Receptor-mediated signaling in *Aspergillus fumigatus*

C. M. Grice*, M. Bertuzzi and E. M. Bignell

South Kensington Campus, Imperial College London, London, UK

---

**INTRODUCTION**

The genus *Aspergillus* is comprised of environmental filamentous mold fungi which utilize decaying organic matter for metabolic energy and nutrition. *Aspergillus fumigatus* is the most pathogenic, and is commonly isolated as an agent of human pulmonary infections (Dagenais and Keller, 2009). In healthy individuals, mucociliary clearance and pulmonary immune defences clear the hundreds of conidia inhaled daily (Balloy and Chignard, 2009). However, medical advances in transplantation and anti-cancer therapies have expanded the immunosuppressed patient population, and the number of individuals infected by opportunistic organisms, such as *A. fumigatus*, has drastically increased (McNeil et al., 2001; Chamilos et al., 2006). For opportunistic fungal pathogens, the phenomenon of "ready-made" virulence has been postulated, whereby traits which evolved for survival in ecological niches also govern survival in susceptible immunocompromised hosts (Casadevall et al., 2003; Rhodes, 2006).

Beyond residual host immune responses, there are additional obstacles to successful colonization of the mammalian lung, including tolerance of host-facilitated stresses, such as iron starvation (Schrott et al., 2004, 2007) and alkaline pH (Peñalva and Arst, 2004; Bignell et al., 2005; Peñalva et al., 2008). The requirement for infecting fungi to detect and respond to such extracellular cues is often essential for infectious growth, and in *A. fumigatus* the fungal receptors through which the extracellular environment is sensed remain largely unknown. This review discusses current knowledge on receptor-mediated signaling in *A. fumigatus* (Figure 1) and catalogues all of the putative seven transmembrane domain (7TMD) sensors encoded by the *A. fumigatus* genome (Table A1). Our analysis exposes the vast numbers of uncharacterized *A. fumigatus* receptor-like proteins.

**G PROTEIN COUPLED RECEPTORS (GPCRs) IN A. fumigatus**

*In silico* analyses of fungal genome sequences have identified genes encoding putative GPCR proteins. In the phytopathogenic fungus *Magnaporthe grisea*, a screen of the predicted proteome using all GPCR sequences at the time available in the GPCR Database (GPCRDB) (Horn et al., 2003) yielded 14 GPCR-like sequences (Kulkarni et al., 2005). A similar exercise applied to *A. fumigatus* identified 15 putative GPCRs (Lafon et al., 2006).

In *Aspergillus*, putative GPCRs are classified by homology, and according to a convention established by Lafon et al. (2006) in *A. nidulans*, into nine groupings. In *A. fumigatus*, Classes 1 and 2 are comprised, respectively, of two putative pheromone receptors GprA (AFUA_3G14330) and GprB (AFUA_5G07880); Class 3 is comprised of two putative carbon sensors GprC (AFUA_7G04800), GprD (AFUA_2G12640); Class 4 is comprised of three putative nitrogen sensors GprE (AFUA_5G04100), GprF (AFUA_1G11900), and GprG (AFUA_1G06840); Class 5 of three putative cAMP receptors GprH (AFUA_5G04140), GprI (AFUA_3G00780), and GprJ (AFUA_3G01750), the latter being unique to *A. fumigatus*; Class 6 is comprised of a single putative GPCR, GprK (AFUA_4G01350) having a regulator of G-protein signaling (RGS) domain, unique to filamentous fungi; Class 7 includes two putative GPCRs with homology to rat growth hormone-releasing factor receptors (Miller et al., 1999) only one of which is found in *A. fumigatus*, GprM (AFUA_7G05130); Class 8 is comprised of three putative GPCRs with identity to yeast Izh zinc regulators.
FIGURE 1 | Receptor-mediated signaling in Aspergillus fumigatus.

(A) G-protein coupled receptor pathways—So far 15 GPCRs have been identified in A. fumigatus, though only two have been characterized (GprC and D).

(B) The pH-response pathway—A shift from acidic to alkaline environmental pH is thought to be detected by the receptor PalH, mediating the phosphorylation, and subsequent ubiquitination of the C-terminally bound arrestin, PalF. This stimulates the proteolytic cleavage of the transcription factor PacC. PacC then undergoes further pH independent cleavage, before translocating to the nucleus.

(C) The histidine kinase pathway—The phosphorelay histidine kinases mediate the transduction of specific external or cytosolic stimuli such as cell wall integrity (Fos-1) and osmotic stress (TcsC), stimulating autophosphorylation upon a conserved histidine residue. The activating stimulus for TcsB remains elusive.

(D) The cell wall stress pathway—Cell wall receptors detect environmental stress such as cell wall damage/heat shock (MidA) and alternative carbon sources (Wsc1); however, the specific stimuli for receptors Wsc2 and 3 remain elusive. (Dotted lines indicate predictions based on studies in other fungi).

(Karpichev et al., 2002; Lyons et al., 2004), two of which are found in A. fumigatus GprO (AFUA_3G10570) and GprP (AFUA_6G07160), and Class 9 is comprised of a single putative GPCR, NopA (AFUA_7g01430) having identity to bacterial opsins. The roles of some of these receptors have been identified in other species though in A. fumigatus little is known (Figure 1).

Among the 15 predicted GPCR-like proteins in A. fumigatus, only two, GprC (AFUA_7G04800) and GprD (AFUA_2G12640), have been characterized (Gehrke et al., 2010). GprC and GprD have been noted as having homology to Gpr1p of Saccharomyces cerevisiae which activates the cAMP pathway in response to glucose, as demonstrated by cAMP enzyme immunoassay (Yun et al., 1998; Kraakman et al., 1999). Furthermore, the A. nidulans GprD homologue mediates increase of intracellular cAMP in response to oxygenated polyunsaturated fatty acids (oxylipins), which act as autocrine and paracrine mediators in eukaryotic organisms (Affeldt et al., 2012). Deletion of A. fumigatus GprC and GprD resulted in significant growth impairment under all tested growth conditions and analysis of virulence revealed significant attenuation of virulence for ΔgprD and delayed mortality for ΔgprC in a murine model of aspergillosis (Gehrke et al., 2010). The remainder of the putative A. fumigatus GPCRs remain to be investigated and nothing is known about their molecular linkages to multi-subunit G-proteins. Unlike most Aspergillus spp., where four predicted Gα subunits occur, only three (GpaA, AFUA_1G13140, GpaB, AFUA_1G12930, and GpaC, AFUA_3G12400) have been identified for A. fumigatus (Liebmann et al., 2003), which presumably act via interaction with the Gβ and Gγ subunits (SfaD, AFUA_5G12210 and GpgA, AFUA_1G05210). In the current absence of other identified G protein subunits, or similar proteins, it is thought that...
the aforementioned five proteins service the entire *A. fumigatus* GPCR repertoire (Figure 1). Undoubtedly the relevance of *A. fumigatus* Gβ and Gγ subunits for viability and vegetative growth is significant as ΔsfaD and ΔgpgA gene deletion mutants are extremely impaired for germination and vegetative growth (Shin et al., 2009).

**GENOME-WIDE in silico PREDICTIONS OF *A. fumigatus* INTEGRAL MEMBRANE PROTEINS**

Kulkarni et al. (2005) noted, based upon membrane topology, that the number of putative GPCR-like proteins encoded by the *M. grisea* genome rose to 76 when the criteria were relaxed to include homologs of the Pth11 receptor (DeZwaan et al., 1999) which is required for *M. grisea* pathogenicity in rice. Applying a more universal approach to *A. fumigatus*, we used the published genome sequence (Nierman et al., 2005) to catalogue all *A. fumigatus* proteins having predicted TMDs (Figure 1). To implement this, we used the TMPRED (Hofmann and Stoffel, 1993) predictive tool to perform an analysis of all 9497 *A. fumigatus* proteins encoded by the reference genome AF293 (Nierman et al., 2005) http://www.cadre-genomes.org.uk/Aspergillusfumigatus/Info/Index. In total we identified 6496 proteins having putative TMDs. Among them, 161 proteins were found to encode seven predicted TMDs (Tables 1 and A1). The majority of the predicted 7TMD proteins are of hypothetical function (Table A1).

**Table 1 | Numbers of predicted *A. fumigatus* TMD proteins, by chromosome.**

| TMDs | Chromosome number |
|------|------------------|
| 1    | 557 519 424 398 428 358 179 138 |
| 2    | 311 404 378 251 288 282 179 161 |
| 3    | 267 257 228 199 206 194 106 114 |
| 4    | 127 233 100 100 141 116 51 57 |
| 5    | 81 85 59 60 56 57 25 40 |
| 6    | 132 38 23 3 22 31 18 17 |
| 7    | 22 26 17 21 16 21 14 8 |
| 8    | 18 22 23 19 28 27 7 16 |
| 9    | 9 10 16 9 10 5 5 4 |
| 10   | 9 13 12 13 15 15 4 9 |
| 11   | 15 23 20 13 16 7 7 12 |
| 12   | 22 22 23 29 21 16 9 18 |
| 13   | 23 25 24 17 18 19 12 9 |
| 14   | 7 6 9 8 7 8 2 1 |
| 15   | 3 2 6 3 5 6 2 3 |
| 16   | 3 1 4 1 2 2 1 1 |
| 17   | 1 0 1 0 2 0 2 4 0 1 |
| 18   | 0 0 0 0 0 0 0 0 0 0 |
| 19   | 0 0 0 0 0 0 0 0 0 0 |
| 20   | 0 0 0 0 0 0 0 0 0 0 |
| 21   | 0 0 0 0 0 0 0 0 0 0 |
| 22   | 1 0 0 0 0 0 0 0 0 0 |

**PalH: A PUTATIVE 7TMD pH SENSOR**

During colonization of the mammalian lung *A. fumigatus* is exposed to a range of microenvironments, of likely differing pHs, not only within the pulmonary niche but also following phagocytosis by macrophages or ingestion by neutrophils and exposure to their vacuole contents (Levitz et al., 1999; Newman, 1999; Reeves et al., 2002; Ibrahim-Granet et al., 2003). Versatility of metabolism and physiology is required to survive such extremes, including appropriate pH-responsive gene expression for nutrient acquisition and survival (Bignell et al., 2005). In the model ascomycete and occasional pathogen *A. nidulans*, the PacC transcription factor governs gene expression in response to extracellular pH (Tilburn et al., 1995; Diez et al., 2002) and is vital for mammalian pathogenicity (Peñalva and Arst, 2004; Bignell et al., 2005; Peñalva et al., 2008). Under alkaline conditions, a signaling cascade involving seven proteins is involved in activation of PacC. A putative pH sensor, PalH, has 7TMDs and a cytoplasmic C-terminus (Negrete-Urtasun et al., 1997, 1999), which interacts with a cognate arrestin encoded by palF (Herranz et al., 2005; Hervas-Aguilera et al., 2010). Unlike canonical GPCR receptors, PalH is not thought to act via interaction with G-protein subunits (Kroeze et al., 2003). When an alkaline response is triggered, PalF is phosphorylated and subsequently ubiquitinat ed in a PalH-dependent manner (Herranz et al., 2005), leading to PalB-mediated, signal dependent, proteolytic cleavage of the pH-responsive transcription factor PacC (Penas et al., 2007; Rodriguez-Galan et al., 2009). Subsequent translocation of the truncated PacC protein, from cytoplasm to nucleus, permits alkaline adaptation via differential expression of genes required to enable growth under alkaline extracellular conditions (Tilburn et al., 1995; Mingot et al., 1999, 2001; Espeso and Arst, 2000; Espeso et al., 2000). In *A. fumigatus* the amino acid residues crucial for PalH and PalF interaction are conserved, and in split-ubiquitin analyses the proteins enter into close proximity (Bertuzzi and Bignell, 2011; Bignell, 2012). We have also recently demonstrated the requirement for *A. fumigatus* PalH for murine infection (Bertuzzi et al., in preparation).

**HISTIDINE KINASE SENSORS IN *A. fumigatus***

Histidine kinases (HK) are phosphorelay protein sensors which transduce extracellular signals. HKS are common in the fungal kingdom, and apparently absent in humans (Wes and Stock, 2001). Amongst archaea, bacteria and fungi, two classes of HK (two-component and hybrid) are found. The former class of two-component receptor systems predominate in bacteria and archaea, whereby autophosphorylation of the HK protein precedes transfer of the phosphoryl group to a conserved aspartate residue in a second protein, termed the response regulator (RR)(Li et al., 2012). HK activities have been associated with both the osmo- and peroxide-regulatory pathways in multiple fungi, and have been most extensively characterized in *S. cerevisiae* (Santos and Shiozaki, 2001). However, RR proteins are not abundantly encoded by fungal genomes; Skn7 and Ssk1 are two examples of such proteins, which in *S. cerevisiae* and *C. albicans*, account for the entire RR cohort of these species (Kaserer et al., 2009; Oide et al., 2010). The fungal phosphotransfer relay can involve three proteins, as exemplified by the *S. cerevisiae* HOG1
MAPK phosphorelay, where an HK (Sln1), a histidine phospho-intermediate (Ypd1) and an RR (Ssk1) collectively mediates a multistep phosphotransfer (Kaserer et al., 2009).

Fungal HKs most commonly fall into the hybrid class of regulators which utilize a single polypeptide. This protein possesses both a Histidine kinase A (HiskA) and a receiver domain (REC) containing a conserved aspartate residue (Li et al., 2012). Other domains, such as the ATP-binding HATPase_c domain (Dago et al., 2012) are also found; however, as these proteins are largely uncharacterized for A. fumigatus, the functional relevance of domain organization is unknown. The composition, and/or relative positioning, of additional domains provides the basis for sub-classification of HKs (Catlett et al., 2003), presented for 12 A. fumigatus HKs in Table A2. Amongst these, only three have been studied: the two-component system proteins A, B, and C (TcsA/Fos-1 AFUA_5G10240, TcsB AFUA_2G00660 and TcsC AFUA_2G03560).

Despite the significance of the HK Fos-1 for detection of extracellular stresses, this hybrid HK has been previously predicted as possessing no TMDs, implying a cytosolic presence (Pott et al., 2000). However, our TMPRED analyses predicted TMDs for all of the three HKs, with Fos-1 possessing a single TMD (Table A2). Deletion of the fos-1 gene leads to a ~66% reduction in conidiation after 48 h in liquid YG medium, as well as heightened resistance to the cell wall-degrading enzyme mix novozym 234, suggesting the role of fos-1 in cell wall assembly (Pott et al., 2000). Δfos-1 mutants were found to have normal morphology, germination, osmotic and oxidative stress tolerance, and antifungal susceptibilities. Subsequent transcriptional analyses found a significant increase in fos-1 expression, relative to in vitro growth, during the first 72 h of infection in a murine model of pulmonary aspergillosis (Zhang et al., 2005), and reduced virulence of A. fumigatus in a systemic murine mode of infection (Clemmons et al., 2002).

In a study addressing the role of oxidative stress in A. fumigatus pathogenicity, Du et al. (2006) characterized the A. fumigatus TcsB protein, a putative homolog of Sln1 in S. cerevisiae. In A. nidulans, TMPRED analysis predicted 2 TMDs for TcsB at the N-terminus (Furukawa et al., 2002), though in A. fumigatus, our prediction extends this to 4TMDs (Table A1). Unlike S. cerevisiae where deletion of sln1 is lethal (Maeda et al., 1994), an A. fumigatus ΔtcsB mutant is viable, demonstrates normal morphology, and is as tolerant as the wild type to increased temperatures, various cell wall damaging agents, and poor nitrogen/carbon sources. The only phenotype discernable for the mutant was a minor sensitivity to SDS (Du et al., 2006). This data suggests a non-essential role for TcsB, or redundancy of function with other, as yet uncharacterized protein(s).

It is believed that group III HK mediate resistance to high osmotic pressure via the high osmolarity glycerol pathway (HOG). For this reason, the characterization of the sole A. fumigatus group III hybrid HK TcsC, classified as such on the basis of putative HAMP (HK, adenyl cyclase, methyl-accepting chemotaxis protein, phosphatase) domains, was investigated (McCormick et al., 2012). The significance of the HAMP domains, based upon studies of other sensor proteins and signaling is postulated as providing the means to couple input and output since HAMP domains of integral membrane hybrid HKs are found in close proximity to the membrane-spanning segment (Parkinson, 2010). It is speculated that in response to extracellular signals, such as altered osmolarity, a conformational rearrangement is triggered which prompts activation of an output domain (Parkinson, 2010). In A. fumigatus, deletion of the tcsC gene resulted in an extended white colony rim and a reduced number of extending hyphae. However, unlike the A. nidulans homologue nikA (Hagiwara et al., 2009), no detrimental effects on sporulation and conidial growth were observed. In the presence of nitrate as a nitrogen source a significant reduction in radial growth was detectable, and furthermore, compared to the control strain, growth of ΔtcsC at 2% O2 abolished sporulation and prompted a dome-shaped morphology indicative of oxygen starvation. A strong inhibition of growth resulted from exposure to hyperosmotic stress (1.2 M sorbitol, 1 M KCl, or 1 M NaCl) but sensitivity to calcofluor white, amphotericin B, posaconazole and caspofungin, extremes of pH/temperature, or oxidative stress were reportedly normal.

In a comparative analysis of wild type and ΔtcsC virulence, no discernable differences in pathogenicity analysis in a murine model of invasive aspergillosis were detected (McCormick et al., 2012).

**CELL WALL RECEPTORS**

The fungal cell wall is essential for viability and an important target of antifungal drugs (Latgé et al., 2005; Latgé, 2007; Walker et al., 2010). In fungi a conserved MAPK signaling module is responsible for maintaining cellular integrity, shape and resilience to environmental stresses (Lee and Levin, 1992; Levin, 2005; Lesage and Bussey, 2006). In S. cerevisiae, such MAPK signaling (Chen and Thorner, 2007) is initiated through stress detection at five integral membrane receptors Wsc1-3, Mid2, and Mtl1 (Lodder et al., 1999). This promotes guanine nucleotide exchange factor (GEFs—Rom1 and Rom2)-mediated nucleotide exchange upon the GTPase Rho1, facilitating the regulation of downstream effectors (Zu et al., 2001). In a quest to find equivalent cell wall sensors in A. fumigatus, Dichtl et al. (2012) performed BLAST analyses to reveal three previously uncharacterized open reading frames with domain structures similar to those of Wsc1-3 (Af Wsc1, AFUA_4G13670, Af Wsc2, AFUA_3G07500, and Af Wsc3, AFUA_5G09020 respectively). Bioinformatic analyses predicted the presence of characteristic cell wall integrity (CW1) sensor N-terminal WSC domains with downstream, though truncated, ser/thr rich regions, and (with the exception of Wsc2) transmembrane domains. In common with the S. cerevisiae sensors a short cytosolic C-terminus was also predicted for two of the sensors (Dichtl et al., 2012).

To discern subcellular localization, ectopically integrating vectors were applied to generate four putative CW1 sensor-GFP fusions, Wsc1-3, and MtlA. From these, localization of all C-terminally tagged sensors was observed at the fungal surface. Additionally a strong presence was observed in vacuoles, though this was dismissed as a by-product of over expression or misfolding. Phenotypic analyses of single and double mutants identified a significant impairment of radial growth in the case of a Δwsc1Δwsc3 double mutant. These findings were further exacerbated in a triple mutant Δwsc1Δwsc3ΔmtlA. Furthermore, in all mutants lacking wsc1, provision of glycerol as
carbon source lead to a significant reduction in radial growth on minimal media (Dichtl et al., 2012).

Previously, mutants lacking members of the CWI MAPK pathway have demonstrated a clear sensitivity to echinocandins and azole antifungals (Fujikawa et al., 2007; Dirr et al., 2010). Extending this analysis to the A. fumigatus mutant phenotypes revealed a single relevant phenotype, namely the heightened sensitivity of theΔwsc1 mutant to the echinocandin, caspofungin (Dichtl et al., 2012).

To study stress-induced activation of intracellular signaling, effects on growth and MpkA phosphorylation were analyzed in the presence of the cell wall perturbing agent, calcoflour white, or following heat shock (48°C). None of the Wsc mutants were found to be sensitive to cell wall perturbation or heat shock, however, ΔmidA was highly sensitive to all of these stresses. In agreement with phenotypic data, calcofluor white-induced MpkA phosphorylation was significantly reduced in the ΔmidA mutant compared with wild type, while phosphorylation of MpkA was not diminished in mutants lacking wsc1 or wsc1 and wsc3 (Dichtl et al., 2012). In S. cerevisiae, the Wsc1 cell wall sensor mediates signaling of alkaline stress via the CWI MAPK module (Serrano et al., 2006); no evidence for such a role in A. fumigatus was obtained. Thus, while Mkk2 null mutants are sensitive to alkalinization of the medium (Dirr et al., 2010), the identity of the activating cell wall sensor remains unknown.

Taken together these findings suggest that MidA is the sole cell wall perturbation sensor, while Wsc1 is required for glucol carbon source assimilation. Furthermore, a compensatory role between Wsc1 and Wsc3 with regards to efficient growth and conidiation has been demonstrated. Despite these observations, a role for Wsc2 has yet to be identified, while the putative CWI pH sensor remains elusive.

RECEPTOR-MEDIATED SIGNALING DURING A. fumigatus INFECTION: RELEVANCE FOR THERAPEUTIC STRATEGY

Drugs which target GPCR function account for >50% of currently licensed drugs (Davies et al., 2008). It therefore follows that fungal GPCRs are likely to be similarly responsive to chemical perturbations. This fact, coupled with the absolute requirement for some GPCRs in fungal growth make a compelling case for these proteins as antifungal drug targets. Although the pharmaceutical market is dominated by GPCR-active molecules, the discovery of most of these agents was made on the basis of functional activity in high throughput screens, only later were the targets and modes of action clarified (Filmore, 2004). In the post-genomic era, with confidence in the pharmaceutical relevance of such proteins, drug discovery can become target-driven. An expanding repertoire of technologies to probe 7TMD protein activities provides the basis upon which to confront functional studies of the uncharacterized receptors in A. fumigatus and to screen for inhibitory molecules. It has recently been suggested that considering seven transmembrane receptors as disordered proteins able to allosterically respond to a number of binding partners, is useful in understanding the plasticity of function exhibited by such proteins (Kenakin and Miller, 2010). Conformational changes which occur in response to extracellular ligands and/or stimuli expose cytosolic signaling domains and present three distinct arenas open to perturbation: extracellular sensing/ligand binding, cytosolic surfaces, and intramembrane domains. In order to prioritise the most promising candidates for drug development, a crucial experiment will be to assess the requirement of such receptors, via regulatable promoters, for sustained viability of established fungal mass in murine models of infection (Gossen and Bujard, 1992).

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Arshad Khan in automating the genome-wide TMPRED analyses. This work was assisted by funding from the Medical Research Council [G0501164 to E. M. Bignell], the Biotechnology and Biological Sciences Research Council [PhD studentship to C. M. Grice, BB/F016239/1], and the Wellcome Trust [WT093596MA to E. M. Bignell].

REFERENCES

Affeldt, K. J., Brodhagen, M., and Keller, N. P. (2012). Aspergillus oxylipin signaling and quorum sensing pathways depend on G protein-coupled receptors. Toxins (Basel) 4, 695–717.
Balloy, V., and Chignard, M. (2009). The innate immune response to Aspergillus fumigatus. Microbes Infect. 11, 919–927.
Bertuzzi, M., and Bignell, E. M. (2011). Sensory perception in fungal pathogens: applications of the split-ubiquitin Membrane Yeast Two-Hybrid (MYTH) technique. Fungal Biol. Rev. 25, 7–7.
Bignell, E. (2012). Conservation of seven transmembrane domain receptors and arrestins required for pH signalling in Aspergillus fumigatus, and implications for drug discovery. Ann. N.Y. Acad. Sci. 1273, 35–43.
Bignell, E., Negrete-Urtasun, S., Calcagno, A. M., Haynes, K., Arst, H. N., Jr., and Rogers, T. (2005). The Aspergillus pH-responsive transcription factor PacC regulates virulence. Mol. Microbiol. 55, 1072–1084.
Casadevall, A., Steinbergen, J. N., and Nosanchuk, J. D. (2003). ‘Ready made’ virulence and ‘dual use’ virulence factors in pathogenic environmental fungi--the Cryptococcus neoformans paradigm. Curr. Opin. Microbiol. 6, 332–337.
Catlett, N. L., Yoder, O. C., and Turgeon, B. G. (2003). Genome analysis of two-component sensor kinases in fungal pathogens. Eukaryot. Cell 2, 1151–1161.
Chamilos, G., Luna, M., Lewis, R. E., Bodey, G. P., Chemaly, R., Tarrand, J. J., et al. (2006). Invasive fungal infections in patients with hematologic malignancies in a tertiary care cancer center: an autopsy study over a 15-year period (1989–2003). Haematologica 91, 986–989.
Chen, R. E., and Thorner, J. (2007). Function and regulation in MAPK signaling pathways: lessons learned from the yeast Saccharomyces cerevisiae. Biochim. Biophys. Acta 1773, 1311–1340.
Clemmons, K. V., Miller, T. K., Seltremnikof, C. P., and Stevens, D. A. (2002). fos-1, a putative histidine kinase as a virulence factor for systemic aspergillosis. Med. Mycol. 40, 259–262.
Dagenais, T. R., and Keller, N. P. (2009). Pathogenesis of Aspergillus fumigatus in invasive Aspergillosis. Clin. Microbiol. Rev. 22, 447–465.
Dago, A. E., Schug, A., Procaccini, A., Hoch, J. A., Weigt, M., and Szurmant, H. (2012). Structural basis of histidine kinase autophosphorylation deduced by integrating genomics, molecular dynamics, and mutagenesis. Proc. Natl. Acad. Sci. U.S.A. 109, E1733–E1742.
Davies, M. N., Secker, A., Halling-Brown, M., Moss, D. S., Freitas, A. A., Timmis, J., et al. (2008). GPCRTree: online hierarchical classification of GPCR function. BMC Res. Notes 1:67. doi: 10.1186/1756-0500-1-67.
DeZwaan, T. M., Carroll, A. M., Valent, B., and Sreevid, J. A. (1999). Magnaporthe grisea Ph11p is a novel plasma membrane protein
Aspergillus fumigatus. Nature 438, 1151–1156.

Oide, S., Liu, J. Y., Yun, S. H., Wu, D. L., Michev, A., Choi, M. Y., et al. (2010). Histidine kinase two-component response regulator proteins regulate reproductive development, virulence, and stress responses of the fungal cereal pathogens Cochliobolus heterostrophus and Gibberella zeae. Eukaryot. Cell 9, 1867–1880.

Parkinson, J. S. (2010). Signaling mechanisms of HAMP domains in chemoreceptors and sensor kinases. Annu. Rev. Microbiol. 64, 101–122.

Peñalva, M., and Arst, H. N. Jr. (2004). Recent advances in the characterization of ambient pH regulation of gene expression in filamentous fungi and yeasts. Annu. Rev. Microbiol. 58, 425–451.

Peñalva, M., Tilburn, J., Bignell, E., and Arst H. N. Jr. (2008). Ambient pH gene regulation in fungi: making connections. Trends Microbiol. 16, 291–300.

Penas, M. M., Hervas-Aguilar, A., Munera-Huertas, T., Reoyo, E., Penalva, M. A., Arst, H. N. Jr., et al. (2007). Further characterization of the signaling proteolysis step in the Aspergillus nidulans pH signal transduction pathway. Eukaryot. Cell 6, 960–970.

Pott, G. B., Miller, T. K., Bartlett, J. A., Palas, J. S., and Selitrennikoff, C. P. (2000). The isolation of FOS-1, a gene encoding a putative two-component histidine kinase from Aspergillus fumigatus. Fungal Genet. Biol. 31, 55–67.

Reeves, E. P., Lu, H., Jacobs, H. L., Messina, C. G., Bolsover, S., Gabella, G., et al. (2002). Killing activity of neutrophils is mediated through activation of proteases by K⁺ flux. Nature 416, 291–297.

Rhodes, J. C. (2006). Aspergillus fumigatus growth and virulence. Mol. Mycol. 44(Suppl. 1), S77–S81.

Rodriguez-Galan, O., Galindo, A., Hervas-Aguilar, A., Arst, H. N. Jr., and Penalva, M. A. (2009). Physiological involvement in pH signaling of Vps24-mediated recruitment of Aspergillus PaB cysteine protease to ESCRT-III. J. Biol. Chem. 284, 4404–4412.

Santos, J. L., and Shiozaki, K. (2001). Fungal histidine kinases. Sci. STKE 2001:re1. doi: 10.1126/stke.2001.98.re1

Schrott, M., Bignell, E., Kragl, C., Joechel, C., Rogers, T., Arst, H. N. Jr., et al. (2004). Siderophore biosynthesis but not reductive iron assimilation is essential for Aspergillus fumigatus virulence. J. Exp. Med. 200, 1213–1219.

Schrott, M., Bignell, E., Kragl, C., Sabiha, Y., Loss, O., Eisendle, M., et al. (2007). Distinct roles for intracellular and extracellular siderophores during Aspergillus fumigatus infection. PLoS Pathog. 3:1195–1207. doi: 10.1371/journal.ppat.0030128

Serrano, R., Martin, H., Casanovar, A., and Arino, J. (2006). Signaling alkaline pH stress in the yeast Saccharomyces cerevisiae through the Wsc1 cell surface sensor and the Slt2 MAPK pathway. J. Biol. Chem. 281, 39785–39795.

Shin, K. S., Kwon, N. I., and Yu, J. H. (2009). Gbg-mediated growth and developmental control in Aspergillus fumigatus. Curr. Genet. 55, 631–641.

Tilburn, J., Sarkar, S., Widdick, D. A., Espeso, E. A., Orejas, M., Munro, C. A. (2010). Fungal echinocandin resistance. Fungal Genet. Biol. 47, 117–126.

West, A. H., and Stock, A. M. (2001). Histidine kinases and response regulator proteins in two-component signaling systems. Trends Biochem. Sci. 26, 369–376.

Yun, C. W., Tamaki, H., Nakayama, R., Yamamoto, K., and Kumagai, H. (1998). Gpr1p, a putative G-protein coupled receptor, regulates glucose-dependent cellular cAMP level and extracellular siderophores during Aspergillus fumigatus infection. Mol. Genet. Genomics 260, 29–33.

Zhang, L., Wang, M. Y., Li, R. Y., and Calderone, R. (2003). Expression of Aspergillus fumigatus virulence-related genes detected in vitro and in vivo with competitive RT-PCR. Mycopathologia 160, 201–206.

Zu, T., Verna, J., and Ballester, R. (2001). Mutations in WSC genes for putative stress receptors result in sensitivity to multiple stress conditions and impairment of Rlm1-dependent gene expression in Saccharomyces cerevisiae. Mol. Genet. Genomics 266, 142–155.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 08 November 2012; accepted: 01 February 2013; published online: 20 February 2013.

Citation: Grice CM, Bertuzzi M and Bignell EM (2013) Receptor-mediated signaling in Aspergillus fumigatus. Front. Microbiol. 4:26. doi: 10.3389/fmicb.2013.00026

This article was submitted to Frontiers in Fungi and Their Interactions, a specialty of Frontiers in Microbiology.

Copyright © 2013 Grice, Bertuzzi and Bignell. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in other forums, provided the original authors and source are credited and subject to any copyright notices concerning any third-party graphics etc.
## APPENDIX

Table A1 | Identity and annotations of *A. fumigatus* proteins having seven predicted transmembrane domains.

| Annotation | ORF Accession number |
|------------|----------------------|
| **CHROMOSOME 1** | |
| Polyketide synthase | AFUA_1G01010 XP_749851.1 |
| Conserved hypothetical protein | AFUA_1G01190 XP_749869.1 |
| High affinity zinc ion transporter/membrane zinc transporter | AFUA_1G01550 XP_749905.1 |
| Conserved hypothetical protein | AFUA_1G01620 XP_749912.1 |
| MFS alpha-glucoside transporter | AFUA_1G03280 XP_750078.1 |
| Peroxisomal ABC transporter (PXA1) | AFUA_1G04790 XP_750227.1 |
| Vacuolar membrane PQ loop repeat protein | AFUA_1G06840 XP_750433.1 |
| Phosphatidate cytidylytransferase | AFUA_1G07010 XP_750449.1 |
| Export control protein CHS7-Like | AFUA_1G07110 XP_750458.1 |
| Rhomboid family protein | AFUA_1G09150 XP_752282.1 |
| Conserved hypothetical protein | AFUA_1G10160 XP_752382.1 |
| COP11-coated vesicle protein SurF4/Erv29 | AFUA_1G11770 XP_752543.1 |
| PQ loop repeat protein | AFUA_1G11900 XP_752556.1 |
| Integral membrane protein Pth11-like | AFUA_1G14080 XP_752778.1 |
| DUF409 domain protein | AFUA_1G14140 XP_752784.1 |
| DUF803 domain membrane protein | AFUA_1G15880 XP_752954.1 |
| Potassium transporter | AFUA_1G16340 XP_753000.1 |
| Fatty acid elongase (Gig30) | AFUA_1G16710 XP_753038.1 |
| Conserved hypothetical protein | AFUA_1G16720 XP_753039.1 |
| **CHROMOSOME 2** | |
| RTA1 domain protein | AFUA_2G00420 XP_749178.1 |
| DUF1275 domain protein | AFUA_2G00530 XP_749189.1 |
| Alpha-amyrase | AFUA_2G00710 XP_749208.1 |
| Cellobiose dehydrogenase | AFUA_2G01180 XP_749254.1 |
| Bax inhibitor family protein | AFUA_2G03220 XP_749457.1 |
| Extracellular threonine rice protein | AFUA_2G03540 XP_749488.1 |
| ZIP metal ion transporter | AFUA_2G08740 XP_755208.1 |
| Nickel transport protein | AFUA_2G08830 XP_755217.1 |
| Serine/threonine protein kinase | AFUA_2G09570 XP_755291.1 |
| ZIP metal ion transporter | AFUA_2G12060 XP_755637.1 |
| Midasin | AFUA_2G12150 XP_755547.1 |
| Integral membrane protein | AFUA_2G12640 XP_755596.1 |
| HEAT repeat protein | AFUA_2G14180 XP_755751.2 |
| Conserved hypothetical protein | AFUA_2G15100 XP_755844.2 |
| Integral membrane protein | AFUA_2G15440 XP_755879.2 |
| DUF92 domain protein | AFUA_2G15640 XP_755899.1 |
| Rhomboid family membrane protein | AFUA_2G16190 XP_755986.1 |
| Integral membrane protein | AFUA_2G16985 XP_001481687.1 |
| Sulfatase domain protein | AFUA_2G17610 XP_756096.1 |
| Integral membrane protein | AFUA_2G17760 XP_756111.1 |
| RTA1 domain protein | AFUA_2G17810 XP_756116.1 |
| RTA1 domain protein | AFUA_2G17890 XP_756125.1 |
| **CHROMOSOME 3** | |
| RTA1 domain protein | AFUA_3G00480 XP_748368.1 |
| DUF1275 domain protein | AFUA_3G00670 XP_748388.1 |
| cAMP receptor (Car4) | AFUA_3G00780 XP_748399.1 |
| Hypothetical protein | AFUA_3G00850 XP_748406.2 |
| RTA1 domain protein | AFUA_3G00920 XP_748413.1 |
| Integral membrane protein Pth11-like | AFUA_3G01200 XP_748441.2 |

(Continued)
| Annotation | ORF | Accession number |
|------------|-----|-----------------|
| RTA1 domain protein | AFUA_3G01630 | XP_748484.1 |
| G protein coupled receptor family protein | AFUA_3G01750 | XP_748496.1 |
| Conserved hypothetical protein | AFUA_3G02450 | XP_748568.1 |
| RTA1 domain protein | AFUA_3G03310 | XP_748650.1 |
| PKS-like enzyme | AFUA_3G03540 | XP_748674.1 |
| UPF0016 domain protein | AFUA_3G07080 | XP_754989.1 |
| Phosphatidylinositol: UDP-GlcNAc transferase PIG-C | AFUA_3G07170 | XP_754889.1 |
| Conserved hypothetical protein | AFUA_3G07420 | XP_754867.2 |
| DUF1275 domain protein | AFUA_3G07550 | XP_754856.1 |
| Sucrose transporter | AFUA_3G08480 | XP_754766.1 |
| Conserved hypothetical protein | AFUA_3G09650 | XP_754654.1 |
| PQ loop repeat protein | AFUA_3G10470 | XP_754572.1 |
| RTA1 domain protein | AFUA_3G10770 | XP_754542.1 |
| RTA1 domain protein | AFUA_3G12830 | XP_754338.2 |
| Nonribosomal peptide synthase | AFUA_3G13730 | XP_754251.1 |
| Mating-type alpha-pheromone receptor PreB | AFUA_3G14330 | XP_754193.1 |
| Conserved hypothetical protein | AFUA_3G14870 | XP_754138.1 |
| Integral membrane protein | AFUA_3G15100 | XP_754114.2 |
| **CHROMOSOME 4** | | |
| Polyketide synthase | AFUA_4G00210 | XP_746435.1 |
| Hypothetical protein | AFUA_4G00580 | XP_746398.1 |
| Hypothetical protein | AFUA_4G01242 | XP_746332.2 |
| Conserved hypothetical protein | AFUA_4G01350 | XP_746323.2 |
| Patatin-like serine hydrolase | AFUA_4G03000 | XP_746486.1 |
| Aquaporin | AFUA_4G03390 | XP_746526.2 |
| Integral membrane protein | AFUA_4G03540 | XP_746541.1 |
| C4-dicarboxylate transporter/malic acid transport protein, putative | AFUA_4G04540 | XP_746640.1 |
| Para-hydroxybenzoate-polyisoprenyltransferase Coq2 | AFUA_4G05970 | XP_752227.1 |
| Longevity-assurance protein (LAC1) | AFUA_4G06290 | XP_752195.1 |
| RNA polymerase II mediator complex subunit Nut1 | AFUA_4G06600 | XP_752166.2 |
| CaaX prenyl protease Ste24 | AFUA_4G07590 | XP_752066.2 |
| Conserved hypothetical protein | AFUA_4G07680 | XP_752057.1 |
| 26S proteasome regulatory subunit Rpn2 | AFUA_4G08480 | XP_751978.1 |
| Conserved hypothetical protein | AFUA_4G10080 | XP_751818.2 |
| Endosomal peripheral membrane protein (Mon2) | AFUA_4G12070 | XP_751624.1 |
| Potassium uptake transporter | AFUA_4G13540 | XP_751477.1 |
| Conserved hypothetical protein | AFUA_4G14210 | XP_751411.1 |
| Low affinity iron transporter | AFUA_4G14840 | XP_751369.1 |
| **CHROMOSOME 5** | | |
| Integral membrane protein | AFUA_5G00100 | XP_748330.2 |
| RTA1 domain protein | AFUA_5G01230 | XP_748219.1 |
| RTA1 domain protein | AFUA_5G01310 | XP_748211.1 |
| Phosphate permease | AFUA_5G01320 | XP_748210.1 |
| Histone acetylase complex subunit Paf400 | AFUA_5G02570 | XP_748085.1 |
| Integral membrane protein | AFUA_5G02860 | XP_7480572 |
| PQ loop repeat protein | AFUA_5G04100 | XP_747934.1 |
| cAMP receptor-like protein | AFUA_5G04135 | XP_001481495.1 |
| Conserved hypothetical protein | AFUA_5G06570 | XP_753976.1 |
| Integral membrane protein | AFUA_5G06670 | XP_753966.1 |
| DUF300 domain protein | AFUA_5G07250 | XP_753909.1 |
| a-pheromone receptor PreA | AFUA_5G07880 | XP_753848.1 |
| PQ loop repeat protein | AFUA_5G08410 | XP_753796.1 |
| Annotation | ORF | Accession number |
|-----------|-----|-----------------|
| Spermine/spermidine synthase family protein | AFUA_5G08500 | XP_753787.1 |
| Beige/BEACH domain protein | AFUA_5G09220 | XP_753717.1 |
| Bax Inhibitor family protein | AFUA_5G09310 | XP_753708.2 |
| RTA1 domain protein | AFUA_5G09900 | XP_753650.1 |
| MFS multidrug transporter | AFUA_5G10140 | XP_753627.1 |
| MHYT domain signaling protein | AFUA_5G11310 | XP_753518.2 |
| 26S proteasome regulatory subunit Mts4 | AFUA_5G11720 | XP_753478.1 |
| Guanine nucleotide exchange factor (Gex2) | AFUA_5G11900 | XP_753461.1 |
| Integral membrane protein (Ptm1) | AFUA_5G12390 | XP_753413.1 |
| Integral membrane protein TmpA | AFUA_5G12520 | XP_753400.1 |
| DUF1275 domain protein | AFUA_5G13060 | XP_753348.1 |
| pH signal transduction protein PalH | AFUA_5G13270 | XP_753327.1 |
| Integral membrane protein | AFUA_5G13725 | XP_753282.2 |
| Integral membrane protein | AFUA_5G14600 | XP_753197.1 |

**CHROMOSOME 6**

| Annotation | ORF | Accession number |
|-----------|-----|-----------------|
| Integral membrane protein | AFUA_6G00320 | XP_731523.1 |
| Cation diffusion facilitator | AFUA_6G00440 | XP_731611.1 |
| Hypothetical protein | AFUA_6G00460 | XP_731509.1 |
| Integral membrane protein | AFUA_6G00640 | XP_731492.1 |
| Signal peptide peptidase | AFUA_6G02150 | XP_747862.1 |
| Hypothetical protein | AFUA_6G03180 | XP_747759.1 |
| Conserved hypothetical protein | AFUA_6G03380 | XP_747738.2 |
| Nonribosomal peptide synthase | AFUA_6G03480 | XP_747729.1 |
| Integral membrane protein (Pth11) | AFUA_6G03600 | XP_747717.1 |
| GTPase activating protein (Tsc2) | AFUA_6G04000 | XP_747677.1 |
| Conserved hypothetical protein | AFUA_6G06950 | XP_750588.2 |
| IZH family channel protein (Izh3) | AFUA_6G07160 | XP_750609.1 |
| 4-hydroxybenzoate polypropenyl transferase | AFUA_6G07240 | XP_750617.1 |
| Integral membrane protein | AFUA_6G07820 | XP_750673.2 |
| Aquaglyceroporin | AFUA_6G08480 | XP_750737.1 |
| RTA1 domain protein | AFUA_6G09550 | XP_750844.1 |
| Plasma membrane hexose transporter | AFUA_6G10460 | XP_750934.1 |
| Cell morphogenesis protein (PAG1) | AFUA_6G11010 | XP_750987.1 |
| Integral membrane protein | AFUA_6G11560 | XP_751039.1 |
| RTA1 domain protein | AFUA_6G11800 | XP_751062.1 |
| GPI transamidase component (GAA1) | AFUA_6G12760 | XP_751154.1 |
| ABC iron exporter Atm1 | AFUA_6G12870 | XP_751165.1 |
| UDP-galactose transporter | AFUA_6G13070 | XP_751184.1 |
| Ferric-chelate reductase | AFUA_6G13750 | XP_751251.1 |
| Integral membrane protein Pth11-like | AFUA_6G13800 | XP_751256.1 |
| Integral membrane protein | AFUA_6G13950 | XP_751270.1 |
| RTA1 domain protein | AFUA_6G14140 | XP_751288.1 |

**CHROMOSOME 7**

| Annotation | ORF | Accession number |
|-----------|-----|-----------------|
| Plasma membrane hexose transporter | AFUA_7G00220 | XP_746907.1 |
| Conserved hypothetical protein | AFUA_7G00280 | XP_746901.1 |
| Squalene-hopene-cyclase | AFUA_7G00300 | XP_746899.1 |
| Conserved hypothetical protein | AFUA_7G04800 | XP_749030.2 |
| Plasma membrane protein Pth11-like | AFUA_7G06130 | XP_748897.2 |
| Conserved hypothetical protein | AFUA_7G06660 | XP_748845.2 |
| Metalloreductase | AFUA_7G07120 | XP_748799.1 |

**CHROMOSOME 8**

| Annotation | ORF | Accession number |
|-----------|-----|-----------------|
| Solute transporter | AFUA_8G00660 | XP_747139.1 |

(Continued)
Table A1 | Continued

| Annotation                      | ORF         | Accession number |
|---------------------------------|-------------|------------------|
| Glycosyl transferase            | AFUA_8G00680| XP_747137.1      |
| Conserved hypothetical protein  | AFUA_8G01300| XP_747076.1      |
| GABA permease                   | AFUA_8G01450| XP_747061.1      |
| NRPS-like enzyme                | AFUA_8G01640| XP_747042.1      |
| Conserved hypothetical protein  | AFUA_8G01840| XP_747022.2      |
| Conserved hypothetical protein  | AFUA_8G02390| XP_746967.1      |
| ZIP family zinc transporter     | AFUA_8G04010| XP_747208.2      |
| Integral membrane protein       | AFUA_8G04560| XP_747263.1      |
| Integral membrane protein       | AFUA_8G05510| XP_747353.1      |
| Chitin synthase F               | AFUA_8G05630| XP_747364.1      |
| RTA1 domain protein             | AFUA_8G05740| XP_747375.1      |
| Cellulose dehydrogenase         | AFUA_8G05805| XP_747382.1      |
| DUF1295 domain protein          | AFUA_8G05810| XP_747383.2      |
| Toxin biosynthesis protein (Tr7)| AFUA_8G05970| XP_747399.1      |
| Metalloreductase transmembrane component | AFUA_8G06210| XP_747422.2      |

Table A2 | Identity and annotations of *A. fumigatus* histidine kinase receptors.

| Annotation                             | ORF         | Accession number | No. of transmembrane domains | Putative conserved domains                                                                 | Putative group no. |
|----------------------------------------|-------------|------------------|------------------------------|-------------------------------------------------------------------------------------------|--------------------|
| CHROMOSOME 2                           |             |                  |                              |                                                                                           |                    |
| Sensor histidine kinase/               | AFUA_2G00660| XP_001481640.1   | 4                            | (1) HiskA (Phospho-acceptor) domain                                                         | 6                  |
| response regulator TcaB/Sin1           |             |                  |                              | (2) Histidine kinase-, DNA gyrase B- and HSP90-like ATPase                                 |                    |
|                                        |             |                  |                              | (3) Histidine kinase-like ATPases                                                           |                    |
|                                        |             |                  |                              | (4) cheY homologous receiver domain                                                         |                    |
| Two-component osmosensing histidine    | AFUA_2G03560| XP_749489.1      | 1                            | (1) Multiple HAMP domains                                                                  | 3                  |
| kinase (Bos1)-TcsC                     |             |                  |                              | (2) His kinase A (Phospho-acceptor) domain                                                  |                    |
|                                        |             |                  |                              | (3) Histidine kinase-like ATPases                                                           |                    |
|                                        |             |                  |                              | (4) cheY homologous receiver domain                                                         |                    |
| CHROMOSOME 3                           |             |                  |                              |                                                                                           |                    |
| Sensor histidine kinase/               | AFUA_3G07130| XP_754893.1      | 3                            | (1) HiskA (Phospho-acceptor) domain                                                         | 7                  |
| response regulator                     |             |                  |                              | (2) Histidine kinase-like ATPases                                                           |                    |
|                                        |             |                  |                              | (3) cheY homologous receiver domain                                                         |                    |
| Sensor histidine kinase/               | AFUA_3G12530| XP_754368.1      | 2                            | (1) PAS domain                                                                            | 5                  |
| response regulator                     |             |                  |                              | (2) HiskA (Phospho-acceptor) domain                                                         |                    |
|                                        |             |                  |                              | (3) Histidine kinase-like ATPases                                                           |                    |
|                                        |             |                  |                              | (4) cheY homologous receiver domain                                                         |                    |
| Sensor histidine kinase/               | AFUA_3G12550| XP_754366.1      | 9                            | (1) Serine/threonine kinase domain                                                         | 10                 |
| response regulator                     |             |                  |                              | (2) AAA ATPase domain                                                                      |                    |
|                                        |             |                  |                              | (3) GAF domain                                                                            |                    |
|                                        |             |                  |                              | (4) HiskA (Phospho-acceptor) domain                                                         |                    |
|                                        |             |                  |                              | (5) Histidine kinase-like ATPases                                                           |                    |
|                                        |             |                  |                              | (6) cheY homologous receiver domain                                                         |                    |
| CHROMOSOME 4                           |             |                  |                              |                                                                                           |                    |
| Sensor histidine kinase/               | AFUA_4G00320| XP_746424.2      | 2                            | (1) GAF domain                                                                            | 2                  |
| response regulator                     |             |                  |                              | (2) HiskA (Phospho-acceptor) domain                                                         |                    |

(Continued)
### Table A2 | Continued

| Annotation | ORF         | Accession number | No. of transmembrane domains | Putative conserved domains                                      | Putative group no. |
|------------|-------------|------------------|-------------------------------|---------------------------------------------------------------|-------------------|
| Sensor histidine kinase/response regulator | AFUA_4G00660 | XP_746390.1       | 1                             | (1) GAF domain (2) HiskA (Phospho-acceptor) domain             | 2                 |
|            |             |                  |                               | (3) Histidine kinase-like ATPases (4) cheY-homologous receiver domain |                   |
| Sensor histidine kinase/response regulator | AFUA_4G02900 | XP_746476.1       | 2                             | (1) GAF domain (2) Phytochrome region (3) HiskA (Phospho-acceptor) domain | 8                 |
|            |             |                  |                               | (4) Histidine kinase-like ATPases (5) cheY-homologous receiver domain |                   |
| Sensor histidine kinase/response regulator | AFUA_4G01020 | XP_746355.1       | 3                             | (1) GAF domain (2) HiskA (Phospho-acceptor) domain             | 2                 |
|            |             |                  |                               | (3) Histidine kinase-like ATPases (4) cheY-homologous receiver domain |                   |
| Sensor histidine kinase/response regulator | AFUA_4G07400 | XP_752086.2       | 0                             | (1) Histidine kinase-like ATPases (2) cheY-homologous receiver domain | 7                 |

**CHROMOSOME 6**

| Annotation | ORF        | Accession number | No. of transmembrane domains | Putative conserved domains                                      | Putative group no. |
|------------|------------|------------------|-------------------------------|---------------------------------------------------------------|-------------------|
| Sensor histidine kinase/ response regulator | AFUA_6G10240 | XP_750913.1       | 1                             | (1) PAS domain (2) HiskA (Phospho-acceptor) domain             | 5                 |
|            | Fos-1/TcsA |                  |                               | (3) Histidine kinase-like ATPases (4) cheY-homologous receiver domain |                   |

**CHROMOSOME 8**

| Annotation | ORF         | Accession number | No. of transmembrane domains | Putative conserved domains                                      | Putative group no. |
|------------|-------------|------------------|-------------------------------|---------------------------------------------------------------|-------------------|
| Sensor histidine kinase/ response regulator | AFUA_8G06140 | XP_747415.2       | 2                             | (1) HiskA (Phospho-acceptor) domain (2) Histidine kinase-like ATPases (3) cheY-homologous receiver domain | 7                 |

HiskA, Histidine kinase A; HAMP, Histidine kinases, adenyl cyclases, methyl-accepting chemotaxis protein, phosphatase; PAS, Per—period circadian protein, Arnt—Al receptor nuclear translocator protein, Sim—single minded protein; GAF, presence in cGMP-regulated cyclic nucleotides PDEs, certain adenyl cyclases and the bacterial transcription factor FhlA; AAA, ATPases associated with diverse cellular activities.