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Genetics

Assignment of the gene for cytoplasmic superoxide dismutase (*Sod-1*) to a region of chromosome 16 and of *Hprt* to a region of the X chromosome in the mouse

[somatic cell hybrids/comparative mapping/Searle's T(X;16)16H translocation/hypoxanthine phosphoribosyltransferase/isoelectric focusing]

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ABSTRACT In the search for homologous chromosome regions in man and mouse, the locus for cytoplasmic superoxide dismutase (SOD-1; superoxide:superoxide oxidoreductase, EC 1.15.1.1) is of particular interest. In man, the *SOD-1* gene occupies the same subregion of chromosome 21 that causes Down syndrome when present in triplicate. Although not obviously implicated in the pathogenesis, *SOD-1* is considered to be a biochemical marker for this aneuploid condition. Using a set of 29 mouse–Chinese hamster somatic cell hybrids, we assign *Sod-1* to mouse chromosome 16. Isoelectric focusing permits distinction between mouse and Chinese hamster isozymes, and trypsin/Giemsa banding distinguishes mouse from Chinese hamster chromosomes. The mouse fibroblasts used were derived from a male mouse carrying Searle's T(X;16)16H reciprocal translocation in which chromosomes X and 16 have exchanged parts. Analysis of informative hybrids leads to regional assignment of *Sod-1* to the distal half of mouse chromosome 16 (16B4 → ter). Because the Chinese hamster cell line (380) used for cell hybridization is deficient in hypoxanthine phosphoribosyltransferase (HPRT; IMP: pyrophosphate phosphoribosyltransferase, EC 2.4.2.8), that part of the mouse X chromosome carrying the complementing *Hprt* gene can be identified by selection in hypoxanthine/aminopterin/thymidine medium and counterselection in 8-azaguanine. Mouse *Hprt* is on the X^T translocation product containing the proximal region X cen → XD.

Comparative gene mapping allows the tracing of chromosome rearrangements during evolution. In mammals, widespread conservation of linkage groups has been observed for closely related species (such as mouse and rat or man and nonhuman primates), for closely linked autosomal gene clusters (such as the major histocompatibility region, heavy chain immunoglobulin genes, and non-α-globin genes), and for genes on the X chromosome [no exceptions have as yet been found in comparing up to 10 X-chromosome gene loci in several mammalian species (1, 2)].

In the mouse, major advances have recently been made in mapping of genes for nonpolymorphic enzyme loci by using Chinese hamster–mouse somatic cell hybrids that segregate mouse chromosomes (3, 4). Homologous loci for enzymes can be identified in different species on the basis of substrate specificity and the formation of enzymatically active heteropolymers in interspecific cell hybrids. When the chromosomal locations of homologous genes are compared in man and mouse, there is evidence for disruption of most autosomal syntenic groups. Homologous enzyme loci on a mouse chromosome may be found distributed over three different human chromosomes and vice versa, reflecting multiple breaks and reorganization of chromosomal material during evolution. However, conservation of several autosomal syntenic groups in mouse and man has recently been reported (4, 5). The largest one involves five gene loci on the distal half of the short arm of human chromosome 1 and on mouse chromosome 4 (4, 6).

Questions about the significance of chromosomal rearrangements—whether they are mediators of evolution or side effects, and whether it is chance or selective advantage that preserved certain chromosome regions over many million years of evolution—may become approachable in the near future.

Progress in this area is expected along two lines of investigation: identification of more homologous chromosome regions in more species and establishment of linear order, map distances, and subregional localizations of loci within conserved syntenic groups. The present report contributes to both areas.

Chromosome 21, the smallest autosome in man, has been intensely studied because trisomy 21 results in a common syndrome of developmental defects and mental retardation (Down syndrome, DS). The genes on chromosome 21 that are responsible for this syndrome when present in the trisomic state have not been specifically identified; however, they have been regionally localized to band 21q22 (7). Triplication of this band alone produces the DS phenotype.

The gene for soluble cytoplasmic superoxide dismutase (SOD-1; superoxide:superoxide oxidoreductase, EC 1.15.1.1) which catalyzes the reaction $O_2^- + O_2^- + 2\,H^+ \rightarrow O_2 + H_2O_2$ has been assigned to human chromosome 21 and to the same band 21q22 (8). Although a gene dosage effect has been demonstrated for SOD activity in cells from DS individuals, it is not clear whether increased SOD activity contributes to the DS phenotype. However, *SOD-1* can be considered a biochemical marker gene for searching in other species for the region that is homologous to the one responsible for the DS phenotype in man. In the present report we assign *Sod-1* to chromosome 16 in the mouse, specifically to the distal half of this chromosome (region 16B5 → ter). Because mouse and Chinese hamster SOD-1 isozymes were difficult to distinguish by conventional electrophoretic methods, an isoelectric focusing procedure was developed.

Cell hybridization studies using several different human X/autosome translocations have led to regional assignments of X-linked genes expressed in cultured cells to the long arm of the human X chromosome in the following order: centromere–phosphoglycerate kinase–α-galactosidase–*HPRT*–glucose-6-phosphate dehydrogenase (9). No regional localizations

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Abbreviations: SOD, superoxide dismutase (EC 1.15.1.1); HPRT, hypoxanthine phosphoribosyltransferase (EC 2.4.2.8); T16, Searle's X/16 reciprocal translocation in the mouse; DS, Down syndrome (trisomy 21); HAT, hypoxanthine/aminopterin/thymidine; 8-AG, 8-azaguanine; 8-AG^R, 8-AG-resistant.

have been established for the homologous loci on the mouse X chromosome. The gene order may well be different, because intrachromosomal rearrangements such as inversions are common pathways in karyotype evolution. For regional mapping of genes on the mouse X chromosome we have hybridized cells from a mouse with T(X;16)16H Searle's translocation [a reciprocal X/autosome translocation involving exchange of the distal third of the X and the distal half of chromosome 16 (10)] to an established Chinese hamster cell line in order to obtain hybrids that segregate mouse chromosomes.

MATERIALS AND METHODS

Cells. A primary fibroblast culture was started from a biopsy sample of the ear of a male mouse with Searle's translocation (T16/Y) obtained from Eva Eicher (The Jackson Laboratory). An established hypoxanthine phosphoriboryltransferase (HPRT; IMP:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8)-deficient, 8-azaguanine (8-AG)-resistant Chinese hamster cell line (380), derived from V79, was donated by Diane Smith (Oak Ridge National Laboratory, Oak Ridge, TN).

Cell Fusion. Cells from both lines were harvested by using trypsin/EDTA, mixed in a ratio of 1:200 (T16/Y to 380) in suspension, and plated into 60-mm dishes at high density. Chinese hamster cells (380) were used as control. After 24 hr, all plates were exposed to inactivated Sendai virus (Microbiological Associates, Bethesda, MD; lot 3-6231; titer, 1:1024; stored at −80°C). Each plate was exposed for 10 min at 4°C to 1000 hemagglutinating units of virus diluted in 0.5 ml of phosphate-buffered saline at pH 8. After two rinses with serum-free medium, regular medium (Ephrussi's F12 with 10% fetal calf serum) was added. The formation of multinucleated cells was observed in the mixed cultures and controls. After 24 hr, cells were transferred 1:30 into medium containing the components of the hypoxanthine/aminopterin/thymidine (HAT) selective system (11). The 380 control cultures and unfused 380 cells in the mixed cultures died within 1 week in HAT medium. The mouse T16/Y fibroblasts were senescent and grew poorly. After 2 weeks, 28 distinct, well-growing colonies of cells with hybrid cell morphology (called "primary clones") were isolated from 11 different original fusion plates.

Counterselection. Five independent primary hybrid clones that contained both translocation chromosomes X^T and 16^T and expressed mouse HPRT activity were counterselected in medium containing 20 μM 8-AG after a period of growth in nonselective medium. Fifteen 8-AG-resistant (8-AG^R) colonies were picked individually, in addition to resistant derivative cell populations (both are called 8-AG^R clones).

Chromosome Analysis. Cells were harvested for chromosome studies and chromosomes were banded by a trypsin/Giemsa procedure as described (3). Between 10 and 31 metaphase spreads from each of 10 clones selected in HAT medium and 19 8-AG^R clones were analyzed in detail and the frequency of each mouse chromosome was determined. Scoring was carried out as described in the legend to Table 1.

Electrophoresis. Cells from subcultures parallel to those used for chromosome analysis were stored as pellets at −70°C for later isozyme analysis. Immediately before electrophoresis the cell pellets were resuspended in 100 μl of lysis buffer (10 mM Tris·HCl/140 mM KCl/2.5 mM $MgCl_2$, pH 7.4) and subjected to three cycles of freeze–thaw treatments. The supernatants obtained by centrifugation (10,000 × *g*, 5 min) were used directly or stored at −20°C for later analysis. Chinese hamster and mouse HPRT and adenine phosphoribosyltransferase were examined on polyacrylamide gels as described (12).

SOD isozymes were separated by isoelectric focusing in horizontal-sheet polyacrylamide gels. The 1.5 mm × 18 cm × 18 cm gels contained 4.8% acrylamide, 0.2% *N,N'*-methylenebisacrylamide, 10% (vol/vol) glycerol, 1.2% Ampholines pH 5–7, and 0.8% Ampholines pH 3–10. The gel solution (50 ml) was degassed before the final additions of 200 μl of 10% ammonium persulfate and 40 μl of *N,N,N',N'*-tetramethylethylenediamine. Focusing was performed at 4°C with platinum strip electrodes. The electrode solutions were 0.03 M H_2SO_4 containing 10% glycerol for the anode and 0.06 M NaOH containing 10% glycerol for the cathode. Filter paper wicks (Schleicher & Schuell 470 A-C) cut into 1 × 18 cm strips were wetted with the appropriate electrode solution and placed under the electrode. A 2-hr prerun was performed at a constant power of 2 W. Individual samples (20 μl of supernatant and 10 μl of 2% pH 3–10 ampholytes containing 10% glycerol) were placed on 0.5 × 0.6 cm pieces of filter paper on the surface of the gel 4 cm from the cathode. Electrofocusing was continued for 12–14 hr, initially at a constant power of 2 W and concluding with a constant voltage of 1000 V and decreasing power. A 1-cm lateral strip of the gel was cut into 1-cm pieces and each piece was placed in 2 ml of distilled H_2O and incubated at 4°C for pH determinations.

A modification of the method described by Weisiger and Fridovich (13) was used for the detection of SOD activity. The gel was incubated in two changes of 50 mM pH 7.8 phosphate buffer for a total of 10 min, nitro blue tetrazolium solution (1 mg/ml) for 10 min, and finally 200 ml of 50 mM pH 7.8 phosphate buffer containing 2 mg of riboflavin and 0.65 g of *N,N,N',N'*-tetramethylethylenediamine for 10 min at 24°C with gentle agitation. Areas of SOD activity remained clear after exposure of the gel to fluorescent light.

RESULTS

Characterization of Hybrids. Of 28 primary hybrid clones between mouse T16/Y fibroblasts and HPRT-deficient Chinese hamster cells (380) isolated in HAT selective medium, all but 1 contained mouse HPRT activity. The exceptional clone contained Chinese hamster HPRT, as determined by polyacrylamide gel electrophoresis, and no recognizable mouse chromosomes. It was considered to be a 380 revertant. The others were true hybrids containing a 2*s* set of 380 chromosomes (described in ref. 14) and a large number of mouse chromosomes including both translocation chromosomes X^T and 16^T.

Ten HAT- and 19 8-AG-selected clones were completely karyotyped, once or repeatedly over a prolonged period in culture. A distinct pattern of nonrandom segregation of mouse chromosomes was observed. Chromosome 11 was consistently absent except in two primary clones at the earliest passage. Chromosome 5 was absent from 23 clones and present at low frequencies in 2 others. On the other hand, mouse chromosomes 12, 15, and 17 were clearly favored with an average representation of more than one copy per cell. Furthermore, mouse chromosomes 1, 2, and 18 were present in all hybrid clones examined. Similar observations have been reported with two other sets of hybrids made with derivatives of the same Chinese hamster cell line (3, 15). This phenomenon did not interfere with our present gene mapping efforts.

SOD-1 has a dimeric structure. Extracts of mouse tissue (BALB/3T3 cultured cells and T16/Y homogenized tissues) and of Chinese hamster cells (380) produced a single major band of cytoplasmic isozyme activity; these bands were distinctly separated by isoelectric focusing (Fig. 1, lanes 1 and 2) after attempts to separate them by using published electrophoretic methods had failed. In mouse–hamster hybrids expressing both forms, a single heterodimeric band of activity was seen in addition (Fig. 1, lane 3). Hybrids having lost the mouse chromo-

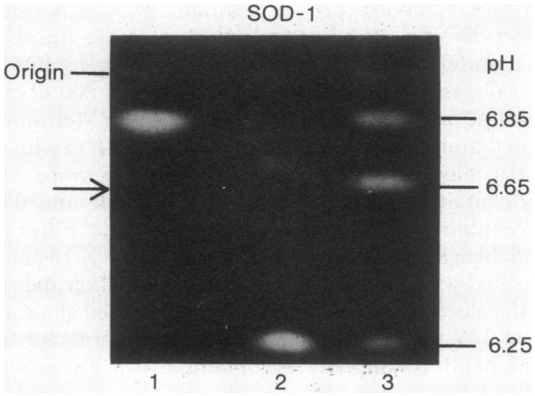

FIG. 1. Isoelectric focusing gel containing tissue extracts from Chinese hamster (380) (lane 1), mouse (BALB/3T3) (lane 2), and mouse–Chinese hamster hybrid (lane 3). The gel was stained for SOD activity. The positions and isoelectric points of the Chinese hamster (6.85), the mouse (6.25), and the mouse–Chinese hamster heterodimer (arrow, 6.65) SOD-1 isozymes are indicated. Only one-fourth of the entire gel length is shown. The additional faint band in lane 2 (at pH 6.7) is the mouse mitochondrial SOD isozyme and is clearly distinguished from the mouse–Chinese hamster heterodimer.

some carrying the gene for SOD-1 produced only the Chinese hamster band.

Assignment of *Sod-1* to Chromosome 16. The occurrence of the mouse band of SOD-1 activity or the mouse–Chinese hamster heteropolymeric band has been compared with the segregation of each mouse chromosome in 29 hybrids (Table 1). The rate of discordancy was 18% or higher for the Y chromosome and for all autosomes except chromosome 16. Because another presumably complete chromosome 16 is contained in the T(X;16)16H translocation, the combined presence of X^T and 16^T was considered equivalent to the presence of a normal 16. The SOD-1 phenotype was found to segregate concordantly with either 16 or X^T and 16^T together. There were no exceptions. These results assign the gene for SOD-1 to either chromosome 16 or X. A normal X chromosome was not present because the hybrid clones were derived from a T16/Y male. By counterselection in 8-AG, we obtained five clones, derived from three independent primary clones, that had lost both T(X;16)16H translocation chromosomes and had retained the normal chromosome 16. All five clones were positive for mouse SOD-1 expression. We therefore feel confident in assigning the gene for SOD-1 to chromosome 16 rather than to the X chromosome. In man and nonhuman primates the gene for SOD-1 is also on an autosome. *Sod-1* is the first biochemical marker gene locus assigned to mouse chromosome 16.

Table 1. Segregation of mouse chromosomes and expression of mouse SOD-1 in T(X;16)16H–Chinese hamster somatic cell hybrids

Chromosome	Total	Number of clones* +/+	+/−	−/+	−/−	% discordancy
1	28	17	11	0	0	39
2	29	18	11	0	0	38
3	28	16	3	2	7	18
4	28	18	8	0	2	29
5	28	3	2	15	8	61
6	28	18	8	0	2	29
7	29	16	8	2	3	34
8	29	17	8	1	3	31
9	29	16	5	2	6	24
10	29	13	9	5	2	48
11	29	2	0	16	11	55
12	29	17	11	1	0	41
13	29	13	3	5	8	28
14	29	17	8	1	3	31
15	29	17	8	1	3	31
17	29	18	11	0	0	38
18	28	18	10	0	0	36
19	29	16	11	2	0	45
Y	28	15	10	3	0	46
(X^T;16^T) or 16	28	18	0	0	10	0

* Shown as ratio of mouse chromosome present/expression of mouse SOD-1. Hybrids were scored "+" if chromosome was present in more than 15% of cells and "−" if chromosome was present in less than 5%. If a chromosome was present in 5–15% of cells, data from this clone were excluded.

Regional Mapping of *Sod-1* to 16B5 → 16ter. Searle's T16 translocation cuts mouse chromosome 16 essentially in half with a breakpoint in band B5 (16). Region cen → B5 with the centromere is contained in the translocation product 16^T, and the distal region B5 → ter is part of X^T. Trypsin/Giemsa banding has allowed a more precise definition of the breakpoints (Fig. 2), but the results are basically consistent with the earlier report based on quinacrine mustard staining (10). Hybrid clones

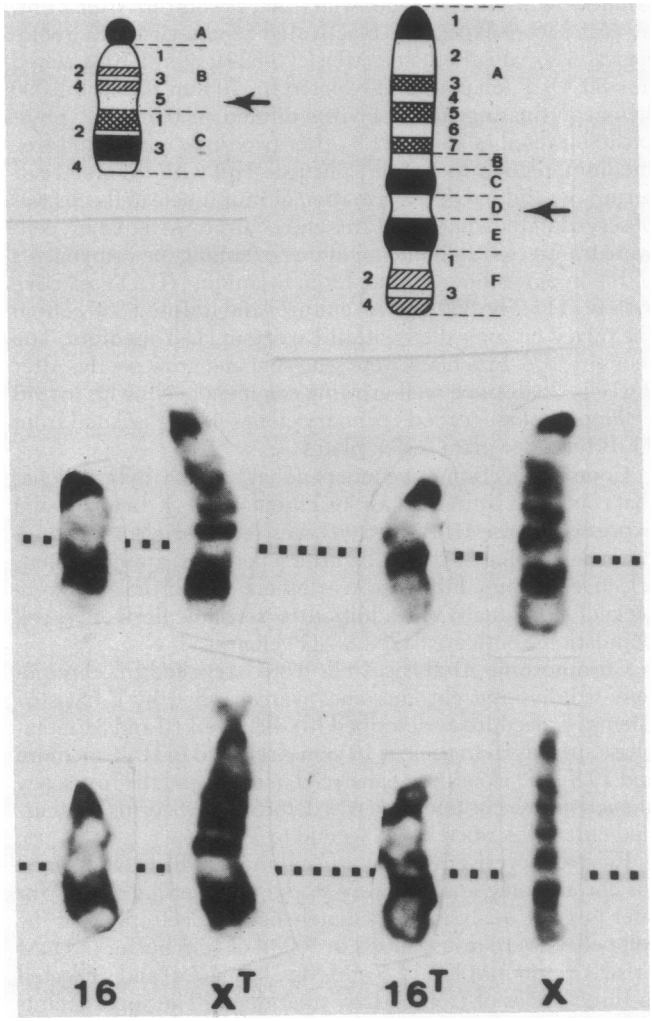

FIG. 2. Searle's T(X;16)16H translocation. Ideograms of mouse chromosome 16 (*Top Left*) and mouse X(*Top Right*) (16). Arrows indicate breakpoints in 16B5 and XD. Translocation products X^T and 16^T result from exchange of the distal regions (below dotted line). Sets of chromosomes 16, X^T, and 16^T are from the same metaphase. The normal X chromosomes are "borrowed" for comparison, because cells in this study were derived from a male T16/Y mouse.

Table 2. Assignment of mouse *Sod-1* to chromosome 16 and regional mapping to 16B5 → 16ter (part of X^T)

No. of hybrid clones (n = 28)	Chromosomes			Mouse SOD-1
	X^T	16^T	16	
7	+	+	+	+
3	−	+	+	+
5*	−	−	+	+
3	+	+	−	+
6†	−	+	−	−
4	−	−	−	−

Scoring for chromosome content was as described in legend to Table 1.

* These five clones are evidence for *Sod-1* assignment to chromosome 16 rather than to X.

† These six clones are informative for regional mapping.

having lost the normal chromosome 16 are informative for regional assignment of genes on chromosome 16, especially after one of the translocation products (X^T) has been selectively eliminated.

Our results with respect to the presence of X^T, 16^T, and 16 in 18 hybrid clones are shown in Table 2. Three primary clones selected in HAT medium contained X^T and 16^T and had lost the normal chromosome 16. Because they expressed mouse SOD-1 activity, we conclude that the break in 16B5 leading to the T16 translocation did not destroy or inactivate the SOD-1 gene. Six 8-AG^R clones derived from two independent primary hybrids had lost X^T and 16 but had retained 16^T in at least 50% of cells (range, 50–90%; mean, 72%). All of these were negative for mouse SOD-1, as were the four controls lacking all three chromosomes of interest. This constitutes evidence for localization of *Sod-1* in region 16B5 → 16ter which is part of X^T rather than in that part of 16 remaining in 16^T. Clones having X^T but no 16 or 16^T would be expected to be positive for mouse SOD-1; however, we did not obtain such a clone. All primary hybrids had both translocation chromosomes, and it was X^T, not 16^T, that was selected against by exposure to 8-AG. Even after prolonged cultivation of hybrids in HAT medium and subcloning, no spontaneous loss of 16^T was observed.

Regional Mapping of *Hprt* on the Mouse X Chromosome. In T16 Searle's translocation, the distal third of the X chromosome (region XD → Xter) has been translocated to chromosome 16^T; the remainder, including the centromere, is contained in X^T. In somatic cell hybrids made with the HPRT-deficient Chinese hamster cell line 380, that part of the mouse X that carries the *Hprt* locus should be retained under HAT selective pressure. In this experiment, all 27 primary hybrids that expressed mouse HPRT, as demonstrated by polyacrylamide gel electrophoresis, had retained both translocation chromosomes. Thus, we decided to select against the HPRT-bearing translocation product by exposure of hybrid cells to the purine analog 8-AG. All 19 8-AG^R clones derived from 5 independent primary clones, had lost X^T, whereas 9 of them had retained 16^T at a frequency of at least 33% (range, 33–90%; mean, 67%), 8 had no 16^T, and 2 had 16^T in between 5 and 15% of cells analyzed and were therefore excluded from studies on X chromosomal regional mapping. These results provide strong evidence for localization of *Hprt* on X^T (region cen → XD).

DISCUSSION

The assignment of *Sod-1* provides a convenient means for the detection of mouse chromosome 16 in interspecific cell hybrids and will promote the assignment of additional genes to this chromosome by demonstration of their concordant segregation with *Sod-1*. It will be of interest to determine whether the mouse homologue of a gene for the interferon receptor that has been assigned to human chromosome 21 is also located on mouse chromosome 16.

The distal mouse chromosome 16 resembles the long arm of human chromosome 21 in that both have a dark G band followed abruptly by a lightly staining region. If that region were conserved, as suggested by regional localization of homologous loci for SOD-1, it might be possible to obtain a mouse with a "Down syndrome equivalent" by breeding appropriate translocation stocks. In the T(2;16)28H translocation the breakpoint on chromosome 16 is distal to that of T16 but the breakpoint on chromosome 2 is near the distal end of the chromosome. It remains to be seen whether viable unbalanced offspring with duplication of the distal region of chromosome 16 can be obtained by the appropriate matings of T(2;16)28H mice.

The work reported here represents an example in the mouse of regional chromosome mapping by somatic cell hybridization of cells carrying a translocation. A large number of mouse translocation stocks are available for this approach. Genetic studies have localized translocation breakpoints and several polymorphic enzyme loci on the linkage map. Genes that can be detected in cultured cells, either by their expression or by nucleic acid hybridization, can be regionally mapped in somatic cell hybrids containing mouse chromosome translocations. Genetic and cytological maps can then be compared with respect to distances between loci and possible influences of translocation breakpoints on recombination. This approach can provide insight into the organization and function of mammalian chromosomes.

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