Proc. Natl. Acad. Sci. USA
Vol. 75, No. 5, pp. 2382–2386, May 1978
Genetics

Homologous genes for enolase, phosphogluconate dehydrogenase, phosphoglucomutase, and adenylate kinase are syntenic on mouse chromosome 4 and human chromosome 1p

(hybrid cells/comparative mapping/enzyme electrophoresis/mouse gene mapping)

PETER A. LALLEY[†‡], UTA FRANCKE[§], AND JOHN D. MINNA[†¶]

[†] NCI-VA Medical Oncology Branch DCT/NCI/NIH, Washington VA Hospital, 50 Irving Street, Washington, D.C. 20422; and [§] Department of Pediatrics, University of California at San Diego, La Jolla, California 92093

Communicated by Victor A. McKusick, March 10, 1978

ABSTRACT **It is possible to generate interspecific somatic cell hybrids that preferentially segregate mouse chromosomes, thus making possible mapping of mouse genes. Therefore, comparison of the linkage relationships of homologous genes in man and mouse is now possible. Chinese hamster × mouse somatic cell hybrids segregating mouse chromosomes were tested for the expression of mouse enolase (ENO-1; EC 4.2.1.11, McKusick no. 17245), 6-phosphogluconate dehydrogenase [PGD; EC 1.1.1.44, McKusick no. 17220], phosphoglucomutase-2 (PGM-2; EC 2.7.5.1, McKusick no. 17190), and adenylate kinase-2 (AK-2; EC 2.7.4.3, McKusick no. 10302). In man, genes coding for the homologous forms of these enzymes have been assigned to the short arm of human chromosome 1. Analysis of 41 primary, independent, hybrid clones indicated that, in the mouse, ENO-1 and AK-2 are syntenic with PGD and PGM-2 and therefore can be assigned to mouse chromosome 4. In contrast, they were asyntenic with 21 other enzymes including mouse dipeptidase-1 (DIP-1, human PEP-C; EC 3.4.11.*, McKusick no. 17000) assigned to human chromosome arm 1q and mouse chromosome 1. Karyologic analysis confirmed this assignment. These data demonstrate that a large autosomal region (21 map units in the mouse and 51 map units in the human male) has been conserved in the evolution of mouse chromosome 4 and the short arm of human chromosome 1. Identification of such conserved regions will contribute to our understanding of the evolution of the mammalian genome and could suggest gene location by homology mapping.**

It has been suggested that, during vertebrate evolution, tetraploidization occurred 2–3 × 10^8 years ago with a resultant doubling of the chromosome number and DNA content and that chromosomal events that tend to preserve ancestral linkage groups such as Robertsonian fusions, inversions, and gene duplications have been among the favored pathways for the development of new species (1, 2). This hypothesis would imply that several ancestral linkage groups of homologous genes should have been conserved among mammalian species widely separated in evolution. Determination of which linkage groups have been conserved or disrupted over a wide range of species could yield information essential to understanding the evolution of the mammalian genome. Although conservation of X-linked genes is the rule in all mammalian species examined (1–5), equivalent genetic data to test this hypothesis for autosomes are not generally available. Progress in this area has been slow because of restrictions inherent in Mendelian genetics and the lack of useful homologous gene markers for comparison.

Interspecific somatic cell hybrids provide an alternative system for comparative gene mapping studies because it is now possible to generate somatic cell hybrids that preferentially segragate the chromosomes of several different species (6–9); a large number of gene markers are available for genetic analysis of somatic cells because many homologous enzymes can be distinguished electrophoretically in several species and concordant segregation of gene markers determines syntenic groups. In addition, concordant segregation of gene markers and specific chromosomes identified by banding techniques allows for gene assignments (10). Therefore, this parasexual approach used so successfully in human gene mapping can be utilized to investigate the linkage relationships of homologous genes in other species.

The genes coding for phosphogluconate dehydrogenase [PGD; 6-phospho-D-gluconate:$NADP^+$ 2-oxidoreductase (decarboxylating), EC 1.1.1.44], enolase-1 (ENO_1; 2-phospho-D-glycerate hydro-lyase, EC 4.2.1.11), phosphoglucomutase-1 (PGM_1; α-D-glucose-1,6-bisphosphate:α-D-glucose-1-phosphate phosphotransferase, EC 2.7.5.1), and adenylate kinase-2 (AK_2; ATP:AMP phosphotransferase, EC 2.7.4.3) are linked and located on the short arm of human chromosome 1 (11–14). In the mouse, the genes coding for PGD and PGM-2 (homologous to human PGM_1) are linked and located on mouse chromosome 4 (7, 15, 16). If this region of human chromosome 1p and mouse chromosome 4 has been conserved in evolution then, in the mouse, *Eno-1* and *Ak-2* should be linked to *Pgd* and *Pgm-2* and located on mouse chromosome 4. To investigate the linkage relationships of these genes, Chinese hamster × mouse somatic cell hybrids that segregate mouse chromosomes were analyzed cytogenetically and enzymatically for the expression of mouse ENO-1, AK-2, PGD, and PGM-2, as well as 21 other gene markers. The data demonstrate that, in the mouse, *Eno-1* and *Ak-2* are syntenic with *Pgd* and *Pgm-2* and can be assigned to chromosome 4. These data indicate that a large autosomal region, represented by a region of mouse chromosome 4 and the short arm of human chromosome 1, has been conserved through the 80 × 10^6 years of evolution that separate man and mouse. These findings will contribute to our understanding of the evolution of the mammalian genome.

MATERIALS AND METHODS

Cells. Primary, independent hybrid clones segregating mouse chromosomes were formed by fusing Chinese hamster (*Cricetulus griseus*) cells (clone E36) deficient in hypoxanthine phosphoribosyltransferase ($HPRT^-$) to normal mouse (*Mus musculus*) spleen and bone marrow cells. The preparation of

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: PGD, phosphogluconate dehydrogenase (EC 1.1.1.44); ENO, enolase (EC 4.2.1.11); PGM, phosphoglucomutase (EC 2.7.5.1); AK, adenylate kinase (EC 2.7.4.3); GDH, glucose dehydrogenase (EC 1.1.1.47) (human).

‡ Present address: Oak Ridge National Laboratory, Biology Division, Oak Ridge, TN 37830.

¶ To whom reprint requests should be addressed.

these hybrids has been described along with our standard tissue culture methods (7). The hybrids were tested 40–60 generations after fusion.

Enzyme Analyses. Mouse and Chinese hamster enzymes were distinguished by starch gel electrophoresis of cell extracts prepared as described (7, 17). All comparisons for one clone were made on a single homogenate preparation. Hybrid clones were analyzed for the enzymes listed in Table 1. PGD, PGM, and AK phenotypes were separated in a Tris/citrate, pH 7.0, buffer system (15) and visualized as described (15, 16, 18). ENO phenotypes were identified by using a citrate/phosphate, pH 6.8, buffer system according to the method of Chen and Giblett (19). When available, mouse enzyme nomenclature is used (20). When not available, human nomenclature has been substituted (21). Where there is disagreement, human nomenclature and the McKusick catalog number (22) are provided.

Chromosome Banding Analysis. Enzyme and chromosome analyses were carried out on parallel cultures of cell hybrids as described (23). Mouse and Chinese hamster chromosomes were identified by using Hoechst 33258 fluorescence and trypsin-Giemsa banding techniques. For each clone, 15–20 metaphases were scored for their mouse chromosome complement, with standard nomenclature (24). Clones (15% of total) with multiple chromosome fragmentation or rearrangements were not used for primary chromosome assignment analysis but are discussed separately. A clone was scored as positive (+) for a given chromosome if the chromosome was present in more than 15% of the metaphases analyzed, negative (−) if the chromosome was present in less than 5% of the cells, and indeterminate if the frequency was between 5% and 15%. The indeterminate class was not used for analysis and represented less than 3% of the total chromosome data.

RESULTS

Forty-one primary independent hybrid clones from five sets of mouse × Chinese hamster hybrids were tested for the presence of 24 mouse enzymes. Figs. 1–3 show the phenotypes used for scoring the hybrid cells for the mouse forms of PGM-2, PGD, ENO-1, and AK-2. The electrophoretic mobilities of mouse PGM and PGD and the assignment of *Pgm-2* and *Pgd* to mouse chromosome 4 have been reported (15, 16). Mouse PGM-2 is homologous to human PGM_1 which has been assigned to human chromosome 1 (11, 16).

Cultured mouse cells expressed one major band of ENO-1 activity that was easily distinguishable from Chinese hamster ENO-1 (Fig. 2). Artificial mixtures of cell extracts demonstrated the two parental bands whereas hybrids positive for mouse ENO-1 exhibited three bands of activity: the two parental forms and a single heteropolymer. This ENO phenotype is similar to that seen in mouse × human or Chinese hamster × human hybrids and indicates that ENO is a dimeric enzyme (11–14).

Starch gel electrophoresis separated AK activity in mouse cells into at least three bands of activity when ADP was used as the substrate (Fig. 3). The faster migrating anodal band is designated AK-1. It is found in mouse tissues and cultured cells as well as in erythrocytes, is inhibited by silver nitrate, and is homologous to human AK_1 (18). The two slower migrating bands are designated AK-2. AK-2 is resistant to silver nitrate inhibition, is not expressed in erythrocytes and is homologous to human AK_2 (18, 25). No heteropolymers were observed in hybrids expressing either AK-1 or AK-2, indicating that both isozymes are monomers as has been shown in man (18, 25). Four mouse AK phenotypes were observed in hybrid clones ($AK\text{-}1^+$, $AK\text{-}2^+$; $AK\text{-}1^+$, $AK\text{-}2^-$; $AK\text{-}1^-$, $AK\text{-}2^+$; and $AK\text{-}1^-$, $AK\text{-}2^-$), indicating that the two isozymes are coded for by two independent and unlinked genes. Recently, we have assigned the gene coding for AK-1 to mouse chromosome 2 (23).

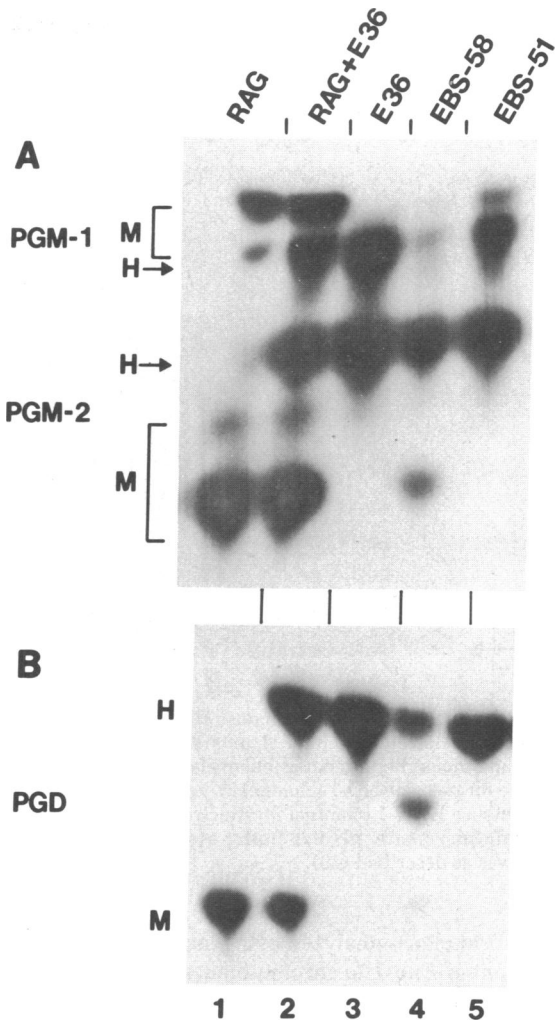

FIG. 1. Starch gel electrophoresis of PGM-1 and -2 (*A*) and PGD (*B*), showing mouse (M) enzymes in RAG cells (channel 1), Chinese hamster (H) enzymes in E36 cells (channel 3), an artificial mixture of mouse and Chinese hamster cell extracts (channel 2), a hybrid clone that expresses mouse PGM-2 and PGD (channel 4); and a hybrid negative for PGM-2 and PGD (channel 5). Electrophoresis was performed in a Tris/citrate, pH 7.0, buffer system; staining for enzyme activity was as described (15, 16).

Assignment of ENO to Chromosome 4. The segregation of mouse ENO-1 was compared to the segregation of 23 different enzyme markers. A positive correlation was noted for the segregation of ENO-1, PGD, PGM-2, and AK-2 (Table 1). Because *Pgd* and *Pgm-2* have been assigned to mouse chromosome 4 (15, 16), these data indicate that *Eno-1* can be assigned to chromosome 4. One discordant clone was noted that was positive for ENO-1 and PGD but negative for PGM-2, indicating that this clone contained a broken chromosome 4. When this clone (EBS-75) was analyzed cytogenetically, no intact chromosome 4 was observed. In addition, EBS-75 contained several mouse chromosome fragments and therefore was not included in the chromosome data for primary gene assignments (23). The results presented in Table 2 demonstrate a perfect correlation between ENO-1 and mouse chromosome 4 and high rates of discordancy between ENO-1 and all other mouse chromosomes

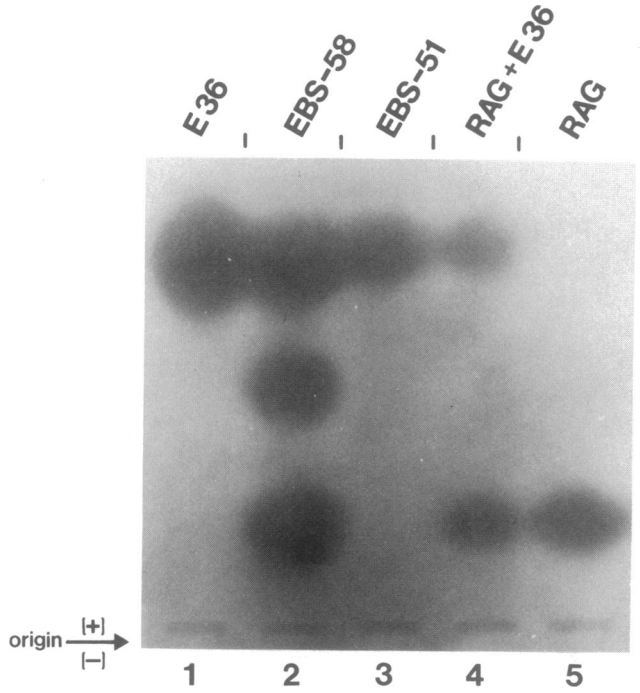

FIG. 2. ENO-1 zymogram of mouse RAG cells (channel 5), Chinese hamster E36 cells (channel 1), an artificial mixture of Chinese hamster and mouse homogenates (channel 4), a hyrbid (EBS-58) that is positive for mouse ENO-1 (channel 2), and a hybrid (EBS-51) that has lost mouse ENO-1 (channel 3). Electrophoresis was carried out in a citrate/phosphate, pH 6.8, buffer system; staining for ENO-1 activity was as described (19).

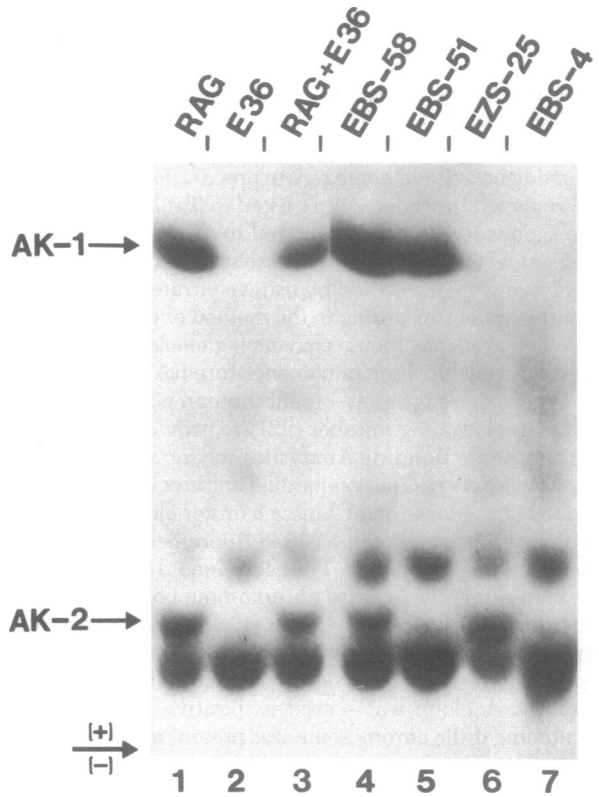

FIG. 3. Electrophoretic pattern of AK-1 and AK-2 after electrophoresis in a Tris/citrate, pH 7.0, buffer system (15) and staining for enzyme activity (18). Channels: 1, mouse AK-1 and AK-2 in RAG cells; 2, Chinese hamster AK in E36 cells; 3, a mixture of mouse and Chinese hamster cell extracts; 4, hybrid clone AK-1$^+$, AK-2$^+$; 5, hybrid clone AK-1$^+$, AK-2$^-$; 6, hybrid clone AK-1$^-$, AK-2$^+$; and 7, AK-1$^-$, AK-2$^-$.

in 25 hybrid clones analyzed cytogenetically, confirming the assignment of *Eno-1* to chromosome 4.

Assignment of AK-2 to Chromosome 4. AK-2 also segregated concordantly with ENO-1, indicating that *Ak-2* may be located on mouse chromosome 4 (Table 1). Only three discordant clones were found. In contrast AK-2 and ENO-1 were highly discordant with all other markers not assigned to chromosome 4. When AK-2 was compared to the segregation of PGD and PGM-2, a similar pattern was observed (Table 3).

The segregation of AK-2 and mouse chromosomes was compared in 23 primary hybrid clones (Table 2). AK-2 segregated discordantly with all the chromosomes except chromosome 4, indicating that *Ak-2* is on chromosome 4. Only two clones were discordant for AK-2 and chromosome 4. One clone was AK-2$^+$ but negative for chromosome 4, PGD, ENO-1, and PGM-2. This discordancy is probably due to chromosome breakage. The other discordant clone, which was AK-2$^-$ but positive for chromosome 4 and for PGD, ENO-1, and PGM-2, may reflect the sensitivity of the AK-2 assay because chromosome 4 was present in only 35% of the cells in this clone. In our system, AK-2 is the least sensitive of the four enzymes. In all clones scored positive for AK-2 and chromosome 4, an intact chromosome 4 was present in at least 55% of metaphases. The frequency for chromosome 4 in the nine positive clones ranged from 55 to 93%.

Table 3 summarizes the phenotypes for all the primary hybrid clones tested for the four enzymes. One discordant clone (PGD$^+$, ENO-1$^+$, PGM-2$^+$, AK-2$^-$) is probably due to a difference in the sensitivity of the enzyme assays; the other two discordancies apparently result from spontaneous fractures of chromosome 4.

DISCUSSION

The utilization of the somatic cell hybrid technique for mouse gene mapping studies is now an established and reliable alternative to Mendelian genetic techniques (7, 9, 23). This approach is particularly useful when genetic variants are not available, as in the case of mouse ENO-1 and AK-2. The data presented in this report demonstrate that *Eno-1* and *Ak-2* are syntenic with *Pgd* and *Pgm-2* and can be assigned to mouse chromosome 4. Genetic data from breeding studies established the gene order as: centromere–*Pgm-2*–*Pgd* (16). The combination of these data with the somatic cell hybrid data from this study suggests a tentative gene order of centromere–*Ak-2*–*Pgm-2*–(*Pgd*, *Eno-1*). However, more chromosome breakage data are needed to establish firmly the gene order of this linkage group in the mouse.

The data reported here are of considerable significance for comparative gene mapping. In man, *PGD*, ENO_1, PGM_1 (homologous to mouse *Pgm-2*), and AK_2 have been assigned to the short arm of human chromosome 1 (11–14). Thus, a large autosomal region has apparently been conserved through the 80×10^6 years of evolution that separate mouse and man. Although in man the linear order of these four genes has not been firmly established, the available evidence indicates the following order from the centromere distally: centromere–PGM_1–AK_2–(ENO_1, *PGD*) (31). The mean (±SD) recombination frequency of *Pgd* and *Pgm-2* in the mouse is 24 ± 4% (16). In the human male the recombination fraction between the two loci is estimated to be 51 centimorgans (cM) by sum-

Table 1. Segregation of ENO-1 with 23 enzyme markers

Enzyme	Chromosome	Enolase/marker, no. of clones +/+	+/−	−/+	−/−	% discordant
DIP-1	1	18	3	10	10	32
ID-1	1	12	4	7	9	34
AK-1	2	17	4	10	9	35
PGD	4	21	0	0	20	0
PGM-2	4	20	1	0	20	2
PGM-1	5	15	2	8	8	20
GPI	7	20	1	12	8	32
APRT	8	18	6	2	13	21
MOD-1	9	16	5	6	14	27
MPI	9	16	5	7	13	29
TRIP-1	10	19	2	7	13	22
ACP-1	12	18	3	12	8	37
ES-10	14	12	9	10	10	46
NP-1	14	11	8	10	10	45
DIP-2	18	12	6	6	14	32
α-GAL	X	15	1	13	3	44
HPRT	X	17	1	17	0	51
AK-2	—	18	2	1	17	8
ID-2	—	10	0	7	7	29
LDH-A	—	20	1	12	8	32
PEP-D	—	19	1	11	7	32
PEP-S	—	13	4	8	12	32
PK-3	—	16	5	7	13	29

A total of 41 primary independent hybrid clones were scored for enolase and 23 other mouse enzyme markers. The chromosome assignments are as previously summarized (19, 22). χ^2 for ENO-1 vs PGD = 47.41; PGM-2 = 41.33; AK-2 = 23.70; $P < 0.001$. The enzyme markers analyzed by starch gel electrophoresis were: α-GAL, α-galactosidase (EC 3.2.1.22); ACP-1, erythrocyte acid phosphatase-1 (EC 3.1.3.2); AK-1 and AK-2, adenylate kinase-1 and -2 (EC 2.7.4.3); APRT, adenine phosphoribosyltransferase (EC 2.4.2.7); ENO-1, enolase-1 (EC 4.2.1.11); ES-10, esterase-10 (ESD in human nomenclature) (EC 3.1.1.1; McKusick no. 13328); GPI-1, glucosephosphate isomerase-1 (EC 5.3.1.9); GR-1, glutathione reductase-1 (GSR in human nomenclature) (EC 1.6.4.2; McKusick nos. 13830); HPRT, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); ID-1 and ID-2, isocitrate dehydrogenase-1 and -2 in mouse nomenclature (IDH-1 and IDH-M in human nomenclature) (EC 1.1.1.42; McKusick nos. 14770 and 14765); LDH-A, lactate dehydrogenase-A (EC 1.1.1.27); MOD-1, malic enzyme soluble form (ME-1 in human nomenclature) (EC 1.1.1.40; McKusick no. 15425); MPI-1, mannosephosphate isomerase-1 (EC 5.3.1.8); NP-1, purine-nucleoside phosphorylase (EC 2.4.2.1); DIP-1, dipeptidase-1 (PEPC in human nomenclature) (EC 3.4.11.*; McKusick no. 17000); DIP-2, dipeptidase-2 (PEPA in human nomenclature) (EC 3.3.11.*; McKusick no. 16980); TRIP-1, tripeptidase-1 (PEPB in human nomenclature) (EC 3.4.11.*; McKusick no. 16990); PEPD, peptidase D (EC 3.4.13.9); PEPS, peptidase S (EC 3.4.11.*); PGD, 6-phosphogluconate dehydrogenase (EC 1.1.1.44; McKusick no. 17220); PGM-1, phosphoglucomutase-1 (PGM_2 in human nomenclature) (EC 2.7.5.1; McKusick no. 17200); PGM-2, phosphoglucomutase-2 (PGM_1 in human nomenclature) (EC 2.7.5.1; McKusick no. 17190); PK-3, pyruvate kinase-3 (PK_{m2} in human nomenclature) (EC 2.7.1.40; McKusick no. 17905) (7, 15, 17–19, 22, 26–30).

mation (5, 31). Whether this difference in map distances represents chromosomal rearrangements, such as inversions, occurring during the evolution of mouse or man or species differences in recombination frequency remains to be determined.

A major problem in comparative gene mapping is identifying the homologous proteins or phenotypes in different species. In the case of enzymes this can be done by comparison of primary amino acid sequences, subunit structure, tissue distribution, intracellular location, formation of heteropolymeric enzymes in hybrid cells, and biochemical characteristics of enzyme activity. The evidence for homology of mouse and human PGD and ENO-1 (ENO_1) (both dimers) is based on heteropolymer formation in hybrid cells. The evidence for homology of mouse PGM-2 and human PGM_1 and of mouse and human AK-2 (AK_2) is based on tissue distribution, substrate specificity, and inhibition studies (15, 16, 18, 25).

Table 2. Segregation of ENO-1, AK-2, and mouse chromosomes in primary hybrid clones

Chromosome	ENO-1 segregation Concordant	ENO-1 segregation Discordant	AK-2 segregation Concordant	AK-2 segregation Discordant
1	15	10	15	8
2	13	12	10	13
3	14	10	11	11
4	24	0	20	2
5	13	9	11	9
6	17	8	16	7
7	15	10	13	10
8	17	7	14	8
9	16	8	15	7
10	15	8	13	8
11	14	11	12	11
12	13	12	13	10
13	15	10	14	9
14	13	9	14	6
15	12	13	12	11
16	15	10	15	8
17	16	9	15	8
18	18	7	14	9
19	18	7	15	8
X	12	13	12	11
Y	15	9	13	9

Standardized mouse chromosome nomenclature is used (24). The concordant column gives the number of primary clones in which the enzyme (ENO-1 or AK-2) and a specific chromosome were either present together or absent together. The discordant column gives the number of primary clones in which only the enzyme (ENO-1 or AK-2) or the chromosome was present.

The discovery that this linkage group is conserved in mouse and man has two important implications. First, it suggests that it is possible to define ancestral linkage groups that have been conserved among mammalian species widely separated in evolution. Evidence for other conserved linkages in divergent species has been reported (1, 3, 30, 32–34).

Second, it suggests that homologous human forms of other mouse genes mapped within this region of mouse chromosome 4 may also be found on human chromosome 1p. Nine mouse genes affecting development, metabolism, or histocompatibility have been mapped within the *Pgm-2–Pgd* linkage group (32). We predict that, if human genes homologous to these mouse genes can be defined, they will be assigned to this region of human chromosome 1p. Conversely, we predict that the mouse

Table 3. Segregation of PGD, ENO-1, PGM-2, and AK-2 in primary hybrid clones

No. of clones	Enzymes PGD	ENO-1	PGM-2	AK-2
18	+	+	+	+
17	−	−	−	−
1	+	+	+	−
1	+	+	−	−
1	−	−	−	+

Thirty-eight independent hybrid clones were scored for the presence (+) or absence (−) of mouse ENO-1, PGD, PGM-2, and AK-2.

genes homologous to other human genes mapping in the PGD-PGM_1 region of human chromosome 1p, such as the genes for uridine monophosphate kinase and α-fucosidase, will be assigned to mouse chromosome 4. It should be noted that recently the gene coding for glucose-6-phosphate dehydrogenase (GDH; D-glucose:NAD(P)$^+$1-oxidoreductase, EC 1.1.1.47) was assigned to human chromosome 1 (35). In the mouse, *Gpd* is closely linked to *Pgd* on mouse chromosome 4 (16), indicating that a fifth gene is conserved in this linkage group.

Comparative mapping by homology is accepted for the X chromosome (5), but few examples are known for conservation of autosomal regions. However, the data presented in this paper suggest that it may be possible to define autosomal regions that are conserved and thus suggest gene assignments by homology mapping for these regions. For example, tightly linked genes (i.e., less than 1 crossover unit apart) have a high probability of being linked in several species, as has been shown for thymidine kinase and galactokinase (36). Womack and Sharp (30) have reported finding a homology between linkage group V in the rat and mouse chromosome 8 which contains a cluster of esterase genes. In addition, homologous human and nonhuman primate genes have been assigned to human and primate chromosomes that are homologous by chromosome banding studies (3, 37).

Lack of conservation of linkage groups also provides useful information. Although the *GPD, PGD, ENO-1, PGM-1, AK-2* complex has apparently been conserved, peptidase C (*PEPC*), assigned to human chromosome 1q (11–14), is carried on mouse chromosome 1 (dipeptidase-1, *Dip-1*, homologous to human PEPC) (26). Using somatic cell hybrids, Finaz *et al.* (37) demonstrated that, in man and baboon, ENO_1, PGM_1 and *PEPC* are syntenic. However, in the African green monkey, ENO_1 and PGM_1 were assigned to chromosome 4 and *PEPC* was assigned to chromosome 13, two telocentric chromosomes that have banding patterns comparable to human chromosomes 1p and 1q, respectively. Taken together, these data suggest that it may be possible to trace genetically the evolutionary divergence of the mammalian gene map by using somatic cell hybrid analysis. Major questions one wishes to ask are: (*i*) Is conservation or rearrangement of linkage groups random (i.e., related to physical distance apart) or functional (i.e., related to selective advantage or disadvantage of a particular arrangement); and (*ii*) Did chromosomal rearrangements proceed independently during the evolution of rodents and primates or did they follow similar rules?

We thank S. Stephenson, T. Gregorio, M. Brown, J. Ivy, and W. Moss for assistance. U.F. is supported by U.S. Public Health Service Grant GM 21110 and by The National Foundation.

1. Ohno, S. (1973) *Nature* **244,** 259–262.
2. Comings, D. E. (1972) *Nature* **238,** 455–457.
3. Warburton, D. & Pearson, P. L. (1976) *Cytogenet. Cell Genet.* **16,** 75–82.
4. Chapman, V. M. & Shows, T. B. (1976) *Nature* **259,** 665–667.
5. McKusick, V. A. & Ruddle, F. H. (1977) *Science* **196,** 390–405.
6. Minna, J. D. & Coon, H. G. (1974) *Nature* **252,** 401–404.
7. Minna, J. D., Marshall, T. H. & Shaffer-Berman, P. V. (1975) *Somat. Cell Genet.* **1,** 355–369.
8. Minna, J. D., Marshall, T. H. & Shaffer-Berman, P. V. (1976) *Cytogenet. Cell Genet.* **16,** 422–426.
9. Kozak, C., Nichols, E. & Ruddle, F. H. (1975) *Somat. Cell Genet.* **1,** 371–382.
10. Ruddle, F. H. (1973) *Nature* **242,** 165–169.
11. Jongsma, A., Van Someren, H., Westerveld, A., Hagemeijer, A. & Pearson, P. L. (1973) *Humangenetik* **20,** 195–202.
12. Douglas, G. R., McAlpine, P. J. & Hamerton, J. L. (1973) *Proc. Natl. Acad. Sci. USA* **70,** 2737–2740.
13. Meera Khan, P., Doppert, B. A., Hagemeijer, A. & Westerveld, A. (1974) *Cytogenet. Cell Genet.* **13,** 130–136.
14. *Baltimore Conference (1975) Third International Workshop on Human Gene Mapping,* Birth Defects: Original Article Series XII 7 (1976) (The National Foundation, New York); and (1975) *Cytogenet. Cell Genet.* **16,** 1–452.
15. Shows, T. B., Ruddle, F. H. & Roderick, T. H. (1969) *Biochem. Genet.* **3,** 25–35.
16. Chapman, V. M. (1975) *Biochem. Genet.* **13,** 849–856.
17. Lalley, P. A., Rattazzi, M. C. & Shows, T. B. (1974) *Proc. Natl. Acad. Sci. USA* **71,** 1569–1573.
18. Wilson, D. E., Povey, S. & Harris, H. (1976) *Ann. Hum. Genet.* **39,** 305–313.
19. Chen, S.-H. & Giblett, E. (1975) *Ann. Hum. Genet.* **39,** 377–380.
20. Womack, J. E. (1976) *Mouse News Lett.* **55,** 6.
21. Giblett, E. (1976) *Cytogenet. Cell Genet.* **16,** 65–82.
22. McKusick, V. A. (1975) *Mendelian Inheritance in Man* (Johns Hopkins University, Baltimore, MD).
23. Francke, U., Lalley, P. A. & Minna, J. D. (1977) *Cytogenet. Cell Genet.* **19,** 57–84.
24. Nesbitt, M. N. & Francke, U. (1973) *Chromosoma* **14,** 145–158.
25. Bruns, G. A. P. & Regina, V. M. (1977) *Biochem. Genet.* **15,** 477–486.
26. Lewis, W. H. P. & Truslove, G. M. (1969) *Biochem. Genet.* **3,** 493–498.
27. Swallow, D., Povey, S. & Harris, H. (1973) *Ann. Hum. Genet.* **37,** 31–37.
28. Nichols, E. A. & Ruddle, F. H. (1973) *J. Histochem. Cytochem.* **21,** 1066–1081.
29. Harris, H. & Hopkinson, D. A. (1976) *Handbook of Enzyme Electrophoresis in Human Genetics* (American Elsevier, New York).
30. Womack, J. E. & Sharp, M. (1976) *Genetics* **82,** 665–675.
31. Cook, P. J. L. & Burgerhout, W. G. (1978) *Cytogenet. Cell Genet.*, in press.
32. Searle, A. G. (1977) *Mouse News Lett.* **56,** 6–23.
33. Lalley, P. A., Brown, J. A., Eddy, R. L., Haley, L. L., Byers, M. G., Goggin, A. P. & Shows, T. B. (1977) *Biochem. Genet.* **15,** 367–382.
34. Minna, J. D., Lalley, P. A. & Francke, U. (1976) *In Vitro* **12,** 726–733.
35. Hameister, H., Ropers, H.-H. & Grzeschik, K.-H. (1978) *Cytogenet. Cell Genet.*, in press.
36. Kozak, C. A. & Ruddle, F. H. (1977) *Somat. Cell Genet.* **3,** 121–134.
37. Finaz, C., Van Cong, N., Cochet, C., Frezel, J. & Grouchy, J. (1977) *Cytogenet. Cell Genet.* **18,** 160–164.