HUMAN GENE EXPRESSION I: AN AMINOACYL-RNA BINDING SYSTEM FROM HUMAN PLACENTA

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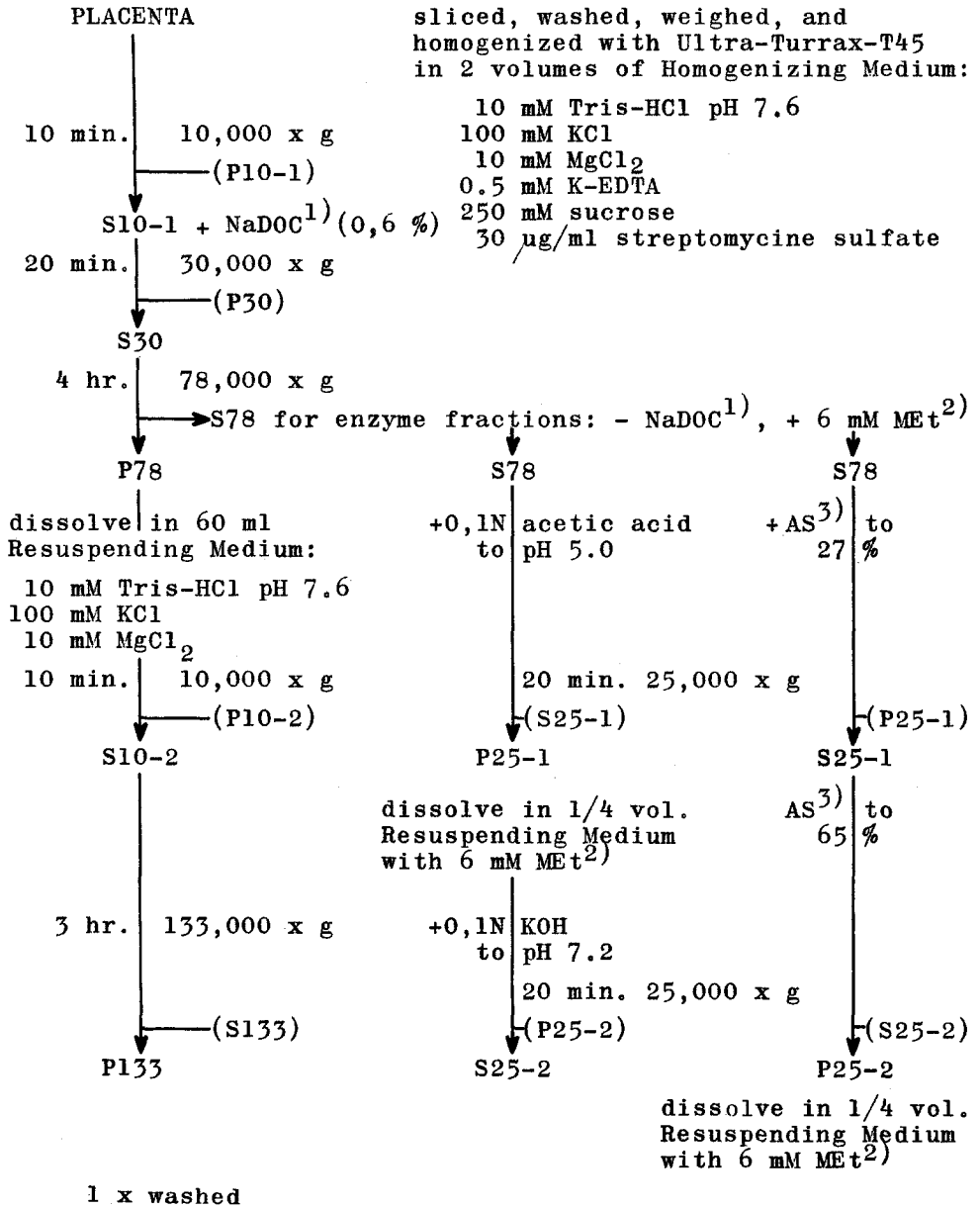
Cell-free systems for the study of protein synthesis can be prepared from human placenta. The preparation of ribosomes, and of pH5- and ammonium sulfate-fractions containing the amino acyl-RNA-synthetases are outlined in table 1. Transfer-RNA (tRNA) is isolated by the method of DELIHAS and STAEHELIN (1966). 45 percent charging with the 20 amino acids is achieved as described in table 2. A first study establishes the conditions for the binding of amino acyl-RNA to human ribosomes specified by polynucleotides of the type XpYpZ...pZ (Schoech,G.K.,and Matthaei,J.H.,1967). As reported in table 3, the system (see legend) works with ribosomes from either human placenta or E.coli. In an attempt to investigate the genetic code in this system, at least 27 codons are found to have the same meaning as in E.coli (Brimacombe et al., 1965; Söll et al., 1965; Matthaei et al., 1965; 1967). Table 4 includes 13 additional codon translations put in parentheses. These are in agreement, too, but need further confirmation. A second communication will present optimized conditions for polypeptide synthetizing systems based on the preparations described here (Abadom et al., 1967).

Ribosomes prepared from human placenta according to table 1 are

TABLE 1: PREPARATION OF HUMAN RIBOSOMES AND ENZYME FRACTIONS



1) Na-deoxycholate 2) ß-mercaptoethanol 3) ammonium sulfate

of similar activity as those from E.coli A19, i.e. different preparations bind 10 to 25 µµmoles of phenylalanyl-RNA(phe-RNA)

TABLE 2: CHARGING OF HUMAN tRNA (%)

Ala	(^{3}H/5000)	2,7	Leu	(^{14}C/150)	1,70
Arg	(^{14}C/155)	3,3	Lys	(^{3}H/3000)	1,50
AsN	(^{14}C/102)	2,0	Met	(^{3}H/1360)	1,50
Asp	(^{14}C/106)	3,2	Phe	(^{3}H/3000)	0,65
Cys	(^{14}C/ 96)	1,3	Pro	(^{3}H/2000)	1,00
GlN	(^{14}C/ 32)	1,6	Ser	(^{14}C/ 87)	4,70
Glu	(^{14}C/125)	0,6	Thr	(^{14}C/133)	2,20
Gly	(^{14}C/ 67)	3,3	Try	(^{3}H/ 875)	4,00
His	(^{14}C/240)	2,3	Tyr	(^{3}H/3000)	0,83
Ile	(^{14}C/174)	1,3	Val	(^{14}C/107)	3,10
Total					45 %

Calculated at: 25 A_{260}-units = 1 mg RNA; MW_{tRNA} = 25.000

Legend to table 2: 1 ml-reaction mixtures contained in 50 mM Tris-HCl pH 7.4 - 50 mM KCl - 10 mM $MgCl_2$ - 6 mM MEt - 2 mM ATP - .5 mM CTP - 7 mM creatine phosphate: 20 µg creatine phosphokinase, 50 mµmoles of each of 20 L-amino acids, one of which labeled with isotope and specific activity indicated in parentheses, 80 mµmoles tRNA, and 2 mg AS-fraction protein (see table 1). They were incubated for 30 min. at 37°C and deproteinized as described (Matthaei et al.,1967a).

per 100 µµmoles of ribosomes without special treatment for removal of indigenous mRNA. They are also very active in the synthesis of both polyphenylalanine from phe or phe-RNA and protein from 20 amino acids (ABADOM et al.,1967). A later communication will show advantages of further purification by sedimentation through sucrose.

Whereas the ph5- and ammonium sulfate-enzyme fractions are prepared from a small part of the same placenta, without the addition of deoxycholate and in the presence of ß-mercaptoethanol, the preparation of tRNA is started from another placenta. The method of DELIHAS and STAEHELIN was the only one of those compared, which resulted in tRNA of a fairly satisfactory

TABLE 3: BINDING OF HUMAN AMINOACYL-RNA ON HUMAN AND E.COLI-RIBOSOMES CODED BY POLYNUCLEOTIDES OF THE TYPE XpYpZ...pZ $_{\sim 100}$

$aRNA^{h}$ 1) + $Ribosomes^{h}$		Codons tested	against aRNA labeled in		$aRNA^{h}$ + $Ribosomes^{c}$ 2)
$\frac{\mu\mu Moles\ aRNA\ bound}{100 \mu\mu Moles\ Ribosomes}$ 3) 4)	% Stimulation			% Stimulation	$\frac{\mu\mu Moles\ aRNA\ bound}{100 \mu\mu Moles\ Ribosomes}$ 3) 5)
1,17 1,04	12,5	UAU.. UUU..	Tyr	34,0	0,67 0,50
2,50 2,00	25,0	GCU.. UUU..	Ala	47,3	2,21 1,50
2,18 1,68	30,0	ACU.. UUU..	Thr	119,2	3,07 1,40
0,78 0,65	20,0	GAU.. UUU..	Asp	187,0	0,43 0,15

1) human
2) from E.coli
3) Calculated at 25 A_{260}-units = 1 mg RNA = 40 mμ moles.
4) " " 13.1 A_{260}-units = 1 mg $ribosomes^{h}$ = 250 μμ moles.
5) " " 16 A_{260}-units = 1 mg $ribosomes^{c}$ = 333 μμ moles.

Legend to table 3: 100 μl-reaction mixtures contained in 10 mM Tris-HCl pH 7.6 - 30 mM KCl - 16 mM $MgCl_2$ the following reactants in μμmoles: 20 ribosomes, approximately 60 polynucleotide chains, and 400 tRNA, 45 % charged with 20 amino acids, one of which labeled with isotope and specific activity given in table 2. Samples were incubated for 20 minutes at 37°C, passed through Millipore-filters and counted by scintillation at counting efficiencies of 16 % for ^{3}H and 68 % for ^{14}C.

acceptor activity. This activity rises sharply by the chromatography on Sephadex G200. Table 2 shows the acceptance for the individual amino acids, which is more homogeneous than in the case of tRNA from E.coli, where we succeeded in reaching 99 percent charging with various preparations (Matthaei et al.,1967a). In the binding assay defined by the legend to table 3, ribosomes and aRNA,* prepared as described above, show the specific stimulations expected with polynucleotides of type XpYpZ... $pZ_{\sim 100}$ (see tables 3 and 4). Although the percent stimulations caused

* aminoacyl-RNA

TABLE 4: CONFIRMATION FOR HUMAN TRANSFER-RNA OF CODON-TRANSLATIONS FOUND IN E.COLI
(Brimacombe et al., 1965; Söll et al., 1965; Matthaei et al., 1965; 1966)

1st	2nd NUCLEOTIDE				3rd
	U	C	A	G	
U	Phe h 1080 (2)		Tyr h 20 (36), c 48 (2)	Cys h 9 (2), c 53 (1)	U
	Phe c 38 (1)		Tyr h 18 (4)		C
		Ser h 22 (2)			A
	(Leu h 7 (2))	(Ser c 13 (1))			G+
C		Pro h 15 (2), c 217 (1)	His h 35 (2), c 14 (1)		U
		Pro h 159 (2)			C
			(Gln c 84 (1))	Arg h 27 (2)	A
			(Gln h 29 (1))	Arg h 16 (1), c 24 (1)	G+
A	(Ile h 8 (2))	Thr h 46 (4), c 113 (1)		Ser c 33 (1)	U
	Ile h 16 (2)	Thr h 18 (2)		(Ser c 23 (1))	C
	Ile h 27 (1), c 16 (1)		Lys h 239 (2), c 35 (1)		A
	(Met h 8 (1), c 7 (1))		(Lys h 6 (2), c 7 (1))	Arg c 33 (3)	G+
G	Val c 90 (1)	Ala h 16 (4)	Asp h 54 (5), c 395 (1)		U
	(Val c 14 (1))	Ala c 47 (2)	Asp c 108 (1)		C
	Val h 30 (2), c 24 (1)	(Ala h 7 (2))	Glu h 17 (1), c 50 (1)	Gly++ c 49 (1)	A
	(Val c 14 (1))	(Ala h 6 (2))	(Glu h 25 (1))	Gly++ h 115 (1), c 35 (2)	G+

Legend to table 4: Average stimulations by 5'-terminal triplets of polynucleotides XpYpZ...pZ$_{\sim 100}$ expressed in percent over the homopolymer-blank. Number of experiments in parentheses. h (c) tested with human (E.coli-) ribosomes.
+ G in third and subsequent nucleotides replaced by inosinic acid (I). ++ primer GpG replaced by IpI.

by these long chains are considerably smaller with human rather than E.coli ribosomes (table 3), repeated experiments confirm the 40 triplet-translations given in table 4. Data put in parentheses needs further confirmation. So far, no differences from the code operating in E.coli have been found. There is also a quantitative correlation in binding efficiency of the codons: Those triplets not yet translated in the human system

are relatively poorly active in the system from E.coli, too. The smaller activity of the 5'-terminal triplets in stimulating human ribosomes may be due to a more completely statistical binding over the entire polynucleotide chain. By using shorter polymers of the same type, we have recently been able to increase the percent stimulations for the homologous human system by a factor of 10. The techniques for direct investigations of the genetic code in man thus appear to be established.

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