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Protein synthesis and environmental changes: a new approach to search for regulatory mechanisms*

Síntesis de proteínas y cambios ambientales: una nueva forma de buscar mecanismos regulatorios

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Aquatic ectothermal animals have evolved homeostatic responses to changes in environmental temperature that occur seasonally, but the biochemical processes involved in this changes are not clear (1-3). Studies of incorporation of radioactive amino acids into protein in fishes have suggested a possible role of the protein synthetic machinery in the acclimation process (4-7). Biosynthesis of proteins in fishes has been studied by isolating and characterizing several of its components (8-11) and by detecting the changes that this process may undergo upon acclimation and acclimatization, and their controlling sites (7, 12, 13). In the liver of the fish *Opsanus tau,* elongation factor 1 (EF-1) is probably the major controllig element (12, 13).

In order to proceed with our studies on fish liver protein synthesis, we have chosen the carp *Cyprinus carpio,* because during different seasons, its habitat undergoes significant changes in temperature. Several studies concerning the biology of this fish have been performed. This knowledge has been helpful to design our experimental approach.

A detailed report about the isolation of tRNA and of a cell free system from the liver of *C. carpio* adapted to summer temperatures and which is active for proteins synthesis is being presented elsewhere (14, 15). Polysomes of the summer adapted fishes (habitat temperature 18°C) (16), isolated by differential centrifugation and sucrose discontinue gradient (17) were subjected to sucrose gradient analysis (18). A typical polysomal pattern was obtained (Fig. 1). Upon ribonuclease treatment, the UV-absorbancy shifted toward the monomer region. The polysomal nature of this preparation was confirmed also by electron microscopy (14). When assayed this polysomes for amino acid incorporation using carp liver tRNA and cell sap as source of soluble factors and aminoacyl tRNA synthetase, optimal polypeptide synthesis was detected (Fig. 2).

Winter adapted carps showed a different behaviour. By applying exactly the same procedure used to obtain structuraly and functionally summer carp liver polysomes, to the liver of winter (habitat temperature 9-10°) adapted

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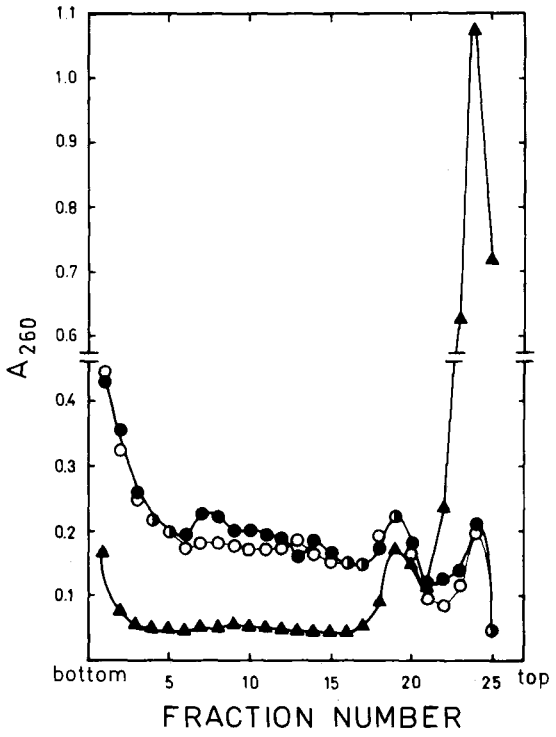
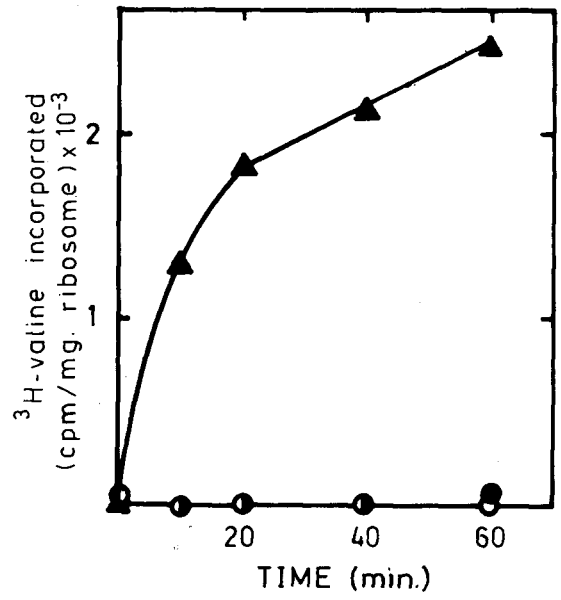


Fig. 1. Sucrose gradient analysis of summer adapted carp liver polysomes. ●——● 0.7 mg of carp liver polysomes; ○——○ 0.7 mg of carp liver polysomes previously incubated at 30° for 30 min and, ▲——▲ 0.7 mg of carp liver polysomes previously incubated with 17 μg of ribonuclease for 30 min at 30°.



carps, the results obtained were quite different. As shown in Fig. 3, the sedimentation pattern obtained by sucrose gradient analysis, shows a high absorbancy peak in the heavy region of the gradient. Treatment with ribonuclease did not change the absorbancy profile. In order to check whether our winter preparations consisted of some special type of polysomes or they represented other kind material, amino acid incorporation assays and electron microscopy analysis were carried out.

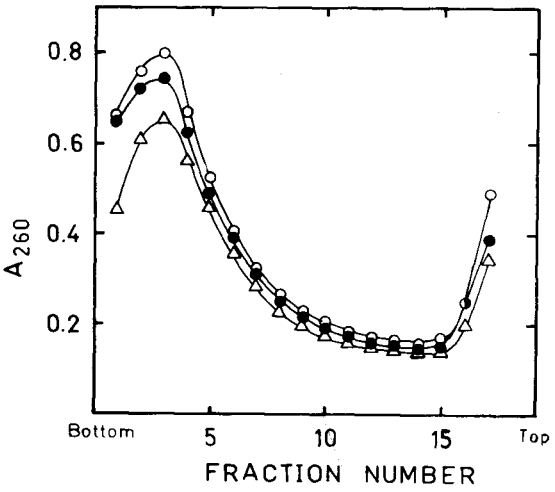


Fig. 3. Sucrose gradient analysis of the product obtained in the attempts to isolate polysomes from winter adapted carp livers. ○——○ untreated material. Treatment with ribonuclease at 30° before the gradient analysis: ●——● 15 min, and △--△ 30 min.

In preparations presenting sedimentation patterns as shown in Fig. 3, none or low amino acid incorporation in polypeptide chains was obtained. The electron microscopical analysis strongly suggested that this preparation mostly consisted of glycogen particles (Fig.

Fig. 2. Amino acid incorporating activity of a cell free system isolated from summer adapted carps. Assay mixtures consisted of 50 mM Tris-HCl buffer pH 7.6; 110 mM KCl; 6 mM MgCl; 2 mM ATP; 1 mM GTP; 8 mM phosphocreatine; creatine phosphokinase (0.04 mg/ml); 0.01 mM labeled amino acid; carp liver tRNA (2.5 A_{260} units/ml); carp liver soluble factors (2 mg/ml) and polysomes (0.88 mg/ml). Temperature of incubation was 18° (14, 15).
▲——▲ with complete system; ●——● without polysomes, and ●——● without soluble factors.

8). Other methods used to isolate polysomes from winter carp livers were performed with similar results. Occasionally, in some winter carps, a small basal polysomal sedimentation pattern was detected by sucrose gradient analysis. Upon ribonuclease treatment, a very small absorbancy peak towards the monomer region appeared.

It has been described that carp liver glycogen content reaches values up to 15-20 mg per 100 mg of wet tissue (19). This is three times higher than the maximum values found in the liver of fed rats (19). The ultrastructural study of the winter carps livers showed that the hepatocytes were loaded with glycogen particles (Fig. 5). These particles were unevenly distributed in the cytoplasm and most frequently they appeared as large patches (Fig. 7). In the cytoplasmic regions, where the glycogen particles were densely packed, no organelles were found. However, after staining the sections with aquous uranyl acetate, particles resembling free ribosomes and small polysomes were observed within the glycogen patch (Fig. 7). The perinuclear localization of the rough endoplasmic reticulum was a consistent finding in the hepatocytes of the winter adapted fishes (Figs. 5 and 7).

A decrease in the liver glycogen content after the administration of insulin to carps has been reported (19, 20).

In order to investigate whether this particular arrangement of the glycogen particles in the hepatocytes of the winter carp livers could be the cause of the difficulties in obtaining active polysomes, several experiments were performed. A single dose of insulin (2,5 i.u./100 g body weight) (19) was administered to several cold acclimatized carps. In a second group of animals the same dose of insulin was repeated three times, every other day. Fig. 4 shows a sucrose gradient sedimentation pattern from a typical polysomes preparation obtained from a 3 dose-insulin treated carp. The absorbancy profile is clearly different from the winter untreated carps (Fig. 3), indicating the presence of a large population of short polysomes, which are degraded after ribonuclease digestion. Fig. 4 shows the shift of the absorbancy to the monomer region upon ribonuclease treatment. When the liver from insulin treated carps were

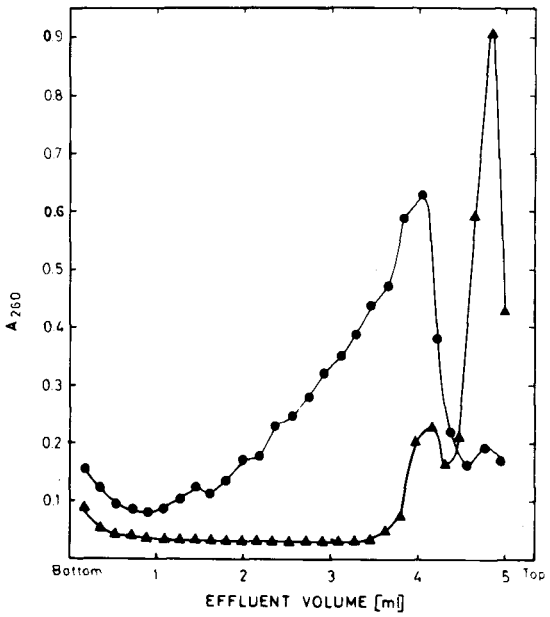


Fig. 4. Sucrose gradient analysis of polysomes from cold adapted carp livers, treated with insuline. ●——● untreated polysomes, ▲——▲ polysomes previously incubated with ribonuclease (30° 30 min).

tested in a cell free protein synthesis assay system with tRNA and cell sap from the same experimental animals, significant amino acid incorporation was clearly detected (21). The study of this preparations under the electron microscopy confirmed the presence of polysomes (Fig. 9). Concurrently, a decrease of the glycogen particles content in the cytoplasm of the insulin treated animals was observed (Fig. 6). Moreover, the hepatocyte cytoarchitecture was radically changed. The rough endoplasmic reticulum was now distributed throughout the cytoplasm and the lumen of both, the rough endoplasmic reticulum cisternae and the nuclear cisternae appeared dilated (Fig. 6).

Our results suggest that glycogen accumulation (during winter temperatures?) may trapp the free ribosomes, thus controlling their availability and therefore, the protein synthesis process. Ribosomes are known to be involved in the mechanisms that control protein synthesis during temperature adaptation. It has been reported that the assembly and disassembly of certain typical ribosomal agregates are

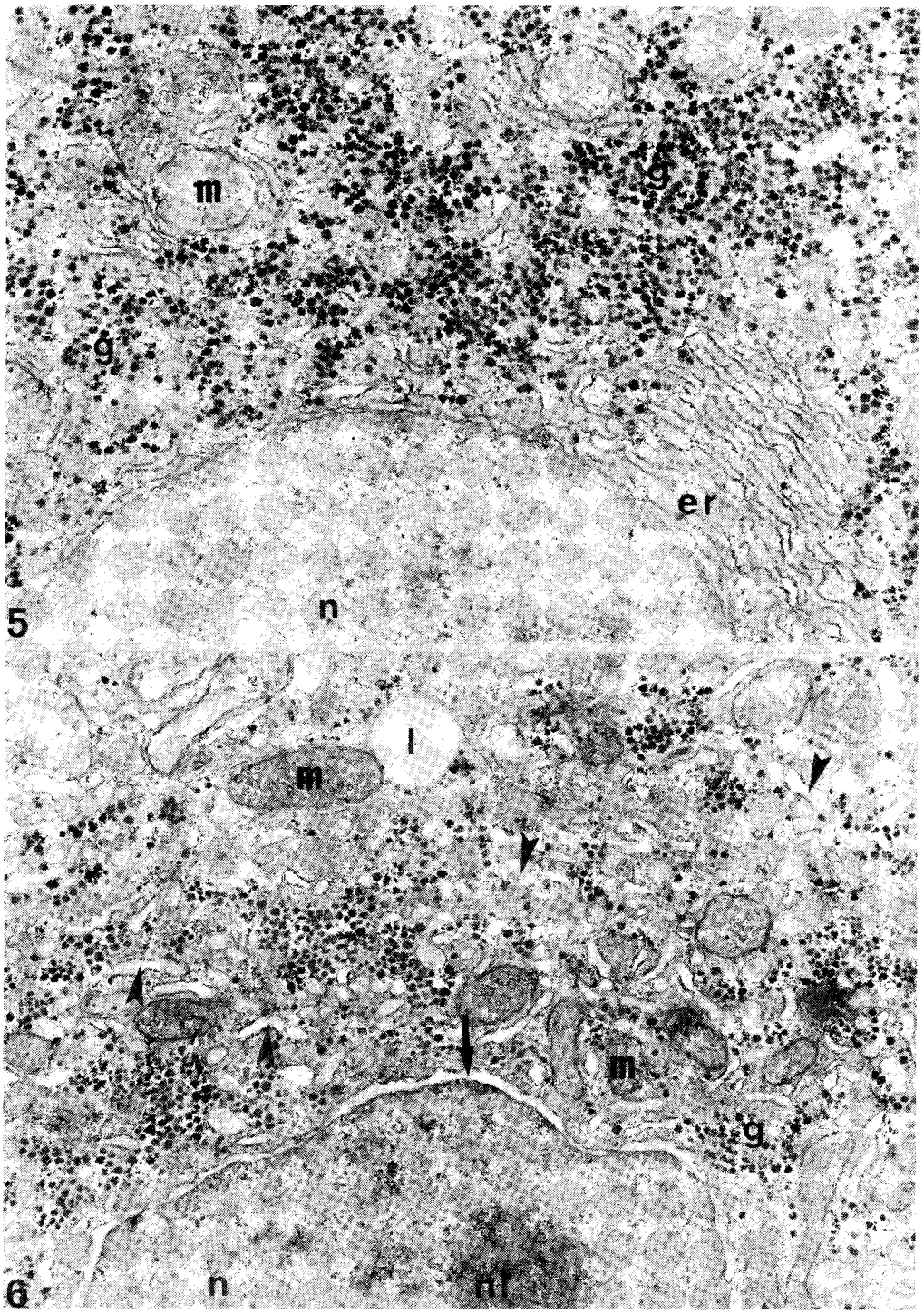
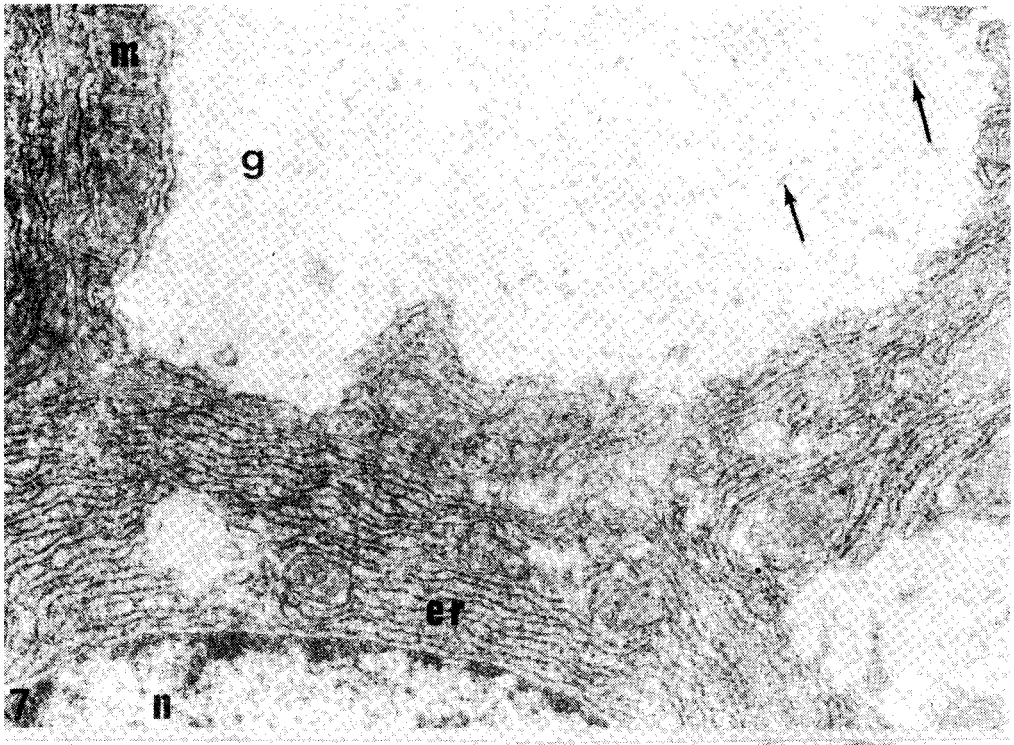


Fig. 5. Hepatocyte from a winter adapted carp showing the large content of glycogen particles (g). n nucleus; m mitochondria; er rough endoplasmic reticulum. Alcoholic uranyl acetate and lead cytrate stain.

Fig. 6. Hepatocyte from a winter adapted carp treated with three doses of insulin. The short arrows point to the dilated cisternae of the rough endoplasmic reticulum, distributed throughout the cytoplasm. The large arrow indicates the dilated nuclear cisternae. l lipid droplet; m mitochondria; n nucleus; nl nucleolus; g glycogen particles. Staining as in Figure 5.



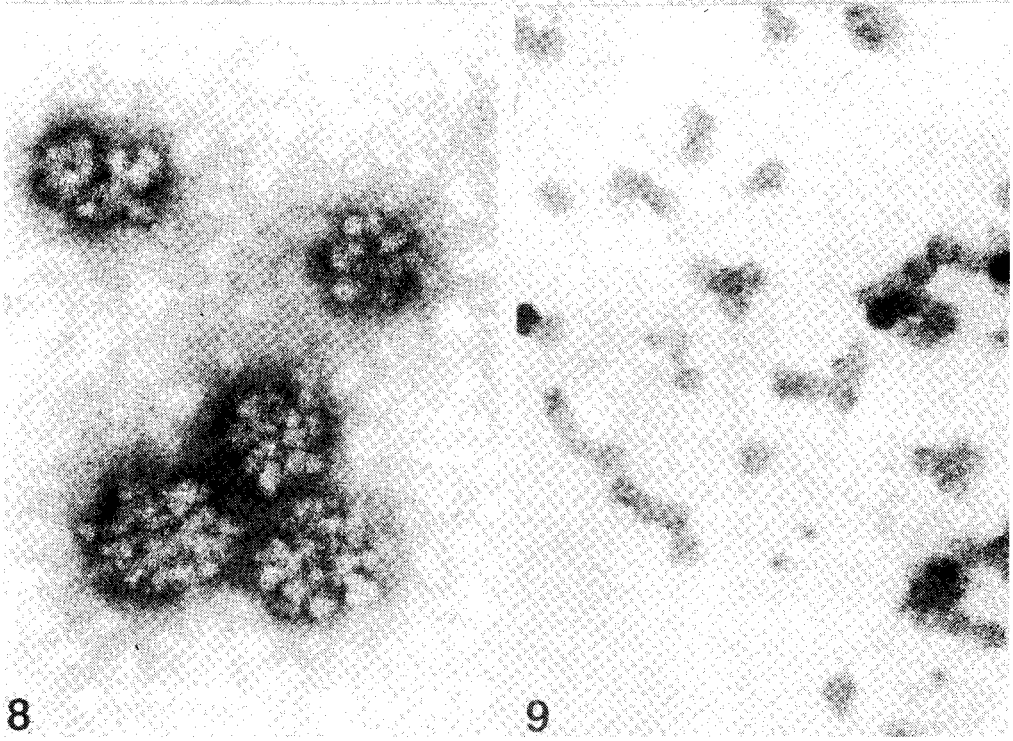


Fig. 7. Hepatocyte from a winter adapted carp showing that glycogen particles (g) and the rough endoplasmic reticulum (er) are circumscribed to two well defined compartments. Glycogen particles appear unstained, thus allowing the visualization of ribosome-like particles within the "glycogen compartment" (arrows). m mitochondria; n nucleus. Aquous uranyl stain.

Fig. 8. Negative staining of isolated glycogen particles from a winter adapted carp.

Fig. 9. Positive staining of isolaed polysomes from a winter adapted carp treated with insulin.

associated with the ability of oocytes and follicle cells from the lizard *Larcerta sícula* to synthesize proteins during hibernation (22).

The results presented in this Symposium are preliminar.

Whether insulin exerts its effect directly upon the polysomal populations or its mechanism of action involves the unpackaging of glycogen particles, thus making polysomes available, it is a matter of current investigation in our laboratory. Also, experiments are in progress, to ascertain the degree and characteristics of glycogen accumulation in the hepatocyte of summer adapted carps.

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