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Purine utilization proteins in the Eurotiales: Cellular compartmentalization, phylogenetic conservation and divergence

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ABSTRACT

The purine utilization pathway has been thoroughly characterized in *Aspergillus nidulans*. We establish here the subcellular distribution of seven key intracellular enzymes, xanthine dehydrogenase (HxA), urate oxidase (UaZ), 5-hydroxy-isourate hydrolase (UaX), 2-oxo-4-hydroxy-4-carboxy ureido imidazoline decarboxylase (UaW), allantoinase (AlX), allantoicase (AaX), ureidoglycolate lyase (UglA), and the fungal-specific α-ketoglutarate Fe(II)-dependent dioxygenase (XanA). HxA, AlX, AaX, UaW and XanA are cytosolic, while UaZ, UaX and UglA are peroxisomal. Peroxisomal localization was confirmed by using appropriate *pex* mutants. The pathway is largely, but not completely conserved in the Eurotiomycetes, noticeably in some species AaX is substituted by an alternative enzyme of probable bacterial origin. UaZ and the urate–xanthine UapA and UapC transporters, are also localized in specific cells of the conidiophore. We show that metabolic accumulation of uric acid occurring in *uaZ* null mutations is associated with an increased frequency of appearance of morphologically distinct colony sectors, diminished conidiospore production, UV resistance and an altered response to oxidation stress, which may provide a rationale for the conidiophore-specific localization. The pathway-specific transcription factor UaY is localized in both the cytoplasm and nuclei under non-inducing conditions, but it rapidly accumulates exclusively to the nuclei upon induction by uric acid.

1. Introduction

Work starting in the 1960s has led to the identification of all the genes encoding the enzymes of the purine utilization pathway in *Aspergillus nidulans* (Gournas et al., 2011 and refs therein). The pathway of purine utilization in *A. nidulans* is shown in Fig. 1. This conforms to the classical purine degradation pathway (Darlington et al., 1965; Vogels and Van der Drift, 1976; Lehninger, 1981), with the addition that xanthine hydroxylation to uric acid can be catalyzed by, besides xanthine dehydrogenase also by an α-ketoglutarate dependent xanthine di-oxygenase, an exclusive fungal enzyme (Cultrone et al., 2005; Montero-Morán et al., 2007). While the pathway has been thoroughly characterized genetically, physiologically and biochemically in this organism (Gournas et al., 2011 and refs therein), the subcellular localization of purine break down is still unknown. This is of considerable interest, as while the biochemical steps are conserved from bacteria to metazoa (with some interesting bacterial exceptions; De la Riva et al., 2008; Pope et al., 2009), orthologous enzymes involved in purine utilization show variable cell localization throughout the evolutionary tree (Hayashi et al., 2000), raising the question of metabolite transport between cellular compartments. *A. nidulans*, as all the members of the Pezizomycotina, is a multicellular organism, which permits to study the distribution of metabolites among specialized cells. The presence of the specific uric acid–xanthine transporter UapA in the metullae, intermediate cells in the development of conidiospores, suggests that indeed purine derivatives can be transported from one cell type to the other in the conidiophore

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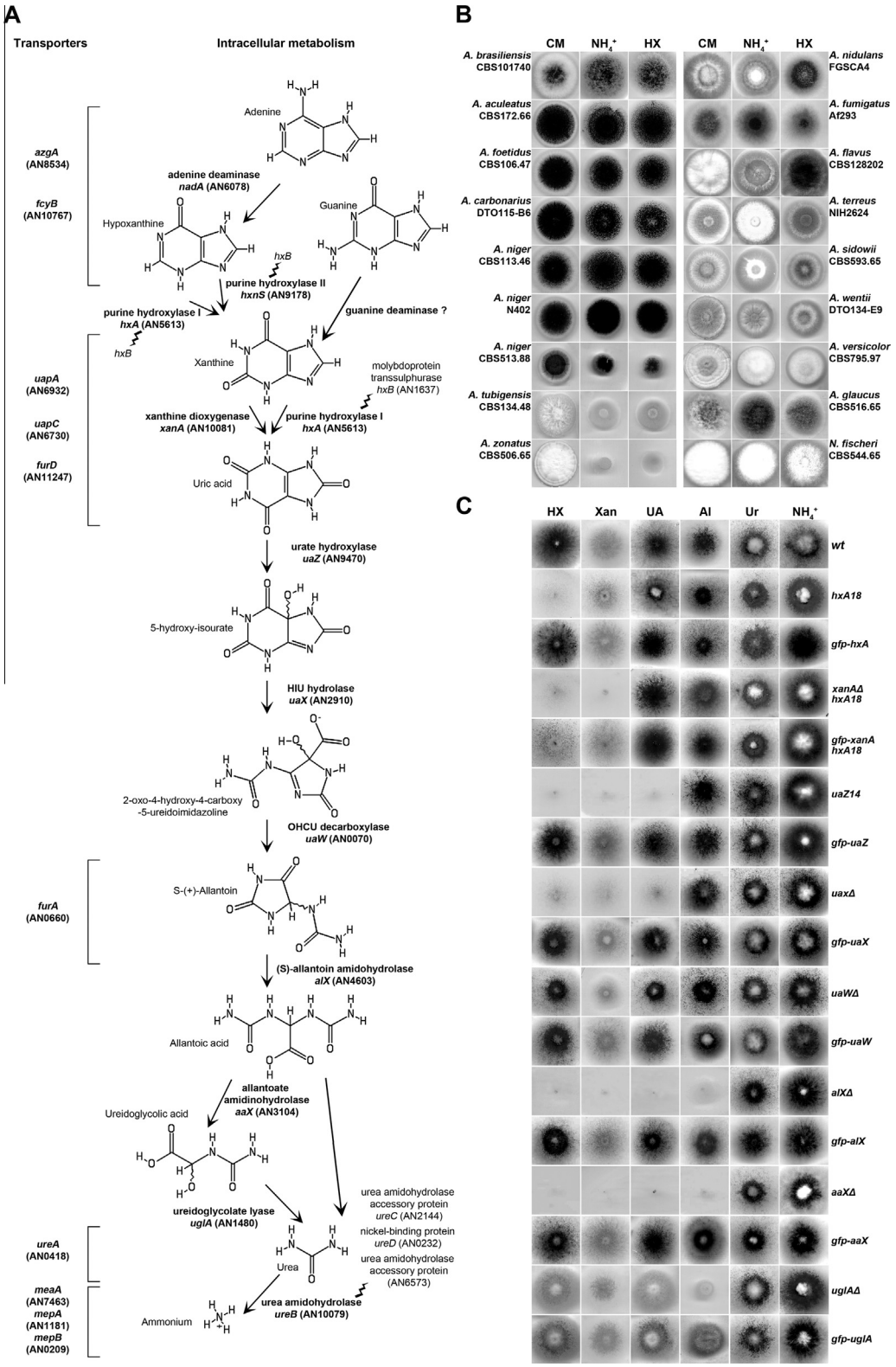

Fig. 1. Gene–enzyme relationships in the purine utilization pathway of Aspergilli and relevant mutant phenotypes in *A. nidulans*. (A) The pathway of purine degradation to ammonium in *A. nidulans* is shown. Adjacent to each arrow the corresponding enzymatic reaction is shown, together with the name and identifier of the cognate gene. The transporters involved in the uptake of different metabolites are also shown to the left of the figure. Connected by a wave-line to the relevant enzymes we show genes and their cognate proteins involved in cofactor synthesis or modification. Experimental identification of each gene is described herein and in a number of publications that are summarized in Gournas et al. (2011). Guanine is a nitrogen source for *A. nidulans* and thus it must be metabolized through this pathway, however, as no experimental work on the conversion of guanine, presumably to xanthine, is extant, nor has a guanine deaminase activity been characterized, a question mark indicates this predicted step. (B) Growth tests of different *wt Aspergillus* species (see Table S3) on complete media (CM) and on minimal media supplemented with ammonium (NH_4^+) or hypoxanthine (HX) as sole nitrogen sources. (C) Growth tests of *A. nidulans* wild-type (*wt*), purine utilization mutants, and purine utilization mutants complemented with *gfp*-tagged versions of the relevant genes, on minimal medium (MM) supplemented with purines or purine-related catabolic metabolites. These are: hypoxanthine (Hx); xanthine (Xan); uric acid (UA); allantoin (Al); urea (Ur) and ammonium (NH_4^+). Growth was carried out at 37 °C, pH 6.8 for 48 h.