Growth of Liver Accompanied by an Increased Binding of Aminoacyl-Transfer Ribonucleic Acids

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Homogenates of rat liver obtained 3 or 14 days after partial hepatectomy were used to prepare the postmicrosomal pH5-supernatant fraction and to prepare salt-wash fractions of the 40S ribosomal subunits and the 80S ribosomes. The factor-dependent binding of methionyl-tRNA^{Met} to ribosomes and the elongation-factor-1-dependent binding of phenylalanyl-tRNA to ribosomes were both increased after 3 days of growth, but not after 14 days of growth. An activity inhibitory to phenylalanyl-tRNA binding that was located in ribosomal wash fractions was decreased after 14 days of growth. Since the decreased inhibitory activity was obtained from the ribosomes and was tested against ribosomes and excess of pH5-supernatant fraction from control rat liver, its action was separate from the phenylalanyl-tRNA binding activities of the pH5-supernatant fractions from sham-operated and regenerating liver.

During liver growth in mice and rats after partial hepatectomy a decreased degradation of proteins accounts for at least part of the increased protein content (Scornik & Botbol, 1976; Swick & Ip, 1974; Hill & Malamud, 1974). Rat liver exhibits an increased incorporation of labelled amino acids into protein *in vitro* 6h after injury or partial hepatectomy, and in the latter case the increase persists up to 48h (Bucher, 1963; Campbell *et al.*, 1967; Majumdar *et al.*, 1967; Scornik *et al.*, 1967; von der Decken, 1968; Schreiber *et al.*, 1971). The time of increased protein synthesis after laparotomy is intermediate between these (Liu & Neuhaus, 1968). An increased liver protein synthesis occurs a few hours after whole-body X-irradiation or γ -irradiation of normal animals, though not after X-irradiation of animals undergoing liver regeneration (Ord & Stocken, 1961; Gerber & Altman, 1970; Hidvégi *et al.*, 1970; Ariyama *et al.*, 1970). Exposure of the liver area of animals to laser irradiation produces a form of partial hepatectomy (Fine *et al.*, 1968) and causes an increased binding of aminoacyl-tRNA to ribosomes, i.e. elongation-factor-1 activity (Nicholls, 1973; Nicholls *et al.*, 1974).

In the kidney during growth there are decreases in the degradation of proteins (Hill & Malamud, 1974), as well as increases in protein synthesis studied *in vivo* and *in vitro* (Coe & Korty, 1967; Nicholls, 1968; Girgis & Nicholls, 1973; Nicholls & Markle,

1974). This increase is accompanied by an increase in the factor-dependent binding of aminoacyl-tRNA to ribosomes (Girgis & Nicholls, 1971; Sendeki *et al.*, 1973; Cappon & Nicholls, 1974b; Nicholls *et al.*, 1975; Cappon *et al.*, 1976; Nicholls & Ng, 1976).

Variations in mRNA concentration and initiation-factor activity appear to be important for the regulation of translation (for a review see Lodish, 1976). For example, in liver responding to methylcholanthrene by synthesizing drug-metabolizing enzymes there was an increase in the activity of the initiation factors IF-M2A and IF-M2B, but not IF-M3 (Hopkinson *et al.*, 1974). Initiation factors prepared from salt washes of hepatoma ribosomes exhibited greater activity than those prepared from control rat liver (Murty *et al.*, 1975).

In our laboratory, salt-wash preparations of rat liver ribosomes have been obtained which promote methionyl-tRNA^{Met} binding *in vitro* (Innanen & Nicholls, 1975). Salt-wash preparations of rat liver ribosomes also contain an inhibitor of aminoacyl-tRNA binding, the activity of which is decreased by agents inducing liver microsomal enzymes (Goodchild & Nicholls, 1976). It was therefore decided to study phenylalanyl-tRNA binding and methionyl-tRNA^{Met} binding during the growth of liver after hepatectomy.

Experimental

Animals

Male rats (140–160g) of the Wistar strain were used and had free access to food (Master Fox Cubes;

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Maple Leaf Milling Co., Georgetown, Ont., Canada) and water throughout the experiments. Partial hepatectomy was performed as described by Waynforth (1969).

Chemicals

L-[U-¹⁴C]Phenylalanyl-tRNA (specific radioactivity 385 mCi/mmol; 0.156 μ Ci/mg of tRNA) containing a mixture of 19 other unlabelled aminoacyl-tRNA species from *Escherichia coli* strain B and [methyl-³H]-methionine (4.0 Ci/mmol) was obtained from New England Nuclear, Lachine, Que., Canada. Sodium fusidate was a gift from the Squibb Institute for Medical Research, New Brunswick, NJ, U.S.A., and from Leo Pharmaceutical Products, Copenhagen, Denmark. The tRNA^{fMet} (*Escherichia coli*) was obtained through the courtesy of Dr. A. D. Kelmers and Dr. F. H. Bergman, National Institute of General Medical Science, Bethesda, MD, U.S.A. Other chemicals, including aurointricarboxylic acid, were from suppliers as previously described (Girgis & Nicholls, 1972; Goodchild & Nicholls, 1976).

Liver ribosomes

A 20% (w/v) homogenate of liver obtained from control rats was prepared in 0.25 M-sucrose dissolved in Buffer I, composed of 50 mM-Tris/HCl (pH 7.8 at 25°C), 80 mM-KCl, 6 mM-MgCl₂ and 10 mM-2-mercaptoethanol as previously described (Cappon & Nicholls, 1974a). The ribosomes were preincubated, treated with 1% (w/v) sodium deoxycholate, and salt-washed as described by Cappon & Nicholls (1974a) to remove the endogenous mRNA and bound elongation factors 1 and 2. The salt-washed ribosomes were sedimented by centrifugation for 2 h at 2°C in a Spinco type-40 rotor at 105 000g (r_{av} , 5.9 cm) through a layer of 0.5 M-sucrose in Buffer I. The liver ribosome pellets were resuspended in 0.25 M-sucrose in Buffer I containing 10% (v/v) glycerol and stored at -20°C.

Liver pH5-supernatant fraction

A 20% (w/v) homogenate prepared in Buffer I was centrifuged for 15 min at 2°C in a Spinco type-40 rotor at 15 000g (r_{av} , 5.9 cm). This postmitochondrial-supernatant fraction (S15) was centrifuged for 2 h at 105 000g to remove the microsomal fraction, and the resultant postmicrosomal supernatant fraction (S105) was used for preparation of the pH5-supernatant fraction, which contained elongation factors 1 and 2 (Girgis & Nicholls, 1972).

The S105 fraction was freed of synthetases and RNA, including tRNA, by adjusting the pH to 5.2 with 1 M-acetic acid. After standing for 30 min, the precipitate was removed by centrifugation at 15 000g for 10 min. The supernatant fluid was adjusted to

pH 7.8 by the addition of 1 M-KOH. This resultant pH5-supernatant fraction, containing a final concentration of 15% (v/v) glycerol, was stored at -20°C.

Preparation of liver elongation factors 1 and 2

The pH5-supernatant fraction of control rat liver was prepared as described above and elongation factor 1 was prepared as described previously by using Sephadex G-200 gel-filtration chromatography (Nicholls *et al.*, 1974; Goodchild & Nicholls, 1976). Elongation factor 2 was prepared by hydroxyapatite chromatography followed by DEAE-Sephadex chromatography (Petryshyn & Nicholls, 1976). The elongation factor-1 and -2 preparations were tested separately for [¹⁴C]phenylalanyl-tRNA incorporation into peptide. The incorporation in both cases was less than 5% of that observed when the same reaction mixture contained both elongation factors 1 and 2. Under the conditions of the incorporation assay (see Table 1), an optimal amount of elongation factor 1 was 180 μ g and an optimal amount of elongation factor 2 was 80 μ g.

Preparation of ribosomal inhibitory salt wash

The postmitochondrial supernatant fraction prepared from a liver homogenate was centrifuged at 105 000g for 3 h to sediment the microsomal fraction. The microsomal pellets obtained as described above were suspended in a volume of Buffer I equal to that of the original S15 fraction. A 20% (v/v) solution of Triton X-100 in water was added to a final concentration of 1%. After stirring for 20 min, the suspension was centrifuged at 105 000g for 3 h. The resulting ribosomal pellets were suspended in 10 mM-Tris/HCl, pH 7.6, containing 2 mM-dithiothreitol, to a concentration of 200 A_{260} units/ml. KCl (3 M) was added slowly with stirring, to a final concentration of 0.5 M-KCl. After stirring for 30 min, the suspension was centrifuged at 105 000g for 4 h. The supernatant was dialysed for 6-8 h against 50 mM-Tris/HCl (pH 7.6)/75 mM-KCl/10 mM-2-mercaptoethanol. This dialysed supernatant was the inhibitory salt wash.

Liver free ribosomes

Rat liver free ribosomes were prepared by the method of Loeb *et al.* (1966) with modifications as described by Innanen & Nicholls (1974).

Factor-dependent binding of methionyl-tRNA^{fMet}

Control rat liver free ribosomes subjected to a 0.5 M-KCl salt wash as described previously (Innanen & Nicholls, 1974) were separated from the salt wash by centrifugation for 8 h at 105 000g through a 'cushion' of 1.0 M-sucrose in Buffer I. The supernatant salt-wash fraction overlying the 1.0 M-sucrose

layer was then dialysed against Buffer I in the cold for 15h and used in the binding studies.

In other experiments control rat liver was homogenized in 0.3M-sucrose in 10mM-Tris/HCl (pH7.5)/5mM-magnesium acetate/1mM-dithiothreitol (Buffer II), as described by Falvey & Staehelin (1970). The postmitochondrial supernatant was prepared as above and the postmicrosomal supernatant was prepared by centrifugation at 105000g for exactly 2.5h. The upper two-thirds of this postmicrosomal-supernatant fraction was then diluted with 2vol. of cold water containing 1mM-dithiothreitol. This fraction was used to provide a liver 40S-subunit preparation as described by Sundkvist *et al.* (1974). After precipitation of the 40S subunits at pH5.1, the subunits were washed for 4h at 2°C with Buffer III, containing 0.2M-sucrose, 20mM-Tris/HCl (pH7.5), 500mM-KCl, 10mM-magnesium acetate, 5mM-dithiothreitol and 25% (v/v) glycerol. The salt-wash preparation was centrifuged for 6h at 105000g, and the resultant supernatant salt-wash fraction was dialysed overnight at 2°C against Buffer III containing 80mM-KCl (rather than 500mM-KCl). The salt-wash preparations were concentrated by ultrafiltration with an Amicon PM 10 membrane and used as a routine in the binding studies, since they were twice as active as the free ribosome preparations described above.

The [³H]formylated methionyl-tRNA^{Met} was prepared as described previously (Innanen & Nicholls, 1974) and the binding to ribosomes was carried out in a manner similar to that of Picciano *et al.* (1973) as described previously (Innanen & Nicholls, 1975). This formylated methionyl-tRNA^{Met} from *Escherichia coli* functions in the factor-dependent binding assay as efficiently as the non-formylated methionyl-tRNA^{Met} from rat liver (Elson *et al.*, 1975; Weissbach & Ochoa, 1976). The reactions were terminated with 3ml of buffer containing 80mM-KCl, 5mM-MgCl₂ and 20mM-Tris/HCl (pH7.5). The diluted mixtures were applied to Millipore filters, and washed with 3×3ml of the above buffer. The filters were dried, dissolved in 2ml of 2-ethoxyethanol and diluted to 10ml with toluene-based scintillation fluid (Nicholls & Girgis, 1970) and counted for radioactivity in a Nuclear-Chicago model 6860 liquid-scintillation spectrometer at 20% efficiency. The results are expressed as d.p.m./mg of salt-wash protein and the binding exhibited a linear dependence on salt wash up to 60μg of protein/tube. These values were corrected by subtraction of values for tubes with no added salt-wash protein, which were approx. 400 d.p.m./tube.

Measurement of protein and RNA

Hot-5%-(w/v)-trichloroacetic acid-insoluble protein was prepared for radioactivity counting as described by Nicholls & Girgis (1970), and the counting

efficiency was 85%. Incorporation and binding were expressed as d.p.m./mg of RNA. These values were corrected by subtraction of values for tubes with no added elongation factors or pH5 supernatant, which were approx. 70 d.p.m./tube. Protein concentration was determined by the method of Lowry *et al.* (1951) and Tombs *et al.* (1959), with bovine serum albumin as a standard. RNA was determined from the *A*₂₆₀ (*A*_{1cm}^{1%} = 230). The results given in the Figures are the mean values for duplicate tubes, representative of those obtained in several separate experiments in which several pairs of rats were studied. Values in the Tables are means ± S.E.M.

Results

Activity of pH5 supernatant

The pH5-supernatant fraction, containing elongation factors 1 and 2, was examined for its capacity to incorporate [¹⁴C]phenylalanyl-tRNA into peptide. In these experiments the incubation contained pre-incubated salt-washed control liver ribosomes and the pH5-supernatant fraction prepared either from liver of sham-operated rats or from liver regenerating for 3 days or 2 weeks. Table 1 shows that there was a statistically significant increase (84%) in the incorporation in liver preparations obtained after 3 days but not after 2 weeks of regeneration. Similar results were found when the postmicrosomal-

Table 1. Incorporation of [¹⁴C]phenylalanyl-tRNA into peptide by using pH5-supernatant fraction obtained from liver during regeneration

An excess of salt-washed control liver ribosomes (94μg of RNA) was incubated with 100μg of pH5-supernatant protein from sham-operated or regenerating liver. The concentration of pH5-supernatant protein was 4.57 ± 0.37 mg/100mg for sham-operated liver and 4.49 ± 0.39 mg/100mg for regenerating liver, so that the amount incubated (100μg) was derived from 2.2mg liver in both preparations. The incubation was for 10 min at 37°C in 0.4 ml of medium containing 50mM-Tris/HCl (pH7.6), 80mM-KCl, 6mM-MgCl₂, 0.2mM-GTP, 10mM-mercaptoethanol, [¹⁴C]phenylalanyl-tRNA (13700 d.p.m.; 40μg of tRNA) and 100μg of poly(U). Results are means ± S.E.M. for three separate experiments in which several pairs of animals were studied. *P* values were obtained by Student's *t* test: **P* < 0.05.

Period of regeneration (days)	10 ⁻³ × Incorporation (d.p.m./mg of RNA)		Increase (%)
	Sham-operated liver	Regenerating liver	
3	13.8 ± 0.86	25.4 ± 1.9*	84
14	15.4 ± 1.8	13.7 ± 0.65	-11

Fig. 1. Effect of concentration of pH5 supernatant and incubation time on the incorporation of [^{14}C]phenylalanyl-tRNA into peptide

The incubation was as described for Table 1. (a) Incubation was for 10 min and pH5-supernatant concentration as indicated; (b) the pH5-supernatant concentration was 100 μg of protein/tube and the incubation time as indicated. ○, Sham-operated liver; Δ, 3-day regenerating liver.

supernatant fraction (S105) was tested. These results confirm, for a 3-day period of regeneration, the observations by Campbell & Greengard (1959) and Campbell *et al.* (1967) on the S105 fraction. Fig. 1 shows that this increase after 3 days was maintained at different concentrations of pH5 supernatant and at various times of incubation. The protein concentration in these fractions remained unchanged at 3 days and also at 2 weeks after hepatectomy.

When the incorporation at 3 days was calculated per g liver weight, the increase was 80% on a wet-weight basis. Since the sham-operated-liver weight was $7.34 \pm 0.17\text{g}$ and the regenerating-liver weight was $5.44 \pm 0.09\text{g}$, the incorporation was 30% greater for the total liver (i.e. per rat) in the hepatectomized animals than it was for the total liver in the sham-operated animals. When the incorporation was calculated on the basis of the DNA concentration reported by Nass (1967) or on the basis of nuclear

Fig. 2. Effect of GTP and poly(U) concentrations on the incorporation of [^{14}C]phenylalanyl-tRNA into peptide. Incubation was as described for Table 1. (a) GTP concentration as indicated; (b) poly(U) concentration as indicated. ○, Sham-operated liver; Δ, 3-day regenerating liver.

concentration given by Linder *et al.* (1970), the increase in the regenerating liver was 126% and 189% respectively. When the incorporation was calculated on the basis of the rRNA concentration reported by Arora & de Lamirande (1967), the increase for regenerating liver was 80%. Further, the increased incorporation is not due to a redistribution of elongation factors between the pH5-supernatant fraction and the pH5-precipitate fraction. The latter fraction contains approximately one-fortieth of the total activity of the pH5-supernatant fraction, and the increased incorporation in the regenerating-liver preparation was similar (93%) to the increase in the pH5-supernatant preparation of regenerating liver.

The addition of increasing amounts of GTP to the incubation mixture did not remove the difference

Fig. 3. Inhibition of peptide analysis by (a) aurintricarboxylic acid and (b) fusidate

Incubations were as described for Table 1, and concentration of aurintricarboxylic acid (in a) or fusidate (b) were as indicated. ○, Sham-operated liver; Δ, 3-day regenerating liver.

were present in the pH5-supernatant fraction. Such an inhibitor has been detected in the microsomal fraction of control liver (Scornik *et al.*, 1967; Nolan & Hoagland, 1971; Rupniak & Quincey, 1973), but not regenerating liver. Moreover, the addition of increasing amounts of poly(U) (Fig. 2b) did not remove the increased activity of the regenerating preparation. If the decreased amount of ribonuclease activity present (Shortman, 1962) during liver regeneration was responsible for the preservation of ribonucleic acid species, including exogenous mRNA, then the addition of excess of poly(U) might be expected to lessen the difference between the pH5-supernatant fractions of control and regenerating liver. As with poly(U) addition, in other experiments the increased activity was still present when either ribosomes or [14 C]phenylalanyl-tRNA contents were changed in the incubation mixture. Thus the decrease in ribonuclease activity cannot account for the increase in the activity of the pH5-supernatant fraction.

Because of the possibility that initiation factors might be solubilized during the preparation of the homogenate and might have a greater activity in the pH5-supernatant fraction from the regenerating liver, experiments were carried out with aurintricarboxylic acid, which is believed to inhibit the binding of mRNA to ribosomes, as well as possibly other steps in initiation (Stewart *et al.*, 1971). Fig. 3(a) shows that no difference in the degree of inhibition produced by aurintricarboxylic acid could be detected in experiments using the supernatant fraction from control sham-operated rats compared with that in experiments using the supernatant fraction from treated rats.

Since elongation factors 1 and 2 were present in the liver pH5-supernatant fraction and not on the salt-washed control liver ribosomes used in these experiments, it was desirable to see whether either of these factors was increased in the pH5 supernatant of the regenerating liver. The addition of various amounts of fusidate, which inhibits the translocation step (Malkin & Lipmann, 1969), did not introduce a differential inhibition of the incorporation of [14 C]phenylalanyl-tRNA into peptide (Fig. 3b). Since fusidate has additional effects (at least in prokaryotes) when an excess of the translocation factor (i.e. elongation factor G), relative to ribosomes, is used (Burns *et al.*, 1974), further experiments were carried out to examine the role of elongation factor 2 (Girgis & Nicholls, 1971).

When the pH5-supernatant fraction of liver obtained from regenerating and control rats was supplemented with increasing amounts of control liver elongation factor 2 (Fig. 4), there was no change in the amount of labelled peptide formed. Since this preparation of elongation factor 2 was fully active when incubated with excess of elongation factor 1,

between the activity of the pH5-supernatant fraction of sham-operated and 3-day regenerating liver (Fig. 2a), as might be expected if a GTP-sensitive inhibitor

Fig. 4. Effect of supplementing the incubation mixture with purified control liver elongation factor 2

The incubation as described for Table 1 except that purified liver elongation factor 2 was added. ○, Sham-operated liver; △, 3-day regenerating liver.

in the absence of pH5 supernatant, these results suggest that excess of elongation-factor-2 activity is present in both preparations and that it is unlikely that elongation-factor-2 activity is altered in liver undergoing repair.

Since elongation-factor-1 activity causes the poly-(U)-directed binding of [¹⁴C]phenylalanyl-tRNA to ribosomes at low Mg²⁺ concentrations, the binding activity of control and regenerating liver preparations was measured by the Millipore-filter assay described by Malkin & Lipmann (1969). The stimulation of binding by the pH5-supernatant fraction from the regenerating liver was significantly greater than that by the pH5 supernatant from control liver (Fig. 5a).

When the reaction mixture was supplemented with control liver elongation factor 1, the addition of 50 μg of protein to the tubes containing control liver pH5-supernatant fraction increased the binding of [¹⁴C]phenylalanyl-tRNA to a value equal to that with unsupplemented pH5 supernatant from the regenerating liver, showing that elongation-factor activity was limiting in the control preparation (Fig. 5b). An increased binding was also observed when control liver elongation factor 1 was added to the pH5 supernatant from regenerating liver, showing that elongation-factor activity was also limiting in this preparation.

Ribosome salt wash

The results shown in Table 2 show the effects of a salt-wash fraction prepared from liver ribosomes that had been freed of membranes by treatment with Triton X-100. This salt-wash fraction markedly inhibited the incorporation of [¹⁴C]phenylalanyl-tRNA into peptide when it was added to a protein-synthesizing system that contained limiting amounts of ribosomes and an excess of the other components of the incubation mixture. Previously we found that the inhibitory activity could be detected in salt-wash

Fig. 5. Binding of [¹⁴C]phenylalanyl-tRNA to ribosomes by using pH5-supernatant fraction obtained from liver on day 3 of regeneration

An excess of preincubated salt-washed liver ribosomes (100 μg of RNA) was incubated with pH5-supernatant protein from control or regenerating liver for 5 min at 37°C in 0.40 ml of medium containing 50 mM-Tris/HCl (pH 7.6), 80 mM-KCl, 6 mM-MgCl₂, 0.2 mM-GTP, [¹⁴C]phenylalanyl-tRNA (13 700 d.p.m., 40 μg of tRNA), 100 μg of poly(U) and 4 mM-fusidate. The reaction was terminated by adding ice-cold buffer, and the samples were washed with three portions of buffer on Millipore filters and counted for radioactivity as described in the Experimental section. Blanks containing all the reactants except supernatant were subtracted. (a) The pH5-supernatant concentration is indicated; (b) elongation factor 1 was added. ○, Sham-operated liver; △, 3-day regenerating liver.

fractions obtained from free and from bound liver ribosomes prepared in a variety of ways (Goodchild & Nicholls, 1976). When a 0.5 M-KCl-wash fraction was prepared from the ribosomes of regenerating liver, there was a significant decrease in the inhibitory activity after 2 weeks, but not after 3 days of liver regeneration (Table 2).

Factor-dependent binding

Previous results in this laboratory showed that rat liver free ribosomes can replace rabbit reticulocyte

Table 2. *Activity of ribosomal inhibitory salt-wash preparation during liver regeneration*

The salt wash was obtained from the Triton-prepared ribosomes of liver of sham-operated and partially hepatectomized rats. Equal amounts of liver were used to prepare the salt wash and the yields of protein were comparable in sham-operated and regenerating liver. The salt wash (150 µg of protein) was added to an incubation mixture containing limiting amounts of ribosomes and excess of pH5 supernatant as described in the Experimental section. Results are means ± S.E.M. for four separate experiments in which several pairs of animals were studied. **P* < 0.05.

Period of regeneration (days)	Inhibition (%)		Decrease (%)
	Sham-operated liver	Regenerating liver	
3	46.1 ± 7.2	37.7 ± 5.3	18
14	47.2 ± 6.3	24.3* ± 4.6	49

Table 3. *Factor-dependent binding of [³H]methionyl-tRNA^{Met} to ribosomes by using salt-wash preparations obtained from liver during regeneration*

The binding reaction was carried out for 3 min at 24°C, and contained (in 50 µl) 80 mM-KCl, 5 mM-MgCl₂, 50 mM-Tris/HCl (pH 7.2), 0.5 mM-GTP, 1.0 mM-dithiothreitol, 10 000 d.p.m. of [³H]methionyl-tRNA^{Met}, 2.0 A₂₆₀ units of ribosomes and approx. 50 µg of salt-wash protein. Equal amounts of liver were used to prepare the salt wash, and the yields of protein were comparable in sham-operated and regenerating liver. Binding is expressed in d.p.m. of [³H]methionyl-tRNA^{Met} bound per mg of protein in the salt-wash preparation. In each 3-day experiment two sham-operated and four partially hepatectomized rats were studied, and in each 14-day experiment two sham-operated and two partially hepatectomized rats were studied. **P* < 0.05.

Period of regeneration (days)	10 ⁻³ × Binding of [³ H]methionyl-tRNA ^{Met} (d.p.m./mg of protein)		Increase (%)
	Sham-operated liver	Regenerating liver	
3	117 ± 5	191* ± 6	63
14	164 ± 14	157 ± 4	-4

ribosomes in the binding of methionyl-tRNA^{Met} (Innanen & Nicholls, 1974, 1975). It was found that tRNA binding at 5 mM-Mg²⁺ is strongly dependent on a preparation of liver ribosomal-wash protein and also on GTP, but not on AUG. At 2 mM-Mg²⁺ there was some lack of specificity in the tRNA binding, possibly owing to a non-specific association of the negatively charged tRNA species to the filters, resulting from low bivalent-cation concentration. In the present experiments a salt-wash preparation obtained from 40S subunits of rat liver promoted the binding of

methionyl-tRNA^{Met} to liver free ribosomes. The salt-wash fraction prepared from 40S subunits of liver regenerating for 3 days was more active than a comparable fraction from the liver of sham-operated rats. At 14 days after hepatectomy the salt-wash fraction of control and regenerating liver exhibited the same activity in the factor-dependent binding of methionyl-tRNA^{Met} (Table 3).

Discussion

The results for 3-day regenerating liver confirm previous results for 2-day regenerating liver, which showed that the postmicrosomal-supernatant fraction had an increased capacity for protein synthesis (Campbell & Greengard, 1959; Campbell *et al.*, 1967). In addition, the present experiments show that it is the aminoacyl-tRNA-binding activity that is increased, confirming a preliminary report of Myron *et al.* (1972). These observations resemble those seen in liver responding to damage by laser irradiation, where a fivefold increase in the activity of purified elongation factor 1 was found, and support the view that laser damage resembles partial hepatectomy, at least in its effect on protein-synthesizing capacity (Fine *et al.*, 1968; Nicholls, 1973; Nicholls *et al.*, 1974). In addition to an increase in [¹⁴C]phenylalanyl-tRNA-binding activity, a significant increase in the factor-dependent binding of methionyl-tRNA^{Met} was found in salt-wash preparations of 40S ribosomal subunits obtained from liver regenerating for 3 days.

It is well known that, in rats, regenerating liver exhibits an increased incorporation of amino acids into protein in cell-free preparations (Campbell *et al.*, 1967; Scornik *et al.*, 1967). The differences between control and regenerating-liver microsomal preparations obtained 36 h after hepatectomy diminished on purification of the microsomal fraction and on addition of GTP and thiol agents, partly owing to a GTP- and thiol-sensitive inhibitor in the control preparations (Nolan & Hoagland, 1971; Rupniak & Quincey, 1973). Interpretation of the results after hepatectomy is complicated further by the decreased alkaline ribonuclease activity located in the soluble supernatant and by changes in the amounts and species of tRNA (Shortman, 1962; Jackson *et al.*, 1970). In the present experiments, carried out 3 days and 14 days after hepatectomy, an excess of GTP, thiol agent and exogenous mRNA were used. In addition, control rat liver ribosomes (that had been salt-washed and preincubated to remove mRNA and the protein factors required for protein synthesis) were used. Moreover, the soluble pH 5-supernatant fraction was used so that mRNA, tRNA species and synthetases were removed from the reaction. Thus the difference observed at 3 days cannot be due to changes

in any of these substances, but is due to a difference in the [^{14}C]phenylalanyl-tRNA binding activity.

An inverse relationship between the rate of growth and the overall rate of protein degradation has been described for skeletal muscle (Goldberg, 1969) and regenerating liver and kidney (Swick & Ip, 1974; Hill & Malamud, 1974; Scornik & Botbol, 1976). Scornik & Botbol (1976) have concluded that a change in the rate of protein degradation is the single most important factor determining the increase in protein content during compensatory growth of liver in the mouse, 36 h after hepatectomy. In measuring the rate of protein synthesis *in vivo*, Scornik (1974) obtained evidence for a 15% increase in the rate of protein synthesis 36 h after hepatectomy, just before the first wave of cell division. The activity for aminoacyl-tRNA binding measured in the regenerating-liver cell-free preparations is increased considerably more than 15%. Thus it must be concluded either that the rat liver 72 h after hepatectomy exhibits greater changes in synthesis than does the mouse liver 36 h after hepatectomy, or else that the liver cell produces an excess of the protein-synthesis factors for initiation and elongation. The latter possibility could easily result from a readjustment in the relative rates of degradation and synthesis as measured by Scornik & Botbol (1976).

An increased initiation-factor activity has been reported for rat hepatoma (Murty *et al.*, 1975) and in rat liver synthesizing drug-metabolizing enzymes (Hopkinson *et al.*, 1974). The increase in the factor-dependent binding of methionyl-tRNA^{fMet} that was observed in the present experiments provides evidence that an increase in initiation-factor activity also occurs in 3-day regenerating liver. The salt wash, obtained from liver free ribosomes or subunits, contained factors that were effective in binding methionyl-tRNA^{fMet} to ribosomes obtained from rat liver as well as rabbit reticulocytes.

The inhibitory activity located in the ribosomal salt-wash preparation of control rats is protein in nature, is directed towards the binding of [^{14}C]phenylalanyl-tRNA to ribosomes and is different from the GTP- and thiol-sensitive inhibitor of microsomal fractions and from ribonuclease (Goodchild & Nicholls, 1976). Its lower activity in ribosomal salt-wash preparations obtained from liver regenerating for 14 days, but not 3 days, suggests that it is not part of a specific mechanism in the growth response after hepatectomy. Tominaga *et al.* (1975), however, have reported a decrease in the activity of an inhibitory material in ribosomes prepared from rat liver regenerating for only 24 h. Such results stress the importance of using salt-washed ribosomes in non-limiting concentrations when studying the activity of protein-synthesis factors.

The evidence obtained shows that the binding of [^{14}C]phenylalanyl-tRNA to ribosomes, before bond

synthesis and translocation, is increased during liver regeneration. The results resemble those obtained in chicken liver after oestrogen treatment (Smith *et al.*, 1976), in rat liver during enzyme induction and after radiation damage (Cappon & Nicholls, 1974a; Nicholls *et al.*, 1974), in rat kidney during growth (Girgis & Nicholls, 1971, 1973; Sendeki *et al.*, 1973; Cappon & Nicholls, 1974b; Nicholls *et al.*, 1975) and in rat spleen after immunization (Willis & Starr, 1971). These observations, and the increased methionyl-tRNA^{fMet} binding activity in regenerating liver, taken together with those of changed activity of translational proteins observed by others (Hopkinson *et al.*, 1974; Pain & Henshaw, 1975; Murty *et al.*, 1975), suggest that there is usually an increased capacity in the activity of the machinery of protein biosynthesis during increased protein production in animals. Evidence for such a view has appeared for prokaryotic cells also (Krauss & Leder, 1975; Furano, 1975). Although variations in mRNA concentration and initiation-factor activity are important for the regulation of translation [for a review see Lodish (1976)], there is a growing body of evidence to support the view that elongation factors may also be involved in regulation (see, e.g., Haschemeyer & Persell, 1973; Nicholls *et al.*, 1974, 1975; Smith *et al.*, 1976).

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