Human Phosphoglycerate Kinase and Inactivation of the X Chromosome

Abstract. *The fibroblasts derived from the skin of a woman heterozygous for an X-linked deficiency of phosphoglycerate kinase represented a mosaic. Two of 22 clones with normal glucose-6-phosphate dehydrogenase activity and hypoxanthine(guanine)phosphoribosyltransferase activity had no phosphoglycerate kinase activity detected by electrophoresis. Because the loci for glucose-6-phosphate dehydrogenase and hypoxanthine(guanine)phosphoribosyltransferase are already known to undergo inactivation and to be on the short arm of the X chromosome and the locus for phosphoglycerate kinase is on the long arm, these observations support the conclusion that the entire human X chromosome can be involved in X inactivation.*

The location of the structural gene for human phosphoglycerate kinase (PGK) (E.C. 2.7.2.3) on the X chromosome was established by pedigree analyses (*1, 2*) and by somatic cell hybridization studies (*3, 4*). The gene was further localized to the distal part of the long arm of the X chromosome (*4*). The PGK locus was recently reported to be X-linked in kangaroos (*5*) and to be involved in X inactivation in a fashion that suggested obligatory paternal inactivation as the usual mechanism for dosage compensation of X-linked genes in marsupials (*6*). Chen *et al.* (*2*) argued that the PGK locus also underwent inactivation in man because electrophoresis of hemolyzates of erythrocytes from women heterozygous for a variant structural allele did not show the hybrid pattern that would be expected if two different subunits of the same enzyme were produced in the same cell. However, because structural (*7*) and somatic cell hybridization studies (*3*) had led to the conclusion that the functional unit of PGK was a monomer, this observation could not be advanced as proof for inactivation of the human PGK locus. The present investigation was designed to provide a conclusive answer by employing the approach originally devised by Davidson *et al.* (*8*) to examine clones obtained from a heterozygous cell strain.

A primary explant of diploid fibroblasts was obtained from the skin of a woman known to be heterozygous for severe PGK deficiency. Previous studies (*9*) had established that she must have received the mutant gene from her mother. She manifested a moderate, but asymptomatic, chronic hemolytic disorder, and had about 80 percent of normal PGK activity in her erythrocytes and about 60 percent in her leukocytes. Her son, affected by a severe nonspherocytic hemolytic disorder and a behavior disorder, had practically no PGK activity in his erythrocytes or leukocytes (*9*). His maternal second cousin had a similar illness and almost no PGK activity in fibroblasts derived from a skin explant (*3*).

Cloning was performed within the first ten subcultures after establishing the primary explant. The procedure described by Puck *et al.* (*10*) was used, but with Eagle's minimal essential medium (MEM) supplemented with nonessential amino acids, 5 percent fetal calf serum, and 5 percent calf serum. When each clone had grown to a convenient size (approximately 10^7 cells), it was analyzed as described below. The remaining cells were stored at −95°C in a mixture of MEM containing 5 percent glycerol and 20 percent fetal calf serum. Subsequent studies were performed on subcultures of these clones within 1 or 2 weeks after thawing.

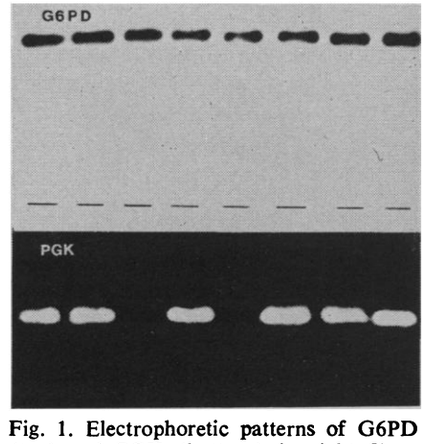

Fig. 1. Electrophoretic patterns of G6PD (top) and PGK (bottom) in eight fibroblast clones derived from the skin of a woman heterozygous for PGK deficiency (PGK^+/PGK^-). The clones in the corresponding channels are the same. The two determinations were performed on the same day with the same cell lysates. Clones in channels 3 and 5 (from the left) have normal G6PD activity, but no PGK activity, as would be expected if they are derived from cells in which the functional X chromosome bears the mutant gene for PGK.

The electrophoretic mobilities of PGK and of another well-known human X chromosome marker, glucose-6-phosphate dehydrogenase (G6PD) (E.C. 1.1.1.49), were determined for each of the 22 clones obtained. The additional X-linked enzyme marker served as an independent proof for the presence of a genetically active X chromosome. This enzymatic activity also permitted evaluation of the state of preservation of the cell lysates because G6PD had been shown to be much more labile than PGK in human fibroblasts. Cells were lysed by sonication. Electrophoresis was performed on cellulose acetate gel strips (Cellogel) as described by Meera Khan (*11*). About 3×10^6 cells from each clone were used for each experiment. Experiments were performed at least in duplicate.

All but two of the clones exhibited normal electrophoretic patterns and normal amounts of PGK and of G6PD activity. These two clones, each with normal G6PD, were completely deficient in PGK activity (Fig. 1). The clones lacking PGK activity were also evaluated for a third X-linked marker, hypoxanthine(guanine)phosphoribosyltransferase (HGPRT) (E.C. 2.4.2.8). Representative samples of single cells from each of these clones incorporated tritiated hypoxanthine normally when examined according to the method of Rosenbloom *et al.* (*12*). The entire series of clones was found to exhibit the same enzymatic properties when reexamined in subcultures that were established anew from samples of cells that had been stored frozen for 3 months. Chromosomal studies of the two PGK-negative clones revealed a normal female diploid complement in all of the ten metaphases analyzed per clone.

Thus, the fibroblastic strain derived from a woman known to be heterozygous for X-linked PGK deficiency was a mosaic composed of cells with normal enzyme activity and cells with complete enzyme deficiency. This finding could not be attributed to sporadic deterioration of the cell lysates in view of the consistent results of enzyme analyses on different cell populations derived from the same clones upon thawing of separate frozen samples. The presence of normal G6PD

and HGPRT activities in the two clones that were PGK-negative excluded the accidental loss of the entire active X chromosome. A partial deletion of the X chromosome, involving only the PGK locus, might be postulated because of the peripheral location of this locus on the long arm of the X chromosome. The finding of a normal karyotype for both PGK-negative clones was at least evidence against the occurrence of a large deletion. In order to account for complete deficiency of enzyme activity in cell lysates from different subcultures of both clones, a deletion should already have been present at the very beginning of the cloning procedure, that is, in two of the individual cells that gave rise to the 22 clones examined. Spontaneous chromosome breaks have never been reported in freshly established diploid cultures of human fibroblasts.

The most obvious interpretation of the data, therefore, is that only one of the two PGK alleles is expressed in each cell. Consequently, the structural gene for human PGK is involved in X chromosome inactivation, as is true with all of the other X-linked loci that have been investigated by cloning [G6PD (*8*), Hunter's locus (*13*), HGPRT (*14*), and α-galactosidase (*15*)]. Contrary to what takes place in marsupials, preferential inactivation of the paternal PGK locus does not appear to occur in man. A conclusion cannot be drawn at this stage, however, as to whether the excess of clones bearing an active paternal X chromosome reflects a situation already existing in vivo or is the result of selection in vitro. This question can best be examined by cloning cells from a heterozygote for two alleles with the same biological fitness, such as the carriers of the electrophoretic variants described by Chen *et al.* (*2*). Unfortunately, these subjects were not available for the present study. The present data and the variable expression of PGK deficiency in other tissues of our heterozygous donor are consistent with the current hypothesis of random X chromosome inactivation at an early stage of embryonic development as the usual mechanism for dose compensation of X-linked genes in man (*16*, *17*).

The demonstration that the PGK locus undergoes inactivation is of particular interest in view of its known chromosomal location. This investigation, to our knowledge, provides the first evidence that the long arm of the normal human X chromosome is involved in X inactivation. Moreover, it seems likely that two of the other loci involved in X inactivation (G6PD and HGPRT) are located on the short arm of the human X chromosome (*4*) at an appreciable distance from one another, as suggested by the high incidence of meiotic recombination between them (*18*) and by their mitotic separation in somatic cell hybrids (*19*). Thus, the conclusion emerges that the entire human X chromosome may, indeed, be involved in the mechanism of X inactivation. This hypothesis (*16*) and its numerous variations (*20*) will be more easily evaluated when a good cytological map of the human X chromosome becomes available.

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Sexual Receptivity: Facilitation by Medial Preoptic Lesions in Female Rats

Abstract. *Lesions in the medial preoptic area of ovariectomized female rats reduced the quantity of estrogen needed to induce sexual receptivity in these animals. In addition, the number of days over which receptive behavior could be elicited after a single initial estrogen injection and with subsequent daily progesterone treatment was significantly increased by lesions in the medial preoptic area. These findings support the view that estrogen acts to reduce an inhibitory action that is tonically exerted by the medial preoptic area on pathways mediating estrous behavior.*

In most mammals female sexual behavior is characterized by a cyclic responsiveness to sexual approaches by the male that vary between receptivity and rejection. For some time it has been known that these changes in female sexual activity are produced by the interaction of varying amounts of steroid hormones and the nervous system. Hormonal, neural, and behavioral factors that influence sexual receptivity have been studied most extensively in the rat. In this species the medial preoptic–anterior hypothalamic region is considered a critical integrative center for mediating sexual receptivity (*1*). Long-term implants of estradiol seem most effective in reinstating behavioral estrus when placed in this area (*2*) and preferential uptake of radioactively labeled estradiol