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Promoter paper

Promoter analysis of the acetate-inducible isocitrate lyase gene (*acu-3*) from *Neurospora crassa*[1]

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Abstract

Analysis of the promoter region of the acetate-induced isocitrate lyase gene (*acu-3*) of *Neurospora crassa* was undertaken. A series of deletions in the 5′ non-transcribed region were constructed and the effects of these mutations on the enzyme levels following growth on sucrose and transfer to acetate were measured. Sequences within the region −603 to −271 relative to the transcription start site appear essential for transcription. The region −950 to −1278 is required for sucrose repression, which is consistent with previous protein/DNA gel retardation results of protein extracts from *N. crassa* cultured on sucrose. Protein extracts from acetate-induced mycelia identify alternative promoter regions apparently involved in acetate-induced gene transcription. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Acetate metabolism; Glyoxylate cycle; Isocitrate lyase; Gene regulation; (*Neurospora crassa*)

Growth of the filamentous fungus *Neurospora crassa* on acetate as a sole carbon source requires the glyoxylate cycle. This cycle involves two reactions catalysed by the enzymes isocitrate lyase (ICL) (EC 4.1.3.1), and malate synthase (MS) (EC 4.1.3.2) [1,2]. The glyoxylate cycle enzymes of *N. crassa* are present at low levels from cells grown on sucrose. However, the transfer of growing mycelium from sucrose to acetate as sole carbon source results in the near co-ordinate induction of the mRNA and synthesis of the enzymes required for acetate utilisation which include ICL and MS [3]. A number of acetate non-utilising mutants (*acu*) have been isolated from *N. crassa* and *Aspergillus nidulans*, several of these mutants demonstrate single enzymes deficiencies and define the genes encoding the specific enzymes involved in acetate metabolism [4–8]. These include the respective structural genes for *A. nidulans* and *N. crassa*: acetyl-CoA synthetase *acuA* (allelic to *facA*) and *acu-5*, ICL *acuD* and *acu-3*, MS *acuE* and *acu-9*, and phosphoenolpyruvate carboxykinase *acuF* and *acu-6*.

Expression of the genes involved in acetate metabolism has been shown to be under distinct regulatory controls. In *A. nidulans* at least two regulatory genes are involved: *facB* responsible for transcriptional acetate-induction and *creA* involved in glucose repression. The *facB* gene encodes a *trans*-acting regulatory protein [9–12] which is required for acetate-dependent induction of the enzymes required for acetate utilisation including ICL, MS [5] and acetami-

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[1] The nucleotide sequence data reported in this paper have been deposited in the EMBL/GenBank Data Library under accession number Z47723.

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