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A regulator gene for acetate utilisation from *Neurospora crassa*

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Abstract The *Neurospora crassa* homologue of the *Aspergillus nidulans* regulatory gene *facB* has been cloned. The gene encodes a putative transcriptional activator of 865 amino acids that contains a DNA-binding domain with a $Zn(II)_2Cys_6$ binuclear cluster, a linker region and a leucine zipper-like heptad repeat. Two internal amino acid sequences are identical to peptide sequences determined from proteolytic fragments of a DNA-binding protein complex specific for genes involved in acetate utilisation and expressed in acetate-induced mycelia of *N. crassa*. Recombinant expression of the predicted DNA-binding domain demonstrates that it is capable of independent recognition of a subset of the promoter sequences that bind the protein complex from *N. crassa*. A duplication-induced mutation in the corresponding gene results in an acetate non-utilising phenotype that is characterised by inefficient induction of the enzymes required for acetate utilisation. The new gene does not fall into any existing complementation group and has been designated *acu-15*.

Keywords *Neurospora crassa* · Transcriptional activator · Acetate utilisation · Glyoxylate cycle · Fungi

Introduction

In order to use acetate as sole carbon source many microorganisms require the presence of the anaplerotic glyoxylate-cycle enzymes and those enzymes essential for gluconeogenesis (Kornberg 1966). The key glyoxylate-cycle enzymes isocitrate lyase (EC 4.1.3.1) and malate synthase (EC 4.1.3.2.) operate via the intermediate acetyl-coenzyme A (CoA), which is produced by the acetate-activating enzyme acetyl-CoA synthetase (EC 6.2.1.1) in *Neurospora crassa* and other filamentous fungi. The transfer of growing *Neurospora* mycelia from sucrose as carbon source to acetate results in the near-coordinate induction of the mRNAs and synthesis of the enzymes required for acetate utilisation (Thomas et al. 1988). Genetic loci encoding these enzymes have been defined in *N. crassa* through the isolation of acetate non-utilising mutants (*acu*) with single enzyme deficiencies: *acu-3* codes for isocitrate lyase, *acu-5* for acetyl-CoA synthetase, *acu-6* for phosphoenolpyruvate carboxykinase (EC 4.1.1.32), *acu-8* for acetyl-CoA hydrolase (EC 3.1.2.1), *acu-9* for malate synthase, and *acu-13* for NAD^+-specific malate dehydrogenase (EC 1.1.1.37) (Flavell and Fincham 1968a, 1968b; Connerton 1990; Connerton et al. 1992; Owen et al. 1992). The genes encoding isocitrate lyase, acetyl-CoA synthetase, acetyl-CoA hydrolase and malate synthase have been isolated and their nucleotide sequences determined (Connerton et al. 1990; Marathe et al. 1990; Gainey et al. 1991, 1992; Sandeman et al. 1991).

In the ascomycete *Aspergillus nidulans* the *facB* gene has been shown to control the induction of a similar set of genes for acetate utilisation (Katz and Hynes 1989). The *facB* gene encodes a DNA-binding protein that contains a $Zn(II)_2Cys_6$ zinc finger, which has been shown to bind nucleotide motifs that reside upstream of the target genes that are up-regulated in response to acetate (Todd et al. 1997a, 1998). In addition to its regulatory role, the *facB* gene product may also have a structural role in acetate metabolism, since temperature-sensitive *facB* alleles render acetyl-CoA synthetase and the enzymes of the glyoxylate cycle thermolabile (Katz and Hynes 1989; Todd et al. 1997b). Hitherto a regulatory gene responsible for the control of these structural genes has not been identified amongst the mutants known to be impaired in acetate utilisation in *N. crassa*. Here we describe a PCR-based method for cloning the analogous gene, demonstrate the DNA-binding function

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