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Genetics

Assignment of genes to regions of mouse chromosomes

[duplication–deficiency mapping/gene duplication/mouse translocations *T(7;19)145H* and *T(X;7)1Ct*/Giemsa banding]

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ABSTRACT **A genetic mapping procedure, called the duplication–deficiency method, is described. This method permits the genetic location of a translocation to be determined within a linkage group without the use of recombination. By utilizing the duplication–deficiency method to define the genetic breakpoints for a series of translocations involving a given chromosome and integrating this information with their cytological breakpoints, obtained by Giemsa banding, a genetic map of the chromosomes is constructed whereby groups of loci are assigned to banded regions.**

Duplication–deficiency mapping and Giemsa banding analysis of the *T(X;7)1Ct* and *T(7;19)145H* translocations together with information from the c^{25H} deletion have permitted mouse chromosome 7 to be divided into six and chromosome 19 into two definable genetic regions.

One of the challenges currently facing mammalian cytogeneticists is the assignment of specific genes to given chromosomal regions. Four methods employed are: hybridization of purified nucleic acid molecules (rRNA, satellite DNA, etc.) to chromosomes (1–6), segregation of translocated chromosomes in somatic cell hybrids (7), deletion mapping (8, 9), and genetic recombination relative to translocation breakpoints (10). A fifth method, here designated the duplication–deficiency method, allows the assignment of specific genes to one side of a translocation breakpoint by comparing the phenotype of individuals with a defined chromosomal imbalance to that of chromosomally balanced individuals. The duplication–deficiency method has been used in the mouse to determine the autosomal breakpoints involved in translocation *T(X;7)1Ct* (11, 12) and has been applied to the *T(14;15)6Ca* translocation (13, 14).

Here we exemplify the use of the duplication–deficiency method in the mouse by locating the genetic breakpoints in the reciprocal translocation *T(7;19)145H*. When this genetic information is integrated with Giemsa (G) banded breakpoint determinations of *T(X;7)1Ct*, *T(7;19)145H*, and c^{25H}, six genetic regions on chromosome (Chr) 7 and two on Chr 19 are assigned to specific banded regions.

MATERIALS AND METHODS

The Duplication–Deficiency Method. It is well established for *Mus musculus* that if one parent is heterozygous for a reciprocal translocation, approximately 50 per cent of the young inherit an unbalanced chromosomal complement and thus are not diploid (15). These chromosomally unbalanced individuals provide the genetic material for the duplication–deficiency mapping method.

The duplication–deficiency method consists of (*a*) mating individuals heterozygous for a translocation and specific loci carried on the translocated chromosomes to normal individuals homozygous for these loci (for examples, see Fig. 3 *top* and *bottom*), (*b*) defining the chromosome complement of any abnormal offspring relative to the translocation involved, (*c*) defining the genotype of the abnormal offspring relative to the segregating loci, (*d*) correlating this genotype with the presence or absence of a translocated chromosome, and thus, (*e*) assigning the loci of interest to one of the translocated chromosomes.

Genes and Chromosomes. In this report, regional assignments within mouse chromosomes 7 and 19 are considered. Linkage and cytological maps of the chromosomes are presented in Fig. 1. A cytological map of the X chromosome is given in Fig. 2 *lower*.

The nomenclature for the chromosomes is as follows. A number (7, 19, etc.) is used to designate a normal intact chromosome or its linkage group. A reciprocally translocated chromosome is represented by two numbers: the first number indicates the chromosome donating the proximal end (region between centromere and breakpoint) and the second number, written as a superscript to the first number, indicates the chromosome donating the distal end (region between breakpoint and telomere). For example, a *T145H* heterozygous individual is chromosomally $7,7^{19},19^{7},19$ (see Fig. 2 *upper*). In the case of the nonreciprocal translocation *T1Ct*, the Chr 7 carrying the deletion is represented 7^{D}, whereas the X with the inserted piece of 7 is represented $X^{(7)}$. Thus, a heterozygous *T1Ct* female is chromosomally $X,X^{(7)},7^{D},7$ (see Fig. 2 *lower*).

The symbol *T145H* is also used when designating a genotype (*T145H*/+ indicates an individual heterozygous for *T145H*), or the position of the breakpoint within a linkage group (*Gpi-1—T145H—p* locates the *T145H* breakpoint in Chr 7 between the *Gpi-1* and *p* loci).

Genotype Determinations. The genotype of individuals was determined by visual inspection for the genes *p*, *bm*, and *ru*, and electrophoretic inspection for the gene *Gpi-1* (see Fig. 3 legend). Each of the alleles p^{J}, *bm*, or *ru* is recessive to its respective wild-type (+) allele. Homozygous p^{J}/p^{J} or *ru*/*ru* mice have nonpigmented eyes, evident as early as 12 days of gestation, and a dilution of coat color, noticeable by one week after birth. Homozygous *bm*/*bm* mice have shortened hind limbs and a domed skull, usually evident by birth. The two alleles at the *Gpi-1* locus, $Gpi\text{-}1^{a}$ and $Gpi\text{-}1^{b}$, are codominant.

The Translocations. *T145H* is a reciprocal translocation between Chr 7 and 19 (17–19). The breakpoint is near *p* on Chr 7 and near *ru* on Chr 19.* *T145H*/+ individuals can be dis-

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Abbreviations; Chr, chromosome; *T145H*, *T(7;19)145H* translocation; *T1Ct*, *T(X;7)1Ct* translocation; G-banding, Giemsa stain banding; *Gpi-1*, glucosephosphate isomerase locus; GPI-1, glucosephosphate isomerase enzyme; *qv*, quivering; *ru-2*, ruby-eye-2 locus or allele; *p*, pink-eyed dilution locus or allele; *tp*, taupe; *c*, albino locus or allele; c^{25H}, deletion covering *c* locus; *Mod-2*, mitochondrial malic enzyme; *sh-1*, shaker-1; *Hbb*, β-chain hemoglobin; *bm*, brachymorphic locus or allele; *ru*, ruby-eye locus or allele; +, wild-type allele for various loci.

* M. F. Lyon and P. Glenister, personal communication appearing in (1971) *Mouse News Letter* **45**, 24.

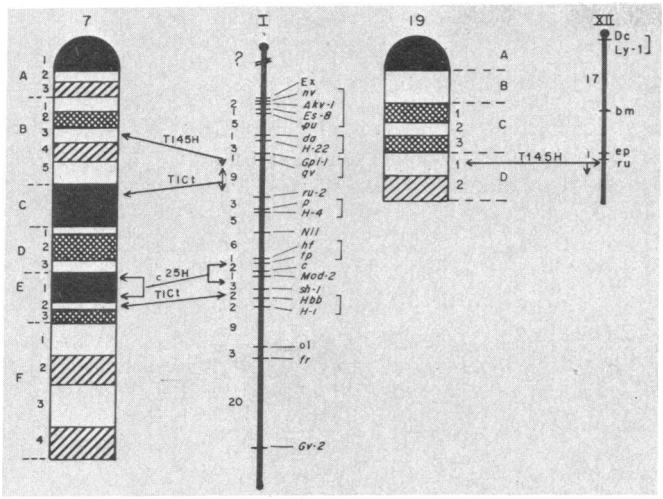

FIG. 1. Cytogenetic map of chromosomes 7 and 19. Banding nomenclature is after Nesbitt and Francke (10). The linkage map of chromosome 7 (formerly linkage group I) is modified from M. C. Green [personal communication appearing in (1975) *Mouse News Letter* **53**, 10], while that of chromosome 19 (formerly linkage group XII) is after Lane *et al.* (16). Distances on the map are in centimorgans. The region labeled c^{25H} is that portion of Chr 7 known to be deleted in this x-ray-induced albino mutation (refs. 8 and 9; E. M. Eicher, L. L. Washburn, S. E. Lewis, S. Gluecksohn-Waelsch, unpublished; E. M. Eicher, S. E. Lewis, T. Gilston, H. Turchin, and S. Gluecksohn-Waelsch, unpublished).

tinguished from their +/+ sibs by the presence of Chr 7^{19} and 19^{7} in mitotic cells (18).

T145H/+ females, received from the Medical Research Council Radiobiology Unit, Harwell, England, in 1972, were mated to $p^J p^J$ males from the C3H/HeJ-p^J strain, and this mating system was continued. After eight generations of mating, the *T145H*/+ females were found to be *Gpi-1*^{a}/*Gpi-1*^{b}. Because the C3H/HeJ-p^J strain carries *Gpi-1*^{b}, and the *T145H*/+ females always inherit their normal Chr 7 from their C3H/HeJ-p^J male parent, *Gpi-1*^{a} must be carried on one of the translocated chromosomes, either Chr 7^{19} or 19^{7}.

T1Ct is a nonreciprocal translocation involving the insertion of a region of Chr 7 (20–22) into the X chromosome (23, 24).

Determination of T145H Breakpoint. The *T145H* breakpoints were determined relative to the loci *Gpi-1* and *p* on Chr 7, and *bm* and *ru* on Chr 19, as follows. Females that were carrying the *T145H* translocation and were heterozygous *Gpi-1*^{a}/*Gpi-1*^{b} and +/p^J (*Gpi-1*^{a} *T145H* +/*Gpi-1*^{b} + p^J) were mated to normal males of the C3H/HeJ-p^J strain that were homozygous *Gpi-1*^{b}/*Gpi-1*^{b} and p^J/p^J (*Gpi-1*^{b} + p^J/*Gpi-1*^{b} + p^J). Fifteen to 19-day-old embryos were removed from the uterus and their eye colors were recorded. Their glucosephosphate isomerase (GPI)-1 phenotypes were determined on erythrocyte lysates by cellulose acetate electrophoresis (method given in legend of Fig. 3). Metaphase chromosomes were prepared from liver and G-banded (see below).

Females carrying the *T145H* translocation were mated to males that were homozygous *Gpi-1*^{b}/*Gpi-1*^{b}, *bm*/*bm*, and *ru*/*ru*. Their *T145H*-carrying daughters (*Gpi-1*^{a} *T145H*/*Gpi-1*^{b} +, ++ *T145H*/*bm ru* +) were mated to males genetically identical to their fathers (*Gpi-1*^{b} +/*Gpi-1*^{b} +, *bm ru* +/*bm ru* +). Embryos were analyzed at term for eye color, hind limb and skull abnormalities, and GPI-1 phenotype. Metaphase chromosomes were prepared from liver and G-banded.

Preparation of Chromosomes from Fetal Liver. Liver tissue from individual embryos was placed in 5 ml of a 0.025% colchicine solution prepared with EDTA buffer (8.0 g of NaCl; 0.2 g of KH_2PO_4; 0.2 g of KCl; 1.15 g of Na_2HPO_4; 0.2 g of EDTA, disodium salt; dissolved in 1 liter of distilled H_2O). A single cell suspension was prepared by gently forcing the tissue back and forth through a 20-gauge needle attached to a 1-ml plastic syringe. The suspension was incubated in the colchicine solution for 10 min at 37°. The cells were then recovered as a pellet by centrifugation for 5 min at 400 × *g*, in a clinical centrifuge (swinging bucket). The EDTA buffer was gently decanted and the pellet was resuspended in 10 ml of a hypotonic solution (0.075 M KCl) using the 20-gauge needle and 1-ml syringe. After incubation for 30 min at 37°, the cells were again pelleted by centrifugation for 5 min (400 × *g*). The hypotonic solution was carefully decanted and the lip of the tube was blotted to remove as much of the remaining solution as possible. Three milliliters of freshly made fixative (3:1 vol/vol, absolute methanol/glacial acetic acid) was gently layered over the pellet with a pasteur pipette. After the pellet turned white, the fixative was carefully decanted and 3 ml of new fixative was added. The tubes were stoppered and placed at 3–5° for a minimum of 30 min to ensure good fixation. The fixative was then decanted, 3 ml of freshly made fixative was added, and the cell pellet was thoroughly suspended with a 20-gauge needle attached to a 1-ml plastic syringe (fixed cells adhere to glass). The sides of the tube were washed down with fixative and the volume of fixative was brought to 5 ml. The cells were centrifuged for 3 min at 800 × *g*, the fixative was decanted, and the cells were resuspended in 5 ml of new fixative with a silicone-treated pasteur pipette. The cells were again centrifuged for 3 min (800 × *g*) and the washing procedure was repeated two more times. Standard air-dried preparations were made and the metaphase chromosomes were banded, as described below.

Cytological Breakpoints. Mitotic chromosomes were prepared from leukocyte cultures (25) of *T1Ct*/+ and *T145H*/+ females. Air-dried preparations were aged at room temperature for 1–3 days and then G-banded as follows. Slides were moved in a constant to-and-fro motion for 8 sec while immersed in 0.25% trypsin (2.5% trypsin, Gibco no. 509 diluted with 0.85% saline), rinsed in saline, and stained for 6 min in Giemsa stain (1 ml of Giemsa, Harleco no. 620, plus 50 ml of 0.05 M phosphate buffer, pH 7.0, Fisher no. SO-B-108). Well-banded metaphases were photographed and karyotypes were prepared. The banding nomenclature used in this report is that of Nesbitt and Francke (10).

RESULTS

Duplication–Deficiency Mapping. In the cross of a *Gpi-1*^{a} *T145H*+/*Gpi-1*^{b} + p^J female by a *Gpi-1*^{b} + p^J/*Gpi-1*^{b} + p^J male, 4% (3 out of 71) of the embryos were runted at term. Two of the runted embryos had nonpigmented eyes and a GPI-1 ABB electrophoretic phenotype compatible only with a *Gpi-1*^{a}/*Gpi-1*^{b}/*Gpi-1*^{b} genotype (Fig. 3 *top* and *middle*). In addition, they had 41 chromosomes, including 7,7,19,19,7^{19}, indicating they had inherited Chr 7, 19, and 7^{19} from their *T145H*/+ mother and thus had three copies of all genes carried on Chr 7^{19}. Because these two offspring expressed the *Gpi-1*^{a} allele when they received Chr 7^{19}, *Gpi-1*^{a} is located on this translocated chromosome. Because these two runted offspring also had nonpigmented eyes, indicating absence of the + allele at the *p* locus, the + allele had to be on the missing translocation product, Chr 19^{7}. Because Chr 7^{19} represents the proximal segment Chr 7 and Chr 19^{7} the distal segment, the order of loci on Chr 7 relative to *T145H* is centromere—*Gpi-1*—*T145H*—*p*.

The third runt had pigmented eyes, a GPI-1AB electropho-

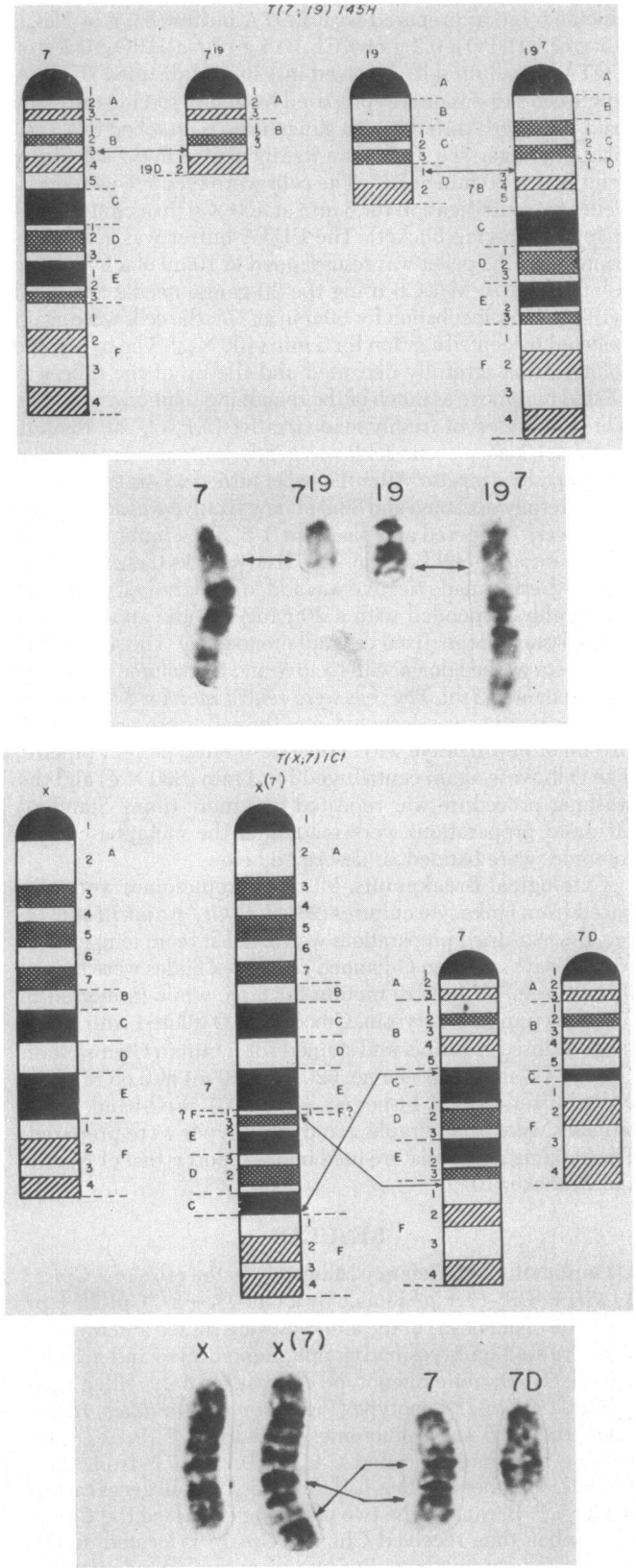

FIG. 2. G-banded preparations and idiogram of the *T(7;19)145H* (*Upper*) and *T(X;7)1Ct* (*Lower*) translocations. Arrows designate breakpoints. It should be noted that, contrary to the published G-banded idiogram of Chr 7 (10), band 7D1 is *narrower* than 7D3. This asymmetry of 7D1 and 7D3 relative to 7D2 provides a visual landmark for defining the relative orientation of this same region in Chr $X^{(7)}$ of the *T(X;7)1Ct* translocation. As can be seen, this 3-band complex is clearly inverted; the wider band 7D3 now lying above 7D2 and the narrow band 7D1 below. The *exact* breakpoint in the X and distal breakpoint in Chr 7 could not be determined because we could not identify the clear band between XE and 7E3 in Chr $X^{(7)}$. This clear band could be derived from the nonstaining band XF1 or 7F1. Because we cannot distinguish between these two possibilities, we place the three breakpoints of *T(X;7)1Ct* as in the proximal one-third of 7C and either on the proximal margin of 7F1 and between XE and XF1, or on the proximal edge of XF1.

retic pattern and 41 chromosomes, including $7,7^{19},19^{7},19,19$, indicating that it had inherited a $7^{19},19^{7}$, and 19 from its *T145H*/+ mother. The presence of pigmented eyes, indicating inheritance of the + allele, supports the assignment (above) of the *p* locus to Chr 19^{7} because this chromosome is now present in this individual's karyotype. (The viability and fertility of both types of runted individuals will be considered in detail in another report.)

From the mating of a *bm ru* +/+ + *T145H*, *Gpi-1*^{b} +/*Gpi-1*^{a} *T145H* female by a *bm ru* +/*bm ru* +, *Gpi-1*^{b} +/*Gpi-1*^{b} + male, two types of runted embryos (3 out of 37) were again noted. Of importance to this study is the type of runt (one embryo) having abnormal hind limbs, nonpigmented eyes, a GPI-1ABB phenotype indicating a *Gpi-1*^{a}/*Gpi-1*^{b}/*Gpi-1*^{b} genotype, and 41 chromosomes, including $7,7,19,19,7^{19}$ (Fig. 3 *middle* and *bottom*). Because this runted individual was *bm*/*bm* and *ru*/*ru* and thus did not receive the + allele for either locus, we conclude that the + alleles for *bm* and *ru* are not carried on Chr 7^{19} but rather on Chr 19^{7}. Therefore, the order of loci on Chr 19 is centromere—*bm*—*ru*—*T145H*.

Cytological Breakpoints. The breakpoints of *T145H* and *T1Ct*, as determined from G-banded chromosomes prepared from cultured leukocytes, are shown in Fig. 2. In this case of *T145H*, the breakpoint in Chr 7 is in band 7B3 and in Chr 19 it is in band 19D1. For *T1Ct*, the breakpoint in the X is between XE1 and XF1 or in the proximal region of band XF1. The first breakpoint in Chr 7 is in the proximal 1/3 of 7C and the second breakpoint is at the distal edge of 7E3 or proximal edge of 7F1. This region of Chr 7 is inserted in an inverted manner into the X chromosome (see Fig. 2 legend).

Quinacrine fluorescence has been used to investigate the *T145H* (17) and *T1Ct* (21, 22) translocations. Nesbitt and Francke (10) have attempted to interpret these results in terms of accepted banding nomenclature. Contrary to our results, they placed the *T145H* breakpoint in Chr 7 in band 7C1. No reference was made to the breakpoint in Chr 19. In relation to *T1Ct*, Nesbitt and Francke suggest that one breakpoint in Chr 7 is in the proximal region of 7C1, which is in agreement with our results, and the other in 7E2 or 7E3 or 7F1. They did not discuss the breakpoint in the X chromosome. Of interest is the fact that quinacrine fluorescence analysis did not allow the inverted insertion of a piece of Chr 7 into the X chromosome to be recognized (21, 22).

Genetic Map. The combined information obtained from the cytological and genetic breakpoints of *T145H* and *T1Ct* as well as the *c*^{25H} deletion (ref. 8; E. M. Eicher, L. L. Washburn, S. E. Lewis, and S. Gluecksohn-Waelsch, unpublished) can now be used to dissect Chr 7 into six regions, as follows: centromere—7B3 (*T145H*), —one-third into 7C1 (*T1Ct*), —most of 7E1 (*c*^{25H}), —7E2 (*T1Ct*), —telomere (see Fig. 1). The *T145H* genetic breakpoint for Chr 7 is between *Gpi-1* and *p*. Previous mapping of *T1Ct* indicates that the order of loci and breakpoints is: centromere—*qv*—*T1Ct*—*ru-2*—*p*—*sh-1*—*T1Ct*—*Hbb* (11, 12, 26). The *c*^{25H} deletion includes the *c* and *Mod-2* loci but not the *tp* or *sh-1* loci (ref. 9; E. M. Eicher, S. E. Lewis, T. Gilston, H. Turchin, and S. Gluecksohn-Waelsch, unpublished). If we assume that mammalian genes are linearly

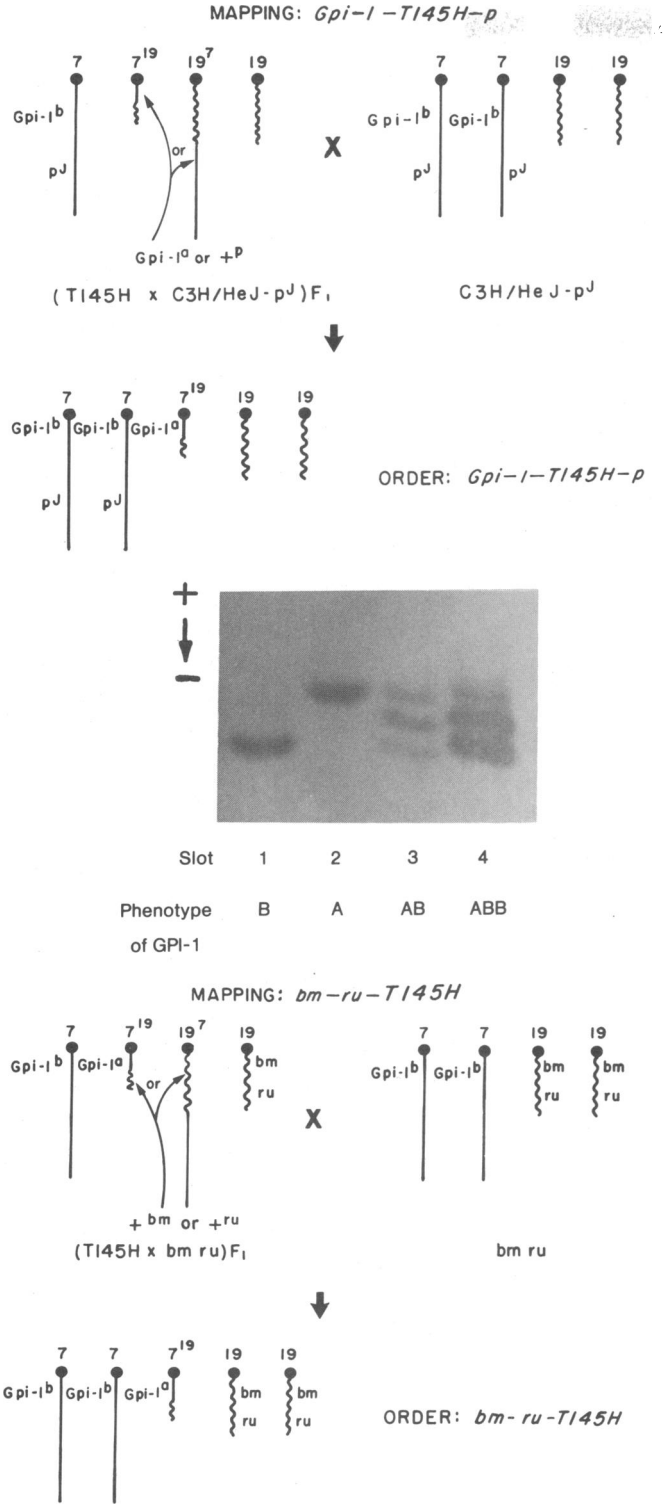

FIG. 3. Duplication–deficiency mapping (*Top* and *Bottom*) of genetic breakpoints of *T145H* relative to Chr 7 and 19. The electrophoretic gel (*Middle*) contains erythrocyte lysates from four different representative individuals: slot 1, *Gpi-1^b/Gpi-1^b*; slot 2, *Gpi-1^a/Gpi-1^a*; slot 3, *Gpi-1^a/Gpi-1^b*; and slot 4, *Gpi-1^a/Gpi-1^b/Gpi-1^b*. Their phenotypes can be designated GPI-1B, GPI-1A, GPI-1AB, and GPI-1ABB, respectively. Lysates for GPI-1 were prepared by adding a 1.6-cm length of packed erythrocytes in a hematocrit tube to three drops of lysis solution (1 g of EDTA, tetrasodium salt, per 1000 ml). The lysate was applied directly to Titan III cellulose acetate plates (Helena Laboratories). Electrophoresis was conducted for 20–35 min, 200 V, anode to cathode in a Tris–glycine buffer, pH 8.5 (3.0 g of Trizma base, 14.4 g of glycine per liter). The stain was applied as an agar overlay and consisted of a mixture of 2 ml of 0.2 M Tris·HCl (pH 8.0), 3 μl of glucose-6-phosphate dehydrogenase, and 0.1 ml each of magnesium acetate (53.6 mg/ml), fructose 6-phosphate (100 mg/ml), phenazine methosulfate (2.5 mg/ml), 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT tetrazolium) (10 mg/ml), and NADP (10 mg/ml) added to 2 ml of agar (360 mg/25 ml, 50–55°). GPI-1 bands of activity were observable within 5 min. (All reagents were obtained from Sigma Chemical Co., St. Louis, MO.)

arranged on the chromosome, the resulting order of the genes and translocation breakpoints of Chr 7 is: centromere—(*Gpi-1, qv*)—*T145H*—*T1Ct*—*ru-2*—*p*—*tp*—*c^{25H}*—*c*—*Mod-2*—*c^{25H}*—*sh-1*—*T1Ct*—*Hbb*—telomere, as diagramed in Fig. 1. All of the known linkage map of Chr 19 (16) is located between the centromere and the *T145H* breakpoint, as shown also in Fig. 1.

DISCUSSION

Genes from a linkage group can be assigned to specific banded regions of a chromosome by determining genetic breakpoints of various translocations whose cytological breakpoints are distributed along the chromosome. The mouse is presently the only mammal that has available the extensive number of inheritable translocations and linkage information necessary to construct such genetic maps.

The genetic localization of mouse translocation or inversion breakpoints has generally involved recombinational analysis. Unfortunately, translocations and inversions can inhibit recombination. Because a breakpoint cannot be genetically defined within a group of loci that do not recombine with it, classical recombination analysis may not allow definitive answers to be obtained.

In contrast, the duplication–deficiency mapping method allows the genetic assignment of a given translocation breakpoint to a region either proximal or distal to a specific locus. The method depends on the availability of translocations for analysis, recovering chromosomally unbalanced individuals, and defining the genotype of these individuals. Unlike the somatic cell hybridization technique that requires the expression of a gene *in vitro*, the duplication–deficiency method can utilize pigment, skeletal, and hair structural loci as well as isozyme loci. Combining neurological, immunological, endocrinological, etc., loci with these other types of genes would allow the entire linkage map of the mouse to be employed for determination of translocation breakpoints. By accumulating genetic and chromosomal breakpoint information from a series of translocations, using duplication–deficiency mapping, a number of questions concerning mammalian chromosomes can be addressed. For example, is there a direct relationship between genetic distance and mitotic chromosome length? If so, one should be able to juxtapose a banded chromosome and its linkage group (as in Fig. 1) and connect corresponding cytological and genetic breakpoints by a series of parallel lines. Any other pattern of lines implies that the genetic and cytological distances are not directly related. Although the amount of information collected on Chr 7 is limited, our results suggest that an absolute one-to-one relationship will not be found.

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