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SCIENCE DIRECT
Fungal Genetics and Biology 43 (2006) 490–502
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

G-protein and cAMP-mediated signaling in aspergilli: A genomic perspective

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Received 20 October 2005; accepted 1 February 2006
Available online 20 March 2006

Abstract

We have carried out an in silico exploration of the genomes of *Aspergillus nidulans*, *Aspergillus fumigatus*, and *Aspergillus oryzae*, and identified components of G-protein/cAMP-mediated signaling. Putative G-protein coupled receptors (GPCRs) were distributed over nine classes. The GPCRs within classes were well conserved among aspergilli but varied in other ascomycetes. As previously observed in *A. nidulans* and other fungi, three Gα, one Gβ, and one Gγ subunits of G proteins were identified in *A. fumigatus*, whereas an additional likely non-functional Gα subunit was present in *A. oryzae*. While most fungal species had five proteins containing the regulator of G-protein signaling (RGS) domain predicted to participate in attenuation of G-protein signaling, *A. fumigatus* and *A. oryzae* had an additional RGS protein (RgsD) related to RgsA of *A. nidulans*. Genes encoding adenylate cyclase, a regulatory subunit and two catalytic subunits of the cAMP-dependent protein kinase, were also identified in the three aspergilli. Finally, regulators of cAMP signaling including low- and high-affinity phosphodiesterases were identified. Taken together, our data indicate a striking diversity at the GPCR level, but little diversity of components at the G-protein and cAMP-signaling level. This may reflect the abilities of these fungi to adapt to various ecological niches and to integrate diverse environmental cues into highly conserved cellular processes.

Keywords: *Neurospora crassa*; *Magnaporthe grisea*; *Fusarium graminearum*; Regulator of G-protein signaling; *Aspergillus*; cAMP; G-protein; GPCR; PKA; Nutrient sensing

1. Introduction

Signal transduction plays a critical role in the biology of all living cells contributing to integration of environmental cues into appropriate cellular activities. In this context, signaling pathways that involve the successive contribution of G-protein coupled receptors (GPCRs), heterotrimeric G proteins, the secondary messenger cAMP, and cAMP-dependent protein kinase (PKA) play a pivotal role. In fungi, these pathways have been shown to regulate various aspects of growth, morphogenesis, mating, and virulence (Lengeler et al., 2000). However, how the components of these pathways are organized and precisely regulate cellular responses remains to be fully understood.

Many of the components of the G-protein and cAMP-signaling pathways have been identified in the model filamentous fungus *A. nidulans* through the use of genetic screens, analyses of expressed sequence tags (ESTs), or partial examination of the genome. In particular, Han et al. (2004a) have identified nine GPCRs (GprA-I) that are distributed among five classes: classes I and II include the GprA (PreB) and GprB (PreA) receptors that are similar to the yeast pheromone receptors and have been shown to contribute to self-fertilization in *A. nidulans* (Seo et al., 2004); class III includes the GprC-E receptors that might be involved in carbon-source sensing on the basis of their high similarity to the *Saccharomyces cerevisiae* Gpr1 receptor (Kraakman et al., 1999; Xue et al., 1998); class IV includes

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doi:10.1016/j.fgb.2006.02.001

the GprF and GprG receptors proposed to participate in nitrogen sensing on the basis of their homology to the *Schizosaccharomyces pombe* Stm1 receptor (Chung et al., 2001); class V includes the GprH and GprI receptors that have homology to the *Dictyostelium discoideum* cAMP receptor cAR1 and as such have been proposed to play a role in cAMP sensing (Galagan et al., 2003). Similar genomic explorations to that of Han et al. (2004a) have been performed in *Neurospora crassa* (Galagan et al., 2003) and *Magnaporthe grisea* (Dean et al., 2005; Kulkarni et al., 2005). These studies suggested the presence of additional classes of GPCRs in other filamentous fungi that may be absent or have been missed in *A. nidulans*.

Heterotrimeric G-protein constituents have also been identified in *A. nidulans* including three Gα subunits (FadA, GanA, and GanB, Chang et al., 2004; Yu et al., 1996), one Gβ subunit (SfaD, Rosen et al., 1999), and one Gγ subunit (GpgA, Seo et al., 2005). While the roles of the FadA/SfaD/GpgA and GanB/SfaD/GpgA in regulating various aspects of germination, hyphal growth, and conidiogenesis have been demonstrated (Lafon et al., 2005; Rosen et al., 1999; Seo et al., 2005; Yu et al., 1996), the function of GanA remains to be uncovered (Chang et al., 2004; Han et al., 2004b). Moreover, proteins with a regulator of G-protein signaling (RGS) domain have been identified (FlbA, RgsA-C), where FlbA and RgsA have been shown to modulate the activity of the FadA and GanB Gα subunits, respectively (Han et al., 2004b; Lee and Adams, 1994, 1995). Finally, genes that encode adenylate cyclase, two catalytic subunits, and one regulatory subunit of PKA, have been identified and the roles of these components in controlling conidial germination, hyphal growth, and conidiogenesis have been examined (Fillinger et al., 2002; Ni et al., 2005; Shimizu and Keller, 2001; A.L., M.K. Chaveroche, S. Fillinger, and C.E., unpublished data). Despite such extensive knowledge, a detailed picture of the interplay of the components of G-protein and cAMP-mediated signaling in *A. nidulans* is still lacking. For instance, the three GPCRs, GprA, GprB, and GprD have been shown to play an important role in regulation of sexual reproduction and yet, the G proteins that act downstream of these GPCRs remain unknown (Han et al., 2004a; Seo et al., 2004). Moreover, studies on the heterotrimeric G proteins, FadA/SfaD/GpgA and GanB/SfaD/GpgA and on adenylate cyclase and PKA have shown their separate involvement in the regulation of spore germination, hyphal growth, and conidiogenesis, but a link between heterotrimeric G proteins and cAMP signaling has only been demonstrated in the case of GanB and in the context of conidial germination (Lafon et al., 2005; Shimizu and Keller, 2001). A further understanding of the role and interplay of these signaling components is crucial and will undoubtedly benefit from detailed knowledge of all the components that are likely to constitute signaling pathways.

Availability of fungal genome sequences provides a means to perform a thorough identification of the components that may be involved in different cellular processes. In this regard, the recent release of the genome sequences of *N. crassa* (Galagan et al., 2003), *M. grisea* (Dean et al., 2005), *Fusarium graminearum* (http://www.broad.mit.edu/annotation/fungi/fusarium/index.html), and of the three *Aspergillus* species *A. nidulans* (Galagan et al., 2005), *A. fumigatus* (Nierman et al., 2005) and *A. oryzae* (Machida et al., 2005) provides a unique opportunity to carry out an extensive comparative analysis that should allow us to identify almost all (if not all) components of the G-protein and cAMP-mediated signaling pathways in *Aspergillus* sp.

Little is known of the components and the nature of G-protein and cAMP signaling in the two latter species. *A. fumigatus* is an opportunistic fungal pathogen responsible for life-threatening disseminated infections in immunocompromised individuals (Latgé, 2001). G-protein and cAMP-mediated signaling have been proposed to regulate virulence in this species although reduced virulence of the mutants that are impaired in such signaling pathway may only reflect a reduction in growth rates (Liebmann et al., 2003, 2004). *A. oryzae* is used in food industries for the production of sake, miso, and soy sauce. A notable difference between this fungus and *A. nidulans* or *A. fumigatus* lies in the genome sizes with that of *A. oryzae* being 36.7 Mb and those of *A. nidulans* and *A. fumigatus*, being 30 and 28 Mb, respectively (Galagan et al., 2005). Genome expansion in *A. oryzae* is associated with an increase in the number of predicted genes. Therefore, it might be of interest to evaluate whether this expansion also affects signaling pathways.

Here, we present a genomic exploration of *A. nidulans*, *A. fumigatus*, and *A. oryzae* with the aim to identify all constituents of the G-protein and cAMP-signaling pathways in these species. Our data extend those of Han et al. (2004a) with identification of four new classes of putative GPCRs in *Aspergillus* sp. and illustrate the diversity that exists between different *Aspergillus* species as well as other species of the Pezizomycotina, a subgroup among the Ascomycetes, with respect to distribution of GPCRs among different classes. Finally, we provide the identification of several regulators of the cAMP-signaling pathway including a cyclase-associated protein and low- and high-affinity phosphodiesterases. This knowledge should facilitate detailed investigation of G-protein and cAMP-mediated signaling in *A. nidulans* and closely related species of medical or industrial interest.

2. Materials and methods

2.1. Source of the data

Genomic sequences and deduced proteomes for *A. nidulans*, *A. fumigatus*, and *A. oryzae* were made available to us in the framework of an international genome annotation program. The genome of *A. nidulans* is available at http://www.broad.mit.edu/annotation/fungi/aspergillus/index.html and through GenBank (Galagan et al., 2005). The genome of *A. fumigatus* is available at http://www.tigr.org/tdb/e2k1/afu1/ and through GenBank (Nierman et al., 2005). The