DECREASE OF PROTEIN SYNTHESIS AND BREAKDOWN OF POLYRIBOSOMES BY ELEVATED TEMPERATURE IN PHYSARUM POLYCEPHALUM

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Summary

The plasmodium of the myxomycete, Physarum polycephalum, which grows rapidly at 27°, was subjected to heat shocks at 40° for periods of either 10 or 30 min. During these shocks, protein hydrolysates labeled with tritium were added to the nutrient media for a 10-min period; with the plasmodia that were shocked for 30 min, the hydrolysate was added during the last 10 min. The heat shock reduced the uptake of amino acids into protein by approximately 40% with the 10-min shock and by more than 70% with the 30-min shock. The heat shock also caused a reduction of polyribosomes of more than 50%. These results are consistent with the suggestion that heat shock interferes with protein synthesis by decreasing the amount of polyribosomes.

Incubation at elevated temperature for short periods delays nuclear division in various living cells including Physarum polycephalum (1) and Tetrahymena (2), and HeLa cells are arrested temporarily in metaphase (3). Although the biochemical mechanisms underlying these effects are not completely understood (2,4), it is known that protein synthesis is reduced and RNA decays at a rapid rate in cultures of Tetrahymena subjected to heat shock (5,6), and it was suggested that destruction of mRNA may be responsible for the decrease in protein synthesis in this organism (7,8). This communication describes the effect of elevated temperature on protein synthesis and polyribosomes in Physarum polycephalum.

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Methods

Cultures: Synchronous stationary cultures of Physarum polycephalum, subline M3C, were grown at 27°, as described previously (9). The time of mitosis was determined by phase-contrast examination of alcohol-fixed smears (10). MIII indicates the third synchronous division following coalescence of microplasmodia.

Heat shock and amino acid incorporation: The incorporation of labeled amino acids into plasmodia subjected to heat shock was performed as follows: Two plasmodia which had completed mitosis III and the following S period were cut in half. One half of each plasmodium was placed in a Petri dish containing 1 ml of a semi-defined growth medium (11), minus the tryptone and yeast extract, and with 0.5 mg hemin per 100 ml of medium and the pH adjusted to 5. In place of the tryptone and yeast extract, the medium contained reconstituted ^{3}H-protein hydrolysate (a mixture of 16 purified ^{3}H-L-amino acids, 0.5 mC per ml, Schwarz BioResearch, Inc., Orangeburg, New York). One ml of this modified medium, containing 12.5 µC of ^{3}H, was distributed evenly on the bottom of a Petri dish. Two halves of the bisected plasmodia were kept at 40°, and the other two served as controls maintained at 27°. After 10 min, the amount of labeled amino acids that had been incorporated was determined, as described later.

Two other plasmodia, similar to those described above, were also bisected, but in this experiment the pieces were placed in Petri dishes containing the usual growth medium (11) for 20 min before they were transferred for a 10-min period to Petri dishes containing the same ^{3}H-protein hydrolysate as described above. During the entire 30-min period, two of the plasmodia halves were maintained at 40° and the other two were the controls kept at 27°.

Following the period of labeling with the ^{3}H-protein hydrolysate, the plasmodia, still attached to the millipore membrane, were washed three times in ice water, scraped from the membrane into 5 ml ice-cold 5% w/v

trichloroacetic acid in 50% v/v aqueous acetone, and carefully suspended. The pellets were washed twice with 5% trichloroacetic acid-acetone-water, and the resultant pellet was dissolved overnight at room temperature in 1 ml 0.4 N NaOH.

Aliquots of 0.2 ml were put on glass fiber filters (Gelman, Ann Arbor, Michigan, Type E, 1-inch diameter), suspended by a common pin. The air-dried filters were counted in a Packard Tri-Carb liquid scintillation counter. Each vial contained 10 ml scintillation reagent consisting of 0.4% diphenyl oxazole (PPO) and 0.005% 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in toluene (Nuclear Chicago, Des Plaines, Illinois). To compensate for the background, a blank of 13 cpm was substracted from the measured counts. Aliquots were taken for protein determination (12). Bovine serum albumin was used as standard.

Preparation of polysomes: Polysomes were prepared from plasmodia frozen in liquid nitrogen essentially as described previously (13). Plasmodial homogenates, prepared in the cold, were centrifuged in a No. 296 rotor of the International centrifuge at 10,000 rpm at -10° for 4 min. The supernatant, free of mitochondria, was then made up to a 0.4% concentration of sodium deoxycholate, and 0.6 ml of this supernatant was layered on 28 ml of a linear sucrose gradient (10 to 50% w/v in 0.025 M KCl, 0.005 M $MgCl_2$, 0.05 M Tris-HCl, pH 7.3 at 20°), which was prepared with a gradient mixer from Buchler Instruments. The mixture was then centrifuged for 150 min in a Spinco SW 25 rotor at 25,000 rpm. The optical density was monitored as described previously (13), and a constant flow rate was obtained by means of a Buchler peristatic pump.

Results

Effect of heat shock on amino acid incorporation: The effect of heat shock on the incorporation of labeled amino acids into protein is shown in Table I. A 10-min period of heat shock reduced the amino acid incorporation

TABLE I

Incorporation of ^{3}H-labeled Amino Acids into Acid-insoluble Material.

Plasmodia were cut in half, and half of the pieces were subjected to temperature shocks of 40° for periods of either 10 or 30 min. The other halves were kept as controls at 27°. All the plasmodia were in contact with labeled amino acids for a period of 10 min. In plasmodia 1 and 2 this coincided with the entire period of the heat shock, but with plasmodia 3 and 4 the labeling was only during the last 10 min of the 30-min period of heat shock.

Plasmodia	Temperature (°C)	Length of heat shock (min)	cpm/mg protein	Incorporation (%)
1a	27	0	180	100
1b	40	10	109	61
2a	27	0	286	100
2b	40	10	160	56
3a	27	0	249	100
3b	40	30	14	6
4a	27	0	192	100
4b	40	30	51	27

TABLE II

Acid-soluble Radioactive Material in the First Trichloroacetic Acid-Acetone-Water Extract of Washed Plasmodia after a 10-Min Pulse with Labeled Amino Acids.

Plasmodia	Treatment of plasmodia	cpm/ total extract
1a	Control	75,000
1b	Heat shock, 10 min	64,200
2a	Control	80,300
2b	Heat shock, 10 min	102,000
3a	Control	94,800
3b	Heat shock, 30 min	46,000
4a	Control	57,000
4b	Heat shock, 30 min	79,800

by about 40%, whereas incorporation was reduced considerably more (70-95%) when the length of the heat shock was increased to 30 min.

Acid-soluble radioactive material not incorporated into protein: To

determine the amount of labeled material not incorporated into protein, the level of activity in the first trichloroacetic acid-acetone-water extract of the washed plasmodia was determined. The total amount of labeled material in the extracts of heat-shocked plasmodia was less than that of the controls in two experiments and greater in two other experiments, as shown in Table II. There is no obvious explanation for this variation, but it is possible that variable amounts of amino acids were adsorbed onto membranes or proteins without being incorporated into protein.

Polyribosomes: The results shown in Table I suggest that the heat shock interferes with the protein metabolism. Since polyribosomes are the major site of protein synthesis, the effect of heat shock on the polyribosome pattern was studied. Immediately following 30 minutes of heat shock (about 2 hr after MIII), the plasmodia were frozen in liquid nitrogen and the sedimentation profiles of the ribosomes determined. The results are shown in Fig. 1. It is obvious that heat shock causes a considerable reduction in the amount of polyribosomes. The quantitative analysis of the

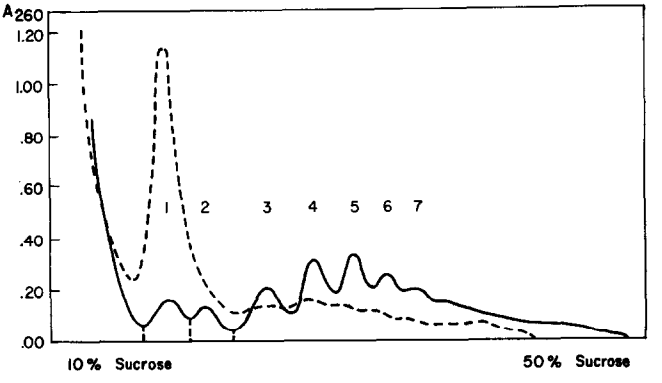

Fig. 1. Sedimentation profiles of Physarum ribosomes and polyribosomes in sucrose gradients. The profiles represent ribosomes of a control plasmodium (solid line) and of a plasmodium subjected to heat shock for 30 min at 40° (dashed line). The procedure for isolating the ribosomes is presented in the section on Methods.

area under the profiles is recorded in Table III. In the control plasmodium 9% of the total ribosomes were found in the first peak, whereas after the

TABLE III

Distribution of Ribosomes and Polyribosomes in the Postmitochondrial Supernatant of Physarum polycephalum.

Numbers are percentages of the total area under the sedimentation profile, consisting of peak 1, peak 2, and polyribosomes.

Area	Percent ribosomes	
	Control plasmodium	Plasmodium after heat shock
Peak 1	9	48
Peak 2	7	14
Polyribosomes	84	38

heat shock this amount increased to almost 50%,with a corresponding decrease in the polyribosomes. This experiment did not show whether the material under peak 1 was ribosomes or a precursor of ribosomes.

The influence of the length of heat shock on the breakdown of polyribosomes is shown in Fig. 2. The change in the polyribosome pattern occurred within a few minutes of heat treatment.

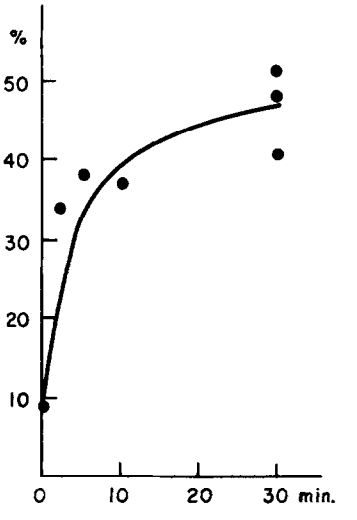

Fig. 2. The effect of the duration of the heat shock (40°) on the sedimentation profile of polyribosomes. The curve shows the rapidity of the increase in peak 1 (ribosomes). Numbers on the ordinate represent areas under peak 1 of the sedimentation profile expressed as a percent of the total area of peaks 1, 2, and polyribosomes.

Discussion

There are at least three possible explanations for the reduced incorporation of labeled amino acids into acid-insoluble material as the result of heat shock: (a) Heat shock may decrease the permeability of the cellular membranes. This does not appear to be the correct explanation, since there was no consistent difference in the amount of labeled material in the first TCA-acetone-water extract of plasmodia that were heat-shocked as compared with the controls. (b) Heat shock may influence the amino acid pool of the plasmodia, thus affecting the amount of incorporation, but again the lack of consistent differences in the amount of label in the TCA-acetone-water extract does not support this suggestion. (c) The most plausible explanation, therefore, is that heat shock decreases protein synthesis by destroying the integrity of the polyribosomes, and this interpretation is consistent with the fact that polyribosomes break down during heat shock. It is not known whether the decrease in the polyribomes is the result of a destruction of mRNA, as suggested by Byfield and Scherbaum (8) in the case of Tetrahymena pyriformis, the result of a simple separation of ribosomes from mRNA, or the result of inhibition of one or more steps in the initiation of protein synthesis. Additional work is needed to answer these questions.

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