Age-related changes in different steps of protein synthesis of liver and kidney of rats

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Protein synthesis in cell-free systems of rat liver and kidney decreases markedly with age. Examination of activity changes of the different steps revealed for both types of organs that reduced binding of aminoacyl-tRNA to ribosomes and reduced peptidyl transfer might be of major importance for the decrease in overall protein synthesis whereas ageing has only little effect on translocation as well as on initiation and termination.

Ageing *Protein biosynthesis* *Elongation* *Elongation factor 1* *Peptidyl transferase*

1. INTRODUCTION

Protein synthesis has been shown to change with increasing age in a wide variety of eukaryotes (review [1]). A marked decrease of protein synthesis in cell-free systems from liver of ageing rats is not due to changes with age in total RNA synthesis [2], in content of mRNA [3], in ribonuclease or protease activity [2,4,5], in free amino acid pools [6,7] or in the loss of an aminoacyl-tRNA synthetase or a tRNA [8], while a report on kidney excluded change in RNase activity [9]. To clarify the contribution of initiation, elongation and termination to the decrease in overall protein synthesis of liver and kidney we used standard assay procedures and cell-free systems from rats aged 1 and 24–28 months.

2. EXPERIMENTAL

The radiochemicals [^{3}H]puromycin hydrochloride (2 Ci/mmol), [^{3}H]phenylalanine (20 Ci/mmol) and [^{3}H]methionine (10 Ci/mmol) were purchased from Radiochemical Centre (Amersham), ATP, GTP, creatine phosphate and creatine phosphokinase from Calbiochem (Giessen), guanylyl imidodiphosphate (GDPNP) from Boehringer (Mannheim), and ApUpG from PL-Biochemicals (St Goar). Poly(A)$^+$ mRNA from rat liver was prepared as in [10]. Rat liver $tRNA_f^{Met}$ was partially purified according to [11] and aminoacylated using *Escherichia coli* synthetase [12].

Male DA/Han inbred rats, 1 month old and 24–28 months old, were obtained from the Zentralinstitut für Versuchstiere (ZfV) (Hannover). All the rats were maintained in barrier-type animal quarters under regulated temperature (22 ± 1°C), relative humidity (55 ± 5%), air change (20 times/h), light intensity (about 300 lux) and light:dark sequence (light schedule 12 h light and 12 h dark). All animals were fed an autoclaved commercial cereal-based diet supplemented with vitamins and minerals (Han:MR3, Eggersmann, Rinteln) ad libitum. Acidified (pH 2.5) and pasteurized tap water was always available. All organs used in this study are macroscopically free of abnormalities, while pathological analysis of other parts revealed alterations: general occurrence of hypophysis adenoma, occurrence of bladder carcinoma and prostatitis; their occurrence generally increases with age.

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Ribosomes, ribosomal 40 S and 60 S subparticles and an extract containing all of the translational factors required for protein synthesis from rat liver and kidney were prepared as in [9,13]. The optimized complete system for protein synthesis contained 40 mM Tris–HCl (pH 7.3), 5.5 mM $MgCl_2$, 60 mM KCl, 3 mM dithioerythritol, 5 mM ATP, 1.5 mM GTP, 12 mM creatine phosphate, 0.1 mg creatine phosphokinase, 2 μg liver poly(A)$^+$ mRNA, 2 μM [^{3}H]phenylalanine, a mixture of the 19 other amino acids at 25 μM each or a ^{14}C-labeled amino acid mix (New England Nuclear, av. spec. act. 265 mCi/mmol) supplemented with asparagine, cysteine, glutamine, methionine and tryptophan and 1 mg extract with equivalent amounts of ribosomes in the case of liver and the test mix [9] in the case of kidney, but using 40 mM Hepes buffer (pH 7.2), 6.5 mM $MgCl_2$ and 100 mM KCl. This slightly increases the efficiency of amino acid incorporation compared to [9]. Incubations in 0.1 ml total vol. were at 28°C for 30 min unless stated otherwise, and samples for determination of amino acid incorporation were processed as in [9]. Initiation of translation was assayed by measurement of the binding of liver [^{3}H]Met-tRNA$_f^{Met}$ to 40 S and/or 60 S ribosomal subunits (30 pmol each) in the presence of ApUpG (0.3 A_{260} unit) as in [14]. There was no measurable binding to the 60 S subunit. Poly(U)-dependent binding of [^{3}H]Phe-tRNA^{Phe} (12 pmol) to ribosomes (1.4 A_{260} units) in the presence of 0.1 mM GDPNP is determined by nitrocellulose filter assay [15]. Assay for peptidyl transferase activity was performed by measurement of formation of peptidyl-[^{3}H]puromycin from nascent ribosomes and [^{3}H]puromycin [16]. The EF2-dependent translocation was determined by poly(U)-dependent [^{3}H]phenylalanine incorporation in the presence of saturating amounts of purified rat liver EF1 from young animals according to [17]. The peptide chain termination was monitored by release of [^{3}H]formylmethionine from [^{3}H]f-Met-tRNA$_f^{Met}$ ribosomes upon addition of poly(UAG) and GTP [18].

3. RESULTS AND DISCUSSION

The changes of the activities in the different steps of protein synthesis do not follow a general scheme of decrease with ageing (table 1). Protein synthesis of cell-free systems from liver and kidney of 24–28 month old rats decreased to a level of

Table 1

Age-related changes in protein synthesis in cell-free systems of rats

Protein synthesis step assayed	% Activity of young rat systems	
	Liver	Kidney
Total protein synthesis	44	33
Binding of [^{3}H]f-Met-tRNA$_f^{Met}$ to 40 S subunits	96	94
Binding of [^{3}H]f-Met-tRNA$_f^{Met}$ to 80 S ribosomes	91	90
Binding of aminoacyl-tRNA to ribosmes[a]	69	58
Peptidyl transfer	84	80
Translocation	102	98
Termination	93	92

[a] Using the method of [29] we similarly conclude that there appears to be no clear temporal correlation between decrease of in vitro synthesis and the predominance of some of the multiple forms of EF1

Each value represents the mean of 6 repetitive measurements using organs from 7 different groups of 2–3 animals. The average coefficient of variation for the different assay procedures is 2.3–3.8% for young rats and 5.1–7.7% for old rats

44% and 33% compared to cell-free systems from 1 month old rats (fig.1). Only slight decreases are noted in binding of [^{3}H]f-Met-tRNA$_f^{Met}$ to ribosomes indicative for initiation. The difference between binding to 40 S subunits and the 80 S initiation complex raises evidence that more than one step in the initiation process decreases, although only slightly. A marked decrease is noted for two steps of peptide chain elongation, the EF1-dependent binding of aminoacyl-tRNA to ribosomes and the peptide bond formation, while the EF2-dependent translocation appeared rather unchanged. The termination stage diminished slightly, in extent comparable to the decrease measured for initiation, what can account for the only slightly lower level of polysomes for these age groups (unpublished, [3,19]). The pattern is similar for both organs, in the case even for animals with different diseases involving other organs.

A significant contribution to the overall decrease in protein synthesis can therefore be attributed to two steps in peptide chain elongation. This is in accord to increased transit times for peptide chains on ribosomes from livers of ageing rat [20], supposed implication of change in EF1-activity for protein synthesis of livers from 30 months, but not 24 months old rats [13], of rat kidney after DDT treatment [21], of rat livers during cirrhosis [22] and of cultured Vero cells after serum addition [16]. The data argue against a major contribution of initiation and/or termination to the overall decrease in protein synthesis in ageing, as was suggested as regulatory event for mammalian cells

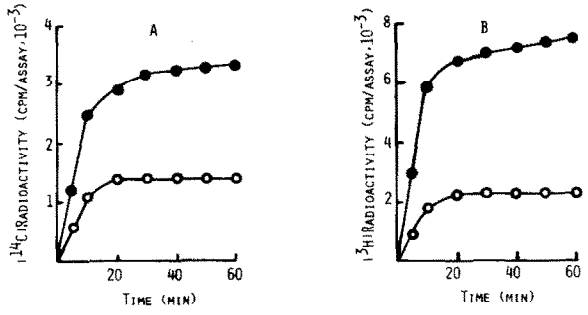

Fig.1. Time course of amino acid incorporation into acid-insoluble material by post-mitochondrial supernatants prepared from livers (A) and kidneys (B) from the young (●) and old (○) rats. Each value represents the mean for the 5 independent measurements on 7 different groups of 2–3 animals.

during mitosis [23] and for resting cells in culture after addition of fresh medium [24,25]. As recently the involvement of decreased binding of aminoacyl-tRNA to ribosomes has been demonstrated to be chiefly responsible for the age-related decline in protein synthesis in *Drosophila melanogaster* [26–28], it appears that deterioration of only certain components of the translational system, namely the EF1-dependent activity and the peptide transfer activity, can be of a more general importance as basis for the wide-spread occurrence of a decreased protein synthesis measured in cell-free systems of senescent multicellular organisms.

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