Effects of liver regeneration on tRNA contents and aminoacyl-tRNA synthetase activities and sedimentation patterns

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The tRNA content and aminoacyl-tRNA synthetases of regenerating liver in the phase of rapid growth were compared with those of livers from both intact and sham-operated rats. At 48 h after hepatectomy, the amount of active tRNA (called 'total acceptor capacity') is significantly higher in regenerating liver than in control livers, owing to a general, possibly not uniform, increase in the various tRNA families, which suggests that it may contribute to the increased protein synthesis and to decreased protein degradation as well. The activities of most, but not of all, aminoacyl-tRNA synthetases in cell sap of regenerating liver tend to be greater than normal. Increased activity of histidyl-tRNA synthetase fits in with the possibility that the mechanisms that control the rate of protein degradation through aminoacylation of tRNA^{His} in cultured cells [Scornik (1983) *J. Biol. Chem.* **258**, 882–886] also operate in the liver and play a role in regeneration. Sedimentation analysis of cell sap in sucrose density gradients shows a shift of prolyl-tRNA synthetase activity toward the high- M_r form in regenerating liver. This change might be related to the positive protein balance and to growth *in vivo*, since it is also observed in the anaplastic Yoshida ascites hepatoma AH 130.

INTRODUCTION

The higher than normal stimulatory activity of cell sap from regenerating liver on protein synthesis in cell-free systems has been ascribed to increased activities of activating enzymes by some workers (Hultin & Von der Decken, 1958; Rendi, 1959) and to increased synthesis of aminoacyl-tRNA by others (Cajone *et al.*, 1980). Those studies were concerned with only a few amino acids. Changes in activity of several aminoacyl-tRNA synthetases are in fact seen in cell sap of regenerating liver, but their relation to growth is not clear, since they are not synchronous (Tidwell *et al.*, 1972). Also, there are still no direct measurements of the amounts of tRNA extracted from regenerating liver and aminoacylated *in vitro* by conventional methods, since previous investigations have focused mainly on the composition of the tRNA pool (Agarwal *et al.*, 1970; Jackson *et al.*, 1970; Tidwell *et al.*, 1972) and bases (Shaw *et al.*, 1978). However, the amount of tRNA could be rate-controlling (see the Discussion section), and we have shown that it is noticeably higher in the liver of developing than of adult rats (Capaccioli *et al.*, 1977).

Comparing regenerating liver in the phase of rapid growth with livers from both intact and sham-operated controls, we have investigated whether partial hepatectomy might elicit an increase in tRNA in the fully differentiated organ of adult rats, up to values found in the perinatal period. Since the aminoacyl-tRNA synthetases are key elements in protein synthesis and may also have other physiological functions (Deutscher, 1984), we also measured the activities of postmicrosomal-supernatant aminoacyl-tRNA synthetases, and we investigated

the sedimentation patterns in sucrose density gradients of prolyl-, aspartyl- and lysyl-tRNA synthetase. Like several other mammalian aminoacyl-tRNA synthetases, those for proline and aspartic acid sediment both free and in complexed forms. In hepatomas, lysyl-tRNA synthetase is in multiple complexes (Perego *et al.*, 1982), and a complex has been isolated containing prolyl-, lysyl- and aspartyl-tRNA synthetase firmly associated with six other aminoacyl-tRNA synthetases, namely those for arginine, methionine, leucine, isoleucine, glutamic acid and glutamine (Perego, 1985; Perego & Del Monte, 1986), as shown by others for CHO cells (Mirande *et al.*, 1985). This association might be related to active protein synthesis, or to growth, or to both, since in CHO cells arrested in G₁ phase by amino acid deprivation, as compared with cells growing exponentially, there is a shift of prolyl-tRNA synthetase activity distribution to lower- M_r forms (Enger *et al.*, 1978).

MATERIALS AND METHODS

Chemicals

L-Amino acids uniformly labelled with ¹⁴C were obtained from Amersham International (Amersham, Bucks., U.K.). This amino acid mixture (code CFB-104) had a specific radioactivity of 54 Ci/g-atom of C. Specific radioactivities of the single amino acids uniformly labelled with ¹⁴C were as follows (in Ci/mol): alanine, 156; arginine, 324; asparagine, 207; aspartic acid, 224 or 229; glutamine, 41; glutamic acid, 290; glycine, 109; histidine, 58; isoleucine, 354; leucine, 311; lysine, 312 or 348; proline, 280; threonine, 208; tyrosine, 468; valine,

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260. Sucrose and phenylmethanesulphonyl fluoride were obtained from Serva, Heidelberg, Germany; dithiothreitol, bovine serum albumin, ATP and CTP were obtained from Sigma, St. Louis, MO, U.S.A. GSH was obtained from BDH, Poole, Dorset, U.K. DEAE-cellulose (type standard, capacity 0.88 mequiv./g) was from Schleicher and Schuell, Keene, NH, U.S.A. All other chemicals were analytical-grade products.

Animals

Male Wistar rats (11–12 weeks old; 285–310 g) were used. They were randomly assigned to the experimental and control groups and used within 1 month. They were fed on a balanced diet (Piccioni) and water *ad libitum* and maintained in light-controlled rooms (light on from 07:00 to 19:00 h). Hepatectomy (Higgins & Anderson, 1931) and sham-operation were carried out between 09:00 and 10:00 h, under light diethyl ether anaesthesia. All the rats were killed by decapitation at the times indicated, after a 13–14 h period of starvation. The Yoshida ascites hepatoma AH 130 was grown intraperitoneally for 6 days (10^7 cells inoculated) in rats of the same age.

RNA extraction and purification

Liver tRNA was prepared quantitatively from the postmitochondrial-supernatant fraction by phenol extraction and DEAE-cellulose chromatography, as described by Capaccioli *et al.* (1977). Homogenization was checked by examining stained smears of low-speed sediments. After deacylation (pH 8.5) and re-precipitation with acetate/ethanol, the tRNA fraction (18–21% of total A_{260} units that had been loaded on a 2 cm $\times$ 16 cm DEAE-cellulose column) was collected on a Millipore (type HA) filter, dried under vacuum, dissolved in 4 mM-MgCl₂/0.4 mM-EDTA and stored at -20°C . The A_{260}/A_{280} ratio of tRNA was 1.9–2.05.

Determination of amino acid acceptor capacity of tRNA

To the standard reaction mixture of Del Monte & Cini (1972), 0.25 mM-CTP was added. The tRNA concentration was 1.2 A_{260} units/ml. In tests with the mixture of labelled amino acids, MgCl₂ was 6 mM. Liver aminoacyl-tRNA synthetases (Yang & Novelli, 1971) were kept at -70°C until used. Reactions were run with two amounts of enzyme (18 and 36 μg of protein in 125 μl final vol.), and samples (50 μl) were taken at 30 and 60 min to verify that aminoacylation was at the plateau, with enzyme in excess. Cold-trichloroacetic-acid-insoluble radioactivity was quantified by scintillation spectrometry with 2,5-bis-(5-t-butylbenzoxol-2-yl)thiophen in toluene as scintillation medium. Hot-acid-insoluble radioactivity (considerable for arginine) was subtracted (Del Monte & Cini, 1972). Aminoacylation was linear with tRNA concentration. Reproducibility was within $\pm 5\%$.

Polyacrylamide-gel electrophoresis of tRNA

This was performed essentially as described by Richards *et al.* (1965). The current was maintained at 6 mA for 6.5 h at 15°C .

Assays of aminoacyl-tRNA synthetase activities

These were done either by the method of Del Monte & Cini (1972) in the presence of 0.25 mM-CTP (Method A), or by the method of Ritter *et al.* (1979), but with a reaction time of 2 min and a reaction volume of 100 μl

(Method B). Enzyme activity is expressed as units/mg of soluble protein. One unit of enzyme activity is defined as the amount of enzyme required to form 1 pmol of aminoacyl-tRNA/min under the specific assay conditions.

Sedimentation analysis of aminoacyl-tRNA synthetase activities

The homogenization medium was 0.35 M-sucrose containing 50 mM-Tris/HCl (pH 7.5), 25 mM-KCl, 5 mM-magnesium acetate and 2 mM-dithiothreitol (or 2 mM-GSH when indicated), which were also present in the sucrose gradient. When present, phenylmethanesulphonyl fluoride was 0.44 mM (Dang & Yang, 1979). The ascites-hepatoma cells were collected by low-speed centrifugation, washed twice and homogenized in a Dounce homogenizer as described by Perego *et al.* (1982). The postmicrosomal-supernatant fraction (Vennegoor & Bloemendal, 1970) was passed through a column (0.6 cm $\times$ 13 cm) of Sephadex G-25 (coarse grade). A sample of 1 ml (10–20 mg protein) was loaded on the top of a 15–35% (w/v) sucrose density gradient of 25 ml, which had been layered on a 60%-sucrose cushion of 6.5 ml (rotor SW27 used in Milan) or of 2 ml (rotor SW25 used in Florence). Centrifugation was at 22 500 (SW27) or at 24 000 (SW25) rev./min for 18 h at 2°C . Then 2 ml fractions were collected by upward displacement, by using ISCO fractionators. The gradient was calibrated reproducibly with several markers covering the range 4–28S (Perego *et al.*, 1982), and no differences were seen between calibration data for gradients that had been run in the two rotors. Starting from the top, the three regions of the gradient closely correspond to 4–11S (1–6 ml), 11–18S (7–12 ml) and $>18\text{S}$ (13–26 ml). Aminoacyl-tRNA synthetase activities were measured in samples (20 μl) from each fraction, by either Method A or Method B, having ascertained that activity values for proline and lysine were similar by the two methods, whereas those for aspartic acid were 4 times higher when measured by Method B.

Assays of total tissue nitrogen and of protein

Tissue N was determined by nesslerization (Umbreit & Burris, 1964) of samples of tissue digested with H₂SO₄/CuSeO₃. Protein was measured by the method of Gornall *et al.* (1949) or by that of Lowry *et al.* (1951) in trichloroacetic acid precipitates (Clark & Jacoby, 1970), with bovine serum albumin as standard.

RESULTS

The results in Table 1 show that, 48 h after partial hepatectomy, the mass of hepatic tissue left *in situ* (determined in a comparable group of rats) was more than doubled in wet weight (from 3 to 7.3 g) and was just doubled in total N (from 118.5 to 243.8 mg). The fall in tissue N per unit wet weight reflects increases in water and fat that, in turn, proportionally dilute the other components of the tissue. Thus tissue N is the best reference basis, although wet weight was also used to facilitate comparisons with data for normal liver in the literature. The concentration of 10.9 A_{260} units of tRNA/g of tissue found for normal liver corresponds to the upper values of the typical range (Shenoy & Rogers, 1977). For regenerating liver, the concentration was significantly higher. When based on tissue N, the increase in tRNA during regeneration was also significantly

Table 1. Effect of sham-operation and partial hepatectomy on liver weight nitrogen and tRNA content, and acceptor capacity of tRNA 48 h after surgery

Results are means $\pm$ S.E.M. for the numbers of animals indicated. Data are for aminoacylation of tRNA with the L-[U- 14 C] amino acid mixture. Significance of differences was evaluated by analysis of variance (nd not determined): $^aP < 0.05$ versus normal control; $^bP < 0.01$ versus normal control; $^cP < 0.05$ versus sham-operated control; $^dP < 0.01$ versus sham-operated control.

	Normal	Sham-operated	Hepatectomized
No. of animals	6	5	5
Liver wt. (g)	9.0 $\pm$ 0.46	9.0 $\pm$ 0.55	7.3 $\pm$ 0.34 nd
Tissue N (mg/g wet wt.)	39.5 $\pm$ 1.1	41.2 $\pm$ 2.0	33.4 $\pm$ 1.6 a,d
tRNA content of tissue (A_{260} units/g wet wt.)	10.9 $\pm$ 0.6	12.4 $\pm$ 1.3	14.2 $\pm$ 0.8 a
(A_{260} unit/mg of tissue N)	0.277 $\pm$ 0.016	0.321 $\pm$ 0.033	0.429 $\pm$ 0.035 a,c
Acceptor capacity of tRNA (c.p.m./ A_{260} unit)	146 300 $\pm$ 5900	98 450 $\pm$ 8550 b	139 950 $\pm$ 12 200 d
Total acceptor capacity (c.p.m./mg of tissue N)	40 700 $\pm$ 3250	31 600 $\pm$ 3850	59 000 $\pm$ 4600 b,d

Table 2. Quantities of individual tRNA families in tRNA from normal, sham-operated and hepatectomized rats

Data are for pools of three preparations each. Acceptor capacity is given as pmol/ A_{260} unit.

Amino acid	Normal		Sham-operated		Hepatectomized	
	(pmol/ A_{260} unit)	(% of total)	(pmol/ A_{260} unit)	(% of total)	(pmol/ A_{260} unit)	(% of total)
Ala	76.2	6.0	51.1	5.7	81.0	7.0
Arg	77.9	6.2	27.1	3.0	59.5	5.2
Asn	70.4	5.6	62.2	6.9	54.5	4.7
Asp	53.9	4.3	29.7	3.3	34.4	3.0
Gln	69.6	5.5	39.2	4.3	48.2	4.1
Glu	46.4	3.7	51.4	5.7	54.9	4.8
Gly	98.3	7.8	65.4	7.2	82.3	7.2
His	59.9	4.8	69.2	7.6	82.3	7.1
Ile	46.0	3.7	31.4	3.5	34.3	3.0
Leu	117.0	9.3	72.5	8.1	110.6	9.6
Lys	70.6	5.6	53.1	5.9	65.0	5.7
Met	49.7	3.9	27.4	3.1	33.0	2.9
Phe	48.3	3.8	37.3	4.1	40.4	3.5
Pro	58.5	4.6	45.6	5.0	50.3	4.4
Ser	106.3	8.4	66.4	7.4	80.0	7.0
Thr	86.3	6.8	71.8	7.8	117.7	10.2
Tyr	31.3	2.5	20.3	2.2	35.8	3.1
Val	94.6	7.5	82.8	9.2	85.7	7.5
Total	1261.2	100.0	903.9	100.0	1149.9	100.0

greater than in sham-operated animals. On the basis of amino acid acceptance (Table 1) and electrophoretic profile (results not shown), tRNA preparations from regenerating liver were closer to those of normal than to sham-operated liver, since the latter displayed significantly lower acceptor capacity and contained proportionally more fast-migrating material.

The data available on acceptor composition of the tRNA pools in regenerating and control (either sham-operated or normal) livers are inadequate, because only low extents of charging of tRNA *in vitro* were reported (Agarwal *et al.*, 1970) or because only percentage acceptor composition was given (Tidwell *et al.*, 1972). Quantities of individual tRNA families in fully (or nearly so) charged liver tRNA and percentage acceptor composition of the tRNA pools of regenerating and control (both sham-operated and normal) livers are summarized in Table 2. Variability between individual animals (Table 1)

was eliminated by using pools of tRNAs from three animals each for aminoacylation. The reproducibility of acylation data within the same batch of tRNA was good, as specified in the Materials and methods section. Furthermore, data in Table 2 for tRNA from normal liver are very close to average values for six other preparations analysed previously (results not shown). From all these data, we conclude that almost all tRNA acceptor families were involved in the decrease of the acceptor capacity of tRNA from sham-operated rats and that the percentage acceptor composition of the tRNA pool from regenerating liver does not undergo dramatic changes, although tRNA^{His} and tRNA^{Thr} were found in somewhat higher amounts in tRNA from regenerating liver than in any normal liver tRNA tested so far. As the result of all the above changes, the amount of active tRNA molecules in the tissue, also called 'total acceptor capacity' (Table 1), tends to be lower after sham-operation, whereas during

Table 3. Activity of aminoacyl-tRNA synthetases from normal and regenerating rat liver 48 h after partial hepatectomy

Data for each experiment refer to the purified cell sap from two pooled livers. Results are means $\pm$ S.E.M. for six experiments with normal liver and five experiments with regenerating liver, and are expressed in enzyme units. Significance of differences was evaluated with Student's *t* test: ^a*P* < 0.1; ^b*P* < 0.02; ^c*P* < 0.01. For other experimental details, see the Materials and methods section.

Aminoacyl-tRNA	Aminoacyl-tRNA synthetase activity (pmol/min per mg of protein)		Activity ratio (R/N)
	Normal (N)	Regenerating (R)	
Ala	117 $\pm$ 16	155 $\pm$ 27	1.32
Arg	828 $\pm$ 115	867 $\pm$ 113	1.05
Asn	105 $\pm$ 13	180 $\pm$ 24 ^b	1.71
Asp	15 $\pm$ 2	14 $\pm$ 3	0.93
Gln	155 $\pm$ 24	241 $\pm$ 42 ^a	1.55
Glu	87 $\pm$ 16	105 $\pm$ 21	1.20
Gly	121 $\pm$ 42	312 $\pm$ 95 ^a	2.58
His	338 $\pm$ 111	561 $\pm$ 237	1.66
Ile	108 $\pm$ 32	283 $\pm$ 43 ^c	2.62
Leu	163 $\pm$ 39	270 $\pm$ 83	1.66
Lys	823 $\pm$ 137	947 $\pm$ 135	1.15
Met	125 $\pm$ 49	129 $\pm$ 73	1.03
Phe	76 $\pm$ 10	75 $\pm$ 4	0.99
Pro	197 $\pm$ 19	251 $\pm$ 47	1.27
Thr	442 $\pm$ 23	497 $\pm$ 60	1.12
Tyr	266 $\pm$ 26	397 $\pm$ 47 ^c	1.49
Val	106 $\pm$ 9	126 $\pm$ 23	1.19

Table 4. Activity of aminoacyl-tRNA synthetases from rat liver 12 h after partial hepatectomy or sham-operation

All rats were operated at 09:00 h and killed at 21:00 h. Data for each experiment refer to the purified cell sap from two or three pooled livers. Results are means $\pm$ S.E.M. for four independent experiments in each group and are expressed in enzyme units. Significance of differences was evaluated with Student's *t* test: ^a*P* < 0.05. Other details are as for Table 3.

Aminoacyl-tRNA	Aminoacyl-tRNA synthetase activity (pmol/min per mg of protein)		Activity ratio (R/S)
	Sham-operated (S)	Regenerating (R)	
Asp	28 $\pm$ 3	47 $\pm$ 7 ^a	1.67
His	317 $\pm$ 74	633 $\pm$ 95 ^a	1.99
Lys	484 $\pm$ 81	698 $\pm$ 77	1.44
Met	111 $\pm$ 33	153 $\pm$ 16	1.37
Phe	68 $\pm$ 10	95 $\pm$ 11	1.39
Pro	257 $\pm$ 2	265 $\pm$ 39	1.03

regeneration it is significantly higher than normal, owing to a general, possibly not uniform, increase in the quantities of the various tRNA acceptor families.

Table 3 shows that 48 h after partial hepatectomy there is a tendency of most, but not all, aminoacyl-tRNA synthetases toward increased activity, already apparent 12 h after partial hepatectomy (Table 4), in which experiments sham-operated rats were used as controls to take into account the short-term effects of surgery.

The results on activity and distribution profiles of free and complexed forms of prolyl-, lysyl- and aspartyl-tRNA synthetase studied by sedimentation analysis of cell sap in sucrose density gradients are summarized in Table 5, which also contains information on the variability in different groups of animals. Regenerating liver, when compared with both normal and sham-operated controls, is characterized by an increase in the high-*M_r* form of

prolyl-tRNA synthetase, which co-sediments with lysyl-tRNA synthetase at >18S, but changes in the distribution of aspartyl-tRNA synthetase are only small, if any. At 48 h after surgery the changes in distribution of prolyl-tRNA synthetase activity from regenerating liver are considerable, whether or not phenylmethanesulphonyl fluoride is added to the system. At this time of regeneration the distribution patterns of low- and high-*M_r* forms of prolyl-tRNA synthetase in both regenerating liver and Yoshida ascites hepatoma (which is a model for uncontrolled growth) appear to be comparable. On the other hand, 24 h after partial hepatectomy [that is, while the first wave of cell division is occurring in the periportal areas of the liver remnant (Grisham, 1962; Bucher, 1967)], only a small increase of the high-*M_r* form of prolyl-tRNA synthetase was observed. This is not surprising, however, since sharp biochemical changes in

Table 5. Sucrose-density-gradient distribution of postmicrosomal aminoacyl-tRNA synthetases specific for proline, lysine and aspartic acid from livers of normal, sham-operated and partially hepatectomized rats and from Yoshida ascites hepatoma AH 130

Homogenates from two or three individual livers or hepatomas were pooled for further fractionation. Postmicrosomal supernatants from control and hepatectomized rats were centrifuged on parallel sucrose gradients. Expts. I-IV were carried out at different times with different groups of rats. In Expt. III the reducing agent was GSH and the enzyme activities were assayed by Method A. In all other experiments the reducing agent was dithiothreitol and the enzyme activities were assayed by Method B. For other details see the Materials and methods section. Values for enzyme distribution in the three regions of the gradient are given as percentages of total enzyme units recovered. Average recoveries of prolyl-, lysyl- and aspartyl-tRNA synthetase activities loaded were 53, 66 and 75% respectively. These results include data obtained for other fractionations carried out in 10–35%-sucrose gradients (details not shown). Abbreviation: PMSF, phenylmethanesulphonyl fluoride.

Aminoacyl-tRNA synthetase tested	Expt. No.	Material	PMSF	Activity distribution in the three regions of the gradient (%)			Specific activity of protein loaded (enzyme units/mg)	Activity recovered (% of loaded)
				4–11S	11–18S	> 18S		
Pro	I	Normal liver	—	39.1	31.3	29.6	172	40
		Sham-operated (48 h)	—	32.1	39.4	28.5	329	53
		Regenerating (48 h)	—	26.2	32.3	41.5	372	65
	II	Normal liver	+	40.8	46.0	13.2	234	51
		Sham-operated (48 h)	+	35.9	37.4	26.7	184	41
		Regenerating (48 h)	+	16.2	34.8	49.0	226	48
	III	Normal liver	—	31.4	48.9	19.7	145	35
		Yoshida hepatoma	—	20.4	29.7	49.9	278	47
	IV	Normal liver	+	37.8	43.5	18.7	454	79
		Sham-operated (24 h)	+	36.3	44.8	18.9	682	63
		Regenerating (24 h)	+	35.2	40.1	24.7	707	62
Lys	II	Normal liver	+	3.2	31.5	65.3	342	63
		Sham-operated (48 h)	+	3.2	36.2	60.6	359	42
		Regenerating (48 h)	+	3.6	37.2	59.2	555	60
	III	Normal liver	—	1.3	26.7	72.0	455	100
		Yoshida hepatoma	—	1.1	31.9	67.0	835	100
Asp	II	Normal liver	+	24.1	28.2	47.7	59	98
		Sham-operated (48 h)	+	27.2	33.9	38.9	60	100
		Regenerating (48 h)	+	34.3	15.4	50.3	81	52
	IV	Normal liver	+	33.8	21.2	45.0	100	60
		Sham-operated (24 h)	+	22.8	26.4	50.8	95	88
		Regenerating (24 h)	+	23.7	23.3	53.0	111	79

one or other zone of the lobule could be obscured by differences in response time to hepatectomy (Ord & Stocken, 1984).

DISCUSSION

In regenerating liver, the total acceptor capacity (Table 1) is close to values found in foetal liver 1 day before birth (Capaccioli *et al.*, 1977). The value of 1261 pmol of total amino acids/ A_{280} unit fixed by tRNA from normal liver of 3–4-month-old rats [the extent of aminoacylation of tRNA may decrease with age (Wust & Rosen, 1972; Mays *et al.*, 1979)] compares well with total acceptor capacity of mixed tRNA from other mammalian systems (Katze & Mason, 1973; Hentzen, 1976), although the composition varies because of the functional adaptation of tRNA pools in specialized systems (Hentzen, 1976).

The decrease in acceptor capacity per A_{280} unit of tRNA from sham-operated rats might be due to changes occurring late, since there was no difference in tRNA from that in regenerating liver 12 h after surgery

(Agarwal *et al.*, 1970), and only a few minor changes in base composition were observed after 18, 24 and 30 h (Shaw *et al.*, 1978). Sham-operated rats could have inherently different nuclease activity, or there may be defective subpopulations of tRNAs and/or other small RNAs of unknown source, such as those that contaminate whole-tissue tRNA preparations from aged animals (Mays *et al.*, 1979). Similar changes might also be present in regenerating liver, where they would be masked, however, by the increase in the amount of active tRNA molecules.

The decreased protein synthesis during starvation, in which condition protein degradation markedly prevails over protein synthesis and the liver mass is dramatically decreased, has been attributed, at least in part, to a decreased total acceptor capacity of the liver cell sap (Shenoy & Rogers, 1977). Conversely, in regenerating liver the increased acceptor capacity might play a role in increased protein synthesis reported by Scornik (1974) as well as in decreased protein degradation (Scornik & Botbol, 1976). The significantly higher concentration of tRNA in regenerating than in normal liver was not shown

in previous studies on cell sap (Hultin & Von der Decken, 1958; Cajone *et al.*, 1980). The discrepancy might be due to differences between tRNA extracted from the postmitochondrial fraction (the present study) and RNA in cell sap in both amount and composition (cf. Katze & Mason, 1973), or could be simply attributable to differences in sensitivity of the methods, in that in previous studies on cell sap colorimetric methods were used to estimate RNA.

Altogether, the data for activity of aminoacyl-tRNA synthetases indicate that large amounts of aminoacylated tRNA, favouring a positive protein balance (Allen *et al.*, 1969; Scornik, 1983), are maintained in regenerating liver. The increase in histidyl-tRNA synthetase activity is of special interest, since protein degradation in cultured CHO cells is enhanced when the ratio of charged to uncharged tRNA^{His} is lowered (Scornik, 1983). Tidwell *et al.* (1972) found that, when they killed (at 09:30 h) rats hepatectomized 18–24 h earlier, the activity of histidyl-tRNA synthetase was 38–48% higher than in normals, but when they killed them (still at 09:30 h) 12 h after hepatectomy the activity was normal. However, in rats killed at 21:00 h and compared with sham-operated rats, we found increased activity of histidyl-tRNA synthetase as early as 12 h post-hepatectomy. Previous data for rat liver and hepatomas suggesting that regulation of histidyl-tRNA synthetase activity is independent from that for most aminoacyl-tRNA synthetases (Del Monte, 1974; Del Monte *et al.*, 1975) indicate a special function of histidyl-tRNA synthetase. Maybe this enzyme is involved in regulation of protein breakdown, as suggested by the recognition of tRNA^{His} as a component of the ubiquitin- and ATP-dependent proteolytic system (Ciechanover *et al.*, 1985). In this connection, from combined data on acceptor capacity of tRNA for histidine and on total acceptor capacity of the tissue, it can be inferred that amounts of tRNA^{His} are higher in regenerating than in control livers.

Shifts in percentage distribution of prolyl-tRNA synthetase to higher- M_r complexes in cell sap from regenerating liver and Yoshida hepatoma, compared with the distribution for normal liver, are consistent with data for CHO cells growing exponentially *in vitro*, compared with cells arrested in G₁ (Enger *et al.*, 1978), if we consider that cell proliferation *in vivo* is not synchronous. Also, taking into account the enzyme activities (Table 5), we think that the shift is due to an increase in the amount of prolyl-tRNA synthetase in the large complex or to changes in enzyme activity, depending on enzymic phosphorylation (Damuni *et al.*, 1982), or to both. There might also be more than one complex sedimenting at >18S. In any case, since this shift is observed whether or not phenylmethanesulphonyl fluoride is added to the system, it is not an artefact, but may have a physiological role, as suggested by Enger *et al.* (1978). For instance, it might favour protein synthesis through increased efficiency of an uptake-activation-loading mechanism located at or near to the cell membrane (Kipnis *et al.*, 1961; see also Airhart *et al.*, 1974; Van Venrooij *et al.*, 1974). This interpretation takes into account the more efficient labelling of newly synthesized proteins in regenerating liver (Scornik, 1974) and is in keeping with models, now receiving experimental support, of the function of the aminoacyl-tRNA synthetase complexes in utilization of extracellular amino acids (reviewed by Deutscher, 1984; Hampel *et al.*, 1984).

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