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The *nadA* gene of *Aspergillus nidulans*, encoding adenine deaminase, is subject to a unique regulatory pattern

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Abstract

The adenine deaminase of *A. nidulans*, encoded by *nadA*, can be considered both as a catabolic and a purine salvage enzyme. We show that its transcriptional regulation reflects this double metabolic role. As all other genes involved in purine utilisation it is induced by uric acid, and this induction is mediated by the UaY transcription factor. However, it is also independently and synergistically induced by adenosine by a UaY-independent mechanism. At variance with all other enzymes of purine catabolism it is not repressed but induced by ammonium. This is at least partly due to the ammonium responsive GATA factor, AreA, acting in the *nadA* promoter as a competitor rather than in synergy with UaY. The *adB* gene, encoding adenylo-succinate synthetase, which can be considered both a biosynthetic and a salvage pathway enzyme, shares with *nadA* both ammonium and adenosine induction.

Keywords: Adenine deaminase; *Aspergillus nidulans*; Purine nucleotide cycle; Purine catabolism; Transcriptional regulation; Ammonium induction

1. Introduction

Deamination of adenine to hypoxanthine, which occurs on most if not all organisms, can take place at the base, nucleoside or nucleotide level. In a given organism, one or more of these reactions may occur (Fig. 1). The adenine deaminases, which act at the level of the purine base, are not universally present. They have not been described in either mammals or plants, but are present in some *Leishmania* species, in bacteria, in archea and in fungi (Murray et al., 1970; Vogels and Van der Drift, 1976; Kidder et al., 1977; Kidder and Nolan, 1979; Worrel and Nagle, 1990; Yabuki and Ashihara, 1991; Deeley, 1992; Nygaard et al., 1996; Matsui et al., 2001).

We have characterised the adenine deaminase genes of three distantly related ascomycetes species, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Aspergillus nidulans* (Ribard et al., 2003). The translated sequences are highly similar. The *A. nidulans* enzyme, over expressed and purified from *E. coli*, shows adenine deaminase but not adenosine deaminase activity (Ribard et al., 2003). These ascomycete adenine deaminases are phylogenetically related to the mammalian and bacterial adenosine deaminases rather than to the previously described bacterial adenine deaminases. The bacterial and fungal adenine deaminases constitute two distinct sub-families of the α/β barrel hydrolases, the bacterial enzymes being related to the ureases and allantoinases rather than to the adenosine deaminases. This implies a convergent evolution towards an adenine deaminase activity within two subfamilies of enzymes built with the same basic α/β barrel scaffold (Ribard et al., 2003).

Fungal adenine deaminases can be distinguished from adenosine deaminases by specific diagnostic residues (Ribard et al., 2003). Orthologues are present in all sequenced *Aspergillus* genomes (including *A. fumigatus*, at

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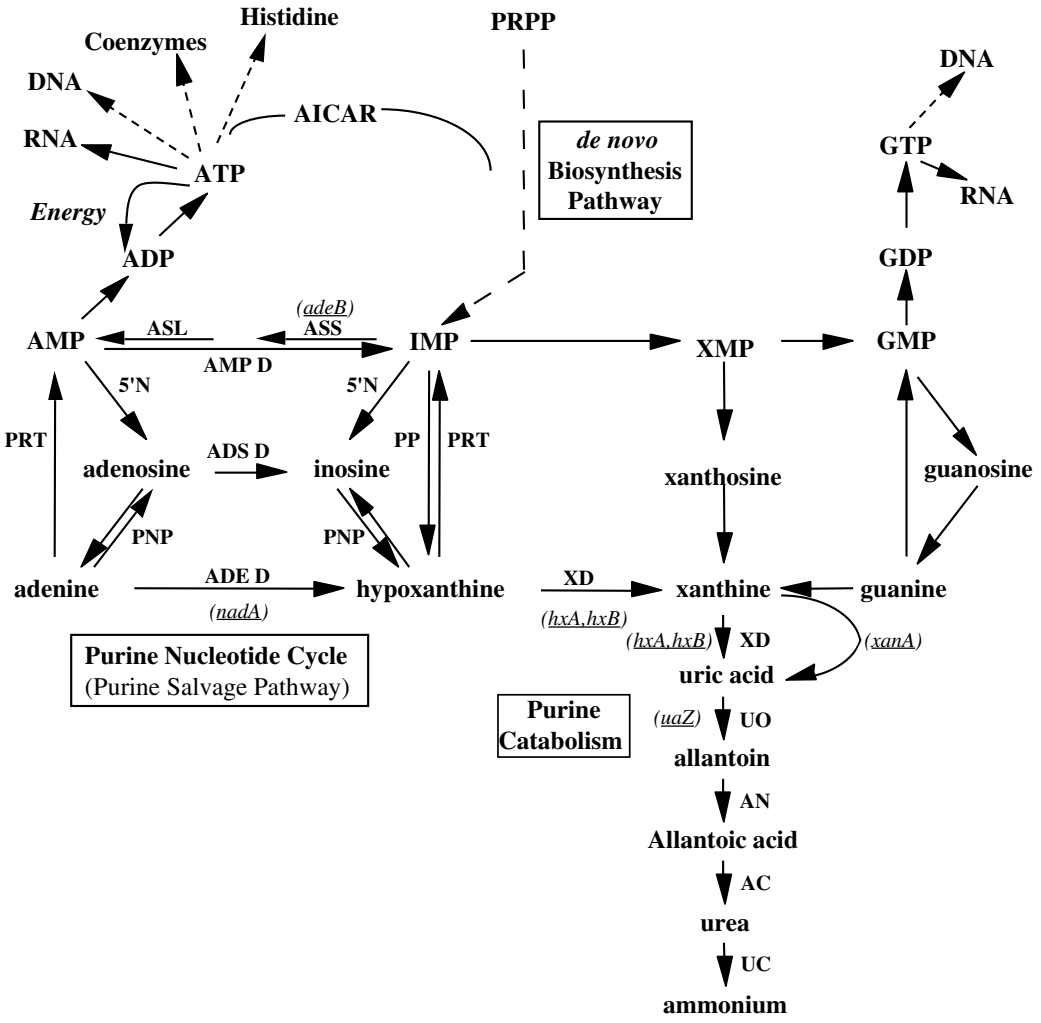

Fig. 1. Purine metabolism. This figure shows an idealised scheme, the different pathways may not exist in all organisms. Purine metabolism includes three related pathways: *de novo* biosynthesis, the purine nucleotide cycle (or purine salvage pathway) and purine catabolism. *A. nidulans* is able to synthesise purines *de novo* and mutants blocked before and after IMP synthesis are available (Foley et al., 1965; Ribard et al., 2001, see http://www.gla.ac.uk/Acad/IBLS/molgen/aspergillus/locia. html2001 for gene/enzyme relationships). Purine catabolism in *A. nidulans* has been described extensively (Scazzocchio, 1994, for review). AC, allantoicase; ADE D, adenine deaminase; ADS D, adenosine deaminase, AMP D, AMP deaminase; AN, Allantoinase; ASL, adenylosuccinate lyase; ASS, adenylosuccinate synthetase; 5′N, 5′ nucleotidase; PNP, purine nucleoside phosphorylase; PP, pyrophosphorylase; PRT, phosphoribosyl transferase; UC, urease; UO, urate oxidase; XDH, xanthine dehydrogenase. Specific nucleobase and nucleoside transporter systems differ widely from one organism to the other and are not indicated. The *A. nidulans* metabolic genes relevant to this work are indicated in italics and underlined. The product of the *hxB* gene is necessary for a posttranscriptional modification of the XDH (encoded by *hxA*) (Amrani et al., 1999). The *xanA* gene encodes a xanthine α ketoglutarate dependent dioxygenase, responsible in *A. nidulans* of an alternative pathway of xanthine oxidation to uric acid (Cultrone et al., 2005). The *uaY* gene (encoding the specific transcriptional activator of purine catabolism in *A. nidulans*) and the xanthine-uric acid transporter gene *uapA* are not included in this figure.

variance with what was found in previous data-base releases, *A. oryzae*, *A. terreus*, *A. niger* and *A. flavus*) and in *F. graminearum*, while they have not been found in the pyromycetes (available genomes *Neurospora crassa*, *Podospora anserina*, *Magnaporthe grisae*) or in the basidiomycetes (*Ustilago maydis*, *Phaenerochete chrysiosporum* and *Coprinus lagopus*).

Adenine and adenosine deaminase can be viewed both as enzymes of the purine utilisation pathway, involved in the utilisation of purine and purine nucleosides as nitrogen sources, and as enzymes of the purine salvage pathway involved in the interconversion of purine nucleotides (Fig. 1). In *S. pombe* and *A. nidulans* adenine deaminase is necessary for the utilisation of adenine as sole nitrogen source. In *S. cerevisiae* the enzyme is only involved in purine interconversion, as this organism lacks all the enzymes leading to the opening of the purine ring (Cultrone et al., 2005, and references therein).

The transcriptional regulation of adenine deaminases has only been cursorily studied. In *Escherichia coli*, the adenine deaminase activity encoded by the *yicP* gene is inducible by adenine (Matsui et al., 2001; Petersen et al., 2002). In *Bacillus subtilis*, the level of adenine deaminase (encoded by the *ade* gene) is reduced when exogenous guanosine serves as a purine source and when glutamine serves as nitrogen source. No effect on the AdE enzyme levels are