LETTERS

Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*

William C. Nierman[1], Arnab Pain[2], Michael J. Anderson[3], Jennifer R. Wortman[1], H. Stanley Kim[1], Javier Arroyo[4], Matthew Berriman[2], Keietsu Abe[5], David B. Archer[6], Clara Bermejo[4], Joan Bennett[7], Paul Bowyer[3], Dan Chen[1], Matthew Collins[2], Richard Coulsen[8], Robert Davies[2], Paul S. Dyer[6], Mark Farman[9], Nadia Fedorova[1], Natalie Fedorova[1], Tamara V. Feldblyum[1], Reinhard Fischer[10], Nigel Fosker[2], Audrey Fraser[2], Jose L. García[11], Maria J. García[12], Arlette Goble[2], Gustavo H. Goldman[13], Katsuya Gomi[5], Sam Griffith-Jones[2], Ryan Gwilliam[2], Brian Haas[1], Hubertus Haas[14], David Harris[2], H. Horiuchi[15], Jiaqi Huang[1], Sean Humphray[2], Javier Jiménez[12], Nancy Keller[15], Hoda Khouri[1], Katsuhiko Kitamoto[16], Tetsuo Kobayashi[17], Sven Konzack[10], Resham Kulkarni[1], Toshitaka Kumagai[18], Anne Lafton[19], Jean-Paul Latgé[20], Weixi Li[9], Angela Lord[2], Charles Lu[1], William H. Majoros[1], Gregory S. May[21], Bruce L. Miller[22], Yasmin Mohamoud[1], Maria Molina[4], Michel Monod[23], Isabelle Mouyna[20], Stephanie Mulligan[1], Lee Murphy[2], Susan O'Neil[2], Ian Paulsen[1], Miguel A. Peñalva[11], Mihaela Pertea[1], Claire Price[2], Bethan L. Pritchard[3], Michael A. Quail[2], Ester Rabbinowitsch[2], Neil Rawlins[2], Marie-Adele Rajandream[2], Utz Reichard[24], Hubert Renauld[2], Geoffrey D. Robson[3], Santiago Rodriguez de Córdoba[11], Jose M. Rodríguez-Peña[4], Catherine M. Ronning[1], Simon Rutter[2], Steven L. Salzberg[1], Miguel Sanchez[12], Juan C. Sánchez-Ferrero[11], David Saunders[2], Kathy Seeger[2], Rob Squares[2], Steven Squares[2], Michio Takeuchi[25], Fredj Tekaia[20], Geoffrey Turner[26], Carlos R. Vazquez de Aldana[12], Janice Weidman[1], Owen White[1], John Woodward[2], Jae-Hyuk Yu[27], Claire Fraser[1], James E. Galagan[28], Kiyoshi Asai[18], Masayuki Machida[29], Neil Hall[2]†, Bart Barrell[2] & David W. Denning[3]

Aspergillus fumigatus **is exceptional among microorganisms in being both a primary and opportunistic pathogen as well as a major allergen[1–3]. Its conidia production is prolific, and so human respiratory tract exposure is almost constant[4]. *A. fumigatus* is isolated from human habitats[5] and vegetable compost heaps[6,7]. In immunocompromised individuals, the incidence of invasive infection can be as high as 50% and the mortality rate is often about 50% (ref. 2). The interaction of *A. fumigatus* and other airborne fungi with the immune system is increasingly linked to severe asthma and sinusitis[8]. Although the burden of invasive disease caused by *A. fumigatus* is substantial, the basic biology of the organism is mostly obscure. Here we show the complete 29.4-megabase genome sequence of the clinical isolate Af293, which consists of eight chromosomes containing 9,926 predicted genes. Microarray analysis revealed temperature-dependent expression of distinct sets of genes, as well as 700 *A. fumigatus* genes not present or significantly diverged in the closely related sexual species *Neosartorya fischeri*, many of which may have roles in the pathogenicity phenotype. The Af293 genome sequence provides an unparalleled resource for the future understanding of this remarkable fungus.**

The genome of *A. fumigatus* Af293 was sequenced by the whole-genome random sequencing method[9] augmented by optical mapping[10]. Genome closure and quality standard attainment was accomplished by directed sequencing and manual editing. (See Table 1 and Supplementary Fig. S1 for genome features.) Sequenced chromosomal arms extend from putative centromeres to the telomere and end in 7–21 tandem repeats of the sequence TTAGGG. The copy number of the mitochondrial genome relative to the nuclear genome is estimated to be 12 based on the redundancy in the assembled

[1]The Institute for Genomic Research, Rockville, Maryland 20850, and The George Washington University School of Medicine, Department of Biochemistry and Molecular Biology, 2300 Eye Street NW, Washington DC 20037, USA. [2]The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK. [3]School of Medicine and Faculty of Life Sciences, The University of Manchester, Stopford Building, Manchester M13 9PT, UK. [4]Departmento Microbiología II. Universidad Complutense de Madrid 28040, Spain. [5]Tohoku University, 1-1 Tsutsumidori-Amamiyamachi Aoba-ku, Sendai 981-8555, Japan. [6]School of Biology, University of Nottingham, University Park, Nottingham NG7 2RD, UK. [7]Department of Cell and Molecular Biology, Tulane University, New Orleans, Louisiana 70118, USA. [8]European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton CB10 1SD, UK. [9]Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546-0312, USA. [10]Max Planck Institute for Terrestrial Microbiology, D-35043 Marburg, Germany. [11]Centro de Investigaciones Biológicas, CSIC, Madrid 28040, Spain. [12]Departmento Microbiologia y Genetica, Universidad de Salamanca, 37007 Salamanca, Spain. [13]Faculdade de Ciencias Farmaceuticas de Ribeirao Preto, Universidade de Sao Paulo, Brazil. [14]Department of Molecular Biology, Innsbruck Medical University, A-6020 Innsbruck, Austria. [15]Department of Plant Pathology, University of Wisconsin at Madison, Madison, Wisconsin 53706, USA. [16]Department of Biotechnology, University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan. [17]Department of Biological Mechanisms and Functions, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464-8601, Japan. [18]Computational Biology Research Center, National Institute of Advanced Industrial Science and Technology (AIST), 2-42 Aomi, Koto-ku, Tokyo 135-0064, Japan. [19]Unité Postulante Biologie et Pathogénicité Fongiques, INRA USC 2019, Institut Pasteur, Paris 75015, France. [20]Unité des Aspergillus, Institut Pasteur, Paris 75015, France. [21]Division of Pathology and Laboratory Medicine, The University of Texas M.D. Anderson Cancer Center, Houston, Texas 77030, USA. [22]Departments of Microbiology, Molecular Biology and Biochemistry, Center for Reproductive Biology, University of Idaho, Moscow, Idaho 83844, USA. [23]Department of Dermatology, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland. [24]Department of Bacteriology, Georg-August-University, D-37077 Gottingen, Germany. [25]Tokyo University of Agriculture and Technology, Saiwai-chou 3-5-8, Fuchu, Tokyo 183-0054, Japan. [26]Department of Molecular Biology and Biotechnology, The University of Sheffield, Sheffield S10 2TN, UK. [27]Department of Food Microbiology and Toxicology, The University of Wisconsin, Madison, Wisconsin 53706, USA. [28]Broad Institute of MIT and Harvard, Cambridge, Massachusetts 02139, USA. [29]Research Center for Glycoscience, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8566, Japan. †Present address: The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, Maryland 20850, USA.

sequence. The protein-coding genes and other genome features were identified by an automated annotation pipeline coupled with manual review.

Several candidate pathogenicity genes have been previously identified by assaying mutants in cultured macrophages or in animal models of invasive aspergillosis. These genes encode proteins involved in central metabolic pathways, signalling, cell wall biosynthesis, pigment biosynthesis and regulation of secondary metabolite production (Supplementary Table S1). This scope of functions suggests that the genomic infrastructure for pathogenicity is complex and integrated with a range of metabolic capabilities. Thus, any computationally based analysis of the genome sequence would not be directly able to identify functions critical for pathogenicity.

A. fumigatus thermotolerance is a trait critical to its ability to thrive in mammalian and avian infections and in the even-higher temperature ranges characteristic of composts (that is, up to 70 °C). To investigate the metabolic adaptation of this fungus to higher temperatures, gene expression was examined throughout a time course upon shift of growth temperatures from 30 °C (representing environments of tropical soil) to 37 °C and 48 °C (representing temperatures in the human body and compost, respectively). Gene expression patterns revealed that comparable numbers of genes were differentially expressed at each temperature, many of them with similar patterns (Fig. 1a). We identified 323 genes (clusters 1 and 2) that showed a higher expression level at 48 °C than at 37 °C, and 135 genes (cluster 3) that were expressed at a higher level at 37 °C than at 48 °C (Fig. 1b, see also Supplementary Table S2). Many of these 323 genes, especially those in cluster 1, which is enriched with heat shock-responsive genes, may have a role in thermotolerance of *A. fumigatus*. These include only 11 (four in cluster 1 and seven in cluster 2) of the 551 homologues of the *Saccharomyces cerevisiae* general stress-response genes, which were shown to be differentially expressed under all stress conditions tested[11]. Cluster 3 also includes a small number of such genes (five), and three of them have the opposite expression patterns from yeast (Supplementary Table S2). These data indicate that high temperature responses in *A. fumigatus* differ from the general stress response in yeast. Except for catalase B, no known genes implicated in pathogenicity showed higher expression at 37 °C than at 48 °C, suggesting that host temperature alone (37 °C) is insufficient to turn on many virulence-related genes.

More allergens (defined by IgE binding) have been characterized from *A. fumigatus* than from all other fungal species combined ($n = 58$)[12]. We identified nine additional predicted allergens in the genome based on similarity with other fungal allergens (Supplementary Table S3), including secreted proteases, glucanases and cellulases. Only *A. fumigatus* encodes the major allergen ribotoxin (Asp f1), which cleaves a single phosphodiester bond of the 28S ribosomal RNA of eukaryotic ribosomes. None of the nine allergens is a spore surface protein, despite a hydrophobin in *Cladosporium herbarum* being allergenic[13]. The allergen Asp f16 has immunoprotective properties[14].

Identification of essential genes may reveal potential targets for drug development. Putative essential genes in the *A. fumigatus* genome were identified by BLASTp search against 131 single-member KOGs (eukaryotic orthologous groups) representing a conserved core of largely essential eukaryotic genes compiled by analysis of seven diverse, completely sequenced eukaryotic genomes[15] (Supplementary Table S4). Only one of the 131 KOGs, KOG3214/DUF701, containing putative Zn ribbon RNA binding proteins, was not found in *A. fumigatus* or other aspergilli, suggesting a lineage-specific gene loss.

A. fumigatus virulence may be augmented by its numerous secondary metabolites, including fumagillin, gliotoxin, fumitremorgin, verruculogen, fumigaclavine, helvolic acid and sphingofungins[4]. Genes controlling fungal secondary metabolites are generally organized in clusters, many of which are species-specific. The *A. fumigatus* genome contains 26 such clusters with polyketide synthase, non-ribosomal peptide synthase and/or dimethylallyl tryptophan synthase genes. Only 13 of the 26 clusters have orthologues in *A. oryzae* and/or *A. nidulans*, and ten of these orthologous clusters are missing many or most of the genes present in the *A. fumigatus* clusters (see 'Selfish Cluster Hypothesis' in Supplementary Information). The unique clusters of *A. fumigatus* are dispersed in the genome with a bias towards telomeric locations. Many of these clusters contain regulatory genes, genes associated with resistance such as transporters involved in efflux[16], and genes with no obvious role in production of the metabolite (Supplementary Table S5). Fifteen of the clusters contain 22 transcriptional regulators, which are probably specific to their cluster because they do not have strong similarity to other proteins in the databases. In contrast to these regulators within the clusters, other global regulators of secondary metabolite synthesis are dispersed in the *A. fumigatus* genome. The genome also contains one copy of *laeA* encoding a global regulator of *Aspergillus* secondary metabolites[17].

Table 2 summarizes the numbers of different classes of secondary metabolite genes for *A. fumigatus*, *A. nidulans* (ref. 18) and *A. oryzae* (ref. 19). (See 'Secondary Metabolites' in Supplementary Information for further discussion.)

Stimulation of the programmed cell death pathway, as reported for *A. fumigatus* and *A. nidulans* during stationary phase and oxidative death[20], presents an opportunity for antifungal drug development.

Table 1 | Properties of the *Aspergillus fumigatus* Af293 genome

Genome	Value
Nuclear genome	
General information	
Size (Mb)	29.4
G+C content (%)	49.9
Gene number	9,926
Mean gene length (bp)	1,431
Per cent coding	50.1
Per cent genes with introns	77.0
Genes of unknown function	3,288
Exons	
Mean number per gene	2.8
Mean length (bp)	516
G+C content (%)	54.0
Introns	
Mean number per gene	1.8
Mean length (bp)	112
G+G content (%)	46.3
Intergenic regions	
Mean length (bp)	1,226
G+C content (%)	46.0
RNA	
tRNA number	179
5S rRNA number	33
Mitochondrial genome	
Size (bp)	31,892
G+C content (%)	25.4
Gene number	16
Mean gene length (bp)	1,189
Per cent coding	44.1
Per cent genes with introns	6.2
tRNA number	33

Table 2 | Secondary metabolite gene types in *A. fumigatus*, *A. nidulans* and *A. oryzae*

Gene type	*A. fumigatus*	*A. nidulans*	*A. oryzae*
Polyketide synthase	14	27	30
Non-ribosomal peptide synthase	14†	14†	18
Fatty acid synthase*	1	6	5
Sesquiterpene cyclase	n.d.	1	1
Dimethylallyl tryptophan synthase	7	2	2

*Includes one primary metabolism fatty acid synthase in each species.
†Includes one hybrid polyketide/peptide synthase.
n.d., not detected.

As with other fungi, *A. fumigatus* lacks homologues of the metazoan upstream apoptotic machinery, whereas the downstream effectors and regulators, both caspase-dependent and caspase-independent, seem to be shared (Supplementary Table S6). *A. fumigatus* possesses a homologue of the key participant of caspase-independent apoptosis in mammals, PARP, which is absent in *S. cerevisiae*. PARP activity was demonstrated previously in *A. nidulans* during sporulation-induced apoptosis[21]. The presence of these proteins in *Aspergillus* is indicative

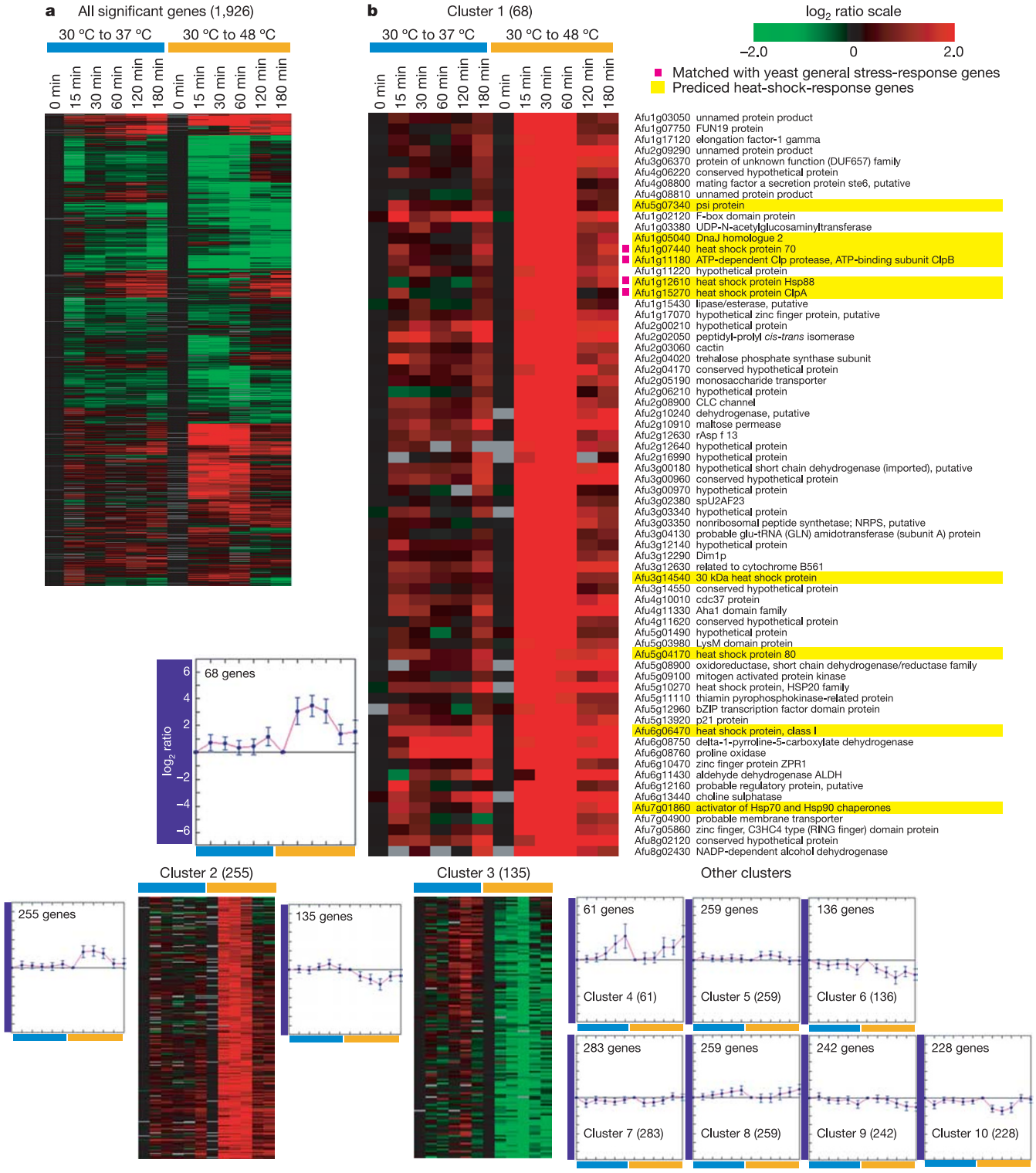


Figure 1 | Gene expression profiles for the temperature shift-responsive genes. The colour bar indicates the range of the expression ratios in the heat map-type figures. **a**, Genes with significantly differentiated expression are shown (see Methods). **b**, The same set of genes grouped into ten clusters. Three clusters of interest (that is, clusters 1 and 2 with genes expressed at a higher level at 48 °C than at 37 °C, and cluster 3 with the opposite pattern) are shown with centroid graphs and heat map-type figures. Cluster 1 enriches heat shock genes that are highlighted in yellow. Homologues to the yeast general stress-response genes are indicated with pink boxes. Vertical bars on graphs represent the data range, and the points in the middle the average.

of the recently identified PARP-dependent programmed cell death pathway and makes these filamentous fungi attractive models in which to study the mechanism and origin of programmed cell death.

As the hyphal cell wall is essential for *A. fumigatus* to penetrate solid nutrient substrates and to resist host cell defence reactions, comprehension of cell wall biosynthesis pathways are important. The *A. fumigatus* cell wall is composed of a fibrillar branched β1,3-glucan core bound to chitin, galactomannan and β1,3-1,4-glucan, embedded in an amorphous cement composed of α1,3-glucan, galactomannan and polygalactosamine[22]. β1,6-glucan and peptidomannan, both present in yeast cell walls, are missing in *A. fumigatus*. The types and numbers of *A. fumigatus* Af293 cell wall-related proteins as compared to other eukaryotes are provided in Supplementary Table S7. Specificity of the polymer organization of the *A. fumigatus* cell wall is reflected at the genomic level in the specificity of the cell wall biosynthetic gene inventory.

In *S. cerevisiae*, certain proteins initially anchored by a glycosyl phosphatidylinositol (GPI) moiety to the plasma membrane, and subsequently cross-linked to β1,3-glucans through β1,6-glucans, are thought to be major participants in yeast cell wall organization[23]. Among 82 putative GPI-anchored proteins identified in *A. fumigatus*, no homologues of these yeast GPI-anchored proteins were found (Supplementary Fig. S2). *A. fumigatus* also lacked homologues of the yeast PIR proteins that are putatively bound to the β1,3-glucans through an alkali-labile bond. It has been hypothesized in yeast that the linkage of proteins to cell wall polysaccharides is important in establishing the three-dimensional polysaccharide network that constitutes the skeleton of all fungal cell walls. On the basis of the comparative analysis reported here, it is more likely that binding to polysaccharides in yeasts is merely a way for certain proteins to remain at the surface of the cell wall to fulfil their biological functions in adhesion and flocculation—events absent in mould biology—and in mating. Hydrophobins, proteins not found in *S. cerevisiae*, are the only cell wall-linked GPI proteins detected in the *A. fumigatus* genome sequence. Hydrophobins have a major role in mould biology, because they are required for attachment to hydrophobic surfaces, formation of aerial structures, air dispersion and survival of conidia.

More than 500 putative *A. fumigatus*-specific genes having no detectable *A. nidulans* or *A. oryzae* homologues were found, mostly annotated as hypothetical proteins. *A. fumigatus*-specific proteins that have functional annotations other than hypothetical are listed in Supplementary Table S8. Most of these seem to have unusual phyletic patterns and are clustered in synteny break locations relative to *A. oryzae* and *A. nidulans* (Fig. 2 in ref. 18). About one-third of the *A. fumigatus*-specific proteins showed significant similarity to other fungal gene products. Furthermore, many seem to be involved in secondary metabolite biosynthesis, such as the developmentally regulated cluster involved in conidial pigment biosynthesis in *A. fumigatus*.

Several of the *A. fumigatus*-specific genes apparently have only bacterial or archaeal homologues, and may confer selective advantage in adapting to environments as diverse as human bodies, compost piles and arsenic-contaminated soil. The most striking finding involves two *A. fumigatus*-specific proteins that show high sequence similarity with the pI258 ArsC superfamily of arsenate reductases,

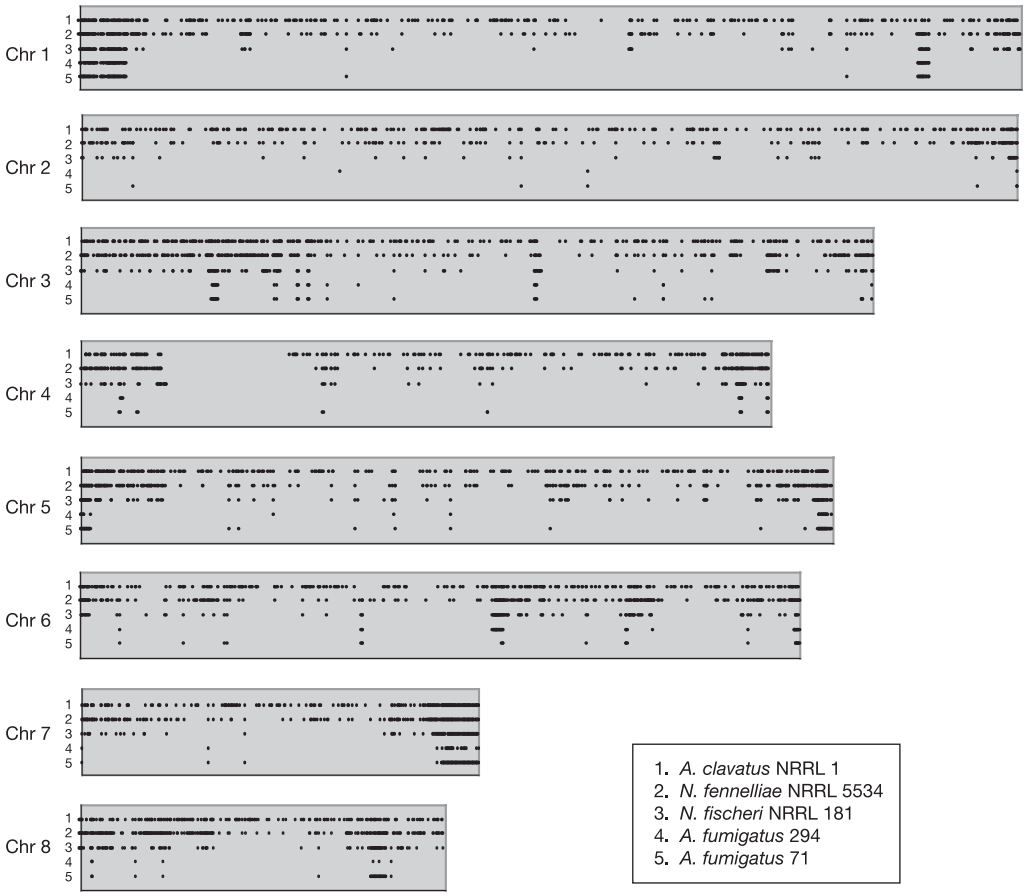


Figure 2 | Spatial distribution of *A. fumigatus* Af293 genes not present or diverged as compared with various (unsequenced) strains. On the basis of the microarray CGH data, *A. fumigatus* Af293 genes (reference) with $\log_2$ ratios equal to or greater than 2 as compared to signals from the query strains are scored as absent or diverged in the query strains. The five query strains are denoted with the numbers 1–5. The locations of *A. fumigatus* genes for which the orthologues are diverged or missing in the query strain are arranged in the order that they appear along the chromosome.

responsible for detoxification of arsenate by reduction to arsenite in bacteria[24]. These two proteins are unrelated to Acr2p of *S. cerevisiae* and are the first instances of the pI258 ArsC-type arsenate reductase in eukaryotes. The corresponding *A. fumigatus* genes are in a duplicated cluster on chromosomes 1 and 5, along with genes encoding an arsenite exporter, an arsenic resistance protein and an arsenic methyltransferase (Supplementary Table S9). It is of particular note that the cluster members seem to have different phyletic patterns. Although all of the significant BLASTp hits for the arsenate reductase and arsenic resistance protein are actinobacterial and proteobacterial proteins, the arsenite exporter appears to be closely related to yeast Acr3p and the methyltransferase has significant similarity to *Neurospora crassa* and archaeal proteins as well as mammalian *S*-adenosyl-L-methionine:AsIII methyltransferase[25]. The selective benefits of the assembly and retention of this cluster may involve the co-regulation of these arsenic resistance genes[26]. Elsewhere in the *A. fumigatus* genome, genes for an arsenite efflux pump and an arsenite translocating ATPase as well as additional copies of the arsenate exporter and arsenic resistance genes have been identified (Supplementary Table S9). This gene complement supports the classification of *A. fumigatus* among the once notorious 'arsenic fungi', organisms that produce the volatile trimethylarsine when grown in arsenate-contaminated environments[27].

The genome sequence of *A. fumigatus* revealed several genes associated with mating processes and sexual development. This topic is discussed further in an accompanying paper[18].

Azoles and allylamines block two sequential steps in the 20-step cascade of ergosterol synthesis. Comparative analysis of the ergosterol synthesis pathway genes revealed variable copy numbers of several genes, including *ERG3* and *ERG11* (Supplementary Table S10). Duplicated genes in the *Aspergillus* ERG pathway may reflect an adaptation strategy modulating the composition and fluidity of the cell membrane.

The comparative analysis of the *A. fumigatus* genome has made good use of the sequences of the *A. nidulans* and *A. oryzae* genomes to study gene and genome evolution among these species (see the accompanying paper[18]). However, within the genus *Aspergillus*, *A. nidulans* and *A. oryzae* are only distantly related to *A. fumigatus*. To explore the association between gene content and phenotype (that is, pathogenicity and related subphenotypes) the much closer taxonomic relationship of *Neosartorya fischeri* and *Neosartorya fennelliae* to *A. fumigatus* provides a more powerful comparative set. *N. fennelliae* is not known to be pathogenic to humans and possesses a sexual cycle. Another closely related species is *Aspergillus clavatus*, a mycotoxin producer that has been implicated in neurotoxicosis in beef cattle as well as respiratory disease in maltworkers[28]. We have used genomic DNA from *N. fischeri*, *N. fennelliae* and *A. clavatus* as well as from two additional strains of *A. fumigatus*, Af294 and Af71, to perform comparative genomic hybridization (CGH) with our Af293 polymerase chain reaction (PCR) amplicon coding sequence (CDS) microarray. The analysis revealed 2,557 total *A. fumigatus* Af293 genes to be absent or diverged in the analysed species. Of these, 1,382 are assigned gene names, including 70 coding for enzymes involved transcriptional regulation, at least 22 in production of secondary metabolites, and 6 encoding proteins for drug resistance transporters. Both of the *arsC* genes were missing or diverged in most of the analysed strains, including *A. fumigatus* strains Af294 and Af71. Figure 2 shows the chromosomal locations of the missing or diverged genes, demonstrating a bias towards subtelomeric locations consistent with the higher density of synteny breaks observed in subtelomeric locations between *A. fumigatus*, *A. nidulans* and *A. oryzae* (Fig. 2 of ref. 18) and suggesting greater genome instability in these regions. The most relevant CGH analysis for phenotypic comparisons, that with *N. fischeri*, revealed 700 genes to be absent or diverged relative to *A. fumigatus* Af293 (Supplementary Table S11). These include at least 13 genes coding for enzymes involved in the production of secondary metabolites, 28 coding for transcriptional regulators and protein kinases, 21 coding for transporters, 199 coding for metabolic and other proteins, and 400 coding for hypothetical proteins. This number of genes is a manageable set to begin the effort of correlating phenotypic differences between these species to gene content.

METHODS

Strain isolates. Af293 was isolated from a patient who ultimately died from invasive aspergillosis[29]. Af71 (NCPF 7098) and Af294 (NCPF 7102) are also clinical isolates. The type strains of *N. fischeri* (NRRL 181), *N. fennelliae* (NRRL 5534) and *A. clavatus* (NRRL 1) were used for CGH.

Sequencing and assembly. The genome of *A. fumigatus* Af293 was sequenced and assembled using the random shotgun method. Closure (finishing) was accomplished by directed sequencing and manual editing of the genome sequence[9]. Sequencing and assembly statistics are provided in Supplementary Methods.

Coding sequence prediction and gene identification. The assembled genomic sequence was processed through the TIGR annotation pipeline, a collection of software known as Eukaryotic Genome Control (EGC) that serves as the central data management system. This pipeline is described in detail in Supplementary Methods.

Microarray methods. The DNA amplicon microarray for *A. fumigatus* Af293 was constructed by designing primers for 9,516 genes (96%) then amplifying these target gene regions from genomic DNA (see Supplementary Methods). The resulting PCR products were purified and spotted in triplicate at high density on Corning UltraGAPS aminosilane-coated microscope slides using a robotic spotter built by Intelligent Automatic Systems and cross-linked by ultraviolet illumination.

For CGH analyses, genomic DNA was prepared from each isolate using the DNeasy Tissue kit (Qiagen). Purified genomic DNA was labelled and hybridized as described[30]. For temperature-shift experiments, conidia (5×10^6 ml^{-1}) from Af293 were incubated in Complete medium for germination (~17 h) at 30 °C. Cultures were then transferred to a water bath of 37 °C or 48 °C for continued growth. Total RNA samples before (that is, 0 min) and after (that is, 15, 39, 60, 120 and 180 min) two temperature shifts (that is, 30 to 37 °C and 30 to 48 °C) were used to profile gene expression. A biological replication of the cell growths and samplings was conducted. Labelling reactions with RNA and hybridizations were conducted as described in the TIGR standard operating procedures found at http://atarrays.tigr.org. The sample from 0 min in each temperature-shift set served as a reference in all hybridizations with samples from later time points within the set. All of the hybridizations with the two biological replicates were repeated in dye-swap sets.

Hybridized slides were scanned and analysed to obtain relative transcript levels (see Supplementary Methods). Normalized data were averaged over replications, and differentially expressed genes at the 95% confidence level were determined using intensity-dependent *Z*-scores (with $Z = 1.96$). The resulting data were organized and visualized using euclidean distance and hierarchical clustering with average linkage clustering method to view the whole data set (Fig. 1a) and *k*-means to group the genes in ten clusters (Fig. 1b) with TIGR MEV (http://www.tigr.org/software).

Received 12 May; accepted 12 October 2005.

1. Casadevall, A. & Pirofski, L. A. Host–pathogen interactions: redefining the basic concepts of virulence and pathogenicity. *Infect. Immun.* **67,** 3703–3713 (1999).
2. Denning, D. W. Invasive aspergillosis. *Clin. Infect. Dis.* **26,** 781–803, 804–805 (1998).
3. Greenberger, P. A. Allergic bronchopulmonary aspergillosis. *J. Allergy Clin. Immunol.* **110,** 685–692 (2002).
4. Latge, J. P. *Aspergillus fumigatus* and aspergillosis. *Clin. Microbiol. Rev.* **12,** 310–350 (1999).
5. Hirsch, T. *et al.* House-dust-mite allergen concentrations (Der f 1) and mold spores in apartment bedrooms before and after installation of insulated windows and central heating systems. *Allergy* **55,** 79–83 (2000).
6. Beffa, T. *et al.* Mycological control and surveillance of biological waste and compost. *Med. Mycol.* **36** (suppl. 1), 137–145 (1998).
7. Ryckeboer, J., Mergaert, J., Coosemans, J., Deprins, K. & Swings, J. Microbiological aspects of biowaste during composting in a monitored compost bin. *J. Appl. Microbiol.* **94,** 127–137 (2003).
8. Zureik, M. *et al.* Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey. *Br. Med. J.* **325,** 411–414 (2002).
9. Nierman, W. C. *et al.* Structural flexibility in the *Burkholderia mallei* genome. *Proc. Natl Acad. Sci. USA* **101,** 14246–14251 (2004).

10. Zhou, S. *et al.* Whole-genome shotgun optical mapping of *Rhodobacter sphaeroides* strain 2.4.1 and its use for whole-genome shotgun sequence assembly. *Genome Res.* **13,** 2142–2151 (2003).
11. Gasch, A. P. *et al.* Genomic expression programs in the response of yeast cells to environmental changes. *Mol. Biol. Cell* **11,** 4241–4257 (2000).
12. Kodzius, R. *et al.* Rapid identification of allergen-encoding cDNA clones by phage display and high-density arrays. *Comb. Chem. High Throughput Screen.* **6,** 147–154 (2003).
13. Weichel, M. *et al.* Immunoglobulin E-binding and skin test reactivity to hydrophobin HCh-1 from *Cladosporium herbarum*, the first allergenic cell wall component of fungi. *Clin. Exp. Allergy* **33,** 72–77 (2003).
14. Bozza, S. *et al.* Vaccination of mice against invasive aspergillosis with recombinant *Aspergillus* proteins and CpG oligodeoxynucleotides as adjuvants. *Microbes Infect.* **4,** 1281–1290 (2002).
15. Koonin, E. V. *et al.* A comprehensive evolutionary classification of proteins encoded in complete eukaryotic genomes. *Genome Biol.* **5,** R7 (2004).
16. Gardiner, D. M., Jarvis, R. S. & Howlett, B. J. The ABC transporter gene in the sirodesmin biosynthetic gene cluster of *Leptosphaeria maculans* is not essential for sirodesmin production but facilitates self-protection. *Fungal Genet. Biol.* **42,** 257–263 (2005).
17. Bok, J. W. & Keller, N. P. LaeA, a regulator of secondary metabolism in *Aspergillus* spp. *Eukaryot. Cell* **3,** 527–535 (2004).
18. Galagan, J. E. *et al.* Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. *Nature* doi:10.1038/nature04341 (this issue).
19. Machida, M. *et al.* Genome sequencing and analysis of *Aspergillus oryzae*. *Nature* doi:10.1038/nature04300 (this issue).
20. Mousavi, S. A. & Robson, G. D. Oxidative and amphotericin-mediated cell death in the opportunistic pathogen *Aspergillus fumigatus* is associated with an apoptotic-like phenotype. *Microbiol.* **150,** 1937–1945 (2004).
21. Thrane, C., Kaufmann, U., Stummann, B. M. & Olsson, S. Activation of caspase-like activity and poly (ADP-ribose) polymerase degradation during sporulation in *Aspergillus nidulans*. *Fungal Genet. Biol.* **41,** 361–368 (2004).
22. Fontaine, T. *et al.* Molecular organization of the alkali-insoluble fraction of *Aspergillus fumigatus* cell wall. *J. Biol. Chem.* **275,** 27594–27607 (2000).
23. Klis, F. M., Mol, P., Hellingwerf, K. & Brul, S. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* **26,** 239–256 (2002).
24. Zegers, I., Martins, J. C., Willem, R., Wyns, L. & Messens, J. Arsenate reductase from *S. aureus* plasmid pI258 is a phosphatase drafted for redox duty. *Nature Struct. Biol.* **8,** 843–847 (2001).
25. Hayakawa, T., Kobayashi, Y., Cui, X. & Hirano, S. A new metabolic pathway of arsenite: arsenic–glutathione complexes are substrates for human arsenic methyltransferase Cyt19. *Arch. Toxicol.* **79,** 183–191 (2005).
26. Wysocki, R., Bobrowicz, P. & Ulaszewski, S. The *Saccharomyces cerevisiae ACR3* gene encodes a putative membrane protein involved in arsenite transport. *J. Biol. Chem.* **272,** 30061–30066 (1997).
27. Bentley, R. & Chasteen, T. G. Microbial methylation of metalloids: arsenic, antimony, and bismuth. *Microbiol. Mol. Biol. Rev.* **66,** 250–271 (2002).
28. Blyth, W., Grant, I. W., Blackadder, E. S. & Greenberg, M. Fungal antigens as a source of sensitization and respiratory disease in Scottish maltworkers. *Clin. Allergy* **7,** 549–562 (1977).
29. Pain, A. *et al.* Insight into the genome of *Aspergillus fumigatus*: analysis of a 922 kb region encompassing the nitrate assimilation gene cluster. *Fungal Genet. Biol.* **41,** 443–453 (2004).
30. Kim, H. *et al.* Use of RNA and genomic DNA references for inferred comparisons in DNA microarray analyses. *Biotechniques* **33,** 924–930 (2002).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements Initial work was funded by the Fungal Research Trust and Burroughs Wellcome Fund. Major funding came from the National Institute of Allergy and Infectious Diseases (NIAID), the Wellcome Trust and the Fondo de Investigaciones Sanitarias. Construction of the Af293 microarray was funded by NIAID. Additional BAC end sequencing was funded internally by the Institut Pasteur. We thank D. Dixon, C. Caulcott, V. McGovern, P. Goodwin and J.-L. Rodriguez-Tudela for their support and encouragement during this project. We also thank C. Staben of the University of Kentucky for intellectual assistance and script development.

Author Information The genome sequence has been submitted to GenBank under the accession numbers NC_007194–NC_007201. All microarray expression data are available through ArrayExpress (http://www.ebi.ac.uk/arrayexpress) with accession numbers A-MEXP-205 (array design) and E-MEXP-332 and E-MEXP-333 (experimental data). Reprints and permissions information is available at npg.nature.com/reprintsandpermissions. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to W.N. (wnierman@tigr.org).

CORRIGENDUM

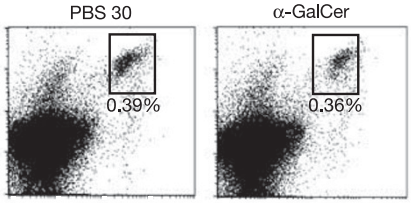
doi:10.1038/nature04475

Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections

Jochen Mattner, Kristin L. DeBord, Nahed Ismail, Randal D. Goff, Carlos Cantu III, Dapeng Zhou, Pierre Saint-Mezard, Vivien Wang, Ying Gao, Ning Yin, Kasper Hoebe, Olaf Schneewind, David Walker, Bruce Beutler, Luc Teyton, Paul B. Savage & Albert Bendelac

Nature 434, 525–529 (2005)

Figure 3 of this Letter contains an inadvertently duplicated panel: the PBS 30 panel is identical to the αGalCer panel (top right). The corrected panels are shown here. Our results and conclusions are unaffected by this oversight.



CORRIGENDUM

doi:10.1038/nature04484

Genome sequencing in microfabricated high-density picolitre reactors

Marcel Margulies, Michael Egholm, William E. Altman, Said Attiya, Joel S. Bader, Lisa A. Bemben, Jan Berka, Michael S. Braverman, Yi-Ju Chen, Zhoutao Chen, Scott B. Dewell, Alex de Winter, James Drake, Lei Du, Joseph M. Fierro, Robin Forte, Xavier V. Gomes, Brian C. Goodwin, Wen He, Scott Helgesen, Chun He Ho, Steve Hutchinson, Gerard P. Irzyk, Szilveszter C. Jando, Maria L. I. Alenquer, Thomas P. Jarvie, Kshama B. Jirage, Jong-Bum Kim, James R. Knight, Janna R. Lanza, John H. Leamon, William L. Lee, Steven M. Lefkowitz, Ming Lei, Jing Li, Kenton L. Lohman, Hong Lu, Vinod B. Makhijani, Keith E. McDade, Michael P. McKenna, Eugene W. Myers, Elizabeth Nickerson, John R. Nobile, Ramona Plant, Bernard P. Puc, Michael Reifler, Michael T. Ronan, George T. Roth, Gary J. Sarkis, Jan Fredrik Simons, John W. Simpson, Maithreyan Srinivasan, Karrie R. Tartaro, Alexander Tomasz, Kari A. Vogt, Greg A. Volkmer, Shally H. Wang, Yong Wang, Michael P. Weiner, David A. Willoughby, Pengguang Yu, Richard F. Begley & Jonathan M. Rothberg

Nature 437, 376–380 (2005)

The following were omitted from the original author listing: Alex de Winter, James Drake, Robin Forte, Steve Hutchinson, William L. Lee, Michael Reifler and David A. Willoughby. These names are included in the revised authorship shown here and either were or are at 454 Life Sciences Corporation, Branford, Connecticut 06405, USA.

CORRIGENDUM

doi:10.1038/nature04572

Genomic sequence of the pathogenic and allergenic filamentous fungus *Aspergillus fumigatus*

William C. Nierman, Arnab Pain, Michael J. Anderson, Jennifer R. Wortman, H. Stanley Kim, Javier Arroyo, Matthew Berriman, Keietsu Abe, David B. Archer, Clara Bermejo, Joan Bennett, Paul Bowyer, Dan Chen, Matthew Collins, Richard Coulsen, Robert Davies, Paul S. Dyer, Mark Farman, Nadia Fedorova, Natalie Fedorova, Tamara V. Feldblyum, Reinhard Fischer, Nigel Fosker, Audrey Fraser, Jose L. García, Maria J. García, Arlette Goble, Gustavo H. Goldman, Katsuya Gomi, Sam Griffith-Jones, Ryan Gwilliam, Brian Haas, Hubertus Haas, David Harris, H. Horiuchi, Jiaqi Huang, Sean Humphray, Javier Jiménez, Nancy Keller, Hoda Khouri, Katsuhiko Kitamoto, Tetsuo Kobayashi, Sven Konzack, Resham Kulkarni, Toshitaka Kumagai, Anne Lafon, Jean-Paul Latgé, Weixi Li, Angela Lord, Charles Lu, William H. Majoros, Gregory S. May, Bruce L. Miller, Yasmin Mohamoud, Maria Molina, Michel Monod, Isabelle Mouyna, Stephanie Mulligan, Lee Murphy, Susan O'Neil, Ian Paulsen, Miguel A. Peñalva, Mihaela Pertea, Claire Price, Bethan L. Pritchard, Michael A. Quail, Ester Rabbinowitsch, Neil Rawlins, Marie-Adele Rajandream, Utz Reichard, Hubert Renauld, Geoffrey D. Robson, Santiago Rodriguez de Córdoba, Jose M. Rodríguez-Peña, Catherine M. Ronning, Simon Rutter, Steven L. Salzberg, Miguel Sanchez, Juan C. Sánchez-Ferrero, David Saunders, Kathy Seeger, Rob Squares, Steven Squares, Michio Takeuchi, Fredj Tekaia, Geoffrey Turner, Carlos R. Vazquez de Aldana, Janice Weidman, Owen White, John Woodward, Jae-Hyuk Yu, Claire Fraser, James E. Galagan, Kiyoshi Asai, Masayuki Machida, Neil Hall, Bart Barrell & David W. Denning

Nature 438, 1151–1156 (2005)

There are two errors in the author listings for this Letter: the surname of Anne Lafon was misspelt as 'Lafton' and the affiliation of Hiroyuki Horiuchi should have been number 16 (and not 15, as was published).

ERRATUM

doi:10.1038/nature04476

Regulated cell-to-cell variation in a cell-fate decision system

Alejandro Colman-Lerner, Andrew Gordon, Eduard Serra, Tina Chin, Orna Resnekov, Drew Endy, C. Gustavo Pesce & Roger Brent

Nature 437, 699–706 (2005)

In Fig. 1b of this Article, the *x* axis of the right-hand plot should be labelled 'α-Factor system output in each cell (CFP F.U. × 10^6)' and not 'ACT1 system output in each cell (CFP F.U. × 10^5)'. In addition, Supplementary Fig. S6 was incorrect as originally published and was replaced on 26 January 2006.