APPLICATION OF THE OVARIAN TERATOMA MAPPING METHOD IN THE MOUSE

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ABSTRACT

Murine ovarian teratomas were used to determine recombination percentages for gene-gene and centromere-gene intervals. Data were obtained utilizing a recombinant inbred strain, LTXBJ, and a number of newly developed LT/SvEi congenic strains. ——Centromere-gene recombination was measured at 11.3 ± 1.2% for the centromere of chromosome 7 – *Gpi-1* interval and 15.8 ± 2.4% for the centromere of chromosome 14 – *Np-1* interval using the ovarian teratoma method. The centromere – *Np-1* interval was measured at 26.5 ± 3.6% using a standard backcross involving the *Rb6Bnr* Robertsonian translocation as a centromere marker. ——To assess the accuracy of the ovarian teratoma mapping method, we compared the recombination frequency obtained for the *Mpi-1–Mod-1* interval on chromosome 9 using the ovarian teratoma method to that obtained using a standard backcross. The recombination percentage was 22.9 ± 5.4 using the ovarian teratoma method and 18.6 ± 3.3 using the backcross method, indicating that the two methods produce equivalent estimates of recombination. In addition, for centromere-gene intervals known to be more than 30 cM in length, the ovarian teratoma method was consistent with classical recombination methods, yielding high recombination percentages. We conclude from these results that the ovarian teratoma mapping method is a reliable method for estimating recombination frequencies and the most accurate method available for estimating centromere-gene recombination frequency in the mouse.

ALTHOUGH more than 450 genes have been positioned on the chromosome map of the laboratory mouse, one region on each of the chromosomes remains poorly defined in terms of recombination frequency: the region between the centromere and nearby genes. To date, two methods have been used to estimate centromere-gene recombination percentages. One method uses cytological centromere markers, such as Robertsonian translocations or centromeric heterochromatin polymorphisms, as codominant genetic (centromeric) markers in conventional linkage crosses (CATTANACH and MOSELEY 1973; LYON, BUTLER and KEMP 1968; EICHER *et al.* 1977; DAVISSON and RODERICK 1975). The centromere-gene recombination percentages obtained using Robertsonian translocations are suspect, however, because these chromosomal aberrations often cause disturbances in the crossing over process. In addition, the effect of Robertsonian translocations on recombination may depend on the chromosomes involved, the origin of the Robertsonian chromosome or both (CATTANACH and MOSELEY 1973; LYON, BUTLER and KEMP 1968). At present there are no data assessing the effect of centromeric heterochromatin on centromere-gene recombination.

The second method for determining centromere-gene recombination percentages utilizes ovarian teratomas. In man and mouse these tumors originate from oocytes that have completed the first meiotic division (MI) but not the second (MII); see Figure 1 (LINDER 1969; LINDER and POWER 1970; EPPIG *et al.* 1977; EICHER 1978, 1981). By determining the frequency of heterozygous teratomas in heterozygous females, we can estimate recombination frequency in a manner similar to that used in Neurospora tetrad and Drosophila attached-X analyses. The ovarian teratoma mapping method has been used to estimate the recombination frequency between the centromere of human chromosome 6 and the phosphoglucomutase-3 (PGM_3) locus (OTT *et al.* 1976a), and the centromere of mouse chromosome 7 and the glucose phosphate isomerase-1 locus (*Gpi-1*) (EICHER 1978).

In this paper, we demonstrate that the ovarian teratoma method and the

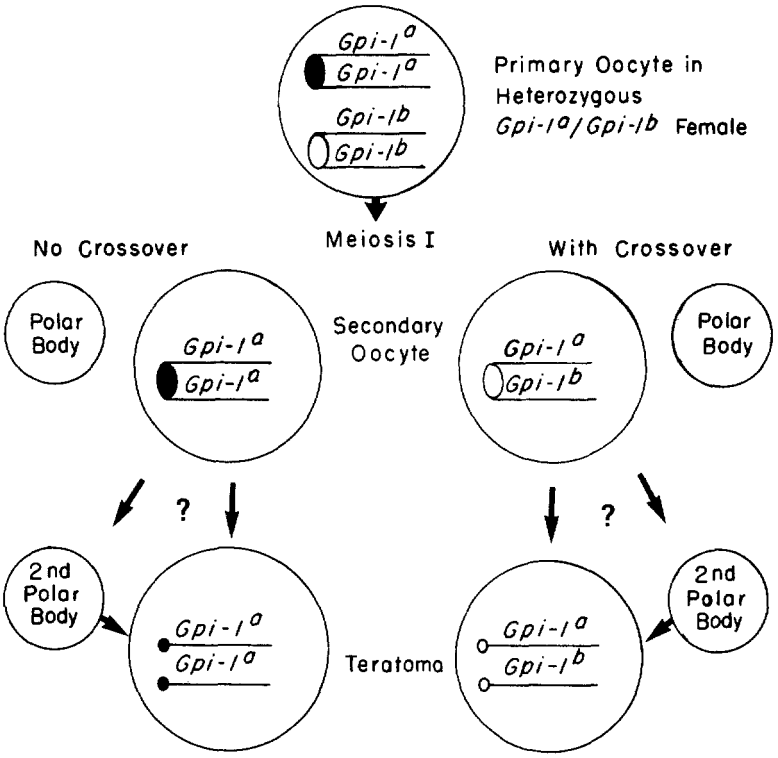

FIGURE 1.—Origin of ovarian teratomas from post-first meiotic division oocytes. If no crossover occurs between the gene *Gpi-1* and the centromere in a heterozygous oocyte ($Gpi\text{-}1^a/Gpi\text{-}1^b$), the secondary oocyte will contain two chromatids carrying either allele $Gpi\text{-}1^a$ or $Gpi\text{-}1^b$. Such a secondary oocyte will develop into a teratoma homozygous for the gene *Gpi-1*. If a crossover occurs in the heterozygous oocyte, the secondary oocyte will contain two chromatids, one carrying allele $Gpi\text{-}1^a$ and one carrying $Gpi\text{-}1^b$. This oocyte will develop into a heterozygous teratoma. Note that the centromere, if cytologically marked, will always be homozygous. Genetic and cytogenetic evidence cannot distinguish between the possible mechanisms by which the secondary oocyte results in a diploid teratoma. Either karyokinesis without cytokinesis or the fusion of the second polar body with the ovum will produce a diploid teratoma.

classical backcross method yield an equivalent gene-gene recombination frequency for the mannose phosphate isomerase-1 (*Mpi-1*)-supernatant malic enzyme (*Mod-1*) interval on chromosome 9. Data are presented that refine the chromosome 7 centromere – *Gpi-1* recombination percentage. In addition, ovarian teratomas are used to estimate the recombination frequency between the centromere of chromosome 14 and the nucleoside phosphorylase-1 (*Np-1*) locus. For comparison, the centromere – *Np-1* recombination frequency is measured using the Robertsonian translocation *Rb(9.14)6Bnr*.

MATERIALS AND METHODS

Mice

To use the ovarian teratoma mapping method, three conditions must be met: (1) females must develop ovarian teratomas at a reasonable frequency, (2) females must be heterozygous for the gene examined and (3) the gene of interest must be expressed and its product detectable in teratoma tissue.

Ovarian teratomas are very rare in the laboratory mouse except in the inbred strain LT/Sv, where 50% of the females develop ovarian teratomas by 3 months of age (STEVENS and VARNUM 1974). To obtain female mice that develop ovarian teratomas and are heterozygous for specific genes, we created a number of new LT/SvEi congenic strains (see FLAHERTY 1981 for review of methods to produce congenic strains). Each congenic strain was made by crossing LT/Sv mice to those of another strain (e.g., C57BL/6J, MA/MyJ) and mating the F_1 progeny back to LT/Sv. Mice of the N_2 and subsequent generations were typed for the genes of interest and appropriate heterozygous mice mated to LT/Sv. At the N_{10} backcross generation, each line was inbred by brother-sister matings to produce congenic strains homozygous for the alternative allele to that carried by LT/Sv. Table 1 lists the LT/SvEi congenic strains produced. Genes placed on the LT/Sv genetic background were codominant isozyme-encoding loci whose products were detectable in ovarian teratomas. In addition, the agouti (*A*) allele and two Robertsonian translocations, *Rb(5.15)4Lub* (*Rb4Lub*) and *Rb(7.18)9Lub* (*Rb9Lub*), were placed on the LT/Sv background.

To produce the data reported in this paper, the following congenic strains were used: LT.B6-*Pep-3*^{a}, LT.CALIF-*Pgd-1*^{a}, LT.CAST-*Pgm-1*^{b}, LT.B6-*Gpi-1*^{b}, LT.CAST-*Mpi-1*^{a}, LT.B6-*Mod-1*^{b}, LT.CAST-*Np-1*^{b} and LT.CAST-*Got-1*^{b}. In addition, a recombinant inbred (RI) strain, LTXBJ, kindly supplied by DR. L. C. STEVENS, was used in crosses to obtain *Gpi-1*^{a}/*Gpi-1*^{b} females. This RI strain, derived from LT/Sv and C57BL/6J, carried the *Gpi-1*^{b} allele.

Matings for determining recombination percentages

When the ovarian teratoma mapping method is employed, all recombination is measured in females. Therefore, to compare the ovarian teratoma method with the backcross method, all backcross progeny were produced using F_1 female parents.

Gene-gene interval (Mpi-1–Mod-1) F_1 females were produced by crossing mice from the LT.CAST-*Mpi-1*^{a} congenic strain to animals from the LT.B6-*Mod-1*^{b} congenic strain. These females were used as the source of ovarian teratomas or mated to LT/Sv (*Mpi-1*^{b} *Mod-1*^{a}/*Mpi-1*^{b} *Mod-1*^{a}) males to produce backcross progeny. Macroscopic ovarian teratomas were dissected from F_1 females 90 days of age and analyzed for *Mpi-1* and *Mod-1*. Backcross progeny were killed at 20–30 days of age and typed for *Mpi-1* and *Mod-1*.

Centromere-gene interval: Ovarian teratoma data for centromere-gene intervals were obtained from two sources: (1) females not needed for breeding during the development of the congenic strains (backcross generations N_2–N_{10}), and (2) females produced in matings between an LT/SvEi congenic strain and either LT/Sv or another LT/SvEi congenic strain. At 90 days of age, both types of females were killed by cervical dislocation and inspected for macroscopic ovarian teratomas. Teratomas were dissected and analyzed for the appropriate isozyme markers. Tissue from each teratoma-bearing female was analyzed to confirm that the female was heterozygous.

The chromosome 14 centromere – *Np-1* recombination frequency was obtained from a backcross using *Rb(9.14)6Bnr* (*Rb6*) as a centromere marker. F_1 females were produced by crossing mice from the LT.CAST-*Np-1*^{b} congenic strain with animals that were *Rb6 Np-1*^{a}/*Rb6 Np-1*^{a}. These females

TABLE 1

LT/SvEi Congenic stains

Gene name or translocation	Gene symbol (chromosome)	LT/SvEi congenic strain designation*
Isocitrate dehydrogenase-1	*Idh-1* (1)	LT.CAST-*Idh-1*^{c}
Peptidase-3	*Pep-3*** (1)	LT.B6-*Pep-3*^{a}
Agouti	*A* (2)	LT.CAST-*A*
Amylase-1	*Amy-1* (3)	LT.CAST-*Amy-1*^{b}
6-phosphogluconate dehydrogenase	*Pgd-1* (4)	LT.CALIF-*Pgd-1*^{a}
Phosphoglucomutase-1	*Pgm-1* (5)	LT.CAST-*Pgm-1*^{b}
Glucose phosphate isomerase-1	*Gpi-1* (7)	LT.B6-*Gpi-1*^{b}
Lactate dehydrogenase-1	*Ldh-1* (7)	LT-*Ldh-1*^{b}
Mannose phosphate isomerase-1	*Mpi-1* (9)	LT.CAST-*Mpi-1*^{a}
Malic enzyme-1 (supernatant)	*Mod-1* (9)	LT.B6-*Mod-1*^{b}
Nucleoside phosphorylase-1	*Np-1* (14)	LT.CAST-*Np-1*^{b}
Esterase-10	*Es-10* (14)	LT.MOL-*Es-10*^{c}
NAD alpha-glycerol-phosphate dehydrogenase-1	*Gdc-1* (15)	LT.CAST-*Gdc-1*^{d}
Glutamic-pyruvic transaminase-1	*Gpt-1* (15)	LT.CAST-*Gpt-1*^{c}
Glyoxalase-1	*Glo-1* (17)	LT.MA-*Glo-1*^{b}
Glutamate oxaloacetate transaminase-1	*Got-1* (19)	LT.CAST-*Got-1*^{b}
Phosphoglycerate kinase-1	*Pgk-1* (X)	LT-*Pgk-1*^{a}
Rb(7.18)9Lub	*Rb9* (7, 18)	LT-*Rb(7.18)9Lub*
Rb(5.15)4Lub	*Rb4* (5, 15)	LT-*Rb(5.15)4Lub*

All LT congenic strains contain the *LT* Y chromosome.

* The abbreviations for the strains of origin for the alleles are as follows: CAST = *Mus musculus castaneous*, B6 = C57BL/6J, MOL = *M. m. molossinus*, MA = MA/MyJ and CALIF = Californian mice. The *Pgk-1*^{a} allele originated in Danish wild mice. The Robertsonian translocations originated in wild European mice from northern Italy near Bergamo (Alpie Orobie).

** Formerly dipeptidase-1 (*Dip-1*).

were mated to LT.CAST-*Np-1*^{b} males to produce backcross progeny. This result was compared with the recombination frequency measured using the ovarian teratoma method.

Isozyme methods

The isozymes used in this study were PEP-3, PGD-1, PGM-1, GPI-1, MPI-1, MOD-1, NP-1 and GOT-1 (see Table 1). Red blood cells (RBCs), liver or teratomas were analyzed. Packed RBCs (1.6-cm length of a hematocrit tube) were lysed with 0.1 ml of hemolysate solution (1 mg of ethylenediamine tetraacetic acid, tetrasodium salt, per ml of distilled H_2O). Liver or teratoma tissue was stored frozen for up to 3 weeks at −20°. Samples were homogenized in distilled H_2O (v/v) and centrifuged at 27,713 × g for 30 min. Electrophoresis was performed directly on liver or teratoma supernatants, or RBC lysates. Electrophoresis was conducted on Titan III (Helena Laboratories) cellulose acetate plates using Helena Zip Zone electrophoresis apparatus. Electrophoretic conditions and staining methods used for GPI-1 were those of EICHER and WASHBURN (1978), for MPI-1 and MOD-1 those of EICHER *et al.* (1980), and for NP-1 those of WOMACK *et al.* (1977).

The tissue samples used to type animals for PEP-3, PGD-1 and PGM-1 were lysed RBCs and for GOT-1 were supernatants from homogenized liver. Tris-glycine buffer, pH 8.5 (3.0 g Trisma Base, Sigma T-1503, 14.4 g glycine, Sigma T-7126, per liter of distilled H_2O) was used for the electrophoresis buffer tanks and to presoak the Titan III cellulose acetate plates (Helena Laboratories) for all enzymes, except PGD-1. For PGD-1, a 0.1 M Tris buffer, titrated to pH 7.6 with citric acid, was used.

Stain components, nicotinamide adenine dinucleotide (NAD) (Sigma N-7004, 660 mg/ml), phenazinemethosulfate (PMS) (Sigma P-9625, 2.5 mg/ml), MTT tetrazolium (Sigma M-2128, 10 mg/ml), and nicotinamide adenine dinucleotide phosphate (NADP) (Sigma N-0505, 10 mg/ml) were prepared in advance and stored frozen. Stain buffers were either 0.2 M Tris, titrated to pH 8.0 with HCl, or a phosphate buffer, pH 7.0 (Fisher SO-B-108). Electrophoresis was conducted cathode to anode at 200 V, and the gels were stained utilizing an agar overlay.

GOT-1 was run for 30 min and then observed after staining with 3 ml of prepared stain mixture (100 ml of pH 7.0 phosphate buffer, 10 mg of pyridoxal 5'-phosphate, Sigma P-9255, 460 mg of L-aspartic acid, Sigma A-9256, and 260 mg of alpha-ketoglutarate, Sigma K-1750, adjusted to pH 7.4 with 2 N NaOH) plus 0.1 ml of saturated solution of fast blue BB (Sigma F-0250) and 2 ml of agar. PEP-3 was run for 30 min and then observed after staining with a mixture of 2 ml of pH 7.0 phosphate buffer, 0.03 ml of $MnCl_2$ (0.25 M), 0.06 ml of *Crotalus adamanteus* snake venom (Sigma V-6875, 10 mg/ml), 0.06 ml of peroxidase (Sigma P-8520, 10 mg/ml), 0.13 ml of peptide-L-leucyl-L-tyrosine (Sigma L-0501, 10 mg/ml) and 0.13 ml of O-dianisidine hydrochloride (Sigma D-3127, 1% solution), plus 2 ml of agar. PGD-1 was run for 15 min and then observed after staining with 2 ml of 0.2 M Tris, pH 8.0, 0.1 ml of $MgCl_2$, 0.1 ml of PMS, 0.1 ml of MTT, 0.1 ml of NADP and 0.2 ml of 6-phosphogluconic acid (Sigma P-6888, 20 mg/ml) plus 2 ml of agar. PGM-1 was run for 45 min and the gel stained with 2 ml of 0.2 M Tris, pH 8.0, 0.13 ml of $MgCl_2$, 0.13 ml of glucose-1-phosphate (Sigma G-7000, 100 mg/ml), 0.06 ml of PMS, 0.06 ml of MTT, 0.06 ml of NADP, 0.02 ml of alpha-D-glucose 1,6-diphosphate (Sigma G-5875, 10 mg/ml), 0.005 ml of glucose-6-phosphate dehydrogenase (Sigma G-8878) and 2 ml of agar.

Mitotic chromosomes

Bone marrow was flushed from the femur and processed as described by EICHER and WASHBURN (1978) for mitotic chromosomes from fetal liver.

Recombination determinations for centromere-gene intervals

Recombination frequency between a gene and its centromere (X) can be estimated from the frequency of heterozygous teratomas (Y) using the relation given by OTT *et al.* (1976a): $X = Y/2$. The quantity Y must be divided by 2 because the chromosomes of a teratoma are equivalent to those of the potential gamete plus the contents of the second polar body. (In a classical recombination experiment, progeny phenotypes are dependent on the contents of only one of the two chromatids present after MI.) The standard error for X is given by GREEN (1981) as:

$$\text{S.E.}_X = (1/2)\sqrt{Y(1 - Y)/N},$$

where N is the total number of teratomas. Because this formula assumes complete interference between the centromere and gene, the recombination fraction obtained is valid only if Y is less than 0.33. As the number of chiasmata between the gene and centromere increases, Y approaches ⅔ (OTT *et al.* 1976b).

Recombination determinations for gene-gene intervals

In the backcross method, the recombination fraction (X) measured between two genes is calculated as: $X = R/N$, where R equals the number of recombinant offspring and N equals the total number of offspring.

In the ovarian teratoma method, the recombination fraction (X) measured between two genes is calculated as: $X = Z/2 = R'/2N$, where Z equals the proportion of chromatids in the ovarian teratomas that are recombinants between the two genes. The quantity Z must be divided by 2 as previously discussed (see centromere-gene intervals). Z is equivalent to R' (the number of teratomas with recombinant chromatids in the gene-gene interval) divided by N (total number of teratomas).

Table 2 shows how to derive R'. R' is the sum of classes III, IV, IVa and twice class V. Because class IVa is phenotypically indistinguishable from class II, the number of teratomas in this class cannot be directly obtained. However, the number of teratomas in class IV should be equivalent to the number of teratomas in class IVa. Class V must be multiplied by 2 because each teratoma of this type represents two crossovers. Therefore, if the number of teratomas in class III equals J, the number of teratomas in class IV equals K, and the number of teratomas in class V equals L, then $R = J + 2(K + L)$. This formula requires that the gene order be known for the two genes relative to the centromere. The standard error for X is given by:

$$\begin{aligned}\text{S.E.}_X &= \sqrt{\text{Var}(f) + \text{Var}(g) + 2(\text{cov}_{fg})} \\ &= \sqrt{f(1 - f)/4N + g(1 - g)/N - fg/N},\end{aligned}$$

where f equals J/N and g equals $(K + L)/N$.

A consideration of multiple crossovers in the centromere-gene and gene-gene region is presented

TABLE 2

Segregation of Mpi-1 *and* Mod-1 *in ovarian teratomas*

Crossover	Class	Chromosomes of teratoma	Phenotype of teratoma
None	I	1/2 o--a--a--- o--a--a---	MPI-1A MOD-1A
		1/2 o--b--b--- o--b--b---	MPI-1B MOD-1B
Between the centromere and *Mpi-1*	II	1/1 o--a--a--- o--b--b---	MPI-1AB MOD-1AB
Between *Mpi-1* and *Mod-1*	III(J)	1/2 o--a--a--- o--a--b---	MPI-1A MOD-1AB
		1/2 o--b--a--- o--b--b---	MPI-1B MOD-1AB
Between the centromere and *Mpi-1* plus *Mpi-1* and *Mod-1*	IV(K)	1/4 o--a--b--- o--b--b---	MPI-1AB MOD-1B
		1/4 o--b--a--- o--a--a---	MPI-1AB MOD-1A
	IVa*	1/4 o--a--a--- o--b--b--- 1/4 o--a--b--- o--b--a---	MPI-1AB MOD-1AB
4-Strand double between *Mpi-1* and *Mod-1*	V(L)	1/2 o--a--b--- o--a--b---	MPI-1A MOD-1B
		1/2 o--b--a--- o--b--a---	MPI-1B MOD-1A

* Note: class IVa and II are phenotypically indistinguishable.

in the APPENDIX. Those probability values are difficult to apply unless a very large sample size is obtained.

RESULTS

Gene-gene interval

It is important to know whether the ovarian teratoma method is a reliable method for estimating recombination frequencies. The *Mpi-1* and *Mod-1* loci on

chromosome 9 (order: centromere – *Mpi-1* – *Mod-1*), were used to test the equivalence of the ovarian teratoma mapping method and the classical recombination mapping method. F_1 females were produced by crossing animals from the LT.CAST-*Mpi-1*^{a} congenic strain with those from the LT.B6-*Mod-1*^{b} congenic strain. These females were used for the teratoma mapping experiment or backcrossed to LT/Sv (*Mpi-1*^{b} *Mod-1*^{a}/*Mpi-1*^{b} *Mod-1*^{a}) males. The recombination percentage between *Mpi-1* and *Mod-1* was 18.6 ± 3.3 (Table 3).

The phenotypic distribution of 59 teratomas from *Mpi-1*^{a} *Mod-1*^{a}/*Mpi-1*^{b} *Mod-1*^{b} females is shown in Table 3. The crossover events that produced these teratoma phenotypes are shown in Table 2. The phenotypes MPI-1A MOD-1A and MPI-1B MOD-1B represent the parental (nonrecombinant) types. The phenotype MPI-1AB MOD-1AB represents crossovers between the centromere and *Mpi-1* plus ½ the crossovers between the centromere and *Mpi-1* and *Mpi-1* and *Mod-1*. A crossover event between *Mpi-1* and *Mod-1* produced the teratoma phenotypes MPI-1A MOD-1AB and MPI-1B MOD-1AB. The MPI-1AB MOD-1A and MPI-1AB MOD-1B phenotypes represent ½ the crossovers between the centromere and *Mpi-1* and *Mpi-1* and *Mod-1*. A four-strand double crossover between *Mpi-1* and *Mod-1* produced the teratoma phenotypes MPI-1A MOD-1B and MPI-1B MOD-1A. The recombination percentage between *Mpi-1* and *Mod-1* is 22.9 ± 5.4, using the formula $X = R'/2N = (J + 2(K + L))/2N = (1 + 26)/118$.

The estimate of recombination for the *Mpi-1–Mod-1* interval using the backcross method was 18.6 ± 3.3%, a value not different from the estimate of 22.9 ± 5.4% for the interval using the teratoma method ($P > 0.5$). We conclude from this experiment that the teratoma mapping method is a valid method for measuring genetic recombination percentages.

Genes unlinked to their centromeres

If the teratoma mapping method produces recombination percentages equivalent to classical recombination methods, we would not expect genes known to be more than 30 cM from the centromere to show linkage to the centromere using the ovarian teratoma mapping method. Table 4 shows data obtained for six genes (*Pep-3*, *Pgd-1*, *Pgm-1*, *Mpi-1*, *Mod-1* and *Got-1*) that meet this criterion. For each gene, the proportion of heterozygous teratomas (Y) obtained from heterozygous host females was greater than 0.50, well above the maximum of 0.33 needed to obtain a meaningful centromere-gene distance. These results are consistent with the known chromosomal location of these genes and suggest that recombination is not inhibited in oocytes that later become teratomas.

Centromere-gene intervals

Centromere-gene recombination frequencies were determined for the centromere of chromosome 7 – *Gpi-1* interval and the centromere of chromosome 14 – *Np-1* interval.

The GPI-1 phenotypes of 241 ovarian teratomas from heterozygous *Gpi-1*^{a}/*Gpi-1*^{b} females are listed in Table 5. Five of these teratomas had a GPI-1A plus GPI-1B band and lacked the heterodimeric band of a GPI-1AB phenotype. This pattern is identical with that produced by mixing GPI-1A with GPI-1B samples,

TABLE 3

Phenotypic distribution of Mpi-1 *and* Mod-1 *in backcross animals and ovarian teratomas*

Backcross:

Mpi-1^a Mod-1^a/Mpi-1^b Mod-1^b × Mpi-1^b Mod-1^a/Mpi-1^b Mod-1^a

Parental phenotypes		Recombinant phenotypes	
MPI-1AB MOD-1A	67	MPI-1AB MOD-1AB	13
MPI-1B MOD-1AB	47	MPI-1B MOD-1A	13
	114		$R = 26$

$$X = R/T = 26/140 = 18.6 \pm 3.3\%.$$

Ovarian teratomas from: *Mpi-1^a Mod-1^a/Mpi-1^b Mod-1^b* females*

Class†	Phenotype	
I	MPI-1A MOD-1A	7
	MPI-1B MOD-1B	1
II	MPI-1AB MOD-1AB	37
III	MPI-1A MOD-1AB	1
	MPI-1B MOD-1AB	0
IV	MPI-1AB MOD-1B	3
	MPI-1AB MOD-1A	7
V	MPI-1A MOD-1B	1
	MPI-1B MOD-1A	2
		59

$$X = Z/2 = R'/2N = (J + 2(K + L))/2N = (1 + 26)/118 = 22.9 \pm 5.4\%$$

* Note that class III (crossover *Mpi-1-Mod-1*) is less than class IV (crossover centromere - *Mpi-1* and *Mpi-1-Mod-1*). This may be a sampling artifact or a peculiar characteristic of chromosome 9 in the centromere - *Mpi-1* region favoring chiasmata in that region.

† See Table 2.

confirming that each was composed of two distinct teratomas. Teratomas of the phenotypes GPI-1AB plus GPI-1B or GPI-1AB plus GPI-1A were not found. The few teratomas composed of two distinct teratomas of like phenotype would not be detectable. However, the calculated recombination percentage would not significantly change because the total number of teratomas typed was large ($N = 241$). In this experiment, the recombination percentage between the centromere of chromosome 7 and *Gpi-1* is 11.4 ± 1.4 (55 recombinants out of 241 teratomas).

In two additional reports (EPPIG *et al.* 1977; EICHER 1978), the chromosome 7 centromere – *Gpi-1* interval was measured using the ovarian teratoma method. The data from these reports plus the present study are presented in Table 5. The combined recombination percentage between the centromere of chromosome 7 and *Gpi-1* is 11.3 ± 1.2 (65 recombinants out of 288 teratomas).

The NP-1 phenotypes of 95 ovarian teratomas from *Np-1^a/Np-1^b* females were distributed as follows: 37 were NP-1A, 28 were NP-1B and 30 were NP-1AB. The recombination percentage between the centromere of chromosome 14 and *Np-1* is 15.8 ± 2.4 based on 30 recombinants out of 95 teratomas.

TABLE 4

Phenotype of ovarian teratomas derived from females heterozygous for genes known to be at least 30 cM from the centromere

Host	Chromosome	Teratoma	Number of teratomas	Y*
PEP-3AB	1	PEP-3A	3	
		PEP-3B	13	0.52
		PEP-3AB	17	
			Total 33	
PGD-1AB	4	PGD-1A	8	
		PGD-1B	8	0.66
		PGD-1AB	31	
			Total 47	
PGM-1AB	5	PGM-1A	5	
		PGM-1B	2	0.65
		PGM-1AB	13	
			Total 20	
MPI-1AB	9	MPI-1A	13	
		MPI-1B	4	0.76
		MPI-1AB	55	
			Total 72	
MOD-1AB	9	MOD-1A	25	
		MOD-1B	8	0.61
		MOD-1AB	51	
			Total 84	
GOT-1AB	19	GOT-1A	6	
		GOT-1B	7	0.52
		GOT-1AB	14	
			Total 27	

* Y is the frequency of heterozygous teratomas.

To compare the centromere-gene recombination percentage obtained using the teratoma method with that obtained using a Robertsonian translocation as a centromeric marker, a backcross was made between *Rb6 Np-1^a*/+ *Np-1^b* females and + *Np-1^b*/+ *Np-1^b* males. Of 147 offspring analyzed, 32 were of the RB6 NP-1AB phenotype, 14 were RB6 NP-1B, 25 were + NP-1AB and 76 were + NP-1B. The recombination percentage for this cross is 26.5 ± 3.6. This backcross gave a different recombination percentage for the centromere-gene interval from the value of 15.8 estimated using the ovarian teratoma method ($P < 0.05$). This example illustrates that genetic recombination percentages obtained using a Robertsonian translocation marker may not be equivalent to those obtained using the ovarian teratoma method.

TABLE 5

Phenotype of teratomas from Gpi-1^a/Gpi-1^b *females*

Data from:	GPI-1 phenotype				Total
	A	B	A + B*	AB	
EPPIG *et al.* 1977	10	11	0	2	23
EICHER 1978	5	7	2 (4)**	8	24
This study	99	77	5 (10)	55	241
	114	95	7 (14)	65	288

Recombination = $(100 \times 65)/(2 \times 288) = 11.3 \pm 1.2\%$

* Each of these teratomas represents two distinct teratomas, one GPI-1A and one GPI-1B, that arose in a single ovary and were not separated when dissected.

** Actual number of teratomas.

DISCUSSION

The ovarian teratoma method is a recently developed method for obtaining recombination frequencies between the centromere and nearby loci. Until now, centromere-gene recombination frequencies in the laboratory mouse have been poorly defined relative to the remainder of the chromosome map. The data reported here demonstrate the validity of the ovarian teratoma mapping method and establish centromere-gene recombination frequencies for two mouse chromosomes.

We determined a gene-gene recombination percentage using the ovarian teratoma method to ascertain whether this mapping method is equivalent to classical recombination measurements. Recombination between *Mpi-1* and *Mod-1* on chromosome 9 was estimated at 18.6 ± 3.3% from backcross data and 22.9 ± 5.4% using the ovarian teratoma mapping method. Thus, the recombination percentage determined for this gene-gene interval is the same whether applying the backcross method or the ovarian teratoma method. We conclude that the ovarian teratoma method is a reliable method for determining recombination percentage.

A further demonstration of the validity of the teratoma method is the behavior of genes known to be far from their centromeres. We found that, in fact, there was no evidence to suggest these genes were linked to their centromeres using the ovarian teratoma method, and thus, the result was consistent with classical recombination measurements. These results also suggest that there is no suppression of recombination in oocytes that later become teratomas.

Centromere-gene recombination percentages were determined for two mouse chromosomes. The recombination percentage between the centromere of chromosome 7 and *Gpi-1* was 11.3 ± 1.2 and between the centromere of chromosome 14 and *Np-1* was 15.8 ± 2.4. In contrast, the chromosome 14 centromere – *Np-1* recombination percentage was 26.5 ± 3.6 using *Rb(9.14)6Bnr* as a cytological centromeric marker.

Map distances can be obtained for centromere-gene intervals using ovarian teratoma data. The recombination fraction (X) for these centromere-gene intervals is Y/2, as previously discussed. A mapping function has been proposed by

OTT *et al.* (1976b) to relate the frequency of heterozygous teratomas (Y) to the map distance (*D*) measured in morgans: $D = \frac{1}{3} \arcsin[(3/2)Y]$. This function agrees well with empirical data from Drosophila and Neurospora and closely follows the Ludwig and Carter-Falconer mapping functions (OTT *et al.* 1976b). The chromosome 7 centromere – *Gpi-1* distance is 11.5 cM and the chromosome 14 centromere – *Np-1* distance is 16.4 cM, using OTT's mapping function.

In summary, we have demonstrated that the ovarian teratoma mapping method is an important tool for determining accurate recombination frequencies for centromere-gene intervals. The method produces recombination fractions comparable to the backcross method and shows the expected high recombination fractions for centromere-gene intervals known to be greater than 30 cM. The ovarian teratoma method has now been used to obtain two centromere-gene recombination frequencies in the laboratory mouse and will be valuable in defining other centromere-gene intervals.

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APPENDIX

A simplified calculation was used in the main text to obtain a gene-gene recombination frequency using ovarian teratoma data. This method assumed that a maximum of one crossover can occur between the centromere and nearest gene. Double recombinations occurring between the two genes are counted in the same way as backcross progeny. On the basis of this assumption, five categories of ovarian teratomas were expected, resulting from:

(1) no crossover in either the centromere-gene or gene-gene region;
(2) no crossover in the centromere-gene region and a single crossover in the gene-gene region;
(3) a single crossover in the centromere-gene region and no crossover in the gene-gene region;
(4) a single crossover in both the centromere-gene and gene-gene region; and
(5) a four-strand double crossover in the gene-gene region.

A more exact method of determining gene-gene recombination frequency using the ovarian teratoma method is presented below. All possible double crossover types (two-, three- and four-strand) are assumed to occur in both the centromere-nearest region and gene-gene region. The calculations provide expected proportions of ovarian teratomas of each phenotype assuming various recombination values for the centromere-gene and gene-gene interval. To use these more exact values, a large number of ovarian teratomas are required so that the smallest class (AAbb, aaBB) is sufficiently represented. This class is 2% or less of the total number of ovarian teratomas.

The following discussion assumes that gene *A* (alleles *A* and *a*) is closer to the centromere than gene *B* (alleles *B* and *b*), that both genes are codominant and that alleles *A-B* and *a-b* are in coupling. The probability of a crossover occurring between the centromere and gene *A* is *q*, and the probability of a crossover occurring between genes *A* and *B* is *p*. It is also assumed that zero, one or two crossovers can occur in each interval (centromere-gene *A* and gene *A*-gene *B*) in all combinations and without chromatid interference. Thus, double crossovers are expected to occur in the ratio 1 two-strand: 2 three-strand: 1 four-strand.

Table 6 shows the crossover types, the corresponding probability of each crossover type and the resulting chromosomes and phenotypes of the ovarian teratomas. The sum of the probabilities for all crossover types is 1.

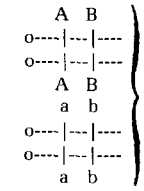
A particular phenotype observed in an ovarian teratoma can arise by several different crossover combinations (see Table 6). In Table 7, the information from Table 6 has been arranged according to the phenotypes of the ovarian teratomas. Each phenotype has been assigned to a class and the probability of that class has been determined. Actual data collection would be in this form.

By assigning values to *q* and *p*, the expected proportions of each ovarian teratoma phenotype are obtained. Table 8 displays expected proportions for ovarian teratoma phenotypes with *q* ranging from 0.00 to 0.50 and *p* ranging from 0.05 to 0.30.

The expected proportions of doubly heterozygous ovarian teratomas (class II, AaBb) do not overlap between values of q less than 0.20. All other cases show overlap in expected proportions.

TABLE 6

Teratoma phenotypes resulting from crossingover in specific regions

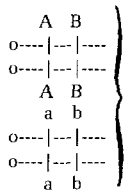


Parental cell at 4-strand stage

Crossover type	Probability		Teratoma chromosomes	Teratoma phenotype
None	$(1-q)^2(1-p)^2$		1/2 o---A---B-- o---A---B--	AABB
			1/2 o---a---b-- o---a---b--	aabb
Single crossover: centromere – gene *A*	$2q(1-q)(1-p)^2$		1/1 o---A---B-- o---a---b--	AaBb
Single crossover: gene *A* – gene *B*	$2q(1-q)^2p(1-p)$		1/2 o---A---B-- o---A---b--	AABb
			1/2 o---a---B-- o---a---b--	aaBb
Single crossover: centromere – gene *A* plus single crossover: gene *A* – gene *B*	$4q(1-)p(1-p)$		1/4 o---A---b-- o---a---b--	Aabb
			1/4 o---a---B-- o---A---B--	AaBB
			1/4 o---A---B-- o---a---b-- 1/4 o---a---B-- o---A---b--	AaBb
Double crossover: gene *A* – gene *B*	$(1-q)^2p^2$	2-strand	1/8 o---A---B-- o---A---B--	AABB
			1/8 o---a---b-- o---a---b--	aabb
		3-strand	1/4 o---A---b-- o---A---B--	AABb
			1/4 o---a---B-- o---a---b--	aaBb
		4-strand	1/8 o---A---b-- o---A---b--	AAbb
			1/8 o---a---B-- o---a---B--	aaBB
Double crossover: centromere – gene *A*	$q^2(1-p)^2$	3-strand	1/2 o---A---B-- o---a---b--	AaBb
		2- or 4-strand	1/4 o---a---b-- o---a---b--	aabb
			1/4 o---A---B-- o---A---B--	AABB

TABLE 6 (*continued*)

Teratoma phenotypes resulting from crossingover in specific regions



Parental cell at 4-strand stage

Crossover type	Probability		Teratoma chromosomes	Teratoma phenotype
Single crossover: centromere – gene *A* plus double crossover: gene *A* – gene *B*	$2q(1-q)p^2$		3/8 o---A---B-- o---a---b--	AaBb
			3/8 o---A---b-- o---a---B--	
			1/8 o---a---b-- o---A---b--	Aabb
			1/8 o---a---B-- o---A---B--	AaBB
Double crossover: centromere – gene *A* plus single crossover: gene *A* – gene *B*	$2q^2p(1-p)$	2- or 4- strand	1/4 o---A---B-- o---A---b--	AABb
			1/4 o---a---B-- o---a---b--	aaBb
		3-strand double	1/8 o---a---B-- o---A---B--	AaBB
			1/8 o---A---b-- o---a---b--	Aabb
			1/8 o---a---b-- o---A---B--	AaBb
			1/8 o---a---B-- o---A---b--	
Double crossover: centromere – gene *A* plus double crossover: gene *A* – gene *B*	q^2p^2		1/16 o---A---B-- o---A---B--	AABB
			1/16 o---a---b-- o---a---b--	aabb
			1/8 o---A---B-- o---A---b--	AABb
			1/8 o---a---b-- o---a---B--	aaBb
			1/8 o---A---B-- o---a---b--	AaBb
			3/8 o---a---B-- o---A---b--	
			1/16 o---a---B-- o---a---B--	aaBB
			1/16 o---A---b-- o---A---b--	AAbb
	$\overline{\sum = 1}$			

TABLE 7

Ovarian teratoma phenotypes, classes and probabilities

Class	Ovarian teratoma phenotypes	Probability
I	AABB, aabb	$(1-q)^2(1-p)^2 + (1-q)^2p^2/4 + q^2(1-p)^2/2 + q^2p^2/8$
II	AaBb	$2q(1-q)(1-p)^2 + 2q(1-q)p(1-p) + q^2(1-p)^2/2 + 3q(1-q)p^2/2 + q^2p(1-p)/2 + q^2p^2/2$
III	AABb, aaBb	$2(1-q)^2p(1-p) + (1-q)^2p^2/2 + q^2p(1-p) + q^2p^2/4$
IV	AaBB, Aabb	$2q(1-q)p(1-p) + q(1-q)p^2/2 + q^2p(1-p)/2$
V	AAbb, aaBB	$(1-q)^2p^2/4 + q^2p^2/8$

Thus, a reasonable value for q can be obtained by comparing the actual proportion of AaBb teratomas detected with the expected proportion of AaBb teratomas, providing the frequency of AaBb teratomas is less than 0.20. Values for q ranging between 0.20 and 0.30 show overlap at the extreme values assigned to p. Thus, this is the least reliable region for p and q determinations. Finally, values of q greater than 0.30 produce expected proportions of AaBb teratomas greater than 0.40. Thus, a frequency of AaBb teratomas greater than 0.40 should be considered evidence that the map distance between the centromere and gene *A* is greater than 30 cM. The measurable centromere-gene recombination frequency using the ovarian teratoma method requires the proportion of heterozygous teratomas to be less than 0.33 (see main text and OTT *et al.* 1976a).

For Table 8 to be of general use, a large number of ovarian teratomas must be obtained so that each class is sufficiently represented. Possibly, with accumulated ovarian teratoma data, the frequency of each class of ovarian teratoma phenotypes observed will allow recombination values to be estimated from such tables.

TABLE 8

Expected proportion of ovarian teratomas of each phenotype

q	p	AABB, aabb	AaBb	AABb, aaBb	AaBB, Aabb	AAbb, aaBB
0.00	0.05	0.9031	0.0000	0.0962	0.0000	0.0006
0.00	0.10	0.8125	0.0000	0.1850	0.0000	0.0025
0.00	0.15	0.7281	0.0000	0.2663	0.0000	0.0056
0.00	0.20	0.6500	0.0000	0.3400	0.0000	0.0100
0.00	0.25	0.5781	0.0000	0.4062	0.0000	0.0156
0.00	0.30	0.5125	0.0000	0.4650	0.0000	0.0225
0.05	0.05	0.8162	0.0916	0.0870	0.0046	0.0006
0.05	0.10	0.7343	0.0873	0.1672	0.0089	0.0023
0.05	0.15	0.6580	0.0834	0.2406	0.0128	0.0051
0.05	0.20	0.5874	0.0799	0.3073	0.0163	0.0090
0.05	0.25	0.5225	0.0767	0.3671	0.0195	0.0141
0.05	0.30	0.4632	0.0739	0.4202	0.0224	0.0203
0.10	0.05	0.7360	0.1761	0.0784	0.0089	0.0005
0.10	0.10	0.6622	0.1679	0.1508	0.0171	0.0020
0.10	0.15	0.5934	0.1604	0.2170	0.0246	0.0046
0.10	0.20	0.5297	0.1536	0.2771	0.0314	0.0082
0.10	0.25	0.4712	0.1475	0.3311	0.0375	0.0127
0.10	0.30	0.4177	0.1421	0.3790	0.0429	0.0183
0.15	0.05	0.6627	0.2534	0.0706	0.0128	0.0005
0.15	0.10	0.5962	0.2417	0.1357	0.0246	0.0018
0.15	0.15	0.5343	0.2309	0.1954	0.0354	0.0041
0.15	0.20	0.4769	0.2211	0.2495	0.0452	0.0073
0.15	0.25	0.4242	0.2123	0.2981	0.0539	0.0115
0.15	0.30	0.3760	0.2046	0.3412	0.0617	0.0165
0.20	0.05	0.5961	0.3237	0.0635	0.0163	0.0004
0.20	0.10	0.5363	0.3086	0.1221	0.0314	0.0017
0.20	0.15	0.4806	0.2949	0.1757	0.0452	0.0037
0.20	0.20	0.4290	0.2824	0.2244	0.0576	0.0066
0.20	0.25	0.3816	0.2713	0.2681	0.0688	0.0103
0.20	0.30	0.3382	0.2614	0.3069	0.0786	0.0149
0.25	0.05	0.5362	0.3867	0.0571	0.0195	0.0004
0.25	0.10	0.4824	0.3688	0.1098	0.0375	0.0015
0.25	0.15	0.4323	0.3523	0.1581	0.0539	0.0033
0.25	0.20	0.3859	0.3375	0.2019	0.0688	0.0059
0.25	0.25	0.3433	0.3242	0.2412	0.0820	0.0093
0.25	0.30	0.3043	0.3125	0.2761	0.0937	0.0134
0.30	0.05	0.4832	0.4427	0.0515	0.0224	0.0003
0.30	0.10	0.4347	0.4221	0.0990	0.0429	0.0013
0.30	0.15	0.3895	0.4034	0.1424	0.0617	0.0030
0.30	0.20	0.3478	0.3864	0.1819	0.0786	0.0054
0.30	0.25	0.3093	0.3713	0.2173	0.0938	0.0084
0.30	0.30	0.2742	0.3579	0.2488	0.1071	0.0120
0.40	0.05	0.3974	0.5331	0.0424	0.0269	0.0003
0.40	0.10	0.3575	0.5084	0.0814	0.0516	0.0011
0.40	0.15	0.3204	0.4859	0.1172	0.0741	0.0025
0.40	0.20	0.2860	0.4656	0.1496	0.0944	0.0044
0.40	0.25	0.2544	0.4475	0.1788	0.1125	0.0069
0.40	0.30	0.2255	0.4316	0.2046	0.1284	0.0099
0.50	0.05	0.3387	0.5950	0.0361	0.0300	0.0002
0.50	0.10	0.3047	0.5675	0.0694	0.0575	0.0009
0.50	0.15	0.2730	0.5425	0.0998	0.0825	0.0021
0.50	0.20	0.2438	0.5200	0.1275	0.1050	0.0038
0.50	0.25	0.2168	0.5000	0.1523	0.1250	0.0059
0.50	0.30	0.1922	0.4825	0.1744	0.1425	0.0084