The Structure and Regulation of Aldehyde Dehydrogenase Encoding Genes in *Aspergillus niger* and *Aspergillus nidulans*.

by

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ABSTRACT.

The primary aim of this research was to determine mechanisms of gene regulation in the filamentous ascomycete fungus *Aspergillus niger*. To this end, a gene encoding an aldehyde dehydrogenase enzyme, designated *ald*A, has been cloned from *A. niger*. This clone and a clone of the *ald*A gene from the related fungus *Aspergillus nidulans* (Pickett *et al.*, 1987) has been used in a comparative study of gene structure and regulation in these organisms.

The complete nucleotide sequence of the *A. niger ald*A gene and its flanking regions, and the structure of its mRNA transcript has been determined. The *ald*A gene of *A. niger* has one major and two minor start points of transcription and 4 sites of polyadenylation. The coding region shows significant similarity to the sequence of the *A. nidulans ald*A gene (Pickett *et al.*, 1987), but contains three introns compared to the two present in the *A. nidulans* gene. Alignment of amino acid sequences of several aldehyde dehydrogenases has implicated two cysteine residues likely to be adjacent at the active site. The promoter region has a general structure similar to other highly expressed fungal genes, including an extensive pyrimidine rich region, TATA and CCAAT box homologies, and several repeated sequences.

Studies of *ald*A mRNA levels in wildtype strains grown under a variety of conditions has revealed that *ald*A expression is subject to induction and repression in both species. However, the relative levels of induction and degree of repression differs markedly between these species in almost all growth conditions tested. Most notably, threonine

induction of *ald*A genes in *A. niger* is insensitive to glucose repression, and in addition to *alc*R mediated control of *ald*A expression in *A. nidulans*, the *amd*R gene (also called *int*A) either directly or indirectly affects *ald*A expression in mycelia grown in fructose or threonine medium.

A strain of *A. niger* carrying a deletion of the *ald*A gene was constructed by gene replacement. In addition to several phenotypes in common with *ald*A mutant strains of *A. nidulans*, it also exhibited some unique phenotypes further suggesting fundamental differences in metabolism.

Heterologous expression of the *A. niger ald*A gene in *A. nidulans* is subject to induction and repression, and induction during growth on ethanol medium requires a functional *alc*R gene product. Expression of the *A. nidulans ald*A in *A. niger* is also controlled by induction and repression, in a similar way, including induction in fructose medium, to that seen for wildtype *A. nidulans*. However, induction in response to growth in threonine medium is, as in wildtype *A. niger*, insensitive to repression by glucose.

The effects of several 5' deletions in the *A. niger ald*A gene have been investigated. Regions required for induction and the high level of expression were identified. Deletion of the putative CCAAT boxes and a sequence common to the promoters of several fungal genes, many of which are subject to carbon catabolite repression, had no observable effects on gene expression. A region containing a silencer of induction during fructose growth was also located, which together with the high level of fructose induced expression of the *A. nidulans ald*A gene in *A. niger*, implies that *A. niger* also has an induction mechanism functionally homolgous to that affected by the *amd*R gene in *A. nidulans*.