Aspergillus fumigatus: saprophyte or pathogen?
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Large-scale genome comparisons have shown that no gene sets are shared exclusively by both Aspergillus fumigatus and any other human pathogen sequenced to date, such as Candida or Cryptococcus species. By contrast, and in agreement with the environmental occurrence of this fungus in decaying vegetation, the enzymatic machinery required by a fungus to colonize plant substrates has been found in the A. fumigatus genome. In addition, the proteome of this fungus contains numerous efflux pumps, including >100 major facilitators that help the fungus to resist either natural aggressive molecules present in the environment or antifungal drugs in humans. Environment sensing, counteracting reactive oxidants, and retrieving essential nutrients from the environment are general metabolic traits that are associated with the growth of the saprotrophic mold A. fumigatus in an unfriendly environment such as its human host.

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Introduction
Aspergillus fumigatus is responsible for invasive aspergillosis (IA), a life-threatening disease that usually only occurs in the immunocompromised patient. The incidence of IA has increased tremendously during the past 10–20 years as medical practices that are now becoming more common, such as immunosuppression in the transplant patient and aggressive anti-cancer chemotherapy, predispose patients to IA. The incidence of IA varies among the patient population and can infect 15% of allogeneic transplant patients — the population at most risk. The mortality rates associated with proven IA infections caused by Aspergillus species range from 60–90%, again depending upon the type of patients infected [1,2,3**].

This review aims to investigate if comparative analysis of the increasing number of sequenced fungal genomes can answer one of the most frequently asked questions in this field of research to date: are there specific A. fumigatus genes that are responsible for human pathogenicity?

How unique is A. fumigatus?
Under the comparative conditions outlined in Box 1, it has been shown that the amount of ancestral duplication in the proteome of A. fumigatus is 40.6%. There are 1111 A. fumigatus–specific proteins that are only present in the A. fumigatus proteome (making up 11.2%). 42% of A. fumigatus proteins are exclusively conserved in Eukaryotes and 27% are ancient proteins (i.e. conserved in Archaea, Bacteria and Eukaryotes). Very few A. fumigatus proteins are exclusively conserved in the archaeal (<0.2%) and bacterial (<0.5%) domains. Domain-specific conservation profiles of each of the considered species can be found at http://www.pasteur.fr/~tekaia/domspec.html.

The number of A. fumigatus proteins that are exclusively conserved in Aspergillus nidulans and Aspergillus oryzae is 178 and 186, respectively, whereas 449 other proteins are exclusively and jointly conserved in the three Aspergillus species. Moreover, 549 proteins are exclusively conserved among A. fumigatus and the filamentous fungi Fusarium graminearum, Magnaporthe grisea, Neurospora crassa, A. oryzae and A. nidulans. No A. fumigatus proteins are shared uniquely with other human pathogens, which suggests that human pathogenic ascomycetous and basidiomycetous yeasts do not share a virulence pathway with A. fumigatus.

As a global approach cannot identify general pathways that are only present in A. fumigatus and other human pathogens, can a comparative analysis of gene sets that control specific pathways, such as those summarized in Figure 1, provide an insight into the basis of human infectivity by A. fumigatus?

A. fumigatus is a grass eater
A. fumigatus is one of the most common inhabitants of the air-borne fungal flora [13]. Its ubiquity in nature suggests that this fungus has a saprophytic lifestyle in decaying organic and plant materials. Growth on plants requires an enzymatic armamentarium that is able to degrade plant cell wall polysaccharides [14]. Indeed, a survey of the A. fumigatus genome has shown that it encodes a wide range of glycosylhydrolases that have the capacity to degrade the major plant cell wall polymers (Table 1).
This genomic survey showed that a similar number of enzyme families are found in these, so-called, saprotrophic or phytopathogenic species and in A. fumigatus, which suggests that the primary ecological niche of A. fumigatus is the plant. Interestingly, no global differences have been demonstrated between the enzymes produced by true phytopathogens (such as M. grisea or F. graminearum) and saprotrophic Aspergilli. Non-phytopathogenic fungi such as the model yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe do not possess these enzymes, which demonstrates that there is a direct correlation between genome characteristics and the ecological niche. However, even though A. fumigatus has genes that encode laccase activity, it does not have genes homologous to lignin peroxidase or to manganese peroxidase that are present in other lignin-degrading fungi such as Phanerochaete chrysosporium [15]. This survey suggests that A. fumigatus plays a major role in leaf but not in hard wood degradation, a result in agreement with its ubiquitous colonization of compost.

Sensing the environment

Two-component phosphorelay systems are a major mechanism by which some organisms sense and adapt to their environment [16,17]. Fungal histidine kinases (HKs) are hybrids, which means that they function in multistep phosphorelays. In these phosphorelays, the phosphate is transferred from the response regulator (RR) domain of the hybrid HK to a second histidine residue in a histidine phosphotransfer domain (HPt), and then to a second RR domain. These systems have been implicated in the regulation of virulence in both plant and animal pathogens. In A. fumigatus, 13 HK genes have been identified with orthologs that are either limited to other Aspergillus and filamentous species or common to all Eukaryotic species. Despite this cross-species conservation, little is known about the signals perceived by and the functions of the orthologous HKs that can vary greatly from species to species. SLN1 is an essential HK in S. cerevisiae and acts as a sensor of the osmotic environment of the cell. SLN1 of Candida albicans, unlike its S. cerevisiae ortholog, is not essential but is involved in hyphal formation and virulence [18]. Currently, the role of the SLN1 homolog in A. fumigatus has not been clearly defined. By contrast, another member of the same cluster, FOS1, is known to be essential for the virulence of A. fumigatus [19,20]. Despite the upstream presence of numerous HKs, A. fumigatus, like other filamentous ascomycetes examined and in contrast to plants and bacteria, contain only one HPt domain protein that is homologous to the yeast YPD1. This organization of HKs should allow the integration of multiple environmental inputs into a single signaling pathway. Future functional characterization should elucidate whether the expansion of the number of HKs can be translated in terms of specific sensing of the host environment by A. fumigatus.

Another mechanism that is involved in A. fumigatus virulence involves the cyclic AMP signaling pathway, which has been shown to be associated with the pathobiology of several human and plant pathogenic fungi [21]. It has been shown that the deletion of GPAB, a G protein α subunit, which is an upstream stimulator of adenylate cyclase, led to drastically attenuated virulence of A. fumigatus in a mouse infection model of IA in the absence of any growth retardation [22]. Conidia of A. fumigatus GPAB mutant were killed more efficiently by human
monocyte-derived macrophages compared to conidia of the wild-type strains.

**Responses to a hostile environment**

Following inhalation of conidia by the immunocompetent host, the innate cellular immune system (comprised of alveolar macrophages and neutrophils) is responsible for the killing of the conidia. The anti-fumigatus activity of phagocytes primarily requires oxidative mechanisms to function [23].

In human pathogenic fungi, melanins have been shown to play a protective role in pathogenicity. In *A. fumigatus*, conidal dihydroxynaphthalene-melanin has been also recognized as a virulence factor [24,25,26]. This hydrophobic pigment, which is present on the conidial surface, quenches reactive oxygen species and protects the pathogen against damage by macrophage and neutrophils [27]. In *A. fumigatus*, a cluster of seven genes has been shown to be involved in the synthesis of this pigment. Comparative genomics has shown that homologs of this cluster were found in most filamentous fungi, including plant pathogens such as *M. grisea*. This result suggested the lack of specificity for the protective role of conidial pigments during infection, but point out its necessary role to withstand ultraviolet light radiations in nature and accessibly reactive oxidants in humans. In addition to their association with pigment biosynthesis, secondary metabolite pathways might play a direct role in fungal virulence, as was shown recently for the global regulator LAEA [28].

Pathogenic microorganisms have also developed a network of oxidoreductases and metabolites to neutralise phagocyte reactive oxygen intermediates (ROIs). The major anti-oxidant molecules and interconnecting pathways that are thought to be active in *A. fumigatus* are shown in Figure 2 [29]. *A. fumigatus* has the entire armamentarium to combat oxidative stress, but no difference can be seen between this and that of non-pathogenic fungi.

Another way in which to withstand the toxic molecules that passively enter the fungal cell is by use of an efficient efflux system. *A. fumigatus* encode >40 ATP-binding cassette transporters. This is more than twice the number of ABC transporter homologs that are present in yeasts.
The genome of \textit{A. fumigatus} also contains >100 genes that encode major facilitator transporters, a family of proteins that is more than five times larger than in yeast and that is absent from higher Eukaryotes. Accordingly, \textit{A. fumigatus} should possess an enhanced capacity for the efflux of toxic metabolites. However, this species does not possess a larger number of transporters than non-pathogenic filamentous fungi. The high number of efflux pumps in \textit{A. fumigatus} could originate from the ecological niche of \textit{A. fumigatus} where it is known that plants and soil (micro)organisms are able to secrete a broad range of toxic compounds. A similar observation was found in the saprophytic \textit{Pseudomonas aeruginosa}, which contains a much higher number of efflux pumps than non-saprophytic bacteria [32]. This high number of efflux pumps and their limited specificity could be the reason for the lack of serious azole-resistance isolates in \textit{A. fumigatus} despite the large quantity of azole fungicides that are sprayed in nature to combat phytopathogenic fungi.

**Thermophyly**

Thermophyly is a requirement for \textit{Aspergillus} pathogenicity. \textit{A. fumigatus} is the most frequently found thermophylic fungus. It is able to grow at 55°C and can survive temperatures of up to 75°C [33,34]. Because of its thermophyly it is an essential component of the compost microflora. Until now only two genes have been directly associated with thermophyly in \textit{Aspergillus}: first, the \textit{THTA} gene that allows the fungus to grow at 48°C but is not important for virulence [35], and second, the \textit{CGRA} gene that is a nucleolar protein involved in ribosome biogenesis [36]. These two proteins are ubiquitous in the fungal world, even among non-thermophylic species. Thermophyly is a requirement for \textit{Aspergillus} pathogenicity. \textit{A. fumigatus} is the most frequently found thermophylic fungus. It is able to grow at 55°C and can survive temperatures of up to 75°C [33,34]. Because of its thermophyly it is an essential component of the compost microflora. Until now only two genes have been directly associated with thermophyly in \textit{Aspergillus}: first, the \textit{THTA} gene that allows the fungus to grow at 48°C but is not important for virulence [35], and second, the \textit{CGRA} gene that is a nucleolar protein involved in ribosome biogenesis [36]. These two proteins are ubiquitous in the fungal world, even among non-thermophylic species. Thermophyly is a requirement for \textit{Aspergillus} pathogenicity. \textit{A. fumigatus} is the most frequently found thermophylic fungus. It is able to grow at 55°C and can survive temperatures of up to 75°C [33,34]. Because of its thermophyly it is an essential component of the compost microflora. Until now only two genes have been directly associated with thermophyly in \textit{Aspergillus}: first, the \textit{THTA} gene that allows the fungus to grow at 48°C but is not important for virulence [35], and second, the \textit{CGRA} gene that is a nucleolar protein involved in ribosome biogenesis [36]. These two proteins are ubiquitous in the fungal world, even among non-thermophylic species.
Phyly could result from amino acid changes as sequence comparisons have shown that amino acid substitutions result in a thermostabilization of the *A. fumigatus* phytase [37]. However, the direct association between a protein and thermophyly as a whole remains unknown. Similarly, in yeast, growth at high temperatures is a complex polygenic trait [36]. However, mutations in homologous genes do not lead to a similar growth temperature phenotype. For example, in *Cryptococcus neoformans*, deletion of calcineurin, α-1,3-glucan synthase, *RAS1*, or MAP kinase (*MPK1*) results in thermosensitive mutants [38–40]. In *Wangiella dermatitidis*, some chitin synthase mutants are unable to grow at 37°C [41]. By contrast, in *A. fumigatus*, none of these mutations lead to a thermosensitive phenotype, which is an indication that thermophyly in fungi is controlled by different genes and that these genes might be differently regulated in *A. fumigatus* than in other fungi.

**Chasing for salts**

Pathogens have developed mechanisms to acquire iron from the host. Blood serum is generally fungistatic because of the presence of transferrin. *A. fumigatus* possess siderophores of the hydroxamate family that are able to remove iron from transferrin in *vitro* and also have a system for reductive iron assimilation. Mutations in the *SIDA* gene that encodes ornithine oxygenase — an essential step in the biosynthesis of the *A. fumigatus* siderophores fericrocin and triacetylfusarinine — resulted in the inability of the deleted strain to grow in low-iron medium as well as in mouse, whereas deletion of the *FETC* and *FTRA* genes, which are responsible for the ferrous assimilation system, does not influence virulence [42,43].

Similarly, *A. fumigatus* requires high amount of magnesium to grow in *vitro*. Acquisition of magnesium in *vitro* in the phagolysosome has indeed been shown to be an essential requirement for bacterial pathogens such as *Mycobacterium tuberculosis* or *Salmonella typhimurium* [44]. Magnesium acquisition is under the control of MgtC, a membrane protein of unknown function that is known to be essential for pathogenicity as mgtC bacterial mutants are unable to grow in the phagocyte. MgtC is one of the few bacterial genes found in *A. fumigatus* and its role is currently being investigated.

Phosphate is another ion that is essential for fungal growth. The amount of phosphate present in the serum, 1 mM, is insufficient for growth of *A. fumigatus*, which require ten times more Pi than this. Many extracellular phosphatases and phospholipases (at least 5 times less genes for both families in yeast than in *A. fumigatus*) that are repressed by the presence of phosphate in the growth medium, have been identified in the genome of *A. fumigatus*. In addition to their use for recovering phosphate, phospholipase can have a direct role in pathogenicity by the perturbance of host membrane integrity [45,46].

Zinc is another macroelement that *A. fumigatus* must acquire from the environment, and this has been shown...
to be important for microbial infection. A family of five zinc transporters, of which two have been recently analysed, are shown to be involved in zinc transport [47]. Knowledge of their role in pathogenicity awaits the results of further animal studies.

Conclusions
Genomic data gathered to date and the biological items pinpointed in Box 2 suggest that A. fumigatus virulence results from the immunosuppression or genetic deficiency of the host rather than from specific and unique fungal determinants. Encountering an immunocompetent host is indeed a dead end for the fungus.

Rather than trying to identify specific fungal virulence factors, perhaps we should consider that the life-threatening A. fumigatus is a saprotrophic fungus that only becomes pathogenic for very simple biological reasons: it is present in high concentrations in the atmosphere, it grows faster than any other airborne fungi at 40 °C and it can overcome the defence of the host not because it has developed specific systems but because the host colonized has a very weak defence immunity. This should direct future studies towards the host rather than the fungus to understand the pathobiology of A. fumigatus.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


3. Steinbach WS, Latgé JP, Stevens DA: Advances against aspergillosis. Med Mycol 2005, 43:S1. The proceedings of the first international scientific meeting on Aspergillus fumigatus. This is essential for anyone that wants to enter the field or for those who need to be refreshed in the area. Both clinical and scientific topics are discussed.


A true textbook for all mycologists working on filamentous fungi, reconstituting molecular biologists and biochemists.


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Box 2 A few biological points of consideration.

1. Resistance of A. fumigatus to host defence reactions is associated with the presence of melanin. Aspergillus niger conidia, however, are more resistant to phagocytosis than those of A. fumigatus, but are not responsible for IA in patients. How significant is resistance to phagocytosis in determination of fungal virulence?

2. Aspergillus flavus is another widely distributed Aspergillus species that is isolated from soil, plant products or insects. This is also a human pathogen. A close phylogenetic association has been found between A. flavus and the ‘generally regarded as safe’ species A. oryzae, which is used in industry. Although recent studies have shown genetic distinctiveness between A. oryzae and most aflatoxin-producing strains of A. flavus, some A. flavus strains are indistinguishable from A. oryzae strains [48]. A. oryzae strains used in the biotechnology industry are able to infect and kill mice when the mice are immunocompromised with cortisone acetate (JP Latgé, unpublished). These data indicate that, in contrast to the generally admitted idea, many molds including the biotech fungus A. oryzae have the enzyme armamentarium to invade lung tissues.

3. Neosartorya fischeri is a food contaminant that is taxonomically closely related to A. fumigatus. It is regarded as a useful comparative genomic tool to identify virulence factors of A. fumigatus as it was not considered to be a human pathogen. However, the finding that Neosartorya is able to infect immunocompromised animals (Latgé, unpublished) and humans [49,50] makes such genomic comparison a questionable method for the identification of virulence genes.

4. Growth rate has been directly correlated to virulence. Genome analyses have not, however, been able to identify the network of regulatory genes that control growth. Differences in growth rate might explain why A. nidulans is less pathogenic than A. fumigatus, as at 40°C A. nidulans grows less than A. fumigatus. 1 log difference is seen in the amount of conidia needed for the two species to reach the same mortality in an experimental IA model. In spite of its lower pathogenicity, A. nidulans has been considered and sometimes proven to be a useful model to understand Aspergillus virulence [51]. The debate regarding the accuracy of the use of A. nidulans is still on.
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A good review about fungal Hks.


The first A. fumigatus mutant that has an attenuated virulence in a mouse model without any growth reduction in vitro.


Understanding how melanin protects the conidia against phagocytic defence reactions.


This study showed that general regulators of secondary metabolite pathways could play a role in pathogenesis.


The first protein shown to be responsible for thermotolerance – a virulence trait in A. fumigatus.


Iron is essential for A. fumigatus to survive in the phagolysosome of the phagocytes. Mutations in iron transporter block A. fumigatus infectivity.


