Vol. 85, No. 2, 1978 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 29, 1978 Pages 756-761

THE INFLUENCE OF TEMPERATURE UPON POLYSOMES OF SPERMATIDS OF RAT TESTES

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Received October 16, 1978

SUMMARY

Incorporation of [^{3}H]phenylalanine into protein by a reconstituted lysate subcellular system (ribosomes plus high-speed supernatant) from rat spermatids was measured at 34°C after 5 minutes preincubation of one component at 0°C while the other component was incubated at temperatures from 30°C to 40°C. Preincubation at temperatures above 34°C inhibits the ribosomal activity but not the high-speed supernatant activity. The incubation of lysate above 34°C results from a dissociation of polysomes to monosomes. These results indicate that ribosomes are the most sensitive component to the increased temperature on protein synthesis in lysate cell free system by spermatids and that the inhibition of protein synthesis in spermatids above 34°C is at least partly explained by the breakdown of polysomes in these cells.

It has long been known that protein synthesis in rat testis is higher at scrotal than at body temperature (1). Indirect evidence suggested that spermatids may be largely responsible for this difference (2). We have recently used elutriation centrifugation to prepare a spermatid fraction which is more than 80 percent pure in rounded (immature) forms (stage < 8) (3,4). Using such purified spermatids, we have shown that the inhibition of protein synthesis by spermatids at body temperature (38°C) is also seen in a subcellular system prepared from these cells (4). Since a subcellular system from spermatids showed less incorporation of amino acids into protein at 38°C than at 34°C (4), the inhibition of protein synthesis by 38°C in whole cells cannot be entirely

0006-291X/78/0852-0756$01.00/0

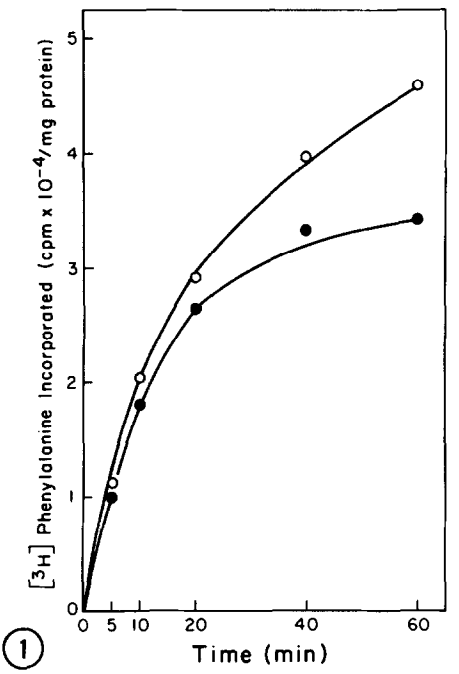
attributable to an effect of transport system. Attempts were made to seek the most sensitive component(s) to the increased temperature in lysate cell free system of spermatids and to correlate changes in polysomal profile with an inhibition of protein synthesis at body temperature, since it is reported that polysomes in the mammalian cells disaggregate and single ribosomes accumulate as the rate of protein synthesis decreases (6).

METHODS

Spermatids were prepared from testes of adult rats (40-50 days old) as described previously (3,4). Spermatids were lysed with hypotonic buffer for 5 minutes at 0°C (5) and lysate was centrifuged at 30,000xg for 20 minutes (S-30). The supernatant was then fractionated (226,000 xg 3 hrs) to prepare ribosomes and high-speed supernatant by an established method (6). Incorporation of [^{3}H]phenylalanine into protein and the sources of various chemicals have also been reported (3,4). The supernatant (S-30) was incubated for 10 minutes at various temperatures and loaded on sucrose density gradients (15-50%) to examine the change in profile of polysomes (6). The proportion of monosomes to polysomes was measured by absorbance at 260 nm in a Gilford recording spectrophotometer.

RESULTS AND DISCUSSION

Figure 1 shows the time course for incorporation of [^{3}H]phenylalanine into protein at 34°C and 37°C by a subcellular system of spermatids. It can be seen that incorporation of [^{3}H]phenylalanine into protein at body temperature was less than that at the other temperature for all times examined. When different fractions from rat testes enriched in Sertoli cells, spermatogonia, and Leydig cells were incubated at 34°C and at 37°C, incorporation of [^{3}H]phenylalanine into protein was greater at 37°C than at 34°C. In spermatocytes, incorpoation of [^{3}H]phenylalanine into protein at 37°C did not decrease significantly compared to that at 34°C. These results indicate that the effect reported



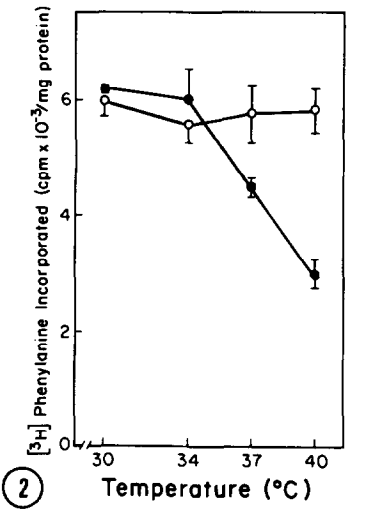


Figure 1. Incorporation of [^{3}H]phenylalanine into protein by S-30 of spermatids. S-30 of spermatids were incubated at 34°C and at 37°C with 5 μCi of [^{3}H]phenylalanine per flask (6). Incorporation of [^{3}H] was measured by trichloroacetic acid precipitation (4). Open circles; 34°C. Closed circles; 37°C.

Figure 2. Incorporation of [^{3}H]phenylalanine into protein by a reconstituted system (ribosomes plus high-speed supernatant) from spermatids. One of two components of the system was kept at 0°C for 5 minutes while the other component was incubated at temperatures shown. The two components were then mixed and [^{3}H]phenylalanine (5 μCi per flask; spc. act. 40 Ci/m mol) and other necessary components were added (6). Incorporation of [^{3}H]phenylalanine into protein was measured by incubating at 34°C for 60 minutes. Incorporation was measured by trichloroacetic acid precipitation (4). Open circles; high-speed supernatant incubated at the temperatures shown. Closed circles; ribosomes incubated at the temperatures shown.

here is specific for spermatids. Lysate (S-30) of spermatids was further fractionated by high speed centrifugation to examine what component in lysate of spermatids is the most vulnerable at body temperature.

Figure 2 shows the incorporation of [^{3}H]phenylalanine into protein at 34°C by a reconstituted subcellular

system (high-speed supernatant plus ribosomes) prepared from spermatids. Ribosomes were kept at 0°C for 5 minutes while the high-speed supernatant was incubated at various temperatures, and the two were mixed prior to the measurement of protein synthesis at 34°C. It was found that in high-speed supernatant higher temperatures were without effect on incorporation of the labeled amino acid into protein (open circles). On the other hand, when ribosomes were incubated for 5 minutes at various temperatures while the high-speed supernatant was held at 0°C, the reconstituted system showed a decreased incorporation of [^{3}H] into protein (closed circles). Clearly it is possible to find conditions under which the ribosomes are adversely affected by 37°C and 40°C without any detectable change in the activity of the high-speed supernatant. Figure 3 shows that the incubation of lysate (S-30) at 40°C for 10 minutes is associated with a breakdown of polysomes to monosomes when compared with lysates held at 0°C and 34°C for the same time.

Evidently, the most sensitive component of the protein synthesizing machinery in spermatids is the ribosomes and that at least part of the defect produced in the ribosomal activity results from a disaggregation of polysomes. Such a change in polysomal profiles is seen when various agents inhibit protein synthesis, e.g. the temperature (6,8,9), and chloral hydrate (7). The changes seen in the present case appear to be general throughout the polysomes profile as opposed to specific loss of a single species of polysomes.

The metabolism of spermatids is characterized by susceptibility to body temperature and by an unusual dependence on glucose as a metabolic substrate. The present studies were performed in the presence of excess ATP and GTP so that the

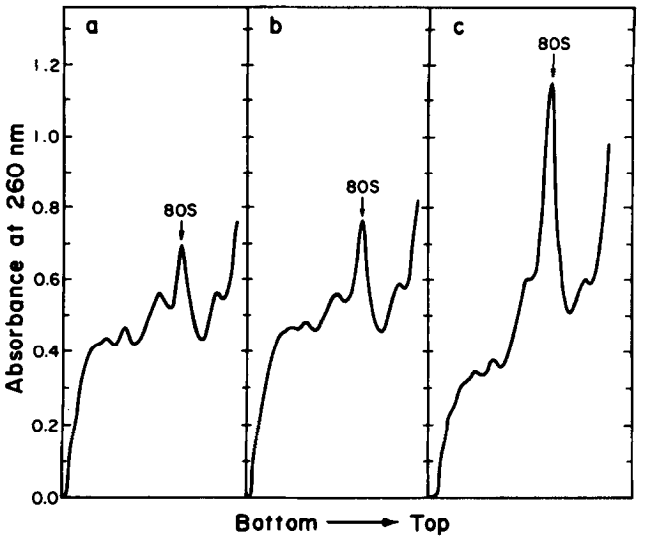


Figure 3. Polysomal profiles from lysate of spermatids. Lysates of spermatids (S-30) were incubated for 10 minutes at 0°C, 34°C, and 40°C (6). Polysomes were then prepared by centrifugation in sucrose density gradients (15-50%) (6). The gradient was pumped into a spectrophotometer and the absorbance was recorded continuously at 260 nm. (a) 0°C. (b) 34°C. (c) 40°C.

breakdown of polysomes observed in these studies cannot be attributed to an effect of the temperature on glucose metabolism.

The present findings do not exclude additional effects of the temperature on spermatids. For example, effects occurring only in the whole cell (transport of glucose and amino acids), additional effects on the ribosome (phosphorylation of ribosomal protein, etc.) or an effect on the high-speed supernatant when the duration of exposure to higher temperatures is prolonged. Moreover, it does not follow that whole cells are as susceptible to such a short exposure to 37°C as lysate, although protein synthesis is inhibited above 34°C in the whole cell system (1-4). Finally, a number of possible mechanisms could explain the effect of temperature

on polysomes - a decrease in available messenger RNA, a defective initiation of protein synthesis, a loss of the stability of polysomes, etc. Further explanation of these various possibilities will require methods for preparing spermatids on a large scale. The importance of understanding the influence of environment on differentiation of the germinal epithelium encourages the development of appropriate methods.

ACKNOWLEDGEMENT

This work was supported by NSF grant No. PCM76-17368. The authors are grateful to Drs. Bob Jones and Isaac Sadnik in the Department of Biological Chemistry, University of California, Irvine, for valuable suggestions during this work.

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