MOLECULAR ANALYSIS OF GENES AND ENZYMES CONTROLLING KEY STEPS IN THE SYNTHESIS OF STORAGE TRIACYLGLYCEROLS IN *BRASSICA*

K. ELBOROUGH*, T. FAWCETT*, W. SIMON*, W. WINZ*, R. SWINHOE*, D. RICE+, T. STUITJE°, M. RAFFERTY+, A. SLABAS*

*Lipid Molecular Biology Group, Department of Biological Sciences, University of Durham, Science Laboratories, South Road, Durham DH1 3LE.

+Krebs Institute, Department of Biochemistry University of Sheffield, PO Box 594, Sheffield S10 2UH.

°Free University of Amsterdam

ABSTRACT

In order to advance our understanding of lipid synthesis and its regulation in rape (*Brassica napus*) we have purified and cloned a number of enzymes involved in this biosynthesis pathway. These include (a) both the cytoplasmic and plastid acetyl CoA carboxylase, (b) the two reductases and (c) acyltransferases involved in triglyceride biosynthesis. The structure and regulation of these components will be described.

INTRODUCTION

The biosynthesis of fatty acids occurs in different subcellular compartments. Key enzymes in the biosynthetic pathway of triglycerides are (a) acetyl CoA carboxylase (ACC) which provides the carbon atoms for chain elongation, (b) the fatty acid synthetase (FAS) which catalyzes the addition of malonyl-ACP to the growing chain and its total reduction, and (c) acyltransferases which are important in decorating the glycerol backbone. Studies on all these components have been undertaken in our laboratory, with collaborators, over the last few years, aimed at a complete elucidation of their structure.

(1) Acetyl CoA carboxylase

Two forms of ACC are present in oil seed rape (a) a multidomain form with a basic 220 kDa polypeptide chain (Elborough *et al.*, 1994). This is cytoplasmic and we have cloned both the cDNA and genomic for this. Currently the structure of the promoter is being investigated, (b) a multisubunit form, some components of which are plastid encoded and others of which are nuclear encoded (Sasaki *et al.*, 1993). This type is found in the plastid of dicots but not monocots. The initial discovery of these two distinct forms of ACC is attributed to Dr's Li & Cronan who noted the presence of the transcarboxylase domain in the chloroplast genome of pea (Li & Cronan, 1992). We have used an Expressed Sequence Tag (EST) from Arabidopsis which resembles the *E. coli* BCCP domain as a probe to clone a full length clone of BCCP from rape.

Both cytoplasmic and plastid carboxylases are now available to measure their importance in metabolic flux using appropriate antisense technology.

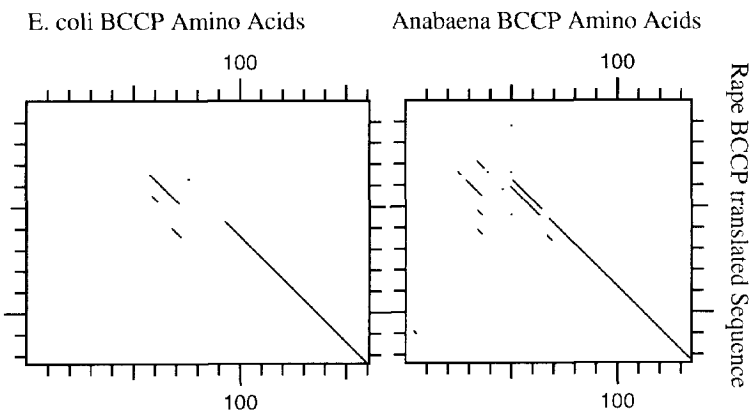

Fig. 1 Dot Matrix Sequence homology of cloned BCCP from rape with (a) *E. coli* and (b) *Anabaena* BCCP

(2) Fatty acid synthetase

We have recently cloned and overexpressed both enoyl ACP reductase (Raffert *et al.*, 1994) and β-ketoacyl ACP reductase (Martinez-Rivas *et al.*, 1994). Both of these proteins have been crystallized and their 3-dimensional structure is being determined at present (Raffert *et al.*, 1994). This will enable us to eventually determine if a FAS complex exists *in vivo*.

(3) Acyltransferases

The acyltransferases of plants are membrane bound so making purification very difficult. An alternative strategy using complementation cloning of an *E. coli* 2-AT mutant has been successfully used in our laboratory to complement this defect (Brown *et al.*, 1994). The isolated clone has been sequenced and shows homology to other acyltransferases.

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