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The role of elongation factors in protein synthesis rate variation in white teleost muscle

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Summary. Protein synthesis-stimulating activity was assayed in the cytosolic fraction of white muscle from teleost fish (rainbow trout, carp) and of rat liver. In vitro protein synthesis-stimulating activity in the cytosolic fraction is reduced by food deprivation. The addition of elongation factors EF1, EF2, or EF1 + EF2 compensates for the starvation-induced loss of protein synthesis-stimulating activity in trout muscle cytosol. The action of EF2 is stronger than that of EF1 in this respect. However, EF1 enhances in vitro protein synthesis-stimulating activity in rat liver cytosol more than EF2. The EF2 concentration in the cytosolic fraction of white muscle from starved trout is significantly lower than in fed specimens.

Key words: Elongation factors – Translation – Starvation – Teleost muscle – *Cyprinus carpio* – *Oncorhynchus mykiss*

Introduction

Proteins (Somero 1983) and their synthesis (Haschemeyer 1978) play a special role in the adaptation of animals to environmental changes. Since the genetic potential of an organism ultimately determines the frame of metabolic responses to changes in environmental conditions, and since gene expression is based upon the synthesis of proteins, adaptation processes are accompanied by qualitative and quantitative changes in protein synthesis, although changes in protein degradation must also be considered (Sidell 1977; Fauconneau 1985; Millward 1989). The kind of protein synthesized is governed by mRNA, whereas its quantity is controlled by the rate of protein synthesis. The latter is determined mainly by the number and activity of the ribosomes present in the cell (von der Decken 1983) and the translation factor concentration (Moldave 1985).

Protein synthesis takes place in three stages: initiation, elongation and termination. Several studies suggest that elongation factors play a key role in the quantitative regulation of translation in eucaryotic organisms (Moldave 1985; Riis et al. 1990). According to Riis et al. (1990) it is becoming clear that the elongation step of protein synthesis is involved in translation regulation. At least the rate of elongation could be expected to influence the translational capacity of the cell (McCarthy and Gualerzi 1990). In eucaryotes, peptide chain elongation is mediated by elongation factors EF1 and EF2. EF1 is composed of a nucleotide-binding protein, EF1α and a nucleotide exchange protein complex, EF1$\beta\gamma$, while EF2 is a single polypeptide chain catalysing the translocation of peptidyl-tRNA on the ribosome (Moldave 1990; Riis et al. 1990).

Adaptive changes during the elongation step can affect EF1, EF2, or both. Few results relating to fish have been published on this topic. The elongation rate is implicated in an organ-specific manner in temperature adaptation of the rainbow trout. Except for the red muscle, the organs of cold-acclimated trout showed higher specific elongation rates than those of warm-acclimated specimens (Simon 1987). Nielsen et al. (1977) found increased levels of EF1 in the livers of cold-acclimated toad fish in summer and winter and reported that in vitro EF1 levels correlated with the in vivo elongation rate. Although results on the influence of feeding conditions on protein synthesis in fish are comparatively plentiful (Fauconneau 1985), the elongation step has not been studied so far. However, using a heterologous in vitro protein synthesis system, a link was established between food quantity and translation activity in rainbow trout muscle cytosol (Junghahn and Jürss 1988; Junghahn et al. 1989).

The purpose of the experiments described here was to determine whether EFs are implicated in nutritionally induced changes in translational activity in rainbow trout muscle. The study was extended to include another teleost species, *Cyprinus carpio*, and rat liver was used for comparison.

Abbreviations: EF, elongation factor(s); SGR, specific growth rate; TCA, trichloroacetic acid

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Materials and methods

Animals. Wistar rats (150–180 g) were fed commercial pellets ad libitum. Polysomes were prepared from the livers of rats after starvation for 18 h. Cytosol fractions were prepared from the livers of rats that had either been starved for 24 h or fed.

Juvenile carp *(Cyprinus carpio)* with a body weight of 100–200 g were held at 21–23 °C for 4 weeks in December/January and were either starved or received commercial pellets several times a day. The fed fish had a SGR of 2.01, whereas the starved carp lost weight.

Juvenile rainbow trout *(Oncorhynchus mykiss)* weighing 65–140 g were held at 12–15 °C for 4 weeks in December and either starved or fed commercial pellets ad libitum once a day. The mean SGR of the fed trout was 0.85.

All fish used for the experiment were kept in fresh water with a natural photoperiod.

Chemicals. L-[^{3}H]Leucine (1.8 TBq · mmol^{-1}) was obtained from UVVVR (Prague, Czechoslovakia); ATP, GTP, CTP and phosphoenolpyruvate from Boehringer (Mannheim, FRG); unlabeled amino acids from Calbiochem (Lucerne, Switzerland); dithioerythrit from Serva (Heidelberg, FRG); (U-^{14}C) nicotinamide adenine dinucleotide (9.8 · 10^6 Bq) from Amersham (Amersham, UK); and GF/A filters from Whatman (London, UK). Pyruvate kinase was prepared as described by Noll et al. (1966). Diphtheria toxin was kindly provided by Professor H. Franz (Staatliches Institut für Immunpräparate und Nährmedien, Berlin, FRG), and elongation factors 1 and 2 (EF1 and EF2) prepared from rabbit reticulocytes were a gift from E.K. Davidova (Institute of Protein Research, Academy of Sciences of the USSR, Pushchino).

Preparation of the cytosolic fraction of fish muscle. The fish were killed by a blow on the head, and the white epaxial muscle between the operculum and dorsal fin was dissected out on one side. Muscle slices weighing 2–4 g each were immediately frozen in aluminium foil between two solid blocks of CO_2 and stored at −80 °C for not more than 5 days as described by Lund and von der Decken (1980).

Muscle slices weighing 2 g each were cut with scissors and homogenized in 4 volumes of solution containing (mmol · l^{-1}): 50 TRIS–HCl (pH 7.5), 250 sucrose, 6 $MgCl_2$, 6 β-mercaptoethanol and 1 EDTA in a Teflon-glass Potter homogenizer. After centrifugation at 10 000 × *g* and 2 °C for 20 min, the postmitochondrial supernatant was centrifuged at 240 000 × *g* and 2 °C for 120 min to spin down the microsomal fraction.

The top two-thirds of the supernatant were taken as the cytosolic fraction. This was passed through a Sephadex G–10 column equilibrated with a buffer containing (mmol · l^{-1}): 50 TRIS–HCl pH 7.3), 1 EDTA and 6 β-mercaptoethanol and 15% glycerol. The top two-thirds of the eluate were then stored in aliquots in liquid nitrogen. Cytosolic fractions of rat liver were prepared in the same manner.

Preparation of rat liver polysomes. Polysomes were prepared from the liver of adult rats (body weight about 180 g) as described by Junghahn und Scholz (1973) with some modifications. The postmitochondrial supernatant was treated with sodium deoxycholate (1.5%) and Triton X–100 (2%) before centrifugation at 105 000 × *g* through a 20% sucrose cushion for 120 min. The crude polysomal pellet was washed free of translation factors by treatment with a solution containing (mmol · l^{-1}): 500 KCl, 250 sucrose, 50 TRIS–HCl buffer (pH 7.3), 5 β-mercaptoethanol, 10 $MgCl_2$ and 25% glycerol, and collected through a 2 *M* sucrose cushion by centrifugation at 240 000 × *g* and 2 °C for 18 h. The polysomes were stored as pellets at −20 °C for at least 6 months with no loss of activity. Such preparations show no endogenous amino acid incorporation when assayed in the absence of the cytosolic fraction.

Cell-free protein synthesis system. The standard system contained washed polysomes from normal rat liver and the cytosolic fraction as a source of tRNA, aminoacyl-tRNA synthetases and translation factors. The incubation mixture contained the following substances per 100 µl final volume of 10% glycerol (mmol · l^{-1}) 50 TRIS–HCl (pH 7.3), 40 CH_3COOK, 4 $Mg(CH_3COO)_2$, 1 ATP, 0.1 GTP, 0.1 CTP, 3 phosphoenolpyruvate, 2 dithioerythrit, plus 1 µg pyruvate kinase, 2 nmol of each the 20 amino acids except unlabelled leucine, L-[^{3}H]leucine (37 · 10^3 Bq), 10 µg of polysomes and cytosolic protein in the amounts shown in the Figures. After incubation for 30 min at 30 °C (fish) and 37 °C (rat), the reaction was stopped by adding trichloroacetic acid (TCA, 5% final concentration). The hot TCA-precipitable material (90 °C, 15 min) was collected on a GF/A glassfibre filter, washed and counted in an LKB liquid scintillation counter.

The microsomal wash fraction from the microsomal rat liver pellet was prepared as described by Bommer et al. (1987). The microsomal fraction was sedimented by centrifugation of the postmitochondrial supernatant at 10 000 × *g* and 2 °C for 120 min. The microsomal pellet was resuspended in a buffer containing (mmol · l^{-1}): 30 TRIS/HCl (pH 7.6), 50 KCl, 6 $MgCl_2$, 0.3 EDTA, 10 2-mercaptoethanol and 20 sucrose. Subsequently, 4 *M* KCl was added to give a final concentration of 0.5 mol KCl · l^{-1}, and the suspension was stirred at 4 °C for 60 min. The microsomes were pelleted overnight at 105 000 × *g*. The top two-thirds of the supernatant was taken as the microsomal wash fraction, passed through a Sephadex G–10 column equilibrated with a buffer containing (mmol · l^{-1}): 20 TRIS/HCl (pH 7.5) 100 KCl, 0.1 EDTA, 10 2-mercaptoethanol and 10% glycerol. Both the cytosolic and microsomal wash fractions were stored in aliquots in liquid nitrogen.

A modified method described in Van Ness et al. (1978, 1980) was used for ADP-ribosylation of EF2 to determine the amount of EF2 in the cytosolic fraction. Samples (100 µl) containing 100–400 µg cytosolic protein were incubated with 1–5 µg diphtheria toxin and 1 pmol [^{14}C]NAD at 30 °C for 14 min. [^{14}C]ADP-ribosyl EF2 was precipitated with 5% TCA and collected on GF/A filters (Whatman) to count radioactivity.

Protein was determined according to Lowry et al. (1951) or by direct measurement at 210 nm, using bovine albumin as a standard.

All measured data are presented as means ± SD. Differences between means were tested for significance by Student's *t*-test if the variances were equal and by the Welch *t*-test otherwise (Rasch et al. 1973).

Results

Starvation effects on protein synthesis-stimulating activity of the cytosolic fraction

Figure 1 depicts the influence of extreme conditions such as starvation and ad libitum feeding on protein synthesis-stimulating activity in cytosolic fractions. Purified polysomes exhibit protein synthesis activity only in the presence of the cytosolic fraction. Tests with increasing amounts of cytosolic protein showed that cytosol from starved specimens had only about half as much amino acid incorporation-stimulating activity as that from fed animals. The same results were obtained for all specimens tested.

Influence of the microsomal wash fraction from rat liver

The effect of added microsomal wash fraction on amino acid incorporation was measured to investigate the role of translation factors in the differences between protein synthesis in the starved and fed states. We tested the influence of microsomal wash fraction from rat liver

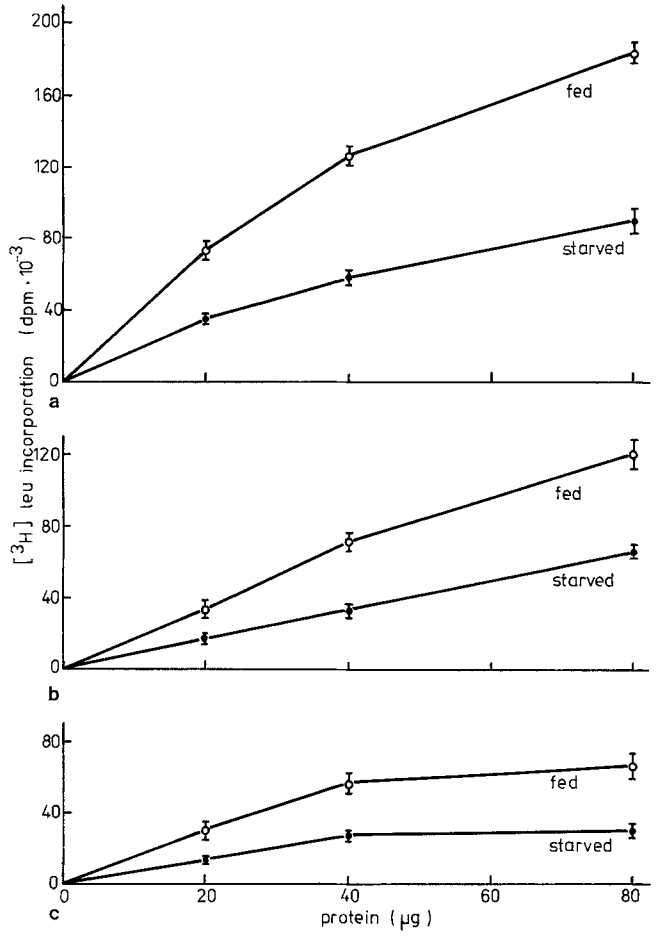

Fig. 1a–c. Stimulation of polysomal protein synthesis by cytosolic fraction from: **a** rat liver ($n=3$); **b** carp muscle ($n=9$); **c** rainbow trout muscle ($n=12$). *Abscissa,* micrograms of protein in cytosolic fraction. Means ±SD

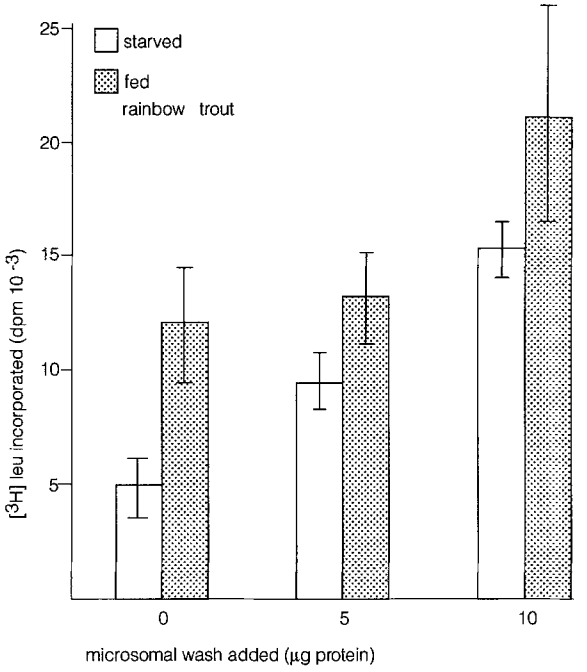

Fig. 2. Effect of addition of microsomal wash fraction on polysomal protein synthesis in the presence of cytosolic fraction (10 µg cytosolic protein) from muscle of starved and fed rainbow trout. *Abscissa,* micrograms of protein in microsomal wash. Means ±SD

containing EF1 and EF2 (although the presence of initiation factors could not be excluded) on polyribosomal protein synthesis in cytosolic fraction from the white muscle of starved and fed rainbow trout. Figure 2 shows that microsomal wash fraction containing the factors essential for protein synthesis, especially EF1 and EF2, stimulates amino acid incorporation in cytosolic fractions from starved and fed rainbow trout. However, the stimulation induced by microsomal wash fraction in experiments with cytosol from starved fish was much higher than in those with cytosol from fed fish. This indicates a partial lack of these factors in cytosol from starved rainbow trout. The addition of only 5 µg microsomal wash protein was sufficient to raise in vitro protein synthesis in muscle cytosol from starved fish to a level similar to that observed in muscle cytosol from fed fish without additional microsomal wash. Therefore, the observations seem to reflect real starvation-induced changes in factor activity.

Effects of elongation factors on in vitro protein synthesis

Purified EF1, EF2 or both were added to the polysomal protein synthesis system (Table 1). The optimal amount of additional EF was ascertained in pilot experiments. As with the microsomal wash fraction, purified EFs have a considerably stronger effect on protein synthesis by muscle cytosol in the cell-free system if the cytosol fraction is obtained from the muscle of starved fish. Nevertheless, tests in the cell-free system always yielded significantly higher values (Table 1) for muscle cytosols from fed rainbow trout than for those from cytosols from starved fish. The difference was smallest when both EFs were added.

Each of the EFs compensated for the starvation-induced loss of in vitro protein synthesis-stimulating activity in trout muscle cytosol. Supplementation of the muscle cytosol from starved fish with both EFs raised protein synthesis activity to levels far beyond that shown by non-supplemented muscle cytosol from fed fish.

Protein synthesis-stimulating activity in fish muscle cytosol is increased by EF1 and EF2 supplementation regardless of the nutritional status of the fish, but the effect of EF2 is greatest. Comparison of the results for fish muscle and rat liver cytosol show that EF2 is unlikely to be a limiting factor for the translation capacity of the latter because EF2 enrichment of rat liver cytosol, unlike EF1 enrichment, had no significant effect on protein synthesis-stimulating activity (Table 1). Since EF2 had the greatest effect on protein synthesis activity in fish muscle cytosol, the levels of cytosol EF2 were measured.

Measurement of EF2 level by ADP ribosylation

Levels of EF2 were lower in both groups of cytosolic fractions prepared from starved and fed trout than in cytosolic fractions from carp muscle or rat liver

Table 1. Stimulation of L-[^{3}H]leucine incorporation (dpm) by addition of elongation factors+

Condition	*n*	Without added EF (control)	+EF–1 (1 µg)	+EF–2 (1 µg)	+EF–1 and EF–2 (1 µg of each)
Cytosolic fraction (10 µg protein) from:					
Trout muscle, starved	12	8796±3246	18041±4370	23210±4218	33361±2267
% of control		100	205	264	379
Trout muscle, fed	12	18950±4463	27075±3682	34792±6390	40541±5321
% of control		100	143	183	214
Carp muscle, fed	3	38831±6123	48761±6900	77821±14000	86271±7071
% of control		100	126	200	222
Rat liver, fed	3	52811±6379	84160±11629	63839±4971	127133±6419
% of control		100	159	121	240

+ *P* values in *t*-test:

Trout muscle, starved	I–II, III, IV	0.001
	II–III	0.01
	II–IV	0.001
	III–IV	0.001
Trout muscle, fed	I–II, III IV	0.001
	II–III	0.01
	II–IV	0.001
Trout muscle	I_{fed}–$I_{starved}$	0.001
	II_{fed}–$II_{starved}$	0.001
	III_{fed}–$III_{starved}$	0.001
	IV_{fed}–$IV_{starved}$	0.001
Carp muscle, fed	I–III	0.02
	I–IV	0.001
	II–III	0.05
	II–IV	0.01
Rat liver, fed	I–II	0.02
	I–IV	0.001
	II–IV	0.01
	III–IV	0.001

(Table 2). Protein synthesis-stimulating activity was also higher in rat liver fractions (Fig. 1). The cytosolic fraction from starved fish, which stimulates protein synthesis less than that from fed fish, also contains significantly less EF2 ($P<0.001$).

Discussion

Food deprivation decreases protein synthesis in animals in a tissue-specific manner (Millward 1989). In fish muscle, protein synthesis response to changes in nutritional status is particularly marked (Smith 1981; Loughna and Goldspink 1984). Food deficits lead to a reduction in both the quantity and activity of ribosomes (Millward 1989). The activity of ribosomes from starved cod, saithe and rainbow trout muscle is depressed in the in vitro protein synthesis system (Lied et al. 1982, 1983; Rosenlund et al. 1984; Rosenlund and Lied 1986). The quantity of monoribosomes in starved fish was high in proportion to polyribosomes (Lied et al. 1982, 1983). For the assay in this study factor-free polysomes (polyribosomes) were used as a constant source of ribosomes and mRNA.

The phenomena reported here thus reflect nutrition-dependent changes in cytosolic factor activity for stimulating polysomal protein synthesis. The results (Fig. 1a–c) show that factors in the cytosolic fraction are implicated in the starvation-induced reduction in protein synthesis. This was not previously known for carp muscle, whereas comparable results have been reported for rainbow trout muscle (Junghahn and Jürss 1988; Junghahn et al. 1989).

Comparison of the results for cytosolic fractions from carp and rainbow trout muscle is striking. The results for rat liver cytosol cannot be included in the comparison, because this assay was performed at 37 °C and the system was homologous (polysomes and cytosol from rat liver). The protein synthesis-stimulating activity of carp muscle cytosol is considerably greater than that of trout muscle cytosol. This is not surprising in the case of fed fish, because carp grew much faster than trout owing to their larger food intake and the higher temperature. This difference in growth was observed earlier by Huisman (1976) in feeding experiments. Since a similar difference exists between the two species under starvation conditions, it must be assumed that protein turnover in carp muscle at 21–23 °C is greater than in trout muscle at 12–15 °C.

The cytosols used and studied in these tests served as a source of aminoacyl-tRNA synthetases, tRNAs, initiation factors and EFs. According to results reported by Simon (1987), protein synthesis in rainbow trout is not

Table 2. Content of elongation factor 2 (EF2) in cytosolic fraction+

	n	EF2 (µg per mg protein of cytosolic fraction)
Cytosolic fraction from:		
Trout muscle, starved	12	0.9±0.14
Trout muscle, fed	12	1.3±0.10
Carp muscle, fed	3	1.8±0.40
Rat liver, fed	3	6.3±0.24

+ Result of *t*-test: Trout muscle (fed compared with starved), $P<0.001$

limited by the acylation rate, so that initiation and elongation factors must be considered the most likely causes of the starvation-induced loss of translation capacity. This is supported by the experiments with the microsomal wash fraction. However, the results do not permit final assessment of the role of the elongation step since the wash fraction also contains initiation factors (Bommer et al. 1987; Ryazanov et al. 1987).

Studies with cytosolic fraction from regenerating rat liver showed that initiation factor 2 is essential for changes in cytosolic activity in the assay. The met-tRNA binding activity of the cytosolic fraction changes during liver regeneration analogously to the protein synthesis-stimulating activity in the polysomal assay (Bommer et al. 1987). Supplementation with EF (Table 1) shows that the elongation step is one of the factors limiting protein synthesis in rat liver and fish muscle. Various authors have shown that EF1 is implicated in adaptive protein synthesis changes in rat liver (Roels-De Schrijver 1985) and fish (Nielsen et al. 1977). EF2 seems to play a special role in protein synthesis regulation in fish muscle. The addition of this factor increases polysomal protein synthesis in fish cytosolic fraction by 183–264%, whereas the effect of EF1 stimulation is somewhat smaller. In contrast, the addition of EF2 had no effect in experiments with rat liver cytosol, whereas EF1 addition increased amino acid incorporation to about 159%. Currently, there is no explanation for the surprisingly strong additive effect of these two factors in the polysomal test with fish muscle cytosol, and it is not clear why each of these factors on its own is able to compensate for the decreased protein synthesis-stimulating activity of muscle cytosol from starved trout.

The reduced EF2 level detected by ADP ribolysation in the muscle cytosol of starved rainbow trout seems to be partly responsible for the decreased protein synthesis activity of this cytosol. However, the following facts must also be taken into account. It is known that EF2 is a specific target of diphtheria toxin (Honjo et al. 1968). The A fragment of the toxin ADP ribosylates EF2, using NAD as an ADP-ribose donor. The unique ADP-ribose attachment site in EF2 is diphthamid, which is a result of post-translational modification of histidin. Diphthamid thus seems to be a site of regulatory EF2 modification. However, this method cannot be used to distinguish between active and inactive EF2. Ryazanov (1987) and Sitikov et al. (1988) found that EF2 can be phosphorylated in animal cells and that EF2 dephosphorylation correlates with protein synthesis activity. In other words, phosphorylated EF2 is significantly less active than dephosphorylated EF2.

In conclusion, the elongation step plays an important role during changes in adaptive translation activity in fish muscle. In contrast to rat liver, in fish muscle EF2 seems to be the more important EF. There is no evidence from the available data that initiation factors such as IF2 play a major role in translation capacity changes in fish muscle.

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