Analysis of the Expression of Two Phosphoglycerate Kinase Genes in a Mouse Cultured Cell Line during Activation and Inactivation of the c-myc Gene

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The effect of a change in the concentration of c-myc protein on the expression of genes for two phosphoglycerate kinase isozymes was investigated. The steady state levels of messenger ribonucleic acids (mRNAs) for sperm-type and non-sperm-type proteins were determined by blot hybridization using the RNA of the mouse cell line 38-2 containing the inducible rat c-myc gene cultured under various conditions. Without inducing the c-myc gene, mRNA for non-sperm-type protein was detected at a level that remained essentially constant during both activation and inactivation of the c-myc gene. mRNA for sperm-type protein was not detected in 38-2 cells cultured under any conditions used. Change in the amount of c-myc protein alone does not appear to bring about a switch of the expression of the two phosphoglycerate kinase genes during spermatogenesis in mouse testis.

Keywords gene expression; transcriptional regulation; isozyme; proto-oncogene; cultured cell

Introduction

A variety of different sperm-specific proteins are known. They are synthesized through the preferential expression of their genes at certain stages during spermatogenesis.[1,2] However, the mechanism of the selective expression of sperm-specific genes during the differentiation of sperm from spermatogonia is poorly understood. Clarification of the mechanism of expression of sperm-specific genes is important for elucidating the means by which spermatogenesis is controlled. Among many genes that show change in their expression during spermatogenesis, some are particularly important, such as the homeobox-containing gene Hox-1.4[3] and several proto-oncogenes such as c-mos,[4,5] int-1,[6] c-abl,[7] N-ras[8] and c-myc.[9,10] This is because their products appear to be involved in cell growth and differentiation, and these proteins are thus likely to be essential to the control of sperm-specific gene expression.

c-myc protein is important to the transformation and differentiation of animal cells.[11] Decrease in the intracellular concentration of c-myc protein has been found to importantly influence differentiation of Friend cells following their induction with dimethyl sulfoxide.[12,13] The c-myc gene shows a marked decrease in its expression after the onset of spermatogenesis, but is actively transcribed in spermatogonia.[9,10] Change in the content of c-myc protein during spermatogenesis may thus possibly be involved in regulating the expression of sperm-specific genes. Phosphoglycerate kinase (PGK), together with other enzymes, is involved in operating the glycolytic pathway, and it has two isozymes, sperm-specific type (PGK2) and non-sperm type (PGK1). The PGK1 gene is an X-linked gene expressed in all somatic and pre-meiotic germ cells.[14,15] The PGK2 gene is an autosomal gene whose expression becomes evident in the late stages of spermatogenesis[14-17] and may possibly be a retroposon.[15,18,19] The switch of expression from the PGK1 to PGK2 gene is considered to occur at a certain stage in spermatogenesis. The study of PGK genes should thus provide some clarification of the mechanism by which sperm-specific gene expression is regulated. As a first step in the clarification of this point, an examination was made to determine whether change in the concentration of c-myc protein in a mouse cultured cell line would affect the expression of PGK genes.

Materials and Methods

Cell Culture The present study used the cell line 38-2, established by introducing a rat c-myc gene attached to a promoter of the human metallothionein IIA gene into mouse erythroleukemia cells.[13] It was cultured in ES medium (Nissui) with 10% calf serum. Expression of the c-myc gene was induced by culturing 38-2 cells in the presence of 0.2 M $ZnCl_2$.

Analysis of Nucleic Acid Deoxyribonucleic acid (DNA) was prepared from 38-2 cells by phenol extraction, and 20 μg of DNA was cleaved with various restriction enzymes. Digested DNA was separated in a 0.7% agarose gel, blotted onto a nitrocellulose filter and hybridized with nick-translated pGKM65b, a complementary DNA clone of the mouse PGK1 gene[20] under standard conditions.[21] Total ribonucleic acid (RNA) was extracted from 38-2 cells or mouse organs by homogenizing them in a buffer containing phenol and was precipitated with ethanol. PolyA^+ RNA was enriched by collecting RNA bound to oligo(dT)-cellulose.[21] RNA was denatured and separated in a formaldehyde-containing agarose gel,[22] and blotted onto a nitrocellulose filter. The filter was pre-hybridized with a solution containing 5X SSC (1X SSC = 0.15 M NaCl, 0.015 M sodium citrate), 20 mM sodium phosphate, pH 7.0, 0.2% bovine serum albumin, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 7% sodium dodecyl sulfate (SDS) and 100 μg/ml denatured salmon sperm DNA at 50 °C for 2 h. Hybridization was then conducted at 50 °C for 12 h after adding 10% dextran sulfate and 5 ng/ml ^{32}P-labeled synthetic oligonucleotides. The filter was washed with a solution containing 1X SSC and 1% SDS at 50 °C for 5 min. The washed filter was exposed to Kodak XAR film using an intensifying screen at −80 °C. Oligonucleotides were synthesized with Beckman System Plus 1. PGK1 oligo contains the sequence, 5′-GGAAAACAGCAGGTTAGCTTAGGAGCACAGGAACCAAAGGCAGGAAAGAA-3′ and PGK2 oligo, the sequence, 5′-TGAGTTGGTTCTGGTCCTGTGCGCAGGAAACAGGAAGCAAGTACATTATG-3′. These sequences correspond to those of the 3′-untranslated region of mouse PGK1[23] and PGK2[18] genes. They were labeled at the 5′-ends with [γ-^{32}P] adenosine triphosphate (ATP) and T4 polynucleotide kinase and used as probes for hybridization.

Results

We first examined the genomic organization of PGK1 and PGK2 genes in 38-2 cells. Cellular DNA was extracted, cleaved with restriction enzymes and analyzed by blot hybridization with a cDNA clone of the mouse PGK1 gene that has been shown to pick up both PGK1 and PGK2

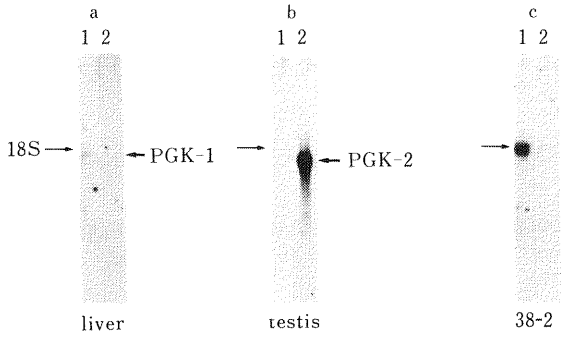

Fig. 1. Blot Hybridization of RNA Prepared from Various Tissues with PGK1 and PGK2 Probes

Nitrocellulose filters containing 16 μg of mouse liver RNA (a), 10 μg of mouse testis RNA (b) and 10 μg of 38-2 cell RNA (c) were hybridized with PGK1 (lane 1) and PGK2 (lane 2) probes. An arrow at the left of each panel indicates the position of 18S ribosomal RNA.

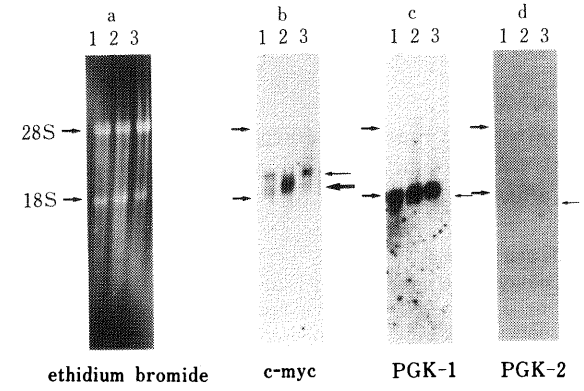

Fig. 2. Blot Hybridization of 38-2 Cell RNA with Various Probes

Lanes 1—3 contain 10 μg of oligo(dT)-bound RNA prepared from 38-2 cells cultured without $ZnCl_2$, for 16 h with $ZnCl_2$, and for 5 h following removal of $ZnCl_2$, respectively. Panel a shows an agarose gel stained with ethidium bromide. Panels b—d show autoradiograms of nitrocellulose filters after hybridization with the probes indicated at the bottom. A plasmid PM2A containing the second exon of rat c-myc gene[13] was used as a probe for detecting c-myc mRNA. Arrows at the left of each panel indicate the positions of 28S and 18S ribosomal RNAs. Arrows at the right show expected positions of each mRNA. Small and large arrows at the right of panel b indicate the positions of mRNAs transcribed from endogenous (mouse) and exogenous (rat) c-myc genes, respectively.

genomic fragments. Both genes retained normal organization in 38-2 cells as previously reported[19] (data not shown). We chose synthetic oligonucleotides as probes to detect messenger ribonucleic acids (mRNAs) for PGK1 and PGK2 individually. When two probes were hybridized to the filter containing RNA of mouse liver and testis tissues, the PGK1 and PGK2 probes specifically picked up an about 1.6 kilobase band from RNA of liver and testis, respectively (Fig. 1a and 1b), indicating both probes to function preferentially. RNA of 38-2 cells was then examined under these conditions. Without inducing the c-myc gene, PGK1 mRNA was found to be predominantly present in 38-2 cells and no PGK2 mRNA could be detected even after a longer period of exposure (Fig. 1c). An examination was then made to determine whether the expression of the PGK1 and PGK2 genes would change in response to that of the c-myc gene in 38-2 cells. Expression of the latter was induced by adding 0.2 M $ZnCl_2$ to the medium. RNA was prepared and analyzed from 38-2 cells cultured without $ZnCl_2$, for 16 h with the metal and for 5 h following removal of the metal. The amount of c-myc mRNA transcribed from the exogenously introduced rat c-myc gene increased after addition of the metal and decreased after washing it out, while mRNA of the endogenous mouse c-myc gene showed no change in its amount (Fig. 2b). PGK1 mRNA appeared to be maintained at an essentially constant level during the increase and decrease in the amount of c-myc mRNA (Fig. 2c). No PGK2 mRNA in 38-2 cells could be detected under any of the culture conditions (Fig. 2d).

Discussion

Expression of the PGK1 gene is considered to cease at about the meiotic stage in spermatogenesis and the PGK2 gene to become active in post-meiotic cells. Cessation of PGK1 gene expression is thought by some investigators to resemble X-chromosome inactivation in the early stages of development.[24] There are many other genes whose expression is altered during spermatogenesis.[2] Clarification of the mechanism that controls the differential expression of two PGK genes in the testis might assist a general understanding of the regulation of mammalian spermatogenic gene expression. Among several proto-oncogenes expressed during spermatogenesis c-myc is noteworthy, because constitutive expression of c-myc gene has been shown to block cell differentiation.[12,13] The mechanism by which c-myc expression controls differentiation could partly involve a function of c-myc protein in regulation of gene expression. Targets of c-myc protein in such a mechanism are, however, not clear. The level of c-myc expression has been found to decrease with the progress of spermatogenesis.[9,10] These observations led us to examine the possibility that a change of c-myc expression affects the expression of the two PGK genes, which behave differently during spermatogenesis.

In the present study, we examined whether a change in the concentration of c-myc protein would induce a switch of expression of the two PGK genes. The data obtained indicate that c-myc protein alone does not affect PGK gene expression. We propose two possible explanations for this. One is that other changes are necessary for this switch in addition to the decrease in c-myc protein. The other is that mouse erythroleukemia cells possess no characteristics of testicular cells. To determine the applicable explanation, a cell line should be used in which other sperm-specific oncogenes can be manipulated as well as the c-myc gene. Another experiment which should be carried out is one where expression of the c-myc gene is altered in teratocarcinoma cells which may differentiate into testicular cells. Analysis of promoters of the two PGK genes is also being carried out to gain some understanding of the mechanism by which differential expression of the two PGK genes is regulated during mammalian spermatogenesis.

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