BIOSYNTHESIS OF NUCLEAR PROTEINS IN EMBRYOS OF RAINBOW TROUT

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SUMMARY

The biosynthesis of nuclear proteins in embryos of rainbow trout occurs in an intracellular compartment either closely associated with or inside the cell nucleus. This synthesis is inhibited by puromycin but only partially inhibited by cycloheximide, which strongly inhibits the synthesis of cytoplasmic proteins.

INTRODUCTION

When isolated nuclei are incubated with radioactive amino acids, nuclear ribosomes become rapidly labelled relative to nuclear proteins other than the residual protein (1,2); furthermore, these ribosomes, which appear to be located in the nucleolus (3), do not carry precursors of nuclear residual protein (4), or ribosomal proteins (3), and can support in vitro protein synthesis (2-7). Although a basic nucleolar protein has been shown to be synthesized by nucleolar ribosomes (3), recent work has clearly shown that the chromosomal histones are made on cytoplasmic ribosomes of mature eucaryotic cells, (8-12) in association with a class of slowly sedimenting polysomes (9). Except for Nemer and Lindsay (12) who studied the sea urchin embryo up to the early blastula stage, and whose evidence is not conclusive, these studies have all dealt with mature cells in tissue culture or trout testis tissue undergoing spermatogenesis. The desirability of studying nuclear protein synthesis in an embryonic system led to the experiments described here.

METHODS

Fertilized eggs of naturally maturing rainbow trout (Salmo gairdnerii) were kept at 10°C until use. The tough chorionic membranes of the eggs were broken by a mechanical slicer, and the contents extruded in Puck's Saline A (13). Cells and nuclei were collected by low speed centrifugation, with special care to avoid shearing which easily disrupted the cells; because of the fragility of embryo tissue most embryo brei preparations contained a mixture of cells contaminated with nuclei.

Nuclei were obtained from embryo preparations by homogenization with a Potter-Elvehjem homogenizer with a glass-reinforced Teflon pestle (Tri-R, Rockville Center, N.Y.); samples were examined by phase contrast microscopy to ensure that cell breakage was complete. Brei or nuclear preparations were washed several times by low speed centrifugation to remove yolk or cytoplasmic proteins, respectively, and the resulting suspensions were dispensed by serological pipettes. After appropriate preincubation of aliquots with cycloheximide or puromycin for 30 to 60 min. at 0°C, ^{3}H-arginine was added and the tubes were placed in a shaking water bath at 10°C for 60 min. Carrier arginine was added (final concentration, at least 10^{-3}M), and after one wash by low speed centrifugation, embryo brei preparations were homogenized as described above. Crude nuclear and cytoplasmic fractions were obtained from the homogenate by centrifugation (650 g, 5 min.). Nuclei (resuspended in Puck's Saline A, two volumes) were purified by centrifugation through a discontinuous sucrose gradient (one volume each of sucrose: 2.3, 1.75 and 1.3 M, each layer also containing $CaCl_2$ 0.003 M) for 1 hour (20,000 rpm, SW25 head) or 30 min (35,000 rpm, SW39 head) in a Beckman L preparative ultracentrifuge. The purified nuclear fraction was washed by centrifugation with 1.5 ml of Puck's Saline A (13).

Basic proteins were extracted from the saline-washed purified nuclear fraction with three portions (0.2 ml) of 0.2 M HCl. After extraction of nucleic acids by 5% trichloracetic acid (90°C, 20 min.), residual and acidic proteins were extracted with 1.0 M NaOH (0.4 ml, 0.2 ml) (14). Aliquots of the extracts were taken for determination of protein by the method of Lowry et al. (15) with bovine serum albumin as a standard, and for precipitation in the presence of carrier protamine sulfate by 5% trichloracetic acid, 0.25% sodium tungstate, pH 2 (16). The precipitate was collected on millipore HA filters, which were dried and counted in toluene scintillator.

In general, samples of the embryos were taken for experiments at three days (high blastula) and 8 days (gastrula stage) after fertilization.

RESULTS AND DISCUSSION

Preparative disc electrophoresis (Canal Industrial Co., Rockville, Md.) of the 0.2 M HCl extract prepared following incubation of an 8 day embryo brei suspension with ^{3}H-arginine was performed in the buffers described by Reisfeld et al. (17), 6.25 M with respect to urea. Although recoveries of both protein and labelled arginine were low, no non-histone contaminants were detected (unpublished data) by this technique; the specific activity of the histones recovered was (on an average) 69% of that measured before this procedure.

The effect of adding the inhibitor cycloheximide to the incubations was tested. Suspensions of cells (approximately 0.085 ml of loosely packed cells in a final volume of 1.0 ml, containing inhibitor

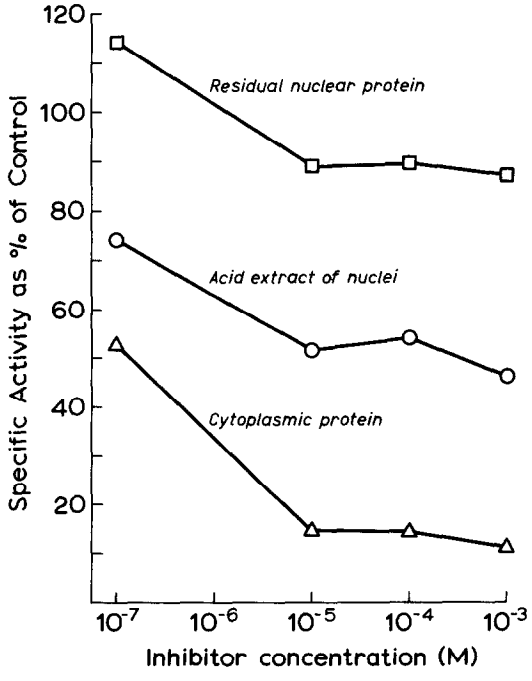

FIGURE 1: Relative specific activity (as a percent of control) of proteins synthesized in different cell fractions as a function of the concentration of cycloheximide. For each fraction the uninhibited control was set at 100%. The fractions, obtained as described in the text, are as follows: △ cytoplasmic protein; ○ proteins of 0.2N HCl extract; □ proteins of 1.0N NaOH extract.

and 10 μc of ^{3}H-arginine) were incubated with different concentrations of cycloheximide and the cells fractionated (Fig. 1). At a concentration of cycloheximide (10^{-7}M) at which protein synthesis is normally significantly inhibited, the incorporation into cytoplasmic proteins was half of the control, whereas the synthesis of both basic and acidic plus residual nuclear proteins was either not significantly affected or only slightly inhibited. Furthermore, at higher molarities of cycloheximide, the incorporation into cytoplasmic proteins decreased still further. In most experiments, almost complete inhibition of cytoplasmic protein synthesis has been observed at concentrations of cycloheximide higher than 10^{-6}M. Perhaps in this case the relatively high level of cytoplasmic protein synthesis was an artefact due to slight contamination of this fraction with small nuclear fragments resulting from homogenization.

The incorporation into both nuclear protein fractions continued even at one thousand times the concentrations of cycloheximide which in ordinary eucaryotic cells would completely abolish protein synthesis (11, 18).

The clear differences in the effect of cycloheximide on nuclear and cytoplasmic protein synthesis indicated that the synthesis of nuclear proteins occurs in a different cell compartment from that in which cytoplasmic proteins are synthesized. It appeared unlikely that this difference was due to differential permeability of this cell compartment to cycloheximide since there was a partial inhibition of nuclear protein synthesis. Glutathione has been found to prevent cycloheximide inhibition, possibly by protecting the enzyme peptidyl transferase II (19). This finding suggested that the cell compartment in which nuclear protein synthesis takes place possibly had a relatively high sulfhydryl concentration; alternatively it could be suspected that the nuclear protein synthesis system has characteristics similar to those of protein synthesis occurring in mitochondria (20). Preliminary experiments have revealed that the normal inhibition of nuclear protein synthesis by cycloheximide occurs at later stages in embryonic development.

To further investigate which intracellular compartment was involved in the biosynthesis of nuclear proteins, nuclei of embryo cells were incubated (0.029 ml of loosely packed nuclei per ml) as described above in Puck's Saline A. In a preliminary experiment, this medium supported incorporation of ^{3}H-arginine approximately 50% better than that used by Allfrey et al. (21) for calf thymus nuclei. Thus this system appears to differ from that described by Allfrey by being active in the presence of isotonic saline solutions.

TABLE I

INCORPORATION OF ^{3}H-ARGININE INTO PROTEINS BY ISOLATED NUCLEI

Sample	Relative Specific Activity		
	Control	Cycloheximide (10^{-2}M)	Puromycin (10^{-3}M)
Day 3			
0.2N HCl extract	100	94.5	13.1
1.0N NaOH extract	100	20.6	10.8
Day 8			
0.2N HCl extract	100	45.5	16.7
1.0N NaOH extract	100	78.0	23.0

The relative specfic activities of the protein fractions extracted from nuclei (purified after incubation) are shown in Table I. Although there was little protein synthesis in the presence of puromycin, even very high concentrations of cycloheximide did not completely inhibit the synthesis of nuclear proteins. The strong inhibition of arginine incorporation in this system by puromycin precludes the possibility that the non-ribosomal arginine transfer reaction (22) is involved in labelling the proteins in this system.

The data presented in Table II indicate that the total incorporation of ^{3}H-arginine into nuclear basic proteins was of the same order of magnitude in both embryo brei and the nuclear preparations from the same source. Since some loss of nuclear material occurred during the homogenization and purification steps, these data can only serve as an approximate estimate of the amount of protein synthesis which occurred. Nevertheless, they indicate that the synthesis of nuclear proteins extracted by 0.2 M HCl proceeds almost unhindered by the loss of cytoplasm. The previous finding that histones were the major labelled basic proteins of the acid extract suggests that histone synthesis may possibly occur in the nucleus of the embryo cell.

It is possible that in primitive undifferentiated embryo cells which have large volumes of cytoplasm it might be advantageous to synthesize nuclear proteins in a cell compartment closely connected to the nucleus, to eliminate the difficulty of transporting the protein into the nucleus. Several groups have shown by histochemical techniques that differences in histone composition occur during the course of development. The changes in histone composition could be

TABLE II

INCORPORATION OF ^{3}H-ARGININE BY EMBRYO BREI AND NUCLEAR PREPARATIONS

	Activity*	
	Day 3	Day 8
0.2 M HCl extract of embryo brei	1225	3600
0.2 M HCl extract of nuclei	932	1308

* Counts/min. in total sample, normalized to a standard amount of tissue added to incubation.

associated with the change in localization of nuclear protein synthesis to a compartment more closely associated with the cytoplasm as differentiation proceeds. Our data can be interpreted to favour this hypothesis.

Further investigation is now indicated to characterize the proteins synthesized and the characteristics of the system, and to study what variations in these properties occur during the development of the trout and chick embryo.

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