Available online at www.sciencedirect.com
ScienceDirect
Fungal Genetics and Biology 45 (2008) 459–472
FUNGAL GENETICS AND BIOLOGY
www.elsevier.com/locate/yfgbi

Characterization and kinetics of the major purine transporters in *Aspergillus fumigatus*

Sophia Goudela [a,1], Utz Reichard [b], Sotiris Amillis [a], George Diallinas [a,*]

[a] *Faculty of Biology, Department of Botany, University of Athens, Panepistimioupolis, Athens 15781, Greece*
[b] *Department of Medical Microbiology and National Reference Centre for Systemic Mycoses, University Hospital of Goettingen, Goettingen, Germany*

Received 17 June 2007; accepted 1 August 2007
Available online 10 August 2007

Abstract

Three genes encoding putative purine transporters have been identified *in silico* in the genome of *Aspergillus fumigatus* by their very close similarity of their translation products to well-studied homologues in *A. nidulans.* Two of these transporters, called *Af*UapC and *Af*AzgA, were found responsible for bulk uptake of purines and studied in detail herein. Genetic knock-out analysis, regulation of transcription, direct purine uptake assays and heterologous expression in *A. nidulans* have unequivocally shown that *Af*UapC and *Af*AzgA are high-affinity, high-capacity, purine/H^+ symporters, the first being specific for xanthine, uric acid and oxypurinol, whereas the second for adenine, hypoxanthine, guanine and purine. The expression of these transporters is primarily controlled at the level of transcription. Transcription of both genes is purine-inducible, albeit with different efficiencies, whereas *AfuapC* is also ammonium-repressible. We characterised in detail the kinetics of the *Af*UapC and *Af*AzgA transporters, both in *A. fumigatus* and in *A. nidulans*, using a plethora of possible purine substrates. This analysis led us to propose kinetic models describing the molecular interactions of *Af*UapC and *Af*AzgA with purines. These models are discussed comparatively with analogous models from other purine transporters from fungi, bacteria and humans, and within the frame of a systematic development of novel purine-related antifungals.

Keywords: Nucleobase; UapC; AzgA; Modelling of substrate interactions

1. Introduction

Aspergillus fumigatus is an opportunistic fungus and the commonest mould pathogen of human beings, unusually causing both invasive disease in immunocompromised patients and allergic disease in patients with atopic immune systems. A ratio of 4% of patients dying in modern European hospitals suffers from invasive Aspergillosis, representing the leading infectious cause of death in leukaemia and bone marrow transplant patients (Denning, 1998; Latge, 1999). Currently, the existing antifungal drugs (amphotericin B, triazoles, echinocandins) against *A. fumigatus* pathogenicity are either lipid- or cell wall-targeted. The efficacy of these drugs is however limited and the frequent occurrence of therapeutic failures due to rapid resistance development emphasize the need for the characterization of new agents.

Nucleobases, nucleosides and their analogues have for long been considered as promising anticancer and antiviral drugs, but less attention was given to their ability as anti-microbial agents. Some of the most striking differences between microorganisms and their mammalian hosts are found in purine metabolism, distinctions that can be exploited to design specific inhibitors for the microbial proteins enzymes (Kraupp and Marz, 1995). An example is the pyrimidine analogue 5-fluorocytosine (5-FC), which was originally developed as a potential antineoplastic agent and later abandoned as anti-cancer drug due to its lack of activity against tumors, that showed however a promising antifungal activity, achieving at the same time low

* Corresponding author. Fax: +30 210 7274702.
E-mail address: diallina@biol.uoa.gr (G. Diallinas).
[1] Present address: Department of Medical Biochemistry and Microbiology, Uppsala University, BMC, Box 582, 75123 Uppsala, Sweden

1087-1845/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.fgb.2007.08.001

toxicity in mammalian cells, due to absence of enzymes (e.g., cytosine deaminase), which convert it to 5-fluorouracil and eventually lead to ''blocking'' of RNA biosynthesis (Vermes et al., 2000). More importantly, the kinetics and specificities of purine transport, the first step in purine salvage, diverge significantly between microorganisms and their mammalian host. Thus, understanding of the mechanisms of purine transport at the molecular level in fungi, as well as, differences in their properties as compared with those of their mammalian hosts, proves to be of apparent significance. Indeed, differences in substrate specificities, inhibition, and affinities for ligands of nucleoside transport between mammalian and protozoan cells (*Trypanosoma, Leishmania*, etc.) have already been used as the basis of anti-parasitic therapies involving the use of cytotoxic purine nucleoside analogues (Wallace et al., 2002).

Among the best-studied purine transporters are those of the model fungus *A. nidulans* (for a review, see De Koning and Diallinas, 2000; Pantazopoulou and Diallinas, in press), an evolutionary close relative of *A. fumigatus*. *A. nidulans* possesses three major purine transporters necessary for growth on purines as sole nitrogen sources, UapA, a high-affinity, high-capacity, uric acid–xanthine transporter (Diallinas and Scazzocchio, 1989; Gorfinkiel et al., 1993; Koukaki et al., 2005; Vlanti et al., 2006; Pantazopoulou and Diallinas, 2006), UapC, a low/moderate-capacity, low/moderate-affinity, general purine transporter (Diallinas et al., 1995, 1998), and AzgA, a high-affinity, high-capacity, hypoxanthine–adenine–guanine transporter (Cecchetto et al., 2004). UapA and UapC belong to the ubiquitous Nucleobase–Ascorbate Transporter family (NAT), also known as the Nucleobase–Cation Symporter family (NCS2), while AzgA defines the AzgA-like family, distantly related to the NAT/NCS2 family and to another fungal nucleobase transporter family, known as NCS1 (De Koning and Diallinas, 2000; Pantazopoulou and Diallinas, in press; www.tcdb.org). Recently, an *A. nidulans* NCS1 homologue, called FcyB, was characterised and shown to be a very low-capacity purine–cytosine transporter (A. Vlanti and G. Diallinas, in preparation). All four *A. nidulans* purine transporters function as substrate/H^+-symporters (Argyrou et al., 2001; Cecchetto et al., 2004; A. Vlanti and G. Diallinas, in preparation).

The genome of *A. fumigatus* has recently been fully sequenced (Nierman et al., 2005) and thus provided the means for the *in silico* identification of the complete set of purine transporters, based on the *A. nidulans* homologous proteins (Pantazopoulou and Diallinas, in press). Single homologues of UapA/UapC, AzgA and FcyB have been identified (see below). Here, we describe the physiological, genetic, molecular and kinetic characterization of the major *A. fumigatus* purine transporters, called *Af*UapC and *Af*AzgA. We also compare the regulation of expression and transport kinetics of *Af*UapC and *Af*AzgA with those of *A. nidulans* and other purine transporters and discuss these observations within the frame of developing novel purine-related antifungals.

2. Materials and methods

2.1. Strains, media and transformations

Standard complete and minimal media (MM) for *A. nidulans* and *A. fumigatus* were used (Cove, 1966; Scazzocchio et al., 1982; www.fgsc.net). In MM, nitrogen sources were used at the following concentrations: urea 5 mM, $NaNO_3$ 10 mM, purines 0.5 mM. Oxypurinol was used at 50 μM. Growth tests were carried out at 30 or 37 °C, at pH 6.8. Supplements were added when appropriate. The *Escherichia coli* K-12 strain used in standard protocols was DH5α. Chemicals and most purines and analogues were purchased from Sigma (St. Louis, MO). Several of purine analogues were gifts from Dr. H. de Koning and Prof. C Scazzocchio. The *A. nidulans* strain used for heterologous expression is the Δ*uapA* Δ*azgA* Δ*uapC argB2 pabaA1* mutant, which has been described previously (Vlanti et al., 2006; Pantazopoulou et al., 2007). This strain carries total deletions of all three major purine transporter genes, and p-aminobenzoic acid and arginine auxotrophies. The *A. fumigatus* wild-type strain used for the gene replacement experiments was D141 (NRRL 6585, US Department of Agriculture, Peoria, IL), a clinical isolate derived from an aspergilloma developing in a 45-year-old human with tuberculosis (Staib et al., 1980). Cloning of the *AfuapC* and *AfazgA* genes was based on the genomic DNA of the wt *A. fumigatus* strain *Af*293, which was used for the genome sequencing project (Nierman et al., 2005). *A. nidulans* transformations were carried out as in Koukaki et al., 2003. *A. fumigatus* transformations were based on modified protocols of Tilburn et al. (1983) for *A. nidulans*, and Paris (1994) for *A. fumigatus*.

2.2. Cloning of AfuapC and AfazgA and expression in A. nidulans

For cloning of the *AfuapC* and *AfazgA*, the coding sequences with their 5′-and 3′-flanking sequences, expected to include the necessary *cis*-acting regulatory elements, were first PCR-amplified using high fidelity PfuUltra™ DNA polymerase (Stratagene, La Jolla, CA), respective oligonucleotide primer pairs A/B or C/D (Table 1) and genomic DNA from the *A. fumigatus* sequencing strain *Af*293 (Nierman et al., 2005) as a template. The PCR followed standard protocols (Innis and Gelfand, 1990) with 35 amplification cycles (for both genes 95 °C: 45 s, 51 °C: 45 s, 72 °C: 120 s). The total amplicon for *AfuapC* was calculated to consist of 2904 bp including 710 bp for a hypothetical promoter region, 1872 bp for the gene and 322 bp for a hypothetical terminator region. For *AfazgA* respective sizes were calculated to be 2964 bp (total), 609 bp (promoter), 1918 bp (gene) and 437 bp (terminator). In each case, PCR yielded a product of the expected total region size of approximately 3 kb. Identity of both products with the respective targeted region was proven by restriction fragment analysis according to standard protocols (Sam-