BIOCHEMICAL LETTERS

Structural distinction of rat GSH transferase subunit 10

We have previously noted a GSH transferase with high GSH peroxidase activity which is prominent in the liver soluble fraction of pre- and post-natal rats. It has a subunit M_r of 25500, intermediate between Yb and Ya, and appears to be closely related to Alpha class subunit 2 (Meyer *et al.*, 1985; Ketterer *et al.*, 1986; Dalton, 1987; Scott & Kirsch, 1987). It may be separated from subunits 1 and 2 by chromatofocusing on PBE 118 (Dalton, 1987). Since its apparent size and activity are distinct from other well-characterized subunits we have given it the designation subunit 10 (Ketterer *et al.*, 1988). A subunit of identical properties was also obtained from adult rat kidney by Hayes (1988, 1990) and termed Y1. To confirm that subunit 10 (Y1) is a distinct gene product we now report that it has a unique primary structure.

GSH transferases were obtained from newborn rat liver cytosol (kindly provided by Dr. G. Yeoh, University of Western Australia) by affinity chromatography on GSH-agarose and the subunits separated by reverse phase h.p.l.c. (Kispert *et al.*, 1989). Subunit 10 was eluted between subunits 2 and 6 and was not completely separated from subunit 2. The subunit 10 fraction was purified further by diluting with 1 vol. of water and re-applying to h.p.l.c. Then 20 μ g of purified subunit 10 was cleaved

with CNBr and the fragments separated by microbore reverse phase h.p.l.c. (Kispert *et al.*, 1989). Uncleaved subunit 10 and CNBr peptides were then subjected to gas phase sequencing using an Applied Biosystems 470A Sequencer. The partial sequence of 113 residues obtained showed 90%, 60% and 57% identity with Alpha class subunits 2, 1 and 8 respectively (see Fig. 1). These data confirm our earlier conclusion that subunit 10 is a unique GSH transferase subunit of the Alpha class and is most closely related to subunit 2. Two residues were obtained in approximately equal yield at position 83, suggesting that micro-heterogeneity exists in subunit 10.

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On the mechanism of induction of chick embryo hepatic δ -aminolaevulinic synthase by translational blockers

Two groups have reported the surprising observation that the addition of cycloheximide (CHX) to the culture medium of chick embryo hepatocytes results in rapid elevations in δ -amino-

Subunit	1	10	20
10	P G K P V L H Y F D G R G R M E P I X X L L A A G V X F		
2			
1	S I A C		
8	E V K . Y Q V T		
10	63 K L V Q T K A I L N A I A T K X N L Y G T L D	70	80
2	 R Y K .		
1	 A R Y D K .		
8	L T R S Y L . A K .		
10	94 Y A E G V A D L E	100	
2			
1	 S/T I L . L T		
8	 D . T Q . . . M		
10	105 V L Y Y P Y	110	
2	 H		
1	I I/M Q L V I		
8	I I G A F		
10	112 P P G E K E A S L A K I K D K A R N R Y F P A Y E K V L	120	130
2			
1	 P D Q . /R K T . L A R T K L F		
8	A Q E L A V K R . K V F I .		
10	170 D P I V G D N F P L X K A L Q T R V	180	
2	 S A L A		
1	 A S L L T S F K S . I		
8	S A P . L S D Q . F K . . I		

Fig. 1. Comparison of partial primary sequence of GSH transferase subunit 10 with those of subunits 1, 2 and 8

The partial primary structure of subunit 10 purified by h.p.l.c. was obtained by gas-phase sequencing as described in the text. The sequences of subunits 2 and 8 are from Telakowski-Hopkins *et al.* (1985) and Ålin *et al.* (1989) respectively; those of the variants of subunit 1 are from Pickett *et al.* (1984) and Lai *et al.* (1984). A dot indicates a residue identical with that of subunit 10.

laevulinate synthase (ALA synthase) mRNA concentrations (Hamilton *et al.*, 1988; Ryan & Ades, 1989). ALA synthase catalyses the production of δ -aminolaevulinic acid, the first committed step in haem production. The activity of the enzyme rises rapidly in chicken embryo hepatocytes incubated in culture with any of a number of porphyrogenic agents, including phenobarbital (PB). Similar increases in ALA synthase activity can be measured in livers of experimental animals exposed to such inducers [reviewed by Ades (1990)]. The rise in activity of the enzyme has been attributed primarily, if not entirely, to transcriptional activation of the ALA synthase gene [Hansen & May (1989) and work of others as reviewed by Ades (1990)]. However, despite extensive studies, the mechanisms of activation of transcription of the ALA synthase gene are not understood, although some of the physical features that make an agent a strong inducer have been catalogued [reviewed by Granick & Sassa (1971)].

To explain the phenomenon seen in hepatocytes incubated with CHX, Hamilton *et al.* (1988) have suggested that the effect might have been due to a rapidly-turning-over haem-dependent ALA synthase repressor protein. The labile protein would disappear from cells incubated with CHX, thus resulting in the transcriptional activation of the ALA synthase gene. On the other hand, we have speculated that CHX might have acted as an inducer of ALA synthase transcription by virtue of its structure (Ryan & Ades, 1989). CHX contains features, such as carbonyl- and nitrogen-containing hydrophobic non-planar structures, characteristic of some inducers of ALA synthase (Granick & Sassa, 1971). To help resolve between the possibilities, we report the results of measurements on the effect of ricin on ALA synthase RNA content in hepatocytes. The toxin, obtained from

Table 1. Effects of protein synthesis inhibitors on ALA synthase RNA in hepatocytes

Primary cultures of hepatocytes from 16-day-old White Leghorn chicken embryos were prepared and maintained as described previously (Ryan & Ades, 1989). At the point where the medium was changed to the serum-free condition, the medium was supplemented as indicated with one or more of the following to the given concentrations: PB at 0.4 mg/ml, CHX at 50 μ g/ml, ricin at 1.0 μ g/ml. The cultures were incubated for 5 h. After the incubation, RNA was isolated from the cells (pooled from 9–12 100-mm culture dishes for each condition) and analysed for ALA synthase sequences by solution hybridization, with 40 μ g of RNA used for the analysis (Drew & Ades, 1986). Values of recovered radioactivity in SI-resistant, acid-insoluble material are indicated in the Table for each condition. Each set of independent determinations was performed using cell cultures prepared from livers pooled from 30–48 embryos. The values obtained with CHX are those from Ryan & Ades (1989) and are included for comparison (means $\pm$ S.D. from three determinations). The conditions were: control, cells incubated for 5 h without any of the agents listed; PB, cells incubated with PB only; PB+inhibitor, cells incubated with PB plus either CHX or ricin; inhibitor, cells incubated with either CHX or ricin. Two independent determinations were performed with cells exposed to ricin (Ricin I and II), and each value is the mean $\pm$ S.D. from a sample of RNA analysed in duplicate.

Condition	Hybridization (c.p.m. of 32 P)		
	CHX	Ricin	
		I	II
Control	440 $\pm$ 10	690 $\pm$ 30	590 $\pm$ 70
PB	2100 $\pm$ 30	3900 $\pm$ 40	2200 $\pm$ 430
PB+inhibitor	5100 $\pm$ 380	4500 $\pm$ 50	4300 $\pm$ 750
Inhibitor	3900 $\pm$ 470	3000 $\pm$ 20	1700 $\pm$ 110

Table 2. Effect of CHX on the stability of ALA synthase RNA

Primary cultures of hepatocytes were prepared as described in the legend of Table 1. At the point where the medium was changed to the serum-free condition, PB was added to the medium (0.4 mg/ml final concentration) and the cells were incubated for 5 h. At the end of that period, the medium was changed to serum-free medium (without PB) containing α -amanitin at a concentration of 1.0 μ g/ml. After 30 min, CHX (50 μ g/ml final concentration) was added to the media of some of the cultures, and the incubation was continued for 4.5 h. RNA was isolated from hepatocytes harvested at different points, and the RNA was analysed for ALA synthase-specific sequences as described in the legend of Table 1. Each value is a mean $\pm$ S.D. from a sample of RNA analysed in duplicate. The results of two independent determinations (I and II) are given.

Conditions	Determination...	Hybridization (c.p.m. of 32 P)	
		I	II
α -Amanitin for 30 min		3700 $\pm$ 10	–
α -Amanitin for 5 h		330 $\pm$ 10	700 $\pm$ 10
α -Amanitin for 30 min, then 4.5 h with α -amanitin and CHX		3900 $\pm$ 400	7400 $\pm$ 200

castor bean, inhibits protein synthesis by functionally inactivating ribosomes (Endo & Tsuguri, 1987). Since ricin is a 66 kDa glycoprotein (Olsnes *et al.*, 1974), it is structurally quite unlike known inducers of ALA synthase.

Primary cultures of hepatocytes were incubated with PB in the presence and absence of either CHX or ricin. We had reported previously (Ryan & Ades, 1989) that CHX potentiated the elevation of ALA synthase RNA concentration caused by PB and by itself led to a rise in the enzyme's mRNA level (Table 1). The pattern seen with ricin was similar to that obtained with CHX. The toxin potentiated the effect of PB, and by itself resulted in a detectable elevation in ALA synthase RNA concentration in the cells (Table 1). We had determined that the concentration of ricin used (1.0 μ g/ml) led to an 80% decrease in the incorporation of [3 H]leucine in hepatocytes.

Clearly then, the rise in ALA synthase RNA due to the translational inhibitors could be attributed to inhibition of protein synthesis. This appeared consistent with the proposal of Hamilton *et al.* (1988) that a labile repressor protein regulates ALA synthase transcription. But could such a protein be one that affects mRNA stability instead? Further analyses suggested that incubation of hepatocytes with CHX indeed did result in an increase in the stability of ALA synthase mRNA. The question was examined with cultures incubated with α -amanitin to inhibit transcription. Hepatocyte cultures were first treated with PB for several hours to raise the starting level of ALA synthase RNA. The cultures were then incubated with α -amanitin to block further transcription. The concentration of α -amanitin used (1.0 μ g/ml) had been found effective for blocking the transcription of the ALA synthase gene (Drew & Ades, 1989). At 30 min after the addition of α -amanitin, CHX was added to some of the cultures. After 4.5 h, RNA was isolated from the hepatocytes and analysed for ALA synthase-specific sequences. As seen in Table 2, in the presence of α -amanitin the concentration of ALA synthase RNA was clearly higher in cells incubated with CHX compared with those without. This implied that CHX significantly decreased the rate of degradation of the enzyme's RNA.

The results suggest the existence of a rather short-lived protein involved in the degradation of ALA synthase mRNA. Drew & Ades (1989) have reported that haem decreases the stability of ALA synthase mRNA in hepatocytes. We therefore propose

that haem requires a labile protein for the haem-stimulated degradation of ALA synthase mRNA. This would be consistent with the observations of Hamilton *et al.* (1988) and the results from our laboratory (Drew & Ades, 1989; Ryan & Ades, 1989).

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Does MAP1B bind to tubulin through the interaction of α -helices?

The amino acid sequences of three brain microtubule-associated proteins, MAP1B, MAP2 and tau, have been recently deduced from the analysis of the corresponding cDNA clones

Fig. 1. Potential MAP1B–tubulin interactions

(a) Possible sequences involved in the interaction MAP1B–tubulin. Their localization, on the respective molecules, is indicated in (c). Tubulin sequences correspond to those present at the C-terminus region of the different molecules of β -tubulin isoforms. The nomenclature is that of Wang *et al.* [9]. (b) Representation, by constructing helical 'wheel diagrams' [10], of the possible α -helices for MAP1B and tubulin sequences. These diagrams have been used to predict helical structures assuming 3.6 amino acids per turn [10]. (c) The localization, within the molecule, of the suggested tubulin-binding MAP1B sequences (in a region that Noble *et al.* [4] described as likely to form several α -helices), is indicated (■). Also, the localization of tubulin sequences (see a) is shown (■). (d) Scheme of the possible interaction between the C-terminal region of a β -subunit and a tubulin-binding site on MAP1b. Tubulin and MAP1B sequences were taken from [9] and [8], respectively.