THE INTRACELLULAR SITE OF SYNTHESIS OF FRUCTOSE 1,6-BISPHOSPHATASE IN RAT LIVER

A thesis presented in partial fulfilment of the requirements
for the degree of Doctor of Philosophy in Biochemistry at
MASSEY UNIVERSITY

JOHN WILLIAM McLEAN
1979

ABSTRACT

Fructose-1,6-bisphosphatase (FBPase, E.C. 3.1.3.11) has been purified from rat liver cytoplasm by a new purification procedure. Monospecific antibodies were raised to FBPase in rabbits to enable the immunochemical isolation and quantitation of FBPase in protein homogenates.

Pulse labelling of rat liver *in vivo* showed that the synthesis of FBPase amounted to 0.89% of total soluble protein synthesis. Newly synthesized FBPase was found almost entirely in liver cytoplasm in contrast to serum albumin which was associated only with microsomes, and amounted to 16.7% of total microsomal protein synthesis.

Isolated free and bound polysomes from liver synthesized almost equal amounts of FBPase when incubated *in vitro*, whereas albumin synthesis was confined to bound polysomes. Premature termination of translation led to the release of partial protein transcripts which were specifically immunoprecipitated.

Affinity-purified ^{125}I-labelled antibodies to FBPase and albumin were used to quantitate nascent protein chains on free and bound polysomes. FBPase antibody bound to both classes of polysome with almost equal affinity but albumin antibody was only associated with bound polysomes.

Poly(A)$^{+}$ RNA isolated from free and bound polysomes by poly(U)-Sepharose chromatography was translated in a cell-free protein-synthesizing system derived from wheat germ. RNA from both classes of polysome synthesized FBPase, but albumin was only synthesized in response to bound polysomal RNA. Some full lengths transcripts corresponding to both proteins were produced in wheat germ extracts, but a large amount of smaller molecular weight material was specifically immunoprecipitated. Total translation products from both classes of polysome also produced considerable abnormally short molecular weight material, but only full length transcripts were produced when the system was programmed with TMV RNA.

Since liver cells contain twice as many bound polysomes as free, it has been concluded that about 70% of intracellular FBPase synthesis takes place on bound polysomes. In contrast, albumin synthesis was almost totally confined (>97%) to bound polysomes and newly synthesized

albumin was segregated into microsomes. The partitioning of synthetic activity for albumin is therefore a result of the distribution of mRNA coding for this protein.

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr John W. Tweedie for his encouragement throughout the course of this study. I also wish to acknowledge the advice and assistance given by Dr Tony Cashmore of the Applied Biochemistry Division, DSIR, Palmerston North. Thanks are also extended to the Soil Science and Dairy Husbandry Departments, Massey University for the use of scintillation and gamma spectrometers.

LIST OF ABBREVIATIONS

AMP	Adenosine 5'-monophosphate
ATP	Adenosine 5'-triphosphate
BPB	Bromophenol blue
CM-	Carboxymethyl-
DE-	Diethylaminoethyl-
DNA	Deoxyribonucleic acid
cDNA	Copy DNA
DTT	Dithiothreitol
EDTA	Ethylenediaminetetra-acetic acid
ER	Endoplasmic reticulum
FBP	D-Fructose 1,6-bisphosphate
FBPase	Fructose-1,6-bisphosphatase
F6P	D-Fructose 6-phosphate
GTP	Guanosine 5'-triphosphate
Hab	Immunoglobulin heavy chain
Hepes	N-2-Hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HK(x)M	50 mM Hepes, x mM KCl, 5 mM $MgCl_2$, pH 7.4
HNa(x)M	50 mM Hepes, x mM NaCl, 5 mM $MgCl_2$, pH 7.4
Lab	Immunoglobulin light chain
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
PB	Phosphate buffer (10 mM Na_2HPO_4, 15 mM NaCl, pH 7.2)
PBS	Phosphate buffered saline (10 mM Na_2HPO_4, 150 mM NaCl, pH 7.4)
PEG	Polyethylene glycol
PFK	Phosphofructokinase
PMSF	Phenylmethyl sulphonyl fluoride
$poly(A)^+$	RNA having a polyadenylate tract at its 3' terminus
$poly(A)^-$	RNA lacking a polyadenylate tract at its 3' terminus
RNA	Ribonucleic acid
mRNA	Messenger RNA
rRNA	Ribosomal RNA
tRNA	Transfer RNA
RNase	Ribonuclease
SDS	Sodium dodecyl sulphate
TCA	Trichloroacetic acid
TEMED	N,N,N",N"-Tetramethylethylenediamine
TMV	Tobacco Mosaic Virus
Tris	tris(Hydroxymethyl)aminomethane

LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS