^5-acetyl-N^5-hydroxyornithine)$_3$ (8). Linking of the hydroxamate groups and amino acid residues for formation of TAFC and DFFC is performed by different nonribosomal peptide synthetases (18, 19) and so far only that required for synthesis of ferricrocin (FC) has been identified. Notably, the gene encoding L-ornithine-N^5-monooxygenase is not clustered with other genes involved in siderophore biosynthesis in *A. nidulans*.

This report demonstrates that *A. fumigatus* employs three iron uptake mechanisms: ferrous iron uptake, reductive iron assimilation, and siderophore-mediated iron uptake. Siderophore-mediated iron uptake but not reductive iron assimilation was found to be essential for virulence in a murine model of invasive aspergillosis.

Materials and Methods

Growth Conditions, Analysis of Siderophore Production, and Uptake. Generally, *A. fumigatus* strains were grown at 37°C in minimal medium (+Fe-*Aspergillus* minimal medium (AMM), iron-replete conditions) according to Pontecorvo et al. (20) containing 1% (wt/vol) glucose as the carbon source, 20 mM glutamine as the nitrogen source, and 10 μM $FeSO_4$. For iron-depleted conditions, iron was omitted. The blood agar was –Fe-AMM containing 5% (vol/vol) sheep blood. Production of conidia of the fungal strains was performed on AMM plates containing 1.5 mM $FeSO_4$. Siderophore production was analyzed by reversed phase HPLC analysis as described previously (21).

cDNA Sequence and Northern Analysis. RNA was isolated using TRI Reagent (Sigma-Aldrich). The cDNA sequences of *sidA*, *ftrA*, and *fetC* were analyzed by reverse-transcribed PCR from total RNA using Superscript (Invitrogen). The 5′- and 3′-ends were determined with the GenRacer method (Invitrogen) using total RNA.

For Northern analysis, 5 μg of total RNA was electrophoresed on 1.2% (wt/vol) agarose–2.2 M formaldehyde gels and blotted onto Hybond N membranes (Amersham Biosciences). The hybridization probes used in this study were generated by PCR using oligonucleotides 5′-AACTACCTCCACCAGAAG and 5′-GAACGGCAATGTTGTAAG for *sidA*, 5′-GGGACAAGAG-CAAGATGC and 5′-CCCAGTAGAGGATGCAAG for *ftrA,* 5′-GTGACCGATCCCAAGAAC and 5′-GGATGGGAATG-TCTTGTG for *fetC,* and 5′-ATATGTTCCTCGTGCCG-TTC and 5′-CCTTACCACGGAAAATGGCA for β-tubulin encoding *tubA*.

Generation of A. fumigatus Mutant Strains. The *A. fumigatus* strains used in this study are summarized in Table I. To enable inactivation of *sidA*, a 5.1-kb fragment of genomic DNA containing *A. fumigatus sidA* was amplified by PCR using primers 5′-TCACCTGCTCGTCATGCGTC and 5′-GGAGTATCTA-GATGCGACACTACTCTC and cloned into pGEM-T (Promega). The resulting plasmid was sequenced and designated pSIDA. To disrupt *sidA* in *A. fumigatus* CEA17 (a *pyrG*$^-$ derivative of CEA10 [22]), a *sidA* internal 1.5-kb SmaI-ClaI fragment (+518 to +2039, stop codon at +1563) was replaced with a 1.9-kb SmaI-ClaI fragment from pAfpyrG containing *pyrG* (23). The resultant 5.5-kb XbaI fragment was gel purified and used to transform *A. fumigatus* CEA17 (24). Uracil prototrophs were selected (23). Transformants were screened by PCR, and single homologous genomic integration was confirmed by Southern blot analysis. A representative *pyrG*$^+$ *sidA*$^-$ strain (Δ*sidA*-CEA17) was selected and used for further analysis. To disrupt *sidA* in *A. fumigatus* American Type Culture Collection (ATCC) 46645, a 2.0-kb BglII-HindIII fragment of pSIDA was replaced with a 4.0-kb BglII-HindIII fragment from pAN7-1 (24) containing the hygromycin B resistance selection marker (*hph*). This results in a *sidA*-null allele, removing –144 to +1844. The resultant 6.9-kb BssHII fragment was gel purified and used to transform *A. fumigatus* ATCC 46645. A representative hygromycin-resistant *sidA*$^-$ strain (Δ*sidA*) was selected and used for further analysis.

To complement *sidA* deficiency in strains Δ*sidA*-CEA17 and Δ*sidA*/Δ*ftrA*-CEA17 (see next paragraph), a single copy of pSIDA was ectopically integrated by transformation to give strains *sidA*^{c}-CEA17 and *sidA*^{c}/Δ*ftrA*-CEA17, respectively. To complement *sidA* deficiency in Δ*sidA*, a *Bpu*1102I site in the 3′ noncoding region of *sidA* in pSIDA was replaced by digestion and blunt ending with a MwoI site. A gel-purified 4.9-kb BssHII fragment from the resultant plasmid was used to transform Δ*sidA*. The MwoI

Table I. *A. fumigatus Strains Used in This Study*

Strain	Genotype	Reference
CEA10	Wild type	22
CEA17	*pyrG*$^-$	22
Δ*sidA*-CEA17	CEA17, Δ*sidA::pyrG*	This work
sidA^c-CEA17	Δ*sidA*-CEA17, (p)::*sidA*	This work
Δ*sidA*/Δ*ftrA*-CEA17	Δ*sidA*-CEA17, Δ*ftrA::hph*	This work
Δ*ftrA*-CEA17	Δ*sidA*/Δ*ftrA*-CEA17, (p)::*sidA*	This work
ATCC 46645	Wild type	American Type Culture Collection
Δ*sidA*	ATCC 46645, Δ*sidA::hph*	This work
Δ*ftrA*	ATCC 46645, Δ*ftrA::hph*	This work
SidA^R	Δ*sidA*, Δ*sidA::sidA**	This work

* indicates a silently mutated version of *sidA* (see Materials and Methods).
^{R} indicates a reconstituted genomic locus.
^{c} indicates the presence of an ectopically integrated, complementing allele.

site allows reconstituted strains to be differentiated from wild-type contaminants. A representative transformant, *SidA*^{R}, was selected for further analysis.

To enable inactivation of *ftrA*, a 5.0-kb fragment of genomic DNA containing *A. fumigatus ftrA* was amplified using primers 5′-GTGGGATTGCTGATGCTG and 5′-AAGATTGATATCAACACCTTTCCCATAAC. The resulting plasmid was sequenced and designated pFTRA. An internal 1.7-kb NheI-HindIII fragment (+395 to + 2082, stop codon at +1322) was replaced with a 3.2-kb NheI-HindIII fragment from pAN7-1 carrying *hph*. A gel-purified 6.5-kb *Eco*RV fragment was used to transform *A. fumigatus* Δ*sidA*-CEA17, *sidA*^{c}-CEA17, and ATCC 46645. Hygromycin-resistant *ftrA*$^-$ transformants were selected, and a representative strain from each background, Δ*sidA*/Δ*ftrA*-CEA17, Δ*ftrA*-CEA17, and Δ*ftrA*, respectively, were selected and used for further analysis.

Virulence Assays. CD1 male mice (18–22 g; Harlan UK Ltd.) were housed in groups of eight in individually vented cages. Animals were allowed free access to food and water containing 1 g/liter tetracycline hydrochloride and 64 mg/l ciprofloxacin as prophylaxis against bacterial infection. Mice were immunosuppressed with i.p. cyclophosphamide (150 mg/kg) on days −3, −1, +2, and every subsequent third day throughout each experiment. A single dose of hydrocortisone acetate (112.5 mg/kg) was administered s.c. on day −1. Conidiospores were harvested in sterile saline from *Aspergillus* complete medium slants (containing 5 mM ammonium (+)−tartrate, 1.5 mM $FeSO_4$, and 200 mM NaH_2PO_4) incubated for 5 d at 37°C. The resultant suspension was filtered through Miracloth (Calbiochem), washed twice with sterile saline, counted using a hemocytometer, and resuspended at a concentration of 1–9 × 10^9 spores/ml. Mice were anesthetized by halothane and infected by intranasal instillation of 4 × 10^6 conidiospores in 40 ml of saline. Mice were weighed at 24-h intervals from day 0. Visual inspections were made twice daily. Mice were killed when predetermined end points, e.g., 20% weight-loss, moribund state, or failure to respond to gentle stimuli, were reached. Survival curves were compared using Kaplan-Meier log rank analysis. Immediately after sacrifice, lungs were removed and fixed in 4% formaldehyde (Sigma-Aldrich). Lungs were embedded in paraffin before sectioning and staining with hematoxylin and eosin and light green Grocott's methenamine silver. Murine infections were performed under UK Home Office Project Licence PPL/70/5361 in dedicated facilities at Imperial College London.

Results and Discussion

SidA Is Essential for Siderophore Biosynthesis. In a first step to studying the role of the siderophore system in iron homeostasis and virulence in *A. fumigatus*, we analyzed production of the hydroxamate-type siderophores TAFC and DFFC in two *A. fumigatus* isolates, ATCC 46645 and CEA10, by reversed phase HPLC (21). During iron starvation, both strains secrete TAFC and accumulate intracellular DFFC (Fig. 1 A). In iron-replete conditions, production of both siderophores is low. *A. fumigatus* siderophore production therefore resembles that of *A. nidulans* (21). Recently, we have shown that L-ornithine-N^5-monooxygenase, which catalyzes the first committed step of hydroxamate-type siderophore biosynthesis, is essential for growth of *A. nidulans* (18). A search in the genome sequence of *A. fumigatus* (http://www.tigr.org) revealed one putative L-ornithine-N^5-monooxygenase encoding gene, termed *sidA*. Comparison of the genomic and cDNA sequences revealed the presence of one intron in *sidA* (sequence data available from GenBank/EMBL/DDBJ under accession no. AY586511). The predicted amino acid sequence of SidA contains 501 amino acids, possesses all signatures typical for hydroxylases involved in siderophore biosynthesis, and has 78% identity to *A. nidulans* SidA. Northern analysis shows that *A. fumigatus sidA* expression is up-regulated by iron starvation (Fig. 1 B).

To analyze the function of *SidA*, we constructed gene deletions in both *A. fumigatus* backgrounds. HPLC analysis showed that the resulting *sidA*-deficient strains lose the

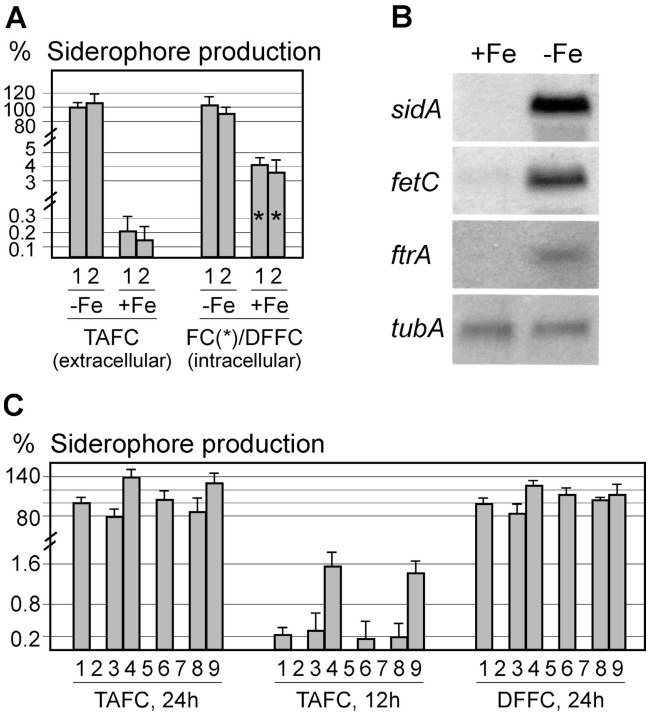

Figure 1. Iron-regulated siderophore production and expression of *A. fumigatus sidA*, *ftrA*, and *fetC*. (A) Siderophore biosynthesis is up-regulated during iron starvation. TAFC, DFFC, and FC levels in both wild-type *A. fumigatus* strains CEA10 (1) and ATCC 46645 (2), following growth for 24 h at 37°C in AMM (20) in iron starvation (−Fe) and iron-replete (+Fe; 10 μM $FeSO_4$) conditions, were determined by reversed phase HPLC analysis as described previously (21). *A. fumigatus* CEA10 siderophore levels under iron starvation conditions were normalized to 100%. (B) *A. fumigatus sidA*, *ftrA*, and *fetC* expression is up-regulated during iron starvation. Total RNA was isolated from *A. fumigatus* CEA10 following growth for 24 h during iron starvation (−Fe) and iron-replete (+Fe) conditions and subjected to Northern analysis. As a control for loading and RNA quality, blots were hybridized with a fragment from the β-tubulin encoding *tubA* gene. (**C**) *A. fumigatus sidA*–deficient strains do not synthesize siderophores, and *ftrA* deficiency brings forward the onset of siderophore biosynthesis. The siderophore production of *A. fumigatus* wild-type (CEA10 [1], ATCC 46645 [6]) and mutant strains (Δ*sidA*-CEA17 [2], *sidA*^{c}-CEA17 [3], Δ*ftrA*-CEA17 [4], Δ*sidA*/Δ*ftrA*-CEA17 [5], Δ*sidA* [7], *sidA*^{R} [8], Δ*ftrA* [9]) was analyzed following growth for 12 or 24 h under iron starvation conditions. *A. fumigatus* CEA10 siderophore levels at 24 h were normalized to 100%.

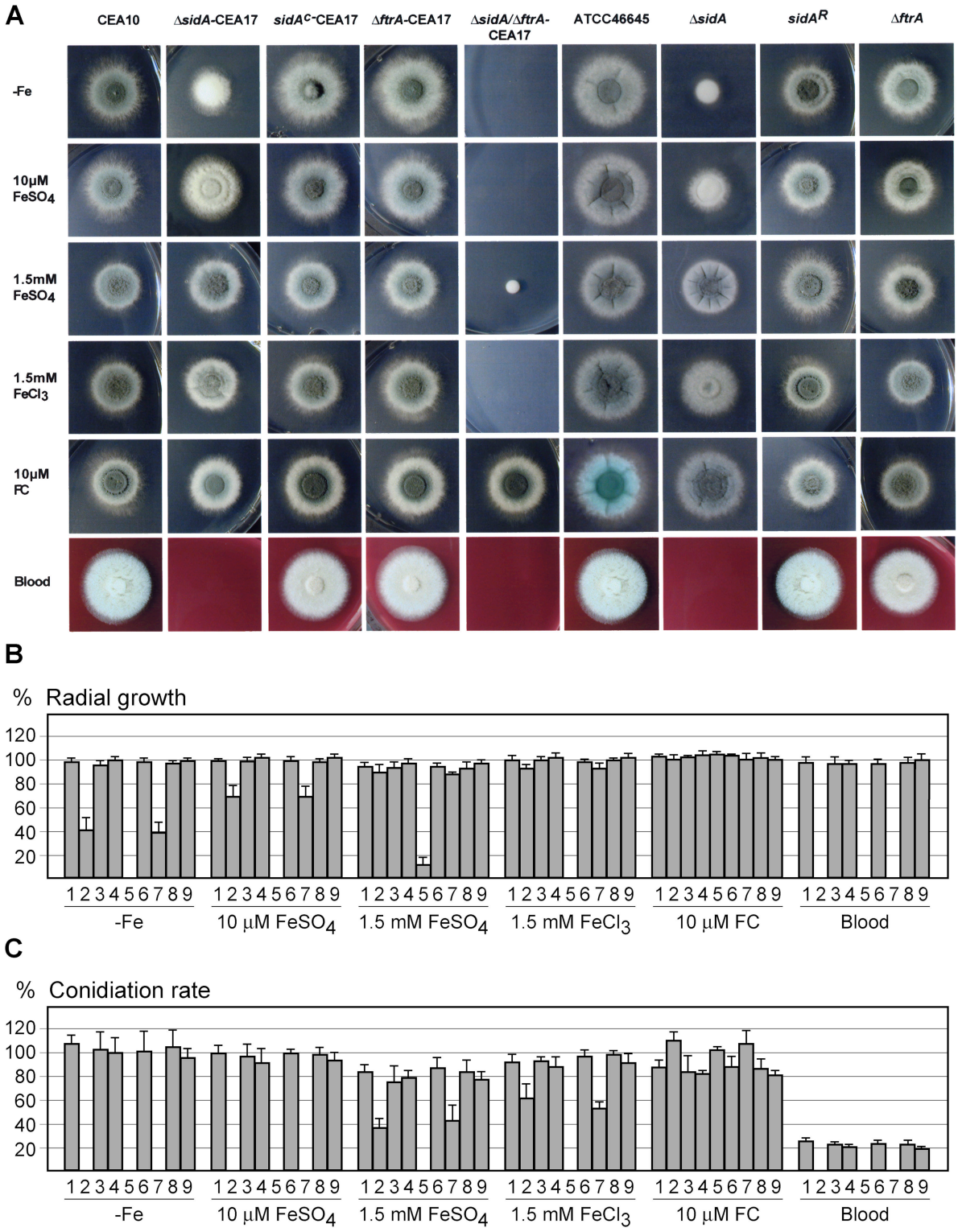

Figure 2. (A) Growth phenotypes of *A. fumigatus* wild-type and mutant strains. SidA and FtrA are components of two independent iron uptake mechanisms. Aliquots of 10^4 conidia of the respective strain were point inoculated on AMM plates containing the respective iron source and incubated for 48 h at 37°C. Blood agar was AMM containing 5% (vol/vol) sheep blood. (B) Quantitative analysis of radial growth of *A. fumigatus* wild-type (CEA10 [1], ATCC 46645 [6]) and mutant strains (Δ*sidA*-CEA17 [2], *sidA*^{c}-CEA17 [3], Δ*ftrA*-CEA17 [4], Δ*sidA*/Δ*ftrA*-CEA17 [5], Δ*sidA* [7], *sidA*^{R} [8], Δ*ftrA* [9]) was performed following growth for 48 h at 37°C. *A. fumigatus* CEA10 radial growth on 10 μM $FeSO_4$ was normalized to 100%. (C) Quantitative analysis of conidiation of *A. fumigatus* wild-type (CEA10 [1], ATCC 46645 [6]) and mutant strains (Δ*sidA*-CEA17 [2], *sidA*^{c}-CEA17 [3], Δ*ftrA*-CEA17 [4], Δ*sidA*/Δ*ftrA*-CEA17 [5], Δ*sidA* [7], *sidA*^{R} [8], Δ*ftrA* [9]) was performed following growth for 120 h at 37°C. *A. fumigatus* CEA10 conidiation rate on 10 μM $FeSO_4$ was normalized to 100%.

ability to produce both TAFC and DFFC (Fig. 1 C), demonstrating that *sidA* is required for *A. fumigatus* siderophore biosynthesis and is highly likely to encode L-ornithine-N^5-monooxygenase. In contrast to a siderophore-negative *A. nidulans* strain, which is almost nonviable without siderophore supplementation (18), *sidA* deficiency in *A. fumigatus* decreases growth rates moderately during iron starvation and only slightly in iron replete conditions (Fig. 2; unpublished data). However, a complete lack of growth was observed on blood agar plates (Fig. 2). This was rescued by addition of FC to the medium. In iron-depleted and replete conditions, the *sidA*-deficient strains had only 1% of wild-type conidiospore production; this increased to ~50% by supplementation with 1.5 mM iron. Supplementation with FC or complementation of *sidA*$^-$ in both backgrounds rescued all observed phenotypes (Fig. 2; unpublished data), e.g., defects in conidiation and growth on blood agar plates, demonstrating that the *sidA*$^-$ phenotypes are a direct result of loss of *sidA*.

A. fumigatus can acquire iron by reductive assimilation and ferrous uptake but lacks specific mechanisms for uptake of host iron compounds. The ability of *A. fumigatus sidA*$^-$ strains to grow on iron-limited media indicate that, in contrast to *A. nidulans*, this fungus possesses at least one additional iron assimilation system. Further inspection of the *A. fumigatus* genome sequence revealed the presence of several putative metalloreductase-encoding genes, one putative ferroxidase *fetC*, and one potential high affinity iron permease–encoding gene *ftrA*. *fetC* and *ftrA* are divergently transcribed from a 1.3-kb intergenic region and comparison of the genomic and cDNA sequences revealed the presence of five introns in *fetC* and three introns in *ftrA* (sequence data available from GenBank/EMBL/DDBJ under accession no. AY586512). The deduced amino acid sequences of FetC and FtrA are 52 and 55% identical, respectively, to the *C. albicans* ferroxidase Fet3 and iron permease Ftr1 (25). Northern blot analysis shows that expression of both genes is up-regulated by iron starvation (Fig. 1 B). Furthermore, *A. fumigatus sidA*$^-$ mutants exhibit increased sensitivity to two inhibitors of the reductive iron uptake system, bathophenanthroline disulfonate, and copper depletion (unpublished data; 26). Together, these data suggest that unlike *A. nidulans*, *A. fumigatus* has the capacity for reductive iron assimilation.

To analyze the potential role of FtrA in iron uptake by *A. fumigatus*, the *ftrA* gene was deleted in both *A. fumigatus* backgrounds. These *ftrA*-deficient strains have essentially wild-type growth and conidiation phenotypes (Fig. 2). However, they display an eightfold increase in TAFC production after 12 h of growth in iron-depleted conditions, demonstrating that lack of FtrA brings forward the onset of siderophore production, possibly to compensate for the lack of reductive iron assimilation.

We also constructed a *ΔsidA/ΔftrA* double mutant in *A. fumigatus* CEA17. On most media, this strain is unable to grow unless supplemented with ferricrocin (Fig. 2), demonstrating that *ftrA* encodes an essential component of a siderophore-independent iron uptake system. Very slow growth occurs in the presence of high levels of ferrous, but not ferric, iron (Fig. 2), suggesting the presence of a specific SidA/FtrA-independent ferrous iron uptake system. In addition, the *ΔsidA/ΔftrA* double mutant failed to grow on blood agar plates (Fig. 2) or on media containing 10 μM of hemoglobin, hemin, holotransferrin, or ferritin as the sole iron source (unpublished data). Thus, as an opportunistic rather than committed pathogen, *A. fumigatus* lacks specific systems for the uptake of host iron compounds.

Siderophore Biosynthesis But Not Reductive Iron Assimilation Is Essential for Virulence of A. fumigatus. To test the requirement for *sidA* and *ftr*A in a murine model of invasive aspergillosis, we compared the virulence of wild-type parental *A. fumigatus* ATCC 46645 and the otherwise isogenic *ΔsidA* and *Δftr*A strains. Groups of 15 neutropenic CD1 mice were intranasally inoculated with equivalent doses (2×10^5) of conidiospores from each strain. The *ΔftrA* mutant was as virulent as the wild-type strain, demonstrating that reductive iron uptake has little or no role to play in *A. fumigatus* virulence (Fig. 3). All mice infected with *A. fumigatus ΔftrA* and ATCC 46645 displayed significant weight loss within 24 h of infection, and weight dropped further throughout the course of the experiment. In vivo, germination of both strains was detectable 40 h postinfection (not depicted) and mice infected with either strain exhibited >90% mortality within 6 d of infection (Fig. 3). *A. fumigatus* was cultured from the lungs of all killed animals. Mock infection with saline resulted in zero mortality (not depicted). In stark contrast, the *ΔsidA* mutant is completely attenuated (Fig. 3). Animals infected with *A. fumigatus ΔsidA* demonstrated modest weight loss within 24 h of infection. This was followed by steady weight gain, despite continued immunosuppression, for the duration of the experiment. All mice survived to at least 15 d postinfection ($P < 0.001$; Kaplan Meier Log Rank Analysis). Reconstitution of *sidA* resulted in restoration of virulence to wild-type levels (Fig. 3), demonstrating that SidA, and thus siderophore biosynthesis, is absolutely required for *A. fumigatus* virulence.

To investigate differences in the fate of *A. fumigatus sidA*-null (*ΔsidA*) and reconstituted (*sidA*^{R}) conidiospores in vivo and assess variation in host immune responses, mice were killed 20, 40, and 60 h postinfection with 2×10^5

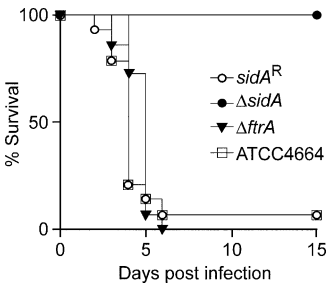

Figure 3. Virulence analysis of *A. fumigatus* iron uptake mutants. Siderophore biosynthesis is essential, whereas reductive iron uptake is unnecessary for *A. fumigatus* virulence. Groups of 15 neutropenic CD1 mice were infected with 2×10^5 conidiospores. Δ*ftr*A (closed triangles) and *sidA*^{R} (closed circles) demonstrate comparable virulence ($P = 0.1656$ and $P = 0.9617$, respectively, by Kaplan Meier log rank analysis) to the wild-type strain ATCC 46645 (open squares). In comparison to *A. fumigatus* ATCC 46645 and *sidA*^{R}, Δ*sidA* (closed circles) is completely attenuated for virulence ($P < 0.0001$).

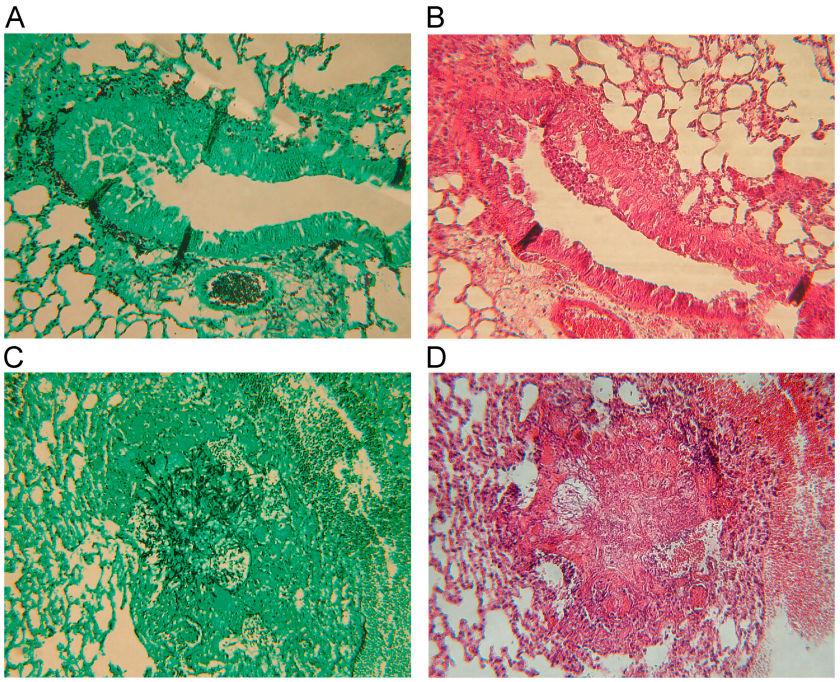

Figure 4. Histological analysis of mice infected with *A. fumigatus* Δ*sidA* and *sidA*^{R} strains. The *A. fumigatus* Δ*sidA* mutant does not germinate in vivo and causes minimal cellular infiltration, whereas the *sidA*^{R} strain germinates, grows profusely, engenders a massive cellular infiltration, and causes major tissue damage. Lungs were surgically removed from mice killed 60 h postinfection with 2×10^5 *A. fumigatus* Δ*sidA* (A and B) or *sidA*^{R} (C and D). Sequential paraffin-embedded murine lung sections were stained with light green and Grocott's methenamine silver (A and C) or hematoxylin and eosin (B and D).

conidia. Lungs were surgically removed and prepared for histology (Fig. 4). Conidia of both strains remain largely ungerminated in the bronchioles 20 h postinfection, and similar levels of cellular recruitment to sites of conidial deposition can be seen. Germination and hyphal extension of the reconstituted *sidA*^{R} conidia are clearly visible 40 h postinfection. Some evidence of epithelial penetration by hyphae, accompanied by increased inflammatory infiltration, is also apparent. At 60 h postinfection, considerable mycelial growth of *A. fumigatus sidA*^{R} can be seen in the lung, characterized by extensive penetration of surrounding tissues, effectively destroying any recognizable lung structure at the foci of infection, and heavy recruitment of inflammatory cells (Fig. 4). In complete contrast, no germination of Δ*sidA* conidia was observed in vivo (Fig. 4). Although conidia are still present in bronchioles 60 h postinfection, only very limited cellular recruitment to foci of infection was seen (Fig. 4). These data suggest that the process of germination plays a role in inducing the host's inflammatory response to infection.

Our data demonstrate that *A. fumigatus* principally employs a siderophore-based iron acquisition system in vivo, in contrast to the reductive iron assimilation system required in the mammalian host by *C. albicans* (*11*). Indeed the *A. fumigatus* reductive iron assimilation system is redundant in vivo. In addition to *A. fumigatus*, many pathogenic fungi produce hydroxamate-type siderophores (27). Since mammals lack SidA, and the hydroxamate-type siderophore system in general, it represents a possible target for the development of antifungal therapies against siderophore-producing fungi.

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No author has a direct competing interest in the work presented. A patent application has been filed on aspects of this work, and some authors potentially have patent rights.

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