Mol Gen Genet (1996) 250: 421–427

ORIGINAL PAPER

Tomoko Mizote · Martha Bibbins
Peter J. Sheffield · Ian F. Connerton

Gel mobility shift scanning of the acetate-inducible promoters from *Neurospora crassa* reveals a common co-inducible DNA-binding protein

Received: 25 January 1995 · Accepted: 10 October 1995

Abstract The promoter regions of four acetate-inducible genes of *Neurospora crassa*, *acu-3*, *acu-5*, *acu-8* and *acu-9*, have been sequenced. Using a scanning gel mobility shift assay particular DNA regions in each promoter have been shown specifically to bind partially purified protein extracted from acetate-induced mycelia. The protein-binding regions so defined have common sequence motifs, elements of which are similar to those required for acetate induction in *Aspergillus nidulans*.

Key words *Neurospora crassa* · Acetate induction · DNA-binding protein · 5′-Upstream regulatory sequence · Glyoxylate bypass

Introduction

The fungus *Neurospora crassa* has the ability to utilise acetate as sole carbon source (Flavell and Fincham 1968a) due to the existence of the glyoxylate bypass, the unique enzymes of which are isocitrate lyase and malate synthase (Kornberg 1966). A number of acetate non-utilising (*acu*) mutants have been isolated; some of these demonstrate single enzyme deficiencies and define the genes encoding the specific enzymes involved in acetate metabolism (Flavell and Fincham 1968a, b; Owen et al. 1992). The genes for the following enzymes have been cloned: acetyl-CoA synthetase (*acu-5*) (Thomas et al. 1988; Connerton et al. 1990), acetyl-CoA hydrolase (*acu-8*) (Marathe et al. 1990), as well as the two genes encoding the glyoxylate bypass enzymes, malate synthase (*acu-9*) (Thomas et al. 1988; Connerton 1990) and isocitrate lyase (*acu-3*) (Gainey et al. 1991, 1992). The synchronisation of regulation exhibited by the acetate-responsive genes of *Neurospora* suggests that they should be regulated by a common mechanism. As a consequence, elements of their promoter regions may contain related sequence motifs as target sites for *trans*-activating proteins.

In the related organism, *Aspergillus nidulans*, the genes encoding a similar set of enzymes related to acetate utilisation are under the control of a pathway-specific transcriptional activator, the structural gene for which is *facB* (Apirion 1965; Armitt et al. 1976; Hynes 1977). The *facB* gene has been cloned and the gene sequence reveals a reading frame that encodes a protein with a functional zinc finger DNA-binding domain (Katz and Hynes 1989; Parsons et al. 1992). A candidate target sequence motif for the product, FacB, has been identified by a *cis*-acting mutation (*amdI9*) in the *amdS* gene (the structural gene for acetamidase; Hynes and Davis 1986), which confers increased *facB*-dependent acetate induction (Hynes et al. 1988). In *N. crassa* a regulatory gene analogous in function to *facB* has not been identified amongst the *acu* mutants isolated (Flavell and Fincham 1968b), and a screen for fluoroacetate resistance/acetate non-utilisation which had been successful with *A. nidulans* (Apirion 1965; Hynes 1977), did not generate regulatory mutants (Owen et al. 1992).

Without the benefit of mutants we therefore undertook a direct molecular approach to investigate potential DNA-protein interactions between acetate-inducible proteins and specific DNA sequences within the promoter regions of cloned *acu* genes from *N. crassa*. Our first goal was the determination of the sequence of the *acu* promoter regions. Based on this information, the *acu-3* gene promoter was initially selected for a scanning gel mobility shift search (Chen and Kinsey 1994) to delimit the protein-binding regions. Following this search, the other available acetate-inducible promoters (*acu-5*, *acu-8* and *acu-9*) were also

Communicated by C. A. M. J. J. van den Hondel

T. Mizote · M. Bibbins · P. J. Sheffield · I. F. Connerton (✉)
Department of Protein Engineering, Institute of Food Research, Earley Gate, Whiteknights Road, Reading, UK, RG6 2EF