78. Synteny of the Genes for Adenylate Kinase and Phosphoglucomutase in the Rat and their Assignment to Rat Chromosome 5

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Somatic cell hybridization has been useful as a technique for studying the assignment of genes in mammals. Most assignments have been made on the basis of the concordant segregation of gene markers and specific chromosomes in interspecific somatic cell hybrids. Individual or syntenic genes can be assigned to certain specific chromosomes by means of chromosome banding techniques useful for the chromosome identification retained in hybrid genomes. A previous study by the author (1978)\(^1\) has demonstrated that genes for enolase (ENO) and phosphogluconate dehydrogenase (PGD) are syntenic in the rat (Rattus norvegicus), and assigned to rat chromosome 5 in rat-mouse somatic hybrid cells in which rat chromosomes segregated. It has been known that in the laboratory mouse (Mus musculus) the genes for ENO and PGD are linked with the genes coding for adenylate kinase-2 (AK-2) and phosphoglucomutase-2 (PGM-2), and that they are located on chromosome 4.\(^2\) Based on the comparative chromosome banding studies of rat and mouse, the evidence has been provided that the banding pattern of rat chromosome 5 is identical to mouse chromosome 4.\(^1,3\) In review of the above evidence, an investigation has been undertaken to determine whether the structural loci for AK-2 and PGM-2 are syntenic with ENO and PGD and located on rat chromosome 5, use being made of rat-mouse somatic hybrids.

Materials and methods. Rat-mouse hybrid clones were obtained from 3 sets of somatic cell hybrid experiments by means of UV-inactivated HVJ mediated cell fusion (cf. Yoshida 1978).\(^1\) In brief, mouse thymidine kinase deficient C1-1D cells were fused with two rat primary fibroblastic lines derived from Fischer (F344) and Wistar (W) strains, as well as with a near diploid rat cell line deficient in hypoxanthine phosphoribosyl transferase established from Dunning leukemia cells of the F344 strain. In addition, rat-Mus caroli somatic cell hybrids were isolated and employed for chromosome and enzyme studies.\(^1\)
For isozyme analysis the cellulose acetate gel electrophoresis for ENO, PGD, AK-2, and PGM-2 was applied.\textsuperscript{2,4,5} Enzyme and chromosome analyses were carried out on parallel cultures of hybrid clones. Rat and mouse chromosomes were identified with 33258 Hoechst and quinacrine mustard fluorescence as described.\textsuperscript{6} A minimum of 20 metaphases were analyzed in each hybrid clone.

Results. Seventeen primary hybrid clones from 3 sets of rat-mouse crosses, and 10 primary clones of rat-\textit{Mus caroli} were analyzed for chromosome complements and isozyme expression. All clones here

![Image of electrophoretic patterns in rat-mouse hybrid cells]

Fig. 1. Electrophoretic patterns in rat-mouse hybrid cells. A. ENO: slot 1, mouse control; slot 2, rat control; slot 3, a hybrid clone negative for rat ENO; slot 4, a hybrid clone expressing rat ENO and a heteropolymer. B. PGD: slot 1, mouse control; slots 2 and 3, hybrid clones expressing rat PGD; slot 4, a hybrid clone with no expression of rat PGD; slot 5, rat control. C. AK-2: slot 1, mouse control; slot 2, an artificial mixture of rat and mouse cell extracts; slot 3, a hybrid clone with rat AK-2; slot 4, a hybrid clone negative for rat AK-2; slot 5, rat control. D. PGM-2: slot 1, \textit{Mus caroli} control; slot 2, an artificial mixture of rat and \textit{Mus caroli} cell extracts; slot 3, rat control; slot 4, a rat-\textit{Mus caroli} hybrid clone with rat PGM-2; slot 5, a hybrid negative for rat PGM-2. "O" indicates origin.
examined were proven to be hybrid, and they lost some chromosomes of the rat genomes. Based on the above features, rat and mouse isozymes could be easily scored following electrophoresis for ENO, PGD, AK-2 and PGM-2 (Fig. 1). The data summarized in Table I have demonstrated that the expression of ENO, PGD and AK-2 in the 17 rat-mouse hybrid clones segregates in concordance with rat chromosome 5. The segregation of PGM-2 as well as of rat chromosomes were investigated in the 10 primary hybrid clones from rat-Mus caroli. It was shown that PGM-2 segregated concordantly with chromosome 5 in these clones (Table I). The above results have shown a concordant segregation of rat chromosome 5, ENO, PGD, AK-2 and PGM-2. Since the genes for ENO and PGD were assigned to rat chromosome 5,1) the evidence presented is thus strongly supplementary for the syntenic relationships of the genes for ENO, PGD, AK-2 and PGM-2 and their assignment to rat chromosome 5.

Discussion. The data presented in this paper provide additional evidence for the assessment of the linkage relationship of homologous genes in different species. The genes for AK-2, ENO, PGD, and PGM-2 which is homologous to human PGM-1 have been shown to be syntenic in man and mouse, and mapped on the short arm of human chromosome 1, as well as on mouse chromosome 4.2),3) Taken together, these data are indicative of that three divergent species of man, mouse and rat have maintained the same linkage relationship of these genes. Since the chromosome 5 of the rat is morphologically similar to chromosome 4 of the mouse in their banding pattern,4),5) the above feature suggests that the banding pattern similarity reflects the conservation of the linkage relationship of homologous genes. In addition, homologous genes between man and nonprimates have been

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Table I. Concordant segregation of rat isozymes and chromosome 5 in rat-mouse primary hybrid clones

<table>
<thead>
<tr>
<th>Rat isozymes</th>
<th>ENO</th>
<th>PGD</th>
<th>AK-2</th>
<th>PGM-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat chromosome 5</td>
<td>+</td>
<td>9 0</td>
<td>9 0</td>
<td>8 0</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>0 8</td>
<td>0 8</td>
<td>0 2</td>
</tr>
</tbody>
</table>

† + and − indicate presence and absence, respectively. * For PGM-2, rat-Mus caroli hybrid clones were examined.

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assigned to human and primate chromosomes with banding pattern similarities.\(^*)\) However, it has remained unknown whether each homologous gene is located in the same order on each region matched for the banding patterns. While genes on the sex chromosome have been known to be conserved in a number of species,\(^7\) there are a few species in which the conservation of genes is shown on certain autosomal regions. The conservation of an autosomal linkage relationship has been demonstrated in tightly linked genes such as thymidine kinase and galactokinase gene pair in different species.\(^8\),\(^*)\) Although either the recombinational distance or the gene order of the four genes here concerned is unknown in the rat, PGM-2 and PDG are 24 map units apart in the mouse\(^10\) with a tentative gene order of centromere — AK-2 — PGM-2 — PDG, ENO.\(^2\) Further, the recombination fraction between the two loci is 51 in the human male\(^*)\) with the gene order of centromere — PGM — AK-2 — PDG, ENO.\(^*)\) Here the suggestion is possibly made that a large autosomal region has been conserved during evolutionary divergency.

**Summary.** In order to assess the syntenic relationship of genes in the laboratory rat (*Rattus norvegicus*) and mouse (*Mus musculus*), rat gene mapping is undertaken in rat-mouse somatic cell hybrids with the rat chromosome segregation. The genes coding for adenylate kinase-2 and phosphoglucomutase-2 are syntenic with enolase and phosphogluconate dehydrogenase, and assigned to rat chromosome 5. The four genes are syntenic in man and mouse, suggesting the conservation of the linkage relationship of homologous genes in the three divergent species.

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**References**


\(^*)\) See the footnote on p. 405.