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Post-meiotic transcription of phosphoglycerate-kinase 2 in mouse testes

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We have used a human phosphoglycerate kinase-1 (PGK-1) cDNA clone to study expression of PGK-2 during mouse spermatogenesis. Hybrid selection, in vitro translation with product identification by 2-D gel electrophoresis demonstrated that the PGK-1 cDNA clone hybridized to PGK-2 mRNA in mouse testes. Northern analyses of RNA purified from separated spermatogenic cells demonstrated a large increase in abundance of PGK-2 mRNA in post-meiotic cells. Thus, post-meiotic transcription of PGK-2 mRNA is demonstrable with cloned DNA probes.

Phosphoglycerate kinase is an essential enzyme in the glycolytic pathway. The isozyme present in all somatic and pre-meiotic germ cells, PGK-1, is coded for by an X-linked locus (1). Phosphoglycerate kinase-2 (PGK-2, EC 2.7.2.3; ATP: 3-phospho-D-glycerate 1-phosphotransferase) is a sperm-specific isozyme that is developmentally regulated in the mouse (27). The appearance of this autosomally-coded form, on chromosome 17 (5), during spermatogenesis can be most easily explained as a necessity due to X-inactivation during spermatogenesis (6, 20, 21). However, the very large increase of a kinetically-indistinguishable form (18) may indicate that the enzyme subserves some new function in post-meiotic cells. Enzymatic activity (16), immunological reactivity (15), and translation (16) of PGK-2 mRNA have only been detected in post-meiotic germ cells. In vitro translational studies of isolated mRNA from purified testicular germ cells demonstrated a marked increase in PGK-2 mRNA in spermatids compared to spermatocytes (8), and the small amount of PGK-2 mRNA found in spermatocytes was not associated with ribosomes in a translational complex (12). However, this mRNA might have been stored in a nontranslatable form, e.g. nonprocessed, and have been transformed at meiosis. Thus, proof of post-meiotic synthesis requires studies with nucleic acid probes which will detect mRNA in any form.

A human PGK-1 cDNA clone was found to hybridize to multiple DNA restriction fragments on Southern blots that showed X chromosome dosage, as well as several bands that did not and which were presumed to be due to cross-hybridization to autosomal loci (23). This is not entirely unexpected since previous studies have shown an immunological cross-reaction between PGK-1 and PGK-2 isozymes (18). A mouse PGK-specific *Eco* RI fragment detected by the human PGK-1 cDNA clone has been mapped to mouse chromosome 17 in appropriate mouse-hamster somatic cell hybrids (A.M.M., unpublished observations). We find that this probe hybridizes to an mRNA in mouse spermatids but not in spermatocytes.

Materials and Methods

Hybrid selection, in vitro translation of testes mRNA with human PGK-1 cDNA clone

Total testicular RNA from CD-1 testes was prepared via a guanadinium thiocyanate extraction procedure (2) and hybridized to nitrocellulose bound *Pst* I inserts of pHPGK-7e DNA under standard conditions (22). The selected RNA was added to a rabbit reticulocyte in vitro translation system (Bethesda Research Laboratories) containing ^{35}S-methionine. Two-dimensional gel electrophoresis was performed according to the method of O'Farrell (24) with minor modifications. Fluorography of the gel was achieved utilizing Enhance scintillation (New England Nuclear) and pre-exposed film.

Northern analyses using the human PGK-1 cDNA clone

Human adult and fetal liver poly(A)$^+$ RNA were prepared as described (25). RNA was prepared from liver and Elutriator-purified (a centrifugal technique) spermatocytes from CD-1 mice as previously described (10). Spermatid RNA was prepared from testes of irradiated CD-1 mice using a proteinase K-phenol/chloroform method with heparin as a nuclease inhibitor, and poly(A)$^+$ RNA was prepared from the spermatocytic RNA on an oligo(dT)-cellulose column (10); RNAs were denatured and electrophoresed in 1% agarose in the presence of formaldehyde, transferred to nitrocellulose, and hybridized with the *Pst* I insert of pHPGK-7e (19,26).

Results

A human PGK-1 cDNA selects PGK-2 mRNA from mouse testes

In order to see if pHPGK-7e, the human cDNA clone to PGK-1 mRNA, would hybridize to PGK-2 mRNA in mouse testes, hybrid selection, in vitro translation was performed. As seen in Fig. 1, this human PGK-1 cDNA selected PGK-2 mRNA from mouse testicular RNA. This newly labelled protein is unambiguously PGK-2 based on its relative mobility compared to the nearby protein (e) synthesized by the reticulocyte lysate system with or without added mRNA. It was not surprising that the clone did not select PGK-1 mRNA, in addition to the PGK-2 mRNA, since the rate of synthesis

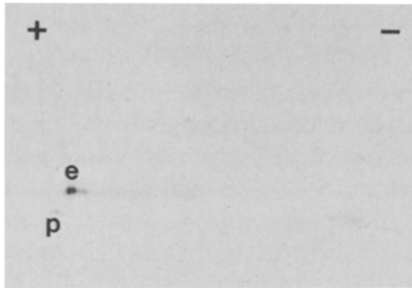

Fig. 1. In vitro translation products following hybridization-selection of testicular mRNA with the *Pst* I insert of pHPGK-7e. The in vitro translation products are: e, the protein produced by an endogenous mRNA of the reticulocyte lysate, as previously described (10); and p, PGK-2 protein, as previously identified using immunoprecipitation with antisera to PGK-2 (16).

of PGK-1 (and, presumably, the relative level of mRNA) is nearly 2 orders of magnitude lower than the rate of synthesis of PGK-2 in mouse testes (16).

Evidence for transcription of PGK-2 in post-meiotic cells

The *Pst* I insert of pHPGK-7e readily hybridized to mouse liver and testicular RNAs (Fig. 2). The human controls show a 2 kb major band in adult and fetal liver; a 2.5 kb minor species is also detected. The overloaded mouse liver sample (lane 4) has a component of similar mobility to the major human liver species without the minor component. Mouse spermatid RNA readily hybridized to the probe, and this mRNA may have a slightly greater electrophoretic mobility than the mouse liver mRNA. Despite the use of poly(A)$^+$ RNA from spermatocytes, neither the 2 nor the 2.5 kb mRNA species could be detected. Therefore, we interpret these data to demonstrate that the 2 kb RNA species detected in Northern gel blots of post-meiotic (haploid) cells is the mRNA corresponding to the PGK-2 structural locus. The lack of detection of PGK-2 mRNA in spermatocytes by the Northern analysis confirms the earlier results of a much greater abundance of PGK-2 mRNA in spermatids than in spermatocytes.

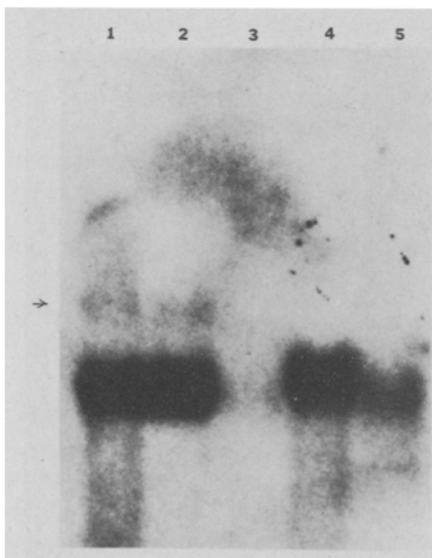

Fig. 2. Northern analysis of RNA from human liver and mouse testicular fractions probed with the *Pst* I insert of pHPGK-73. *Lane 1*: 4 μg human adult liver poly(A)$^+$ RNA; *lane 2*: 2 μg human fetal liver poly(A)$^+$ RNA; *lane 3*: 7.5 μg mouse spermatocyte poly(A)$^+$ RNA; *lane 4*: 25 μg total mouse liver RNA; *lane 5*: 7.5 μg total mouse spermatid RNA; *arrow*: 2.5 kb.

Discussion

Previous experiments using functional assays for mRNA (in vitro translation of poly(A)$^+$ mRNAs) have shown that mRNA for prtamine-like histone (8), PGK-2 (8), and a large number of proteins detected by 2-D gel electrophoresis (16) increase after meiosis. The data we have now obtained by Northern analysis using a human cDNA clone to PGK-1 further demonstrate post-meiotic transcription. mRNA for PGK-2 first appears during meiosis and appears in a non-polysomal fraction. It then greatly increases in amount in round spermatids, where it is found in the polysomal fraction (12). The enhanced relative abundance of PGK-2 mRNA in spermatids cannot be explained by selective degradation of other mRNAs since the amount of mRNA per amount of DNA only decreases by about 30% in spermatids (13). The RNA cannot have been stored in spermatocytes since the RNA has been purified from possibly complexing proteins and the probe would hybridize to nonprocessed or otherwise immature transcripts. Other studies have also demonstrated post-meiotic expression using cloned probes (3,4,11,14), and new testes-specific probes are becoming available (17).

The major reason for our interest in post-meiotic gene expression is its relevance to transmission ratio distortion by *t*-alleles (9). *t*-Alleles are complex chromosomal alterations in mice which are transmitted by males, but not females, to their offspring in non-Mendelian ratios. A variety of data (reviewed in ref. 9) indicate that post-meiotic expression must be involved. It is interesting that PGK-2 maps close to the *t*-complex and is post-meiotically expressed but our studies of PGK-2 enzymatic activity in spermatozoa of T/t^n males have not disclosed altered expression (7).

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