RESEARCH ARTICLE
INTERNATIONAL MICROBIOLOGY (2006) 9:281-287
www.im.microbios.org

Tracy Power
Montserrat Ortoneda
John P. Morrissey
Alan D. W. Dobson*

Microbiology Department and Environmental Research Institute, University College Cork, National University of Ireland, Cork, Ireland

Differential expression of genes involved in iron metabolism in *Aspergillus fumigatus*

Summary. The ability of fungi to survive in many environments is linked to their capacity to acquire essential nutrients. Iron is generally complexed and available in very limited amounts. Like bacteria, fungi have evolved highly specific systems for iron acquisition. Production and uptake of iron-chelating siderophores has been shown to be important for certain human bacterial pathogens, as well as in fungal pathogens such as *Cryptococcus neoformans* and *Fusarium graminearum*. This system also enables the opportunistic fungal pathogen *Aspergillus fumigatus* to infect and subsequently colonize the human lung. In this study, advantage was taken of genome sequence data available for both *Aspergillus nidulans* and *A. fumigatus* either to partially clone or to design PCR primers for 10 genes putatively involved in siderophore biosynthesis or uptake in *A. fumigatus*. The expression of these genes was then monitored by semi-quantitative and quantitative real-time PCR over a range of iron concentrations. As expected, the putative biosynthetic genes *sidA*, *sidC* and *sidD* were all strongly up-regulated under iron starvation conditions, although the variable degree of induction indicates complex regulation by a number of transcriptional factors, including the GATA family protein SreA. In contrast, the gene *sidE* shows no iron-regulation, suggesting that SidE may not be involved in siderophore biosynthesis. The characterisation of the expression patterns of this subset of genes in the iron regulon facilitates further studies into the importance of iron acquisition for pathogenesis of *A. fumigatus*. [**Int Microbiol** 2006; 9(4):281-287]

Key words: *Aspergillus fumigatus* · iron metabolism · siderophore · real-time PCR · GATA factor

Received 10 August 2006
Accepted 15 October 2006

*Corresponding author:
A.D.W. Dobson
Microbiology Department
National University of Ireland
Cork, Ireland
Tel. +353-214901946. Fax +353-214901932
E-mail: a.dobson@ucc.ie

Introduction

Aspergillus fumigatus is the most common airborne fungal pathogen of humans, causing life-threatening invasive disease in immunocompromised patients, such as bone marrow and stem cell transplant patients [8]. The survival of *A. fumigatus* in the host indicates that this fungus has mechanisms for obtaining essential nutrients for its growth and reproduction [7]. One of these essential nutrients is iron. Iron levels in the human body are very low. For example, free-iron concentration in serum is 10^{-18} M. This is due to the iron being complexed with various iron-binding molecules, including ferritin and heme compounds within the cell or with transferrin or lactoferrin in extracellular fluids [2,16]. Fungi have developed various high-affinity mechanisms of iron acquisition [6]. Under iron starvation, most fungi synthesize and excrete siderophores, which are low molecular-weight, ferric iron-specific chelators [12]. In addition to iron acquisition, siderophores play a role in intracellular iron storage in most fungi [11]. However, in some yeasts such as *Saccharomyces cerevisiae*, siderophores play a minor role in iron acquisition.

Siderophore production in *Aspergillus nidulans* has been well studied, and the fungus is known to produce two major

siderophores. It excretes triacetylfusarine C (TAFC) and contains ferricrocin (FC) intracellulary [3,13]. Subsequent to chelating Fe(III), the siderophore-Fe(III) complex is taken up by MirB, a transporter belonging to the major facilitator protein superfamily [6]. After uptake, TAFC is cleaved by an esterase, and products excreted. Fe(III) is then transferred to FC [13]. Recent reports indicate that siderophore biosynthesis is essential for viability of *A. nidulans* [4]. Two genes involved in siderophore biosynthesis have been characterised in *A. nidulans*, namely *sidA* (L-ornithine-N^5-oxygenase), which catalyzes the first committed step in siderophore biosynthesis, and *sidC*, which encodes a non-ribosomal peptide synthetase (NRPS) involved in FC synthesis [4]. In *A. nidulans*, siderophore biosynthesis and uptake are negatively regulated by the GATA transcription factor SREA. This iron responsive transcriptional repressor is known to regulate a number of siderophore biosynthetic genes, including *sidA*, *sidB*, *sidC* and *atrH*, as well as the utilization genes *mirA*, *mirB*, *mirC* and *estA*. In addition, the *sodA*, *sodB* and *catB* genes encoding superoxide dismutases and catalase have been reported to be up-regulated under iron-replete conditions in an SREA mutant [5].

Work on the siderophore system in *A. nidulans* has facilitated the characterisation of the equivalent system in *A. fumigatus*, with the siderophore system and in particular the *sidA* gene, which has been reported as being essential for virulence in *A. fumigatus* [19]. Thus, given the potential role of iron metabolism on fungal pathogenicity, and the fact that siderophore biosynthesis and uptake represent possible targets for antifungal therapy [5], it is important that we gain a fuller understanding of the regulation of key genes involved in iron metabolism in *A. fumigatus*. We report here the differential regulation of a number of genes involved in iron metabolism in *A. fumigatus* when the fungus is grown in a range of iron concentrations. These genes appear to be regulated in a similar manner to their homologous genes in *A. nidulans*, with increased expression levels being observed under low iron conditions, and expression levels decreasing as iron concentrations increase.

Materials and methods

Fungal strain and culture media. *A. fumigatus* strain AF293 was cultured in *Aspergillus* minimal medium (AMM) containing different concentrations of $FeSO_4$ (1, 10, 100, 1000 μM). For iron depleted conditions, iron was omitted. *A. fumigatus* cultures were set up in 250 ml conical flasks at 150 rpm. Medium was inoculated with *A. fumigatus* conidia at a final concentration of 1×10^7 spores/ml (spore concentration was calculated using a haemocytometer, Agar Scientific Ltd., Essex, UK), and flasks incubated at 37°C. After 24 h, mycelia were harvested. Mycelium was filtered through sterilised miracloth (Calbiochem), washed with phosphate buffer saline (PBS) and dried using Whatmann filter paper. Mycelia were then stored at –70°C until needed for RNA extraction. The culture supernatants were kept and used for determination of iron concentration and the evaluation of siderophore production using the chrome azurol S (CAS) assay [20].

Determination of iron concentration. The culture supernatants and un-inoculated control medium for each of the iron concentrations were tested for iron concentration using Flame Atomic Absorption (Perkin-Elmer 2280 AAS), and the utilization of iron by the fungus was established.

Evaluation of siderophore production. A modification of the CAS assay was used to detect siderophore production in *A. fumigatus*. Culture supernatants were collected after 48 h and 100 μl was added to wells that were cut out of the CAS agar plates. Plates were incubated at 37ºC for 5 h. The change in color or the presence of a halo around the well after incubation indicated siderophore production.

Bioinformatic analysis. PCR primer sets (Table 1) were designed to amplify parts of *A. fumigatus* genes likely to be involved in iron metabolism using sequence data available in the NCBI Genbank [http://www.ncbi.nlm.nih.gov/]. Sequence data for *b-tubulin*, *sidA*, *sidC*, *sidD*, *sidE*, *ftrA* and *fetC*, *mirB*, *amcA*, *catB* and *lysF* can be found under the accession numbers AY048754, AY586511, DQ011870, DQ013888, DQ011871, AY586512, NW876231, NW876227, U87850 and NW876238, respectively. For *mirC*, which was not available in the NCBI Genbank at that time, sequence data from searches in the [http://www.genedb.org] databases were used. Whole genome shotgun sequences of *A. fumigatus* were subjected to BLAST algorithm to identify similarity to *mirC* in *A. nidulans.* For *sreA*, an alignment of *A. nidulans*, *Ustilago maydis*, *Neurospora crassa*, *Penicillium chrysogenum*, *Botrytis cinera*, *Candida albicans* and *Schizosaccharomyces pombe* gene sequences were used to identify highly conserved regions, which were then

Table 1. PCR primers used in this study

Gene		Primer sequence (5′-3′)	Product size (bp)
β-tubulin	FP	ACAACTGGGCCAAGGGTCACTACA	224
	RP	GGGGGAGGAACAACGGAGAAG	
sidA	FP	GGGGAAGAGGTGGTCGAAGTG	223
	RP	GGGCCGGCAGCGTTGTG	
sidC	FP	AGGGAGGCTGCTTGAAATA	222
	RP	AGAATGAGGCCTTGCGAACTG	
sidD	FP	CAACTGCCAGCACGCGGTCTC	232
	RP	CGGTACGGGTCTGGTCATTCA	
sidE	FP	AGGCCTTCTTGCGGACTGGTG	274
	RP	GCCTTGAGCTTCTTGCGATTGG	
mirB	FP	CCACCGCCGGCTACATCACCA	261
	RP	AGGCGGCCAGCACAGCAACC	
mirC	FP	GCGCTGGGGCTACGGGATGT	238
	RP	CGCAGCAAGAGTAAGAGGCAC	
ftrA	FP	GATCACTTCGCTAGCACGGAG	233
	RP	ACGGTGATGAAGGGGAGGAGA	
fetC	FP	GTCGCCGCTAGCCCTGAATCC	230
	RP	TGACCCCAGCAACCACACGAC	
amcA	FP	TGCGCCTCCAATCACAACCATC	255
	RP	CGGAAGCGGCACCACTGAACA	
sreA	FP	CACCGACAGGGGCGATGATTT	287
	RP	GCCTCGAGCAGCAGACTTGTA	
catB	FP	CCCCGAGAGCTGATGATGAAG	244
	RP	CTGCGGGGTAATCCAAGCCAC	
lysF	FP	AGAGGAGGCGGGTGACTGG	254
	RP	CTTCGGGGCGCTTGTAGAT	

used for primer design in *A. fumigatus*. The identity of each gene fragment was confirmed following sequence analysis of each PCR product. Based on the deduced amino acid sequence, high levels of identity (65% SreA and 37% MirC) were observed with the *A. nidulans* homologs. These partial gene sequences can now be found under the accession numbers XM748430 and XM744609, for *sreA*, and *mirC*, respectively.

RNA preparation and cDNA synthesis. The harvested mycelia were pulverized to a fine powder using a mortar and pestle in the presence of liquid nitrogen. Total RNA was isolated, using RNeasy plant mini-kit (Qiagen). Extracted RNA was treated with 2 µl DNase I, RNase free (Roche) and 2 µl $MgCl_2$. A clean up step was then carried out as per Rneasy plant mini-kit instructions. RNA integrity was assessed using 1.0% TBE agarose gel electrophoresis and subsequently stored at –70°C. PCR was carried out to ensure that there was no DNA contamination of the RNA sample. From each of the iron growth conditions of *A. fumigatus*, cDNA was synthesised using Expand reverse transcriptase and random hexamer primers (Roche).

Real-time PCR (RT-PCR). Standard PCR reactions were performed using cDNA as a template. As a control, primers specific for the *β-tubulin* of *A. fumigatus* gene were used to monitor expression of this constitutively expressed gene. The PCR consisted of the following steps: 95°C denaturation for 30 s, optimum annealing temperature for 30 s, 72°C extension for 30–45 s × 30 cycles; and 72°C extension for 4 min. RT-PCR was carried out in 20 µl volumes on a Lightcycler™ (Roche) as previously described [15]. Reactions were prepared in Lightcycler™ glass capillaries (Roche), which were incubated in pre-cooled centrifuge adapters prior to sample preparation and loading. The fast start SYBR Green master kit (Roche) was used for all LightCycler (LC)-PCR reactions. Primers were specifically designed to have similar annealing temperatures and size. Conditions were optimized for the real time PCR as recommended by the manufacturer. This included optimizing $MgCl_2$ concentration, primer concentration and annealing temperatures. The final optimized conditions for *β-tubulin*, *sidA*, *sidC*, *sidD*, *sidE*, *mirB*, *fetC*, *ftrA*, *amcA*, *sreA*, *lysF* and *catB* genes consisted of an 18 µl of a master mix: 0.5 µl each primer, $MgCl_2$ (25 µM stock), Faststart DNA Taq polymerise, reaction buffer, dNTP mix, SYBR Green I dye and 2 mM $MgCl_2$, and H_2O (PCR grade, Roche). To this master mix was added 2 µl of undiluted template to create a total reaction volume of 20 µl. The contents were placed in glass capillaries, capped, briefly centrifuged (Eppendorf centrifuge 5415 C) and placed in the Lightcycler. Cycling involved an initial denaturing step (95ºC for 10 s), amplification (59ºC for 15 s × 35 cycles), melting curve analysis (69ºC for 15 s) and a cooling step (40ºC for 30 s). LC assay data was carried out using second derivative maximum method of the Roche LC software (version 3.53). Relative gene expression was obtained using the $2^{-\Delta\Delta C_T}$ method [9]. To verify that the light cycler PCR products were of the correct size, a 10 µl aliquot of each reaction was run on a 1.5% agarose gel stained with ethidium bromide and visualised under UV light. Each experiment was performed in triplicate and standard deviations calculated to ensure statistical accuracy.

Results and Discussion

Siderophore production. As expected, after five hours higher levels of siderophores were produced in the absence of free iron (Fig. 1), as indicated by the presence of a large orange halo. A smaller halo was produced from fungal cultures grown at 1 µM $FeSO_4$, while no siderophore production was detected at concentrations higher than 1 µM $FeSO_4$. Similar methods have been used to detect siderophore production in other *Aspergillus* species [10], thus this method also proved useful to monitor production in *A. fumigatus.*

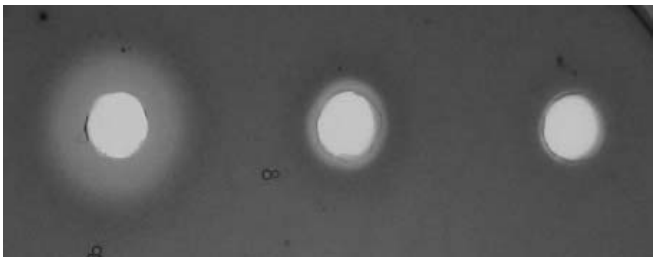

Fig. 1. Evaluation of siderophore production by *Aspergillus fumigatus* under iron concentrations (0, 1 and 10 µM). Maximum siderophore production was induced when *A. fumigatus* was grown in the absence of iron. This can be seen by the presence of an orange halo.

Analysis of gene expression. These PCR primers were then used to monitor expression of these iron metabolism genes in *A. fumigatus* using RT-PCR, when the fungus was grown in the presence of different iron concentrations ranging from 0 to 10,000 µM $FeSO_4$ (Fig. 2). From Fig. 2B it is clear that expression of the majority of the genes examined was up-regulated under low iron conditions, with expression levels decreasing as the iron concentration in the growth medium increased. This is particularly true for the siderophore biosynthetic genes *sidA*, *sidC*, and *sidD*, the putative siderophore transport gene *mirB*, the high affinity permease gene *ftrA* and the putative mitochondrial carrier for the siderophore precursor ornithine *amcA*. The highest levels of expression in each case were observed at 0 µM $FeSO_4$. Expression of the putative siderophore transport gene *mirC* however, appeared to be largely unaffected by iron, suggesting that it might form part of a different regulon within *A. fumigatus*.

In contrast to many of the other genes expression of *sidD* appears to be repressed at 10 µM $FeSO_4$ while the expression of the putative GATA-type transcription factor gene *sreA* is repressed at 0 µM $FeSO_4$, with expression levels increasing as iron concentrations increase (Fig. 2B). The *sreA* gene product SREA functions as a repressor of siderophore biosynthesis in *A. nidulans*, with *sreA* expression being reported to be repressed at low iron concentrations [14]. Our data therefore support the idea that the *sreA* gene might play a similar role in regulating siderophore biosynthetic genes in *A. fumigatus* and *A. nidulans*.

Table 2 shows the differential expression of some of these iron-regulated genes in *A. fumigatus*. Using 10 µM $FeSO_4$ as the standard iron concentration, the siderophore biosynthetic genes *sidA*, *sidC* and *sidD* were 21-, 17- and 96-fold up-regulated respectively under low iron conditions. Haas and coworkers [19] have previously reported the up-regulation of *sidA* expression in *A. fumigatus* under low iron conditions using Northern analysis. Overall patterns of *sidC* and *sidD*

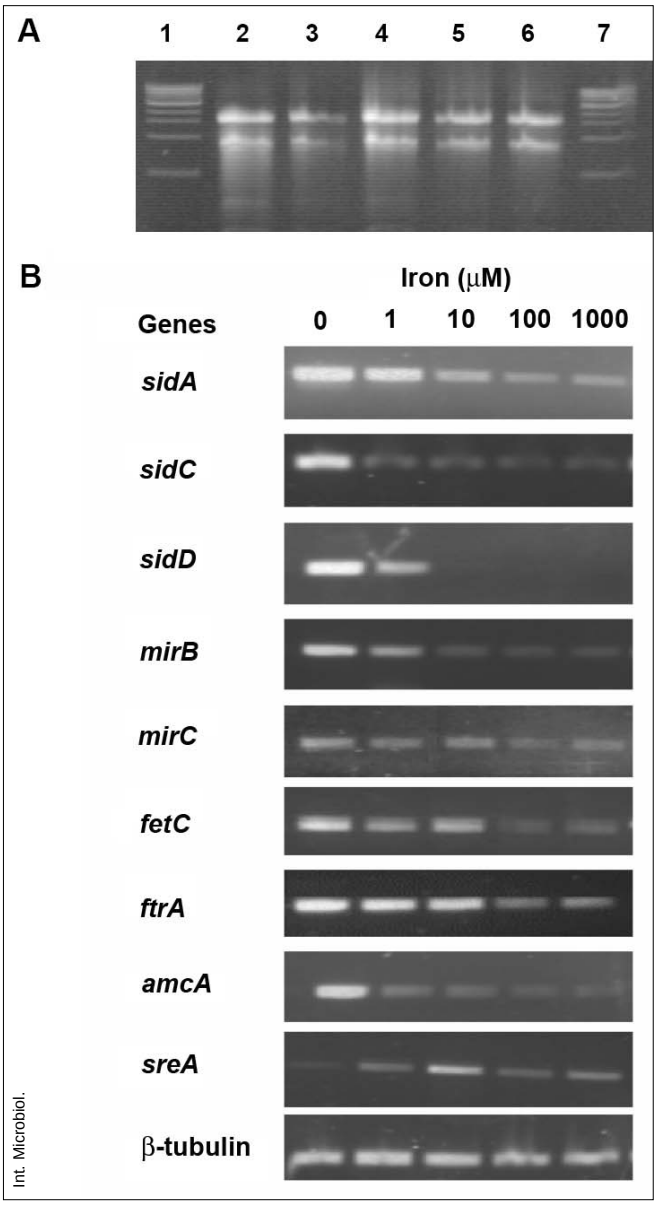

Fig. 2. (**A**) Total RNA from Aspergillus fumigatus. Lane 1 and 7: 1 kb ladder, Lanes 2, 3, 4, 5 and 6 represent RNA extracted from cultures grown in 0, 1, 10, 100, 1000 μM FeSO4. (B) Real-time PCR analysis of *sidA, sidC, sidD, mirB, mirC, ftrA, fetC, sreA*, and *amcA* gene expression in *Asperigillus fumigatus* under different iron conditions. *β-tubulin* gene expression was monitored as a control.

expression were similar at the different iron concentrations tested. However, *sidD* was up-regulated to a greater extent (96-fold) than *sidC* (17-fold) at 0 μM $FeSO_4$ and repressed to a greater extent (0.43-fold) than *sidC* (1.28-fold) at 1000 μM $FeSO_4$. Previously, Reiber and co-workers have also reported similar trends with *sidD* expression decreasing from 25–60% under low iron conditions, and up to 90% under high iron concentration, when compared to expression in the absence

Table 2. Relative fold induction of iron metabolism related genes under differential iron conditions using real-time PCR

	Iron concentrations (μM)				
Gene	0	1	10	100	1000
sidA	21.30 ± 11.0[a]	4.88 ± 2.31	1	0.87 ± 0.31	1.32 ± 0.50
sidC	17.66 ± 13.2	1.00 ± 0.08	1	1.92 ± 1.47	1.28 ± 0.75
sidD	96.3 ± 7.40	13.41 ± 14.6	1	0.44 ± 0.07	0.43 ± 0.50
sidE	0.26 ± 0.1	0.38 ± 0.2	1	0.54 ± 0.05	0.59 ± 0.03
mirB	38.2 ± 4.47	6.81 ± 0.90	1	0.24 ± 0.08	0.33 ± 0.17
ftrA	7.36 ± 7.37	1.51 ± 0.94	1	0.60 ± 0.50	0.90 ± 0.90
fetC	2.11 ± 0.51	0.83 ± 0.19	1	0.35 ± 0.08	0.39 ± 0.13
amcA	20.8 ± 7.08	1.291 ± 0.50	1	0.56 ± 0.14	0.52 ± 0.16
sreA	0.12 ± 0.03	0.49 ± 0.07	1	0.70 ± 0.04	0.66 ± 0.13
catB	0.12 ± 0.04	0.18 ±0 .02	1	0.40 ± 0.13	0.60 ± 0.17
lysF	0.39 ± 0.30	1.04 ± 0.43	1	0.91 ± 0.47	1.05 ± 0.89

[a]Values represent the mean of each triplicate sample ± standard deviation.

of iron, using RT-PCR [17]. Thus, although it appears that the siderophore biosynthetic gene *sidA*, and the two putative non ribosomal peptide synthase (NRPS) genes *sidC* and *sidD* may be co-regulated by iron in *A. fumigatus*, they are likely to be regulated by a number of distinct systems given that different transcript levels were observed for each of these genes at different iron concentrations. Note that the expression of the other putative NRPS gene *sidE* was largely unaffected by iron, which suggest that it may not be involved in siderophore biosynthesis in *A. fumigatus*, but could perform additional functions in addition to siderophore biosynthesis, which may explain why iron does not affect its expression.

Expression of *amcA*, which is likely to encode a mitochondrial carrier for the siderophore precursor ornithine in *A. fumigatus*, also appears to be markedly up-regulated (20-fold) at 0 μM $FeSO_4$, with expression decreasing (0.5-fold) at higher iron concentrations. Expression of *amcA* in *A. nidulans* appears to be regulated in a similar fashion, with transcript level being high during iron depletion and significantly lower during iron replete growth [14]. Given that *amcA* expression is directly regulated by SREA in *A. nidulans*, it seems likely that *amcA* expression may also be regulated in a similar fashion in *A. fumigatus* specifically to meet the increased ornithine demand, as this amino acid is the key precursor for all hydroxamate sidrophores. Expression levels of *mirB*, a putative siderophore transporter, which is known to transport triacetylfusarine C in *A. nidulans*, *ftrA*, a putative permease and *fetC*, a putative ferroxidase are also up-regulated at 0 μM $FeSO_4$ being 38-, 7- and 2-fold up-regulated respectively (Table 2); while expression of both *mirB* and *fetC* were also down-regulated at higher concentrations of iron (100 and 1000 μM).

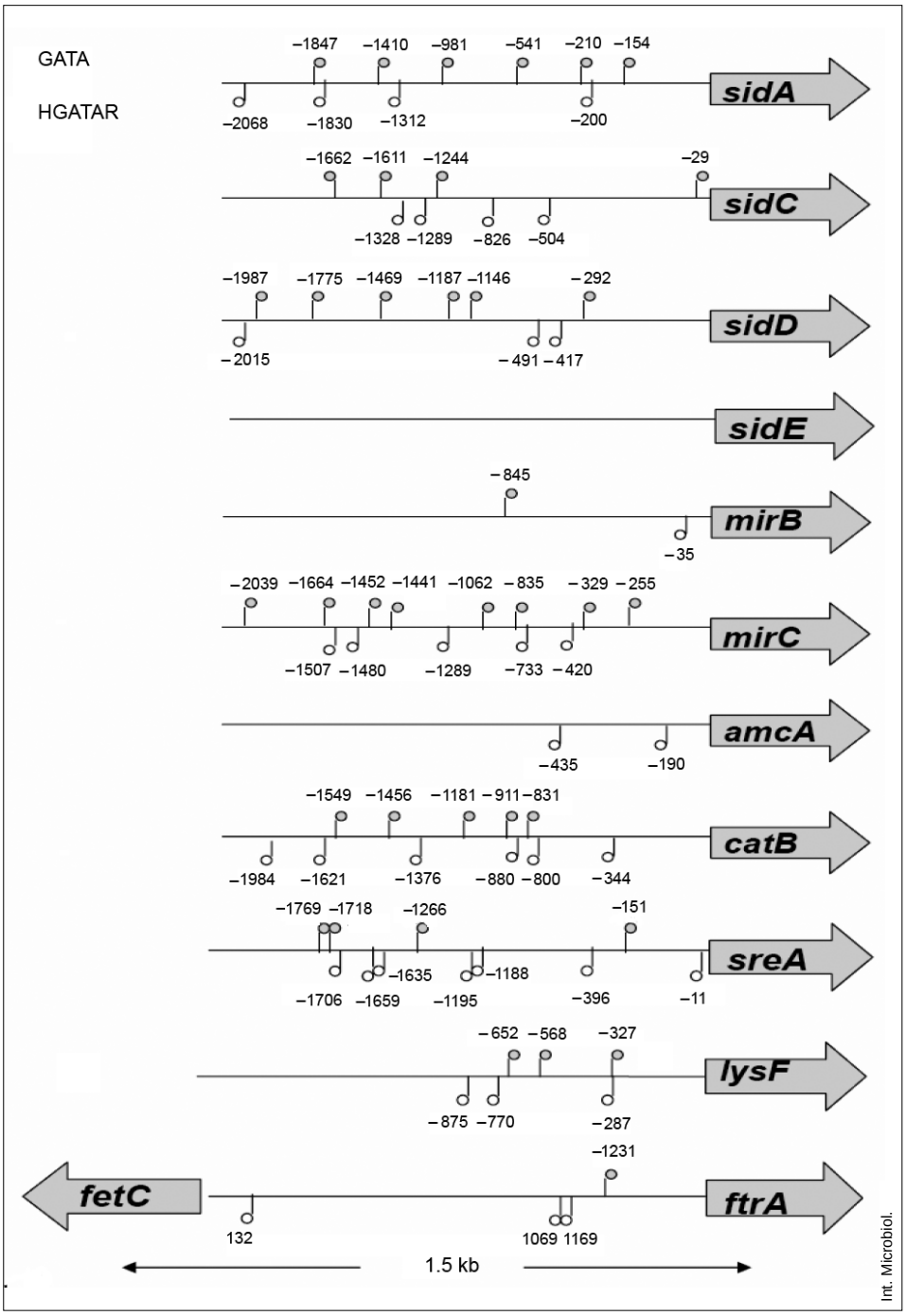

Fig. 3. Localization of putative binding sites for GATA transcription factors in *Aspergillus fumigatus* (H = A, C or G, R = A or G), in the promoter regions of the *sidA, sidC, sidD, sidE, mirB, mirC, amcA, catB, sreA, lysF, ftrA* and *fetC* genes.

Finally, in contrast to the other iron metabolism genes, in which iron depletion led to up-regulation of gene expression, the opposite trend was observed in the expression levels of *sreA*, *catB* and *lysF*, which were down-regulated 0.1-, 0.1- and 0.4-fold, respectively, at 0 μM $FeSO_4$. The repression of *sreA* expression is interesting with our results showing that as iron concentration increases (100 and 1000 μM) *sreA* expression decreases. Previous reports on *A. nidulans* had shown that, in *sreA* deletion strains, extracellular siderophore production was de-repressed but still regulated negatively by iron availability, indicating the presence of an additional iron-regulatory mechanism [13]. The same may be true in *A. fumigatus* given that at 100 and 1000 μM $FeSO_4$ SREA levels may be sufficiently low not to cause repression of siderophore production, yet siderophore production ceases in *A. fumigatus* (see Fig. 1).

Analysis of promoter regions. Analysis of the promoter region of each of the genes was then performed to determine whether a correlation between expression and the presence of putative *sreA* transcription factor binding sites could be established (Fig. 3). Note that a number of HGATAR motifs, which represent putative binding sites for GATA factors such as SREA [18], were observed in many of the promoter sequences analysed. In addition, a number of GATA sequences were also present in a number of the promoter sequences analysed. These GATA sequences which do not follow the HGATAR consensus motif have also been found to serve as binding sites for GATA factors in vivo such as in the *sid1* promoter of *Ustilago maydis*, which facilitates binding of the SREA homologue URBS1 [1]. While either HGATAR or GATA sequences were present in the promoter regions analysed, one exception was the *sidE* gene promoter, which did not contain either of these potential SreA binding sites, indicating that *sidE* may not be under the control of *sreA*. This correlates with the results obtained from real time PCR, which indicated that its expression was unaffected by iron. Finally, SreA itself might be auto-regulated, given that a number of GATA sequences are present in the promoter region of *sreA* gene.

In summary, it appears that a number of genes that are probably involved in iron metabolism in *A. fumigatus* are regulated in a similar manner to their homologous genes in *A. nidulans* [5,6,14]. It also seems probable that many of these genes in *A. fumigatus* are regulated by the GATA transcription factor SREA as is the case in *A. nidulans*, but also that some factor other than SREA must be involved in regulating the system in *A. fumigatus*. Thus, the well characterized siderophore biosynthetic and uptake systems in *A. nidulans* should provide us with a useful model with which to further dissect iron metabolism in *A. fumigatus*, thereby offering further insights into iron regulation of gene expression in this pathogenic fungus.

Acknowledgements. This work was financially supported by the Irish Research Council for Science and Engineering Technology (IRCSET), by Enterprise Ireland (SC/02/517; IP/2005/0268) and by the Irish government under the National Development Plan 2000-2006.

References

1. An Z, Mei B, Yuan WM, Leong SA (1997) The distal GATA sequences of the *sid1* promoter of *Ustilago maydis* mediate iron repression of siderophore production and interact directly with *Urbs1*, a GATA family transcription factor. EMBO J 16:1742-1750
2. Byers BR, Arceneaux JE (1998) Microbial iron transport: iron acquisition by pathogenic microorganisms. Met Ions Biol Syst 35:37-66
3. Charlang G, Ng B, Horowitz NH, Horowitz RM (1981) Cellular and extracellular siderophores of *Aspergillus nidulans* and *Penicillium chrysogenum*. Molec Cell Biol 1:94-100
4. Eisendle M, Oberegger H, Zadra I, Haas H (2003) The siderophore system is essential for viability of *Aspergillus nidulans*: functional analysis of two genes encoding L-ornithine N^5- monooxygenase (*sidA*) and a non-ribososmal peptide synthase (*sidC*). Mol Microbiol 49:359-375
5. Haas H (2003) Molecular genetics of fungal siderophore biosynthesis and uptake: the role of siderophores in iron uptake and storage. Appl Microbiol Biotechnol 62:316-330
6. Haas H, Schoeser M, Lesuisse E, Ernst JF, Parson W, Abt B, Winkelmann G, Oberegger H (2003) Characterisation of the *Aspergillus nidulans* transporters for the siderophores enterobactin and triacetylfusarinine C. Biochem J 371:505-513
7. Hissen AHT, Chow JMT, Pinto LJ, Moore MM (2004) Survival of *Aspergillus fumigatus* in serum involves removal of iron from transferrin: the role of siderophores. Infect Immun 72:1402-1408
8. Kontoyiannis DP, Bodey GP (2002) Invasive aspergillosis in 2002: an update. Eur J Clin Microbiol Infect Dis 21:161-172
9. Livak JK, Schmittgen TD (2001) Analysis of relative gene expression data using real-time-quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. Methods 25:402-408
10. Machuca A, Milagres AMF (2003) Use of CAS-agar plate modified to study the effect of different variables on the siderophore production by *Aspergillus*. Lett Appl Microbiol 36:177-181
11. Matzanke BF, Bill E, Trautwein AX, Winkelmann G (1987) Role of siderophores in iron storage in spores of *Neurospora crassa* and *Aspergillus ochraceus*. J Bacteriol 169:5873-5876
12. Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270:26723-26726
13. Oberegger H, Schoeser M, Zadra I, Abt B, Haas H (2001) SREA is involved in regulation of siderophore biosynthesis, utilization and uptake in *Aspergillus nidulans*. Mol Microbiol 41:1077-1089
14. Oberegger H, Schoeser M, Zadra I, Schrettl M, Parson W, Haas H (2002) Regulation of *freA*, *acoA*, *lysF* and *cycA* expression by iron availability in *Aspergillus nidulans*. Appl Environ Microbiol 68:5769-5772
15. O'Callaghan J, Stapleton PC, Dobson ADW (2006) Ochratoxin A biosynthetic genes in *Aspergillus ochraceus* are differentially regulated by pH and nutritional stimuli. Fungal Genet Biol 43:213-221
16. Ratledge C, Dover LG (2000) Iron metabolism in pathogenic bacteria. Annu Rev Microbiol 54:881-941
17. Reiber K, Reeves EP, Neville CM, Winkler R, Gebhardt P, Kavanagh K, Doyle S (2005) The expression of selected non-ribosomal peptide synthetases in *Aspergillus fumigatus* is controlled by the availability of free iron. FEMS Microbiol Lett 248:83-91
18. Scazzocchio C (2000) The fungal GATA factors. Curr Opin Microbiol 3:126-131
19. Schrettl M, Bignell E, Kragl C, Joechl C, Rogers T, Arst HN Jr, Haynes K, Haas H (2004) Siderophore biosynthesis but not reductive iron assimilation is essential for *Aspergillus fumigatus* virulence. J Exp Med 200:1213-1219
20. Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160:47-56

Expresión diferencial de genes del metabolismo de hierro en *Aspergillus fumigatus*

Resumen. La capacidad de los hongos de sobrevivir en muchos ambientes está ligada a su capacidad de adquirir nutrientes esenciales. El hierro, por lo general, se encuentra unido a alguna molécula y disponible sólo en cantidades muy limitadas. Como las bacterias, los hongos han desarrollado un sistema muy específico para incorporar hierro. Se ha demostrado que la producción y la incorporación de sideróforos quelantes de hierro es importante para ciertas bacterias patógenas humanas, así como en los hongos patógenos *Cryptococcus neoformans* y *Fusarium graminearum*. Este sistema también permite al hongo patógeno oportunista *Aspergillus fumigatus* infectar y colonizar el pulmón humano. En este estudio se aprovechó la información disponible de la secuencia genómica de *Aspergillus nidulans* y *A. fumigatus* para clonar parcialmente o diseñar cebadores de PCR para 10 genes posiblemente implicados en la biosíntesis o incorporación de sideróforos en *A. fumigatus*. Se siguió la expresión de estos genes usando PCR semi-cuantitativa y cuantitativa en tiempo real en un intervalo dado de concentraciones de hierro. Tal como se esperaba, los genes putativos biosintéticos *sidA*, *sidC* y *sidD* estaban fuertemente regulados en condiciones de carencia de hierro, aunque el grado variable de inducción indica que existe una regulación compleja debida a varios factores de transcripción, como la proteína SreA de la familia GATA. En cambio, el gen *sidE* no muestra regulación por hierro, lo que sugiere que la proteína SidE quizá no intervenga en la biosíntesis de sideróforos. La caracterización de los patrones de expresión de este subconjunto de genes en el regulón del hierro facilita futuros estudios sobre la importancia de la incorporación de hierro para la patogénesis de *A. fumigatus*. [**Int Microbiol** 2006; 9(4):281-287]

Palabras clave: *Aspergillus fumigatus* · metabolismo de hierro · sideróforo · PCR en tiempo real · factor GATA

Expressão diferencial de genes do metabolismo do ferro em *Aspergillus fumigatus*

Resumo. A habilidade de um fungo para sobreviver em vários ambientes é relacionada com sua capacidade de adquirir nutrientes essenciais. O ferro emgeral encontra-se unido a alguma molécula e em quantidades muito limitadas, por isso os fungos, da mesma forma que as bactérias, desenvolveram sistemas altamente específicos para adquiri-lo. Demonstrou-se que a produção e absorção de sideróforos quelantes de hierro é importante para algumas bactérias patógenas humanas, tal como *Cryptococcus neoformans* e *Fusarium graminearum*. Este sistema também permite ao patógeno fúngico oportunista *Aspergillus fumigatus*, infectar e colonizar o pulmão humano. Neste estudo, aproveitou-se a informação disponível da seqüência genômica de *Aspergillus nidulans* e *A. fumigatus*, para clonar parcialmente em alguns casos, ou desenhar iniciadores da PCR em outros, para 10 genes supostamente envolvidos na biossíntese ou absorção de sideróforos em *A. fumigatus*. Monitorou-se a expressão destes genes usando PCR semi-quantitativa e quantitativa em tempo real em uma margem de concentrações de ferro. Com esperado, os genes putativos biossintéticos *sidA*, *sidC* e *sidD* estavam fortemente regulados em condições de depleção de ferro, mesmo que o grau de indução variável indique uma complexa regulação por uma série de fatores de transcrição, incluindo a proteína SreA da família GATA. Pelo contrário, o gene *sidE* não mostrou regulação de ferro, o que sugere que a proteina SidE você não participa da biossíntese de sideróforos. A caracterização dos padrões de expressão deste subconjunto de genes no regulón de ferro, facilita futuros estudos sobre a importância da aquisição de ferro para a patogénese de *A. fumigatus*. [**Int Microbiol** 2006; 9(4):281-287]

Palavras chave: *Aspergillus fumigatus* · metabolismo de ferro · sideróforo · PCR em tempo real · fator GATA